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Erythrocyte stearidonic acid and other *n*-3 fatty acids and CHD in the Physicians' Health Study

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Abstract

Intake of marine-based *n*-3 fatty acids (EPA, docosapentaenoic acid and DHA) is recommended to prevent CHD. Stearidonic acid (SDA), a plant-based *n*-3 fatty acid, is a precursor of EPA and may be more readily converted to EPA than α-linolenic acid (ALA). While transgenic soyabeans might supply SDA at low cost, it is unclear whether SDA is associated with CHD risk. Furthermore, associations of other *n*-3 fatty acids with CHD risk remain inconsistent. The present ancillary study examined the association of erythrocyte SDA as well as other *n*-3 fatty acids with the risk of CHD. In a prospective nested case–control study of the Physicians' Health Study, we randomly selected 1000 pairs of incident CHD with matching controls. Erythrocyte fatty acids were measured using GC. We used conditional logistic regression to estimate relative risks. Mean age was 68·7 (SD 8·7) years. In a multivariable model controlling for matching factors and established CHD risk factors, OR for CHD for each standard deviation increase of log-SDA was 1·03 (95 % CI 0·90, 1·18). Corresponding values for log-ALA and log-marine *n*-3 fatty acids were 1·04 (95 % CI 0·94, 1·16) and 0·97 (95 % CI 0·88, 1·07), respectively. In conclusion, the present data did not show an association among erythrocyte SDA, ALA or marine *n*-3 fatty acids and the risk of CHD in male physicians.

Key words: Stearidonic acid: *n*-3 Fatty acids: α-Linolenic acid: CHD

Although rates of CHD mortality have decreased substantially over the last 3 decades[1], CHD still remains a major public health problem in the USA and other nations around the world. About 48 % of the reduction in CHD deaths has been attributed to improvement in modifiable lifestyle and dietary risk factors[2]. Therefore, identifying novel lifestyle and dietary factors that could further lower the risk of CHD remains important in CHD prevention. It has been several decades since cardioprotective effects of fish consumption were reported. An inverse association between intake of marine-based *n*-3 PUFA, EPA (20 : 5 *n*-3), DHA (22 : 6 *n*-3), docosapentaenoic acid (22:5 *n*-3), and perhaps the plant-based *n*-3 fatty acid (FA) α-linolenic acid (ALA; 18:3 *n*-3) and CHD has been reported[3–5]. Based on those findings, the American Heart Association recommends consumption of at least two fish meals per week for primary prevention and 1 g of EPA + DHA/d for secondary prevention of CHD[6]. However, findings on the effects of these *n*-3 FA on CHD have not been consistent[7,8]. Stearidonic acid (SDA; 18 : 4 *n*-3) is an *n*-3 FA found in small concentrations in certain plants and may be more readily converted to EPA than ALA[9]. A previous investigation reported that increased intake of SDA may increase erythrocyte EPA concentration[9]. Recently, transgenic soyabeans, which can supply SDA at low cost, have been developed. However, it is unclear whether erythrocyte SDA is associated with a lower risk of CHD. Hence, the present ancillary study examined the association of erythrocyte membrane SDA as well as other *n*-3 FA with CHD risk in a nested case–control study within the Physicians' Health Study (PHS).

Subjects and methods

Study population

The PHS 1 was a randomised, double-blind, placebo-controlled trial designed to test the effects of low-dose aspirin and β-carotene on CVD and cancer among 22 071 US male physicians. The PHS 2 was a randomised trial

Abbreviations: ALA, α-linolenic acid; FA, fatty acid; PHS, Physicians' Health Study; SDA, stearidonic acid.

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designed to test the benefits and risks of vitamins E and C, β-carotene and multivitamins in the prevention of cancer, CVD, age-related eye diseases and cognitive function among 14 642 US male physicians aged ≥50 years at baseline. A detailed description of both studies has been published previously[10,11].

Using a prospective nested case–control design, we randomly selected 1000 incident CHD cases that provided blood samples between 1995 and 2001 for the present ancillary study. For each case, we used a density sampling technique to randomly select one control subject who was alive and free of confirmed CHD at the time of the index case diagnosis and matched on age at blood collection (within 1 year), year of birth (within 2 years) and time of blood collection (within 3 months). Each case was eligible to serve as a control before CHD diagnosis. Similarly, each control was eligible to later become a CHD case to assure that controls were representative of a total population that gave rise to the CHD cases.[12] Ultimately, twenty-seven controls later developed CHD after enrolment and served as cases in the present study. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki. Each participant gave written informed consent, and the Brigham and Women's Hospital (Boston, MA, USA) Institutional Review Board approved the study protocol.

Blood collection and storage

For the present project, blood was collected between 1995 and 2001. Detailed description of methods of blood collection and storage has been published previously[13].

Measurement of erythrocyte fatty acid profiles

Baseline erythrocyte samples from all cases and controls were handled identically throughout sample collection, long-term storage, sample retrieval and assays. All investigators and laboratory personnel were unaware of participants' case–control status. The FA content of erythrocyte membranes was determined as follows: after osmotic haemolysis, the erythrocyte membranes were washed three times with NaCl, an internal standard (heptadecanoate) was added to the cell pellet and total lipids were extracted according to the Folch method[14], followed by saponification and methylation[15]. The resultant FA methyl esters were dried down under N_2, re-suspended in 100 μl of hexane, transferred into amber GC vials and stored at −20°C until the time of analysis[16,17]. The erythrocyte FA methyl esters were analysed using an Autosystem XL gas chromatograph (Perkin Elmer) equipped with a 100 m × 0·25 mm inner diameter (film thickness 0·25 μm) capillary column (SP-2560; Supelco). Peaks of interest were identified by comparison with authentic FA standards (Nu-Chek Prep, Inc.) and expressed as molar percentage proportions of FA relative to the internal standard. Relative values of inter-assay CV were <4·5 % for the *n*-3 FA reported here.

Ascertainment of incidence of CHD

We obtained information on the occurrence of major diseases including CHD through annual follow-up questionnaires. CHD was the primary outcome and was defined as non-fatal myocardial infarction, fatal myocardial infarction, percutaneous transluminal coronary angioplasty, coronary artery bypass graft, coronary death and sudden death. Fatal CHD consisted of fatal myocardial infarction, coronary death and sudden death. All cardiovascular events in the PHS have been adjudicated by an endpoint committee[10]. The diagnosis of myocardial infarction was confirmed by using WHO criteria[18]. Revascularisation procedures were confirmed by hospital records.

Other variables

Information on age, height, body weight, BMI, cigarette smoking, exercise, fish consumption, alcohol consumption, hypertension, diabetes mellitus and hypercholesterolaemia was collected at baseline through annual questionnaires and semi-quantitative FFQ. While the FFQ was not validated in the PHS, it has been validated elsewhere[19].

Statistical analysis

The distributions of each erythrocyte FA were skewed to the right (e.g. SDA: skewness; 3·63, kurtosis; 17·73, Shapiro–Wilk test; $P< W < 0·0001$). Thus, we used the natural logarithm to normalise their distributions and created tertiles of each erythrocyte FA based on the respective distribution in the control series for SDA, ALA and marine *n*-3 FA (sum EPA, DHA and docosapentaenoic acid). Baseline CHD risk factors were compared according to tertiles of each erythrocyte FA, using ANOVA for means and χ^2 tests for proportions. Spearman correlation coefficients between erythrocyte and dietary FA were evaluated. We used conditional logistic regression to compute multivariable-adjusted OR (95 % CI) for CHD for each standard deviation increase of log-transformed FA. The initial model only controlled for matching factors and a final model also controlled for BMI, smoking status (never, current and past smokers), exercise (vigorous exercise <1 time/week, ≥1 time/week), alcohol consumption (<1 time/week, 1–4 times/week, 5–7 times/week, >7 times/week), hypertension, diabetes mellitus and hypercholesterolaemia. In a secondary analysis, we examined the association between erythrocyte FA and fatal CHD. The present ancillary study was designed to have 80 % power to detect a relative risk of 0·74 for the primary outcome (total CHD), with a two-sided α level of 0·05. *Post hoc* power calculations indicated that with 165 fatal CHD events, we had 80 % power to detect a relative risk of 0·50 for CHD death (two-sided $\alpha = 0·05$). All analyses were completed using SAS (version 9.2; SAS Institute). All statistical tests were two-sided and $P<0·05$ was considered significant.

Results

Mean age was 68·7 (SD 8·7) years. Baseline characteristics of the participants by tertiles of each erythrocyte *n*-3 FA are

Table 1. Baseline characteristics of the 2000 study participants according to levels of erythrocyte *n*-3 fatty acids in the Physicians' Health Study
(Mean values and standard deviations)

	Tertiles of SDA				Tertiles of ALA				Tertiles of marine *n*-3			
	1st (*n* 643)		3rd (*n* 706)		1st (*n* 645)		3rd (*n* 686)		1st (*n* 686)		3rd (*n* 650)	
Baseline characteristics	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Erythrocyte SDA (%)	0·01	0·01	0·07*	0·06	0·04	0·06	0·04*	0·04	0·05	0·06	0·02*	0·02
Erythrocyte ALA (%)	0·17	0·05	0·2*	0·09	0·12	0·02	0·26*	0·07	0·2	0·09	0·17*	0·05
Erythrocyte marine *n*-3 (%)	6·37	2	5·27*	1·90	5·9	2·07	5·85*	1·83	4·07	1·12	8·14*	1·29
Age (years)	67·7	9·0	69·1*	8·5	68·5	8·5	68·5	8·9	69·1	8·8	68·7	8·9
BMI (kg/m^2)	25·6	3·5	25·9	3·3	26	3·4	25·7	3·2	26·4	3·6	25·1*	3·0
Energy consumption												
kcal	1715	527	1680	515	1694	511	1679	509	1686	501	1656	519
kJ	7176	2205	7029	2155	7088	2138	7025	2130	7054	2096	6929	2171
Current smoker (%)	3·4		2·7		2·2		2·8		4·4		1·4*	
Past smoker (%)	47·9		46·9		48·8		47·2		46·9		49·9	
Never smoker (%)	48·7		50·4		49·0		50·0		48·7		48·8	
Frequent alcohol drinking, ≥5 times/week (%)	33·9		34·4		33·8		35·0		30·8		37·1*	
Current exercise, ≥1 time/week (%)	64·7		59·6*		57·5		60·6		59·1		62·9	
Fish intake, <1 time/week (%)	24·7		31·6		28·8		27·8		42·7		14·3*	
Fish intake, 1–4 times/week (%)	72·5		66·9		69·8		70·0		56·3		81·5*	
Fish intake, ≥5 times/week (%)	2·8		1·5		1·4		2·2		1·0		4·3*	
History of hypertension (%)	44·8		46·2		43·1		44·9		48·0		43·7	
History of diabetes (%)	10·1		6·9		9·0		9·8		8·5		8·8	
History of hypercholesterolaemia (%)	24·9		23·9		23·1		28·1		21·4		26·6*	

SDA, stearidonic acid; ALA, α-linolenic acid; marine *n*-3, sum of EPA, DHA and docosapentaenoic acid.
* Value was significantly different from that for the 1st tertile ($P<0·05$, ANOVA for continuous variables, and χ^2 test was used for categorical variables).

summarised in Table 1. Erythrocyte marine *n*-3 FA were associated with lower BMI, non-smoking status and frequent alcohol and fish consumption. Erythrocyte marine *n*-3 FA were inversely correlated with erythrocyte SDA and erythrocyte ALA, and Spearman correlation coefficients were −0·27 ($P<0·001$) and −0·05 ($P=0·02$), respectively. In contrast, SDA was positively correlated with ALA (r 0·10, $P<0·001$). The correlation coefficients between dietary marine *n*-3 FA and erythrocyte marine *n*-3 FA were greater than that of ALA. For example, the Spearman correlation coefficient between dietary EPA and erythrocyte EPA was 0·33 (n 1674, $P<0·001$); the corresponding value for DHA was 0·36 (n 1674, $P<0·001$) and was 0·02 (n 1674, $P=0·53$) for ALA.

In a conditional logistic regression adjusting for matching factors, the OR (95 % CI) for CHD for each SD higher log-SDA was 1·04 (95 % CI 0·91, 1·18). The corresponding value per SD increase in log-ALA was 1·06 (95 % CI 0·96, 1·17) and for log-marine *n*-3 FA, it was 0·92 (95 % CI 0·84, 1·00; Table 2). Additional adjustment for BMI, alcohol intake, smoking, physical activity, history of hypertension, diabetes and hypercholesterolaemia did not alter these results (Table 2). Only log-EPA in a model adjusting for matching factors showed significant inverse association with the risk of CHD. However, after adjustment for other risk factors, this association was no longer significant (Table 2). In a secondary analysis, each erythrocyte FA was not associated with fatal CHD (multivariable-adjusted OR (95 % CI) were 1·05 (95 % CI 0·75, 1·45) for each SD increase of log-SDA; 1·19 (95 % CI 0·89, 1·60) per SD increase in log-ALA; and 0·98 (95 % CI 0·76, 1·25) per SD increase in log-marine *n*-3 FA (Table 2). We also did not observe an association between combined erythrocyte EPA + DHA and CHD risk in the present study. Mutual adjustment for other erythrocyte FA (i.e. adjusting for ALA, EPA, DHA and docosapentaenoic acid when assessing association of SDA with CHD) did not alter the results. The OR for CHD for each SD higher log-SDA was 1·03 (95 % CI 0·90, 1·18); the corresponding values were 1·04 (95 % CI 0·94, 1·16) for log ALA and 0·99 (95 % CI 0·94, 1·04) for log-marine *n*-3 FA.

Discussion

Summary of main findings

In the present prospective nested case–control study, we found no evidence for associations between erythrocyte SDA, ALA or marine *n*-3 FA and the risk of total or fatal CHD in the US male physicians.

Erythrocyte membrane stearidonic acid/α-linolenic acid and risk of CHD

To the best of our knowledge, the present study is the first and largest study to assess the association between erythrocyte SDA and the risk of CHD. The lack of a meaningful association between SDA and CHD as well as ALA and CHD merits some comments. The conversion of ALA to EPA requires Δ-6 desaturase, a rate-limiting enzyme[20]. While such a conversion of ALA to EPA in humans is negligible (0·2–7 %), SDA is readily converted to EPA[21–23]. A negative correlation (r −0·27; $P<0·0001$) observed between erythrocyte SDA and marine *n*-3 FA is supportive of this conversion in the present data. Although not readily available, there is a future possibility that consumption of plant-based SDA from genetically engineered soyabean oil may make SDA a cost-effective way to enrich the diet with plant-based *n*-3 FA that can be converted to EPA. A lack of an association between SDA and CHD could be partially explained by the small contribution of SDA to the total erythrocyte membrane FA (range 0·03–0·04 % of total erythrocyte membrane FA). Alternatively, the fact that all participants were male physicians with optimal dietary intake[24] might have made it very difficult to detect a small effect size. It is also possible that SDA may not be associated with CHD risk despite the reported increase in ω-3 index after consumption of SDA[9]. Lastly, we examined total CHD as the primary outcome of the present study which was heavily driven by non-fatal CHD. We cannot exclude differential associations of SDA with other types of CHD. Additional studies are needed to clarify such hypotheses.

Table 2. Total CHD and fatal CHD according to erythrocyte *n*-3 fatty acids per 1 standard deviation increase in each erythrocyte fatty acid in the Physicians' Health Study

(Odds ratios and 95 % confidence intervals)

	Total CHD (n 1000)				Fatal CHD (n 165)			
	Model 1*		Model 2†		Model 1*		Model 2†	
	OR	95 % CI	OR	95 % CI	OR	95 % CI	OR	95 % CI
Per SD increase of								
Log SDA	1·04	0·91, 1·18	1·03	0·90, 1·18	1·04	0·76, 1·41	1·05	0·75, 1·45
Log ALA	1·06	0·96, 1·17	1·04	0·94, 1·16	1·11	0·85, 1·45	1·19	0·89, 1·60
Log marine *n*-3	0·92	0·84, 1·00	0·97	0·88, 1·07	0·91	0·73, 1·14	0·98	0·76, 1·25
Log EPA	0·90	0·81, 0·98	0·94	0·85, 1·03	0·86	0·69, 1·08	0·96	0·74, 1·23
Log DPA	0·92	0·83, 1·01	0·96	0·87, 1·06	0·91	0·72, 1·14	0·99	0·77, 1·27
Log DHA	0·94	0·85, 1·02	0·99	0·90, 1·10	0·98	0·79, 1·22	0·99	0·78, 1·26

SDA, erythrocyte stearidonic acid; ALA, erythrocyte α-linolenic acid; marine *n*-3, sum of erythrocyte EPA, docosapentaenoic acid (DPA) and DHA.

* Conditional logistic regression matched for age, the date of blood kit returned, the same population, the age at blood test.

† Adjusted for BMI, smoking status, exercise level, alcohol consumption, history of hypertension, history of diabetes and history of hypercholesterolaemia.

British Journal of Nutrition

ALA is another plant-based *n*-3 FA that is relatively inexpensive and more widely available than marine *n*-3 FA. However, we did not observe a significant association between erythrocyte ALA and the risk of CHD or fatal CHD. Findings from previous studies do not show a consistent association of ALA with CHD risk[25]. Some investigators have reported that high tissue ALA, including erythrocyte ALA, may be positively associated with CHD risk and sudden death[26]. The reason for such a positive relation with CHD risk is unclear. As the conversion of ALA to EPA and then to DHA depends not only on dietary factors, but also on the metabolic processes of Δ-5 and 6 desaturases, it is possible that variation in erythrocyte ALA may be partially influenced by genetic differences in enzymatic activity of Δ-5/6 desaturases; this may help account for some of the inconsistencies reported on associations of dietary ALA consumption and erythrocyte ALA with CHD risk[26]. Unfortunately, we did not have similar data on the conversion of ALA to EPA in the present study for comparison.

Erythrocyte membrane marine n-3 fatty acids (EPA, DHA and docosapentaenoic acid) and risk of CHD

The present study did not show a significant association between erythrocyte marine *n*-3 FA and the risk of total or fatal CHD. This finding is also consistent with those reported for the Nurses' Health Study[3]. In contrast, several observational studies reported inverse associations between marine *n*-3 FA and either CHD or fatal CHD[27,28]. In an earlier report by Albert *et al.*[27] using the Physicians' Health cohort, an inverse association between marine *n*-3 FA and sudden cardiac death was documented. These apparent divergent findings from the same cohort merit some comments. First, in the present nested case–control study, the focus was on total CHD as the primary outcome and not on sudden death, as in the previous paper by Albert and colleagues. Hence, subjects included in the present paper are different from those included in the earlier PHS paper. Second, sudden death was not a primary outcome for the present analysis and we do not have enough power to detect the association between marine *n*-3 FA and sudden cardiac death due to the small number of sudden deaths in the present study (*n* 41). Third, we used erythrocyte FA in the present study, while Albert *et al.* used whole-blood FA in their study. It has been shown that erythrocyte *n*-3 FA are highly reproducible and will assure accurate exposure classification[29]. Fourth, the time of blood collection in the present study is more than 15 years (maximum 20 years) later than in the earlier PHS paper. It is possible that dietary patterns of the present study population has changed over time, given the American Heart Association guidelines recommending intake of fish and marine *n*-3 FA were announced after Albert's publication[6]. The association of marine *n*-3 FA with CHD could differ by background levels of marine *n*-3 FA in the population. In a Japanese trial (Japanese EPA Lipid Intervention Study; JELIS), where participants consume fish five times more than the US participants and other countries, EPA intervention did not show significant effect on sudden cardiac death, possibly due to high background levels of EPA and other marine *n*-3 FA[30]. The association between marine *n*-3 FA and total CHD, fatal CHD or other cardiovascular outcomes may also differ. Further studies are needed for clarification.

Strengths and limitations

The present study has some limitations. First, we have only one baseline measurement of erythrocyte FA. Thus, we were not able to account for change in erythrocyte FA during the follow-up period. Second, as the present study is an observational study, we cannot exclude residual and unmeasured confounding as a partial explanation of the present findings. Third, the sample used in the present study consists of highly educated male physicians; thus, the findings from the present study may not apply to other socio-economic or ethnic groups and women. Nevertheless, the present study has several strengths, including a large sample size, matching on key confounders to minimise confounding, prospective study design, validation of incident CHD and the use of reproducible biomarkers (erythrocyte FA) to assess *n*-3 FA.

Conclusions

In conclusion, the present findings did not show evidence for an association among erythrocyte SDA, ALA and marine *n*-3 FA and CHD risk in US male physicians.

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