Changes in Erythrocyte Membrane *Trans* and Marine Fatty Acids between 1999 and 2006 in Older Americans[1–3]

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Abstract

Over the last several years, national programs to lower the content of industrially produced (IP) C18:1 and C18:2 *trans* fatty acids in foods have been implemented, but whether this has resulted in lower blood *trans* fatty acid levels is unknown. Likewise, an increased perception of the health benefits of fish oils rich in EPA and DHA may have resulted in an increase in consumption and blood levels of these fatty acids. To explore these issues, we analyzed the changes in RBC fatty acid composition between the 7th (1998–2001) and 8th (2005–2007) examination cycles in a random sample of the Framingham Offspring cohort. This was a retrospective cohort study of 291 participants from whom blood was drawn at both examinations and for whom complete covariate data were available. Overall, the proportion of *trans* fatty acids in RBC changed by −23% (95% CI: −26 to −21%). RBC EPA+DHA proportions increased by 41% (95% CI: 31 to 52%) in 38 individuals who were taking fish oil supplements at examination 8, but in 253 participants not taking fish oil, the proportion of RBC EPA+DHA did not change. In conclusion, in a random subsample of Framingham Offspring participants with serial observations over 6.7 y, the proportion of *trans* fatty acids in RBC decreased. Those of EPA+DHA increased in people taking fish oil supplements. These changes could potentially translate into a lower risk for cardiovascular disease. J. Nutr. 142: 1297–1303, 2012.

Introduction

The 2 types of dietary fatty acids that are likely to affect their blood levels most are the *trans* and long-chain (n-3) fatty acids, in great part because neither is synthesized to a large extent in vivo. *Trans* versions of C18:1 and C18:2 derive largely from industrially produced (IP)[10], partially hydrogenated vegetable oils (1). A small proportion (~20%) of the total *trans* fatty acid intake comes from ruminant-produced (RP) fats (with *trans* C16:1 as a marker) (2). The marine (n-3) fatty acids [EPA,

docosapentaenoic acid, and DHA] are derived almost exclusively from fish and fish oils (3). Both of these classes of fatty acids have been associated with risk for cardiovascular disease: beneficially with the (n-3) class (4) and adversely with industrially produced *trans*-fatty acid (IP-*trans*) (5). The risk posed by ruminant-produced *trans*-fatty acid (RP-*trans*) remains unclear (6). Consequently, changes in blood/tissue levels of these fatty acids may be expected to alter cardiovascular risk, either directly or via changes in surrogate risk markers.

Beginning in the late 1990s, efforts to remove IP-*trans* fatty acids from the food supply were underway in the US (1) and other countries (7), most notably Denmark, where they were effectively banned in the early 2000s (8). The effects that these efforts have had on IP-*trans* fat intakes in the US are unknown due to the inability of major nutrient databases to keep abreast of the constant changes in food composition. However, if blood levels of IP-*trans* fatty acids decreased over the last decade, then it would be reasonable to conclude that the national efforts to remove IP-*trans* fats from the food supply were at least somewhat successful, as suggested by a recent report from the NHANES survey (9).

Just as efforts to decrease *trans* fatty acids in the diet were growing, recommendations to increase the intake of the long-chain (n-3) fatty acids were being made by the AHA (3) and other governmental agencies around the world (10). As with the

trans fatty acids, the most reliable indicator that such advice was being heeded would be changes in circulating levels of the marine (n-3) fatty acids in the population. The RBC membrane has been used by several investigators to quantify relatively long-term dietary exposure to *trans* and marine (n-3) dietary fatty acids (11–13) and has been shown to correlate with outcomes (e. g., sudden cardiac death) better than dietary questionnaire-derived estimates of (n-3) fatty acid intake (13). The purpose of this study was to track serial changes in RBC fatty acid content in a well-characterized cohort of American adults between the late 1990s and mid-2000s to determine the extent to which fatty acid intakes may have changed.

Methods

Study sample. Children (and their spouses) of the original Framingham Heart Study cohort, recruited in 1971, constitute the Framingham Offspring Study (14). For this study, 299 participants were randomly selected who had RBC available at both examination cycles 7 (1998–2001) and 8 (2005–2007). The study was approved by the institutional review board of Boston University Medical Center.

Laboratory methods. Methods for the analysis of non-HDL and HDL cholesterol and creatinine were previously described (14). The fatty acid content of unwashed, packed RBC was measured in cells isolated from blood drawn after a 10- to 12-h fast and frozen at $-80°C$ immediately after collection. Methanol containing 14% boron trifluoride (Sigma-Aldrich) and hexane (EMD Chemicals) were sequentially added (250 μL each) to a 25-μL aliquot of RBC. The vial was briefly vortexed and then placed in an aluminum bead hot bath at 100°C for 10 min. After cooling, 250 μL of HPLC grade water was added, the tubes were recapped, vortexed, and centrifuged for 3 min at 1500 × *g* to separate layers. A 50-μL aliquot of the hexane (upper) layer was transferred to a GC vial. FAME analysis was carried out using a GC2010 Gas Chromatograph (Shimadzu) equipped with a SP2560, 100-m fused silica capillary column (0.25-mm i.d., 0.2-μm film thickness; Supelco). Run conditions were: carrier gas, hydrogen; linear velocity, 22 cm/s; injector temperature, 230°C; oven program, initial temperature of 140°C hold for 5 min, ramp temperature at a rate of 4°C/min to 240°C and hold for 15 min (total run time, 45 min); and detector temperature, 240°C. FAME were identified by comparison with a standard mixture of fatty acids characteristic of RBC (GLC 727, NuCheck Prep), which was also used to determine individual FAME response factors. Fatty acid composition was expressed as a percentage of total identified FAME. The chromatographic conditions used in this study were sufficient to isolate the C16:1 *trans* isomers [marker for RP *trans* (15)] and the C18:2 Δ 9*t*-12*c*, 9*t*-12*t*, and 9*c*-12*t* isomers. However, each individual C18:1 *trans* molecular species (i.e., C18:1 Δ6 thru Δ13) could not be segregated but appeared as 2 blended peaks that eluted just before oleic acid. The areas of these 2 peaks were summed and referred to a C18:1 *trans*. The sum of all C18:2 and C18:1 *trans* species is here termed IP-*trans* (16), although these 2 classes were also separately analyzed due to their potential differential associations with cardiovascular disease (11). The CV were 3% for EPA + DHA and 7% for total *trans* fats.

Diet assessment. (n-3) and *trans* fatty acid intakes at examinations 7 and 8 were estimated by FFQ (17). The validity of this instrument for fatty acid intake has been documented by comparison with adipose tissue fatty acid composition (18,19). A single nutrient database that was released in 2002 was used to calculate the fatty acid composition of diets at both examinations, because 2 separate databases reflecting food composition during 1998–2001 and 2005–2007 did not exist. The composition of foods containing (n-3) fatty acids was largely stable from 1998 to 2007, so using a single nutrient database provided relatively accurate estimates of intake at both examinations (although only the sum of EPA+DHA was available, not the individual fatty acids nor docosapentaenoic acid). However, due to the continual reformulation of cooking fats and oils during the study period in anticipation of new federal labeling regulations, which were to take effect in January 2006, the *trans* fat data are more likely to reflect food composition at examination 7 than at examination 8. Therefore, estimates of the change in *trans* fat intakes between these examinations is likely to be biased and was not attempted; the baseline (1999) intake estimates should be reasonably accurate and are thus reported. Fish oil supplement use was self-reported under Medications as "fish oil" or "omega-3" but not "flaxseed oil" and was available at both examinations.

Statistical methods. Samples from 299 participants who had RBC available at examinations 7 (1998–2001) and 8 (2005–2007) were randomly selected to study temporal changes in fatty acid composition. One participant was excluded due to gastric by-pass surgery between examinations and 7 participants were missing covariates, leaving 291 participants for analyses. Differences between examinations 7 and 8 in fatty acids and patient characteristics were tested using paired *t* test for continuous and McNemar's test for categorical variables.

Changes between examinations 7 and 8 in RBC content of C18:1t + C18:2t (IP-*trans* species) and EPA+DHA [omega-3 index (21)] were analyzed as the dependent variables in adjusted linear models that included age, sex, and the baseline RBC fatty acid level at examination 7. PROC GLMSELECT was used with stepwise variable selection and Schwarz Bayesian Criterion (20) to select the best model (21) from 14 candidate variables (fish oil use at examination 8, changes in the following variables: non-HDL and HDL cholesterol, TG, statin use, dietary fatty acid intake, serum creatinine, diastolic and systolic blood pressure, BMI, glasses of wine per week, initiated hypertension treatment, newly diagnosed diabetes, and smoking cessation). Residual diagnostics were conducted to verify model assumptions.

The critical level α was set to 0.05 for the a priori hypotheses and Bonferroni adjustment was used for univariate differences in participant characteristics (0.05/15 = 0.0033) and individual fatty acids (0.05/22 = 0.0023). Analyses were performed using SAS software (version 9.2; SAS Institute).

Results

Potential explanatory baseline variables (examination 7) and their differences at examination 8 were identified (**Table 1**). At baseline, 97% of the *trans* fatty acid intake was from IP-*trans*. The intake of RP-*trans* did not change (*P* = 0.86), but, as discussed earlier, the change in IP-*trans* intake could not be reliably calculated. The dietary EPA+DHA intake increased by ~20% between the examinations (*P* = 0.002).

At examination 7, IP-*trans* fatty acids constituted 93% of the total RBC *trans* fatty acids (**Table 2**). At examination 8, total *trans* fatty acid content was reduced by 21%, with the IP-subset accounting for 98% of the total change. At examination 7, EPA +DHA accounted for 67% of the total marine (n-3) fatty acids in RBC membranes and 71% of the change in total marine (n-3) fatty acids was from increases in EPA and DHA. The changes in proportions of (n-3) fatty acid between examinations 7 and 8 were driven predominantly by the 38 participants who reported taking fish oil at examination 8. In these participants, the omega-3 index increased by 41% (**Fig. 1**), whereas it did not significantly change in the 253 that were not taking fish oil. The 23% decrease in the proportion of IP-*trans* in RBC was unaffected by fish oil supplementation status (Fig. 1).

With respect to which fatty acids may have replaced those that changed between examinations, the 0.57% decrease in total *trans* fatty acids appears to have been predominately offset by small increases in stearic and oleic acids (Table 2). The 0.52% increase in the marine (n-3) fatty acids appears to have been compensated for by similar decreases in the 20- and 22-carbon, highly unsaturated (n-6) fatty acids.

We further explored factors associated with changes in the IP-*trans* and EPA+DHA using multivariable linear regression and

TABLE 1 Changes in variables potentially affecting proportions of RBC fatty acids in a random sample of the Framingham Offspring cohort between 1996 and 2007[1]

Variable	Examination 7	Examination 8 − Examination 7		
			n initiating/n discontinuing	P value[2]
Statin use, n (%)	58 (20)		70/15	<0.0001
Serum non-HDL-C, $mmol/L$	3.81 ± 0.93	−0.52 ± 1.01		<0.0001
Serum TG, $mmol/L$	1.48 ± 0.81	−0.17 ± 0.71		<0.0001
Serum HDL-C, $mmol/L$	1.45 ± 0.47	0.05 ± 0.26		0.0034
Hypertension treatment, n (%)	97 (33)		60/4	<0.0001
Diastolic, $mm Hg$	75 ± 9.5	−2.6 ± 10		<0.0001
Systolic, $mm Hg$	127 ± 18	2.5 ± 17		0.013
Diabetes, n (%)	31 (11)		16/0	<0.0001
Serum creatinine, $\mu mol/L$	76 ± 16	3.5 ± 14.1		0.0001
Current smoker, n (%)	26 (9)		1/7	0.034
BMI, kg/m^2	28.1 ± 5.1	0.2 ± 2.1		0.13
Wine, $glass/wk$	2.2 ± 3.5	−0.2 ± 2.7		0.22
Fish oil supplementation, n (%)	9 (3)		33/4	<0.0001
Dietary intake,[3] g/d				
16:1 *trans*	0.09 ± 0.05	0.00[4] ± 0.05		0.86
18:1 *trans*	2.00 ± 1.13	—[5]		
18:2 *trans*	0.49 ± 0.26	—		
EPA+DHA	0.30 ± 0.24	0.06 ± 0.29		0.0019

[1] Values are mean ± SD, n = 291 unless otherwise indicated. Age at examination 7 was 62 ± 9 y and the group was 41% male. HDL-C, HDL cholesterol.
[2] Bonferroni significance is 0.05/15 tests or <0.0033.
[3] Dietary intake had ~9 and 15% missing data for individual exams and paired differences, respectively.
[4] <0.01 g/d.
[5] Nutrition database was released in 2002 and dietary intakes of C18 *trans* fats are valid for only examination 7.
due to subsequent reformulation of foods and cooking oils.

allowed the variables listed in Table 1 to enter the models. The changes in the omega-3 index were directly related to fish oil supplementation, dietary EPA+DHA intake, changes in wine consumption, and starting statins and inversely related to baseline EPA+DHA level (**Table 3**). In the adjusted model, the omega-3 index increased by ~2 percentage points for participants who used fish oil supplements at examination 8, regardless of their RBC baseline level. The negative slope estimate for the change in the index compared with baseline values illustrates regression to the mean regardless of supplementation status (**Fig. 2**). Changes in the proportions of IP-*trans* were directly related to baseline age and male gender and to changes in non-HDL cholesterol (non-HDL-C) and were inversely related to changes in HDL-C and baseline IP-*trans* levels (again, regression to the mean) (Table 3). The multivariable models explained 33 and 44% of the variability in changes of IP-*trans* and EPA+DHA, respectively. As noted above, the increases in the omega-3 index were due mainly to the participants who were taking fish oil supplements at examination 8; however, these participants also significantly increased their dietary EPA+DHA by 241 ± 275 mg/d (means ± SD). There was no significant change in dietary EPA+DHA intake in the participants that were not supplementing at examination 8 (30 ± 287 mg/d).

Discussion

In this study, we sought to determine to what extent and in what manner RBC membrane fatty acid composition might have changed over a median 6.7-y period in a random subsample of Framingham Offspring Study participants. We observed a 21% decrease in the proportion of RBC *trans* fatty acids, virtually all (98%) from reduced levels of those *trans* species that are produced by the commercial hydrogenation of vegetable oils. Based on emerging evidence from the 1990s that *trans* fats increased risk for cardiovascular disease (5), the Institute of Medicine of the National Academy of Sciences recommended in 2002 that dietary *trans* fat intake be reduced as much as possible (22). In 2003, the FDA announced, and the press widely reported, that beginning in January of 2006, nutrition labels on packaged foods must list the amount of *trans* fats per serving. Anticipating such a regulation, fat and oil producers had begun to reformulate their products to be able to list "0" on the label's "*trans* fat per serving" line. In mid-2006, the AHA launched a national campaign to educate the public about the health hazards of *trans* fats, and in December of 2006, the New York City Board of Health voted to remove artificial *trans* fats in public restaurants beginning in July of 2007. These official actions were taking place during the time when the Offspring examination 8 blood samples were drawn and likely heightened the public awareness of the dangers of consuming *trans*-fat rich foods. But, as noted earlier, the food industry had for many years been reformulating their products in order to be able to comply with the impending regulations. Hence, our findings of a 21% reduction in RBC levels of IP-*trans* fatty acids [along with similar findings from NHANES (9)] suggest that these cumulative efforts were at least somewhat successful. The lack of meaningful change in the RP-*trans* species (−0.01 percentage points, a 6% decrease) similarly suggests that the reductions in total *trans* were due to reformulations of foods with partially hydrogenated oils, not reductions in the intake of dairy products or meat.

Baseline RBC levels of *trans* fats were similar in this study to those reported in the Nurses' Health Study in samples collected between 1989 and 1990 (23) and in the King County Primary

TABLE 2 Changes in proportions of RBC fatty acids in a random sample of the Framingham Offspring cohort between 1999 and 2006[1]

Fatty acid	Examination 7	Examination 8 − Examination 7 paired change	
	% total fatty acids		P value[2]
18:1 *trans*	2.15 ± 0.63	−0.47 ± 0.53	<0.0001
18:2 *trans*	0.35 ± 0.09	−0.09 ± 0.10	<0.0001
16:1 *trans*	0.18 ± 0.04	−0.01 ± 0.05	0.0019
20:5(n-3)	0.60 ± 0.28	0.17 ± 0.43	<0.0001
22:5(n-3)	2.59 ± 0.34	0.15 ± 0.48	<0.0001
22:6(n-3)	4.76 ± 1.31	0.20 ± 1.15	0.0030
14:0	0.34 ± 0.09	−0.04 ± 0.11	<0.0001
16:0	21.5 ± 1.27	−0.13 ± 1.22	0.067
18:0	17.7 ± 0.92	0.18 ± 1.04	0.0030
24:0	0.44 ± 0.12	−0.01 ± 0.19	0.43
16:1	0.38 ± 0.18	0.01 ± 0.20	0.21
18:1	13.8 ± 1.02	0.18 ± 0.88	0.0006
20:1	0.23 ± 0.04	0.04 ± 0.10	<0.0001
24:1	0.43 ± 0.13	0.01 ± 0.20	0.31
18:3(n-3)	0.20 ± 0.09	−0.02 ± 0.13	0.013
18:2(n-6)	11.0 ± 1.46	0.28 ± 1.90	0.012
18:3(n-6)	0.06 ± 0.03	0.04 ± 0.09	<0.0001
20:2(n-6)	0.29 ± 0.04	−0.01 ± 0.04	0.015
20:3(n-6)	1.65 ± 0.36	−0.06 ± 0.22	<0.0001
20:4(n-6)	16.8 ± 1.31	−0.23 ± 1.38	0.0056
22:4(n-6)	3.83 ± 0.77	−0.17 ± 0.71	<0.0001
22:5(n-6)	0.70 ± 0.17	−0.05 ± 0.17	<0.0001

[1] Values are mean ± SD, n = 291.
[2] Bonferroni significance is 0.05/22 tests or <0.0023.

Cardiac Arrest study in samples collected between 1988 and 1999 (11). The only other study that has tracked *trans* fat levels in biological samples during this period of heightened concern about (and regulation of) *trans* fats was from Canada. Similar to the decrease observed here, levels of *trans* fatty acids in human breast milk decreased by 35% between 1998 and 2005 (24). Likewise, there was a decrease in adipose tissue *trans* fat levels documented by Clifton et al. (25) in Australia after a reformulation of vegetable oils in the mid 1990s.

It has been estimated that reducing U.S. *trans* fat intakes from ~2% of energy to 1% could reduce the proportion of preventable coronary heart disease events by >10% (5). The mechanisms responsible could be via effects on serum lipids, inflammatory markers, adipose tissue metabolism, and endothelial function, as outlined by Mozaffarian et al. (5). Non-HDL-C (directly) and HDL-C (inversely) were selected as predictor variables into the IP *trans* model (Table 3). However, the converse was not true, i.e., these changes in lipoprotein cholesterol were better explained by other known factors (e.g., changes in statin use, TG, BMI, and the prevalence of diabetes) than by changes in IP-*trans* levels (**Supplemental Table 1**).

Proportions of marine (n-3) fatty acids in RBC did not change between examinations in those participants who did not start taking fish oil supplements, increased substantially in those who did, and decreased in the 4 who stopped, confirming the reliability and modifiability of this marker. The percent of the U.S. population reportedly taking fish oil supplements increased from ~2% in the late 1990s to 14% in 2006 (26), which is generally consistent with our observation that the proportion of Framingham participants who reported taking supplements increased from 3 to 12% between 1999 and 2006. The 40% increase in the omega-3 index in those who started taking fish oil supplements

was due to a combination of supplementation and concomitantly increasing dietary intake of EPA+DHA. It is not surprising that those who chose to begin fish oil supplementation also chose to add more fish to their diets. Initiating statin use was associated with an increase in the omega-3 index independent of
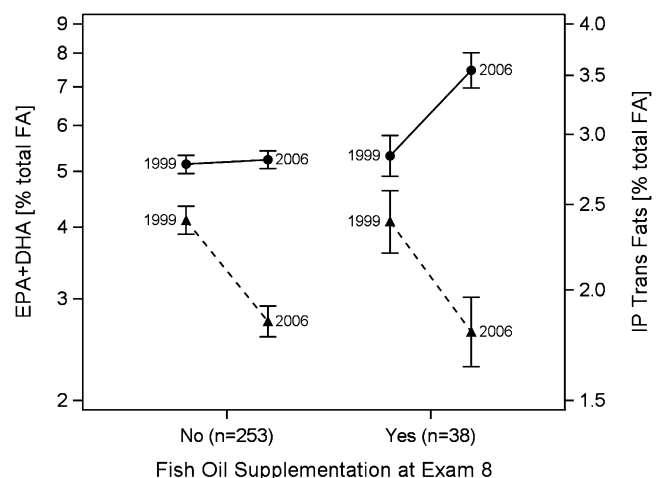

FIGURE 1 Changes between 1999 and 2006 in proportions of IP-*trans* (C18:1t+C18:2t, dashed lines) and EPA+DHA (solid lines) in RBC from a random sample of the Framingham Offspring cohort by use of fish oil supplements at examination 8. Values are geometric mean (95% CI). For IP-*trans*, the −23% (−26 to −21%; P < 0.0001) was independent of fish oil use. For EPA+DHA, levels increased by 41% (95%CI: 31 to 52%; P < 0.0001) in those supplementing at examination 8 but did not change (P = 0.46) in those not taking fish oil. IP-*trans*, industrially produced *trans*-fatty acid.

TABLE 3 Variables associated with changes in proportions of RBC fatty acids in a random sample of the Framingham Offspring cohort between 1999 and 2006

	Estimate	SE	P value	Lower	Upper
				95% CI	
18:1 *trans* + 18:2 *trans* (n = 291)					
Sex (male)[1]	0.13	0.06	0.029	0.01	0.25
Age (10 y)[1]	0.10	0.03	0.0007	0.04	0.16
Baseline RBC 18:1*t* + 18:2*t*[1]	−0.46	0.04	<0.0001	−0.54	−0.38
Change in non-HDL-C (1 SD = 1 mmol/L)	0.09	0.03	0.0032	0.03	0.15
Change in HDL-C (1 SD = 0.26 mmol/L)	−0.07	0.03	0.0085	−0.13	−0.02
EPA + DHA (n = 246)[2]					
Sex (male)[1]	0.15	0.14	0.30	−0.13	0.42
Age (10 y)[1]	0.01	0.08	0.94	−0.14	0.16
Baseline RBC EPA + DHA[1]	−0.31	0.05	<0.0001	−0.40	−0.22
Fish oil supplementation at examination 8	2.04	0.20	<0.0001	1.64	2.45
Change in EPA+DHA dietary intake (1 SD = 294 mg)	0.22	0.07	0.0024	0.08	0.36
Started statins	0.48	0.16	0.0031	0.16	0.80
Change in drinking wine (+1 glass/wk)	0.06	0.03	0.016	0.01	0.11

[1] Forced into Multivariable Linear Regression Models.
[2] The sample size was reduced from 291 to 246 due to missing dietary information.

changes in supplement use or fish consumption. Rise et al. (27) reported that in vitro incubation with statins increased levels of EPA and DHA in a human acute leukemia cell line, suggesting increased desaturase activity, but this was not observed in hepatocytes. In humans, statin treatment did not affect EPA or
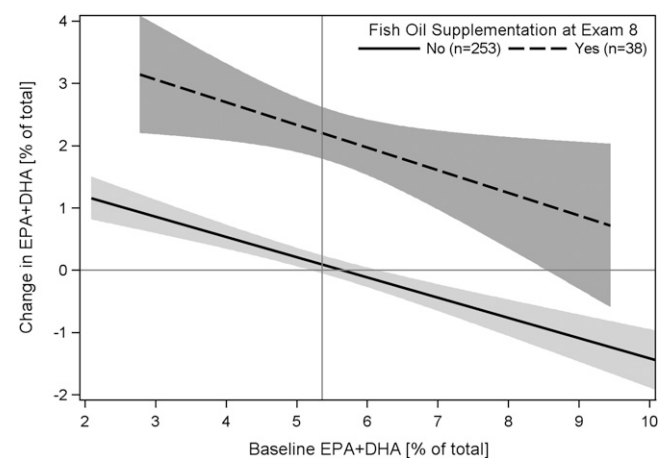

FIGURE 2 Changes from 1999 to 2006 in proportions of EPA+DHA in RBC relative to proportions at baseline in a random sample of the Framingham Offspring cohort by use of fish oil supplements at examination 8. Linear regression with 95% CI bands shows that EPA+DHA increased by 2.04 (1.64, 2.45; P < 0.0001) percentage points for participants who used fish oil supplements at examination 8, regardless of their baseline level. The slope estimate for the baseline value of −0.31 (−0.40, −0.22; P < 0.0001) shows that proportions of EPA+DHA tended to increase in those with low levels at baseline and tended to decrease in those with high baseline levels (i.e. regression to the mean).

DHA levels in plasma cholesteryl esters, but it did increase arachidonic acid levels (28). The effect of statins on RBC marine fatty acid levels has not been reported. Another factor independently associated with an increase in the omega-3 index was increased wine intake. This confirms the association observed between wine intake and both plasma (29) and RBC (30) EPA+DHA levels in 2 European cohorts. Whether unmeasured confounding factors or some biological mechanism is responsible for this association is not clear.

We observed no effect of aging per se on the omega-3 index. Previous cross-sectional studies reported significant direct relations between these fatty acids and age, even when controlling for the intake of oily fish and fish oil supplements (31–38). Although a biological explanation for this phenomenon is possible (e.g., continued tissue accretion even with small intakes), it may also be explained by "the attrition of the susceptible," i.e., because a lower (n-3) fatty acid content has been associated with increased risk for death in both randomized trials (39–41) and prospective cohort studies (42–44), older cohorts would ordinarily be enriched with individuals having higher concentrations. Hence, aging per se does not raise blood (n-3) fatty acid concentrations but higher concentrations may promote longevity (43,45).

With the increase in RBC (n-3) fatty acids, we found an associated decrease in long-chain (n-6) fatty acids consistent with the inhibition of Δ-5 desaturase by EPA (46), which results in a presumed substitution of an (n-3) for an (n-6) fatty acid in the sn-2 position of membrane phospholipids.

The omega-3 index is highly correlated with cardiac EPA+DHA levels (47,48) and have been proposed as a potential new risk factor for coronary heart disease mortality (49). Indeed, RBC fatty acid patterns were recently reported to be superior to the standard risk factors in discriminating acute coronary syndrome patients from controls (50). The 2.2-percentage point increase in the omega-3 index in those who supplemented might be expected to reduce risk for sudden cardiac death by ~50% based on prospective data from the Physicians' Health Study (44). The potential effects of a change in RBC *trans* fat levels (particularly C18:2 *trans*) on risk for sudden cardiac death may be estimated from the King County Study to be of a similar magnitude (11). Hence, the RBC fatty acid changes observed in the Framingham study, both *trans* and (n-3), may have substantial clinical relevance. Because proportions of both (n-3) and *trans* fatty acids in RBC are influenced by dietary intake, the observations made here indicate that the fatty acids consumed changed in Framingham Offspring participants between 1999 and 2006. For the (n-3) fatty acids, these changes were intentional (initiation of fish oil supplementation, choosing to eat more fish); however, for *trans* fatty acids, the changes were most likely unintentional and resulted from shifts in the fatty acid composition of the conventional food supply.

Limitations

This study was conducted with a small (~10%) but random subset of the Framingham Offspring population. Accordingly, we did not discuss changes in traditional risk factors, which may not be representative of the full cohort. As originally constituted, this cohort was ethnically homogeneous and thus our findings may not be applicable to non-white groups. In addition, the participants may be unlike other cohorts of similar age and race owing to their potentially heightened attentiveness to healthy lifestyles as a result of having participated in extensive examinations every 4–8 y as part of this well-known study. Indeed, the

mean intake of marine (n-3) fatty acids in this cohort was ~300 mg/d, which is higher than that in several other cohorts [165 mg/d (51), 188 mg/d (52), 214 mg/d (53), 226 mg/d (54), and 231 mg/d (55)], but lower than that in the Cardiovascular Health Study [452 mg/d (56)]. We could not explore in more detail which fatty acids may have replaced the *trans* and marine (n-3) fatty acids, because the sample size is small (~300) for multivariate analysis considering a 22-dimensional fatty acid profile and the changes were small on the order of 0.5% percentage point.

In conclusion, we observed that RBC *trans* fat levels decreased in a random sample of the Framingham cohort in parallel with national efforts to reduce the *trans* fatty acid content of the American diet. The omega-3 index was unchanged in participants who did not change their intake of marine (n-3) fatty acids but rose substantially with increased intakes from diet and supplements. Taken together, these 2 changes in fatty acids would suggest a potential for reduced risk for coronary heart disease in this community-based sample.

Acknowledgments

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