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Omega-3 fatty acids are associated with a lower prevalence of autoantibodies in shared epitope-positive subjects at risk for rheumatoid arthritis

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Abstract

Objectives—Previously, we found that omega-3 fatty acids (n-3 FAs) were inversely associated with anti-cyclic citrullinated peptide (anti-CCP) positivity in participants at risk for future

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Competing interests None declared.

Patient consent Obtained.

Ethics approval The study protocol was approved by the following institutional review boards (IRBs) at each SERA site: Colorado Multiple IRB, University of Nebraska Medical Center IRB, Benaroya Research Institute at Virginia Mason IRB, Cedars-Sinai Medical Center's IRB, North Shore-LIJ IRB and the Chicago Biomedicine IRB.

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rheumatoid arthritis (RA). We investigated whether n-3 FAs were also associated with rheumatoid factor (RF) positivity and whether these associations were modified by shared epitope (SE) positivity.

Methods—The Studies of the Etiology of RA (SERA) cohort includes RA-free participants who are at increased risk for RA. We conducted a nested case-control study (n=136) to determine the association between RF and anti-CCP2 positivity and n-3 FA percentage in erythrocyte membranes (n-3 FA% in red blood cells (RBCs)). Additionally, in the baseline visit of the SERA cohort (n=2166), we evaluated the association between reported n-3 FA supplement use and prevalence of RF and anti-CCP2. We assessed SE positivity as an effect modifier.

Results—In the case-control study, increasing n-3 FA% in RBCs was inversely associated with RF positivity in SE-positive participants (OR 0.27, 95% CI 0.10 to 0.79), but not SE-negative participants. Similar associations were seen with anti-CCP2 positivity in SE-positive participants (OR 0.42, 95% CI 0.20 to 0.89), but not SE-negative participants. In the SERA cohort at baseline, n-3 FA supplement use was associated with a lower prevalence of RF positivity in SE-positive participants (OR 0.32, 95% CI 0.12 to 0.82), but not SE-negative participants; similar but non-significant trends were observed with anti-CCP2.

Conclusions—The potential protective effect of n-3 FAs on RA-related autoimmunity may be most pronounced in those who exhibit HLA class II genetic susceptibility to RA.

INTRODUCTION

Multiple studies demonstrate that in a majority of patients with seropositive rheumatoid arthritis (RA) there appears to be a period of disease development that is characterised by elevated circulating autoantibodies, including rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP), prior to the development of clinically apparent synovitis.¹⁻⁶ These studies suggest an emerging disease paradigm where both genetic and environmental factors initiate a preclinical autoimmune state, perhaps initiated at mucosal sites,^{7,8} followed by ongoing genetic and environmental factors that propagate this preclinical autoimmune state to clinically apparent RA.⁹⁻¹²

The strongest genetic predictor of seropositive RA is the shared epitope (SE) defined by human leucocyte antigen - antigen D related (HLA-DR) alleles.¹³⁻¹⁵ As genetic susceptibility does not entirely predict who will develop RA,¹⁶ environmental factors likely contribute to a meaningful proportion of remaining risk. Cigarette smoke, a well-known risk factor for RA,^{17,18} interacts with SE to modify the risk for seropositive (anti-CCP/RF) RA,¹⁸⁻²² where the strongest association was observed in SE-positive smokers.¹⁸⁻²² Furthermore, environmental factors like smoking may be more important in the initial generation of anti-CCP, while SE may be more important on the transition from anti-CCP positivity to clinically apparent RA.²³ Exploration of additional interactions between genes and environmental factors could offer clues to underlying mechanisms of RA pathogenesis and discovery of potential preventive factors.

Omega-3 fatty acids (n-3 FAs) could be a preventive factor, given their anti-inflammatory properties.²⁴⁻²⁸ Epidemiological studies report a significant inverse association between fatty fish consumption and RA,²⁹⁻³¹ suggesting that longer-chain n-3 FAs found in fatty fish

(eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) may protect against RA development. In support of this hypothesis, a case-control study found RA cases had significantly lower levels of EPA, and EPA+DHA in their red blood cell (RBC) membranes (a biomarker of n-3 FA status) compared with controls.³² Furthermore, we found both n-3 FA supplement use and n-3 FA levels in RBC membranes were inversely associated with anti-CCP2 positivity in individuals without RA, but at risk for future RA.³³ However, it remains unclear whether n-3 FA exposure is associated with other RA-related autoantibodies, such as RF, and whether the association between n-3 FAs and RA-related autoantibodies is modified by SE, similar to the effect modification observed between SE and smoking on RA.

We used a nested case-control design within the Studies of the Etiology of RA (SERA) cohort to identify associations between the biomarker, n-3 FA % in RBCs, and RF and anti-CCP2 status. We then used the entire SERA cohort to determine whether reported n-3 FA supplement use was associated with the prevalence of RF and anti-CCP2 at baseline. In both study designs, we evaluated SE as an effect modifier.

METHODS

Description of the SERA cohort

SERA is a multicentre prospective study following participants who do not have RA, but are at increased risk for RA. Participants were enrolled between 2002 and 2012 from two at-risk populations. The first at-risk group consisted of 1769 first-degree relatives (FDRs) of probands with 1987 American College of Rheumatology (ACR) classifiable RA³⁴ or had been diagnosed by a board-certified rheumatologist. FDRs were recruited from study sites located in Denver, Seattle, Nebraska, New York City, Los Angeles and Chicago. The second at-risk group consisted of 634 parents of children who participated in the Diabetes Autoimmunity Study in the Young (DAISY); these children were recruited in Denver based on possessing type 1 diabetes risk alleles, which includes HLA-DR4. Thus, the parents of DAISY children have a higher proportion of the RA risk allele HLA-DR4. All participants (FDRs and DAISY) received a 68-count joint examination performed by trained study personnel,³⁵ with no evidence of RA by 1987 ACR criteria³⁴ at their baseline study visit, where they are given questionnaires to assess environmental factors and are tested for anti-CCP2 and RF. Following their baseline visit, SERA participants are informed of their autoantibody status and are invited to return for follow-up visits for further assessment of environmental factors, anti-CCP2 and RF autoantibodies, and joint signs.

SE positivity was determined by screening participant DNA for HLA-DR4 and HLA-DR1 subtypes using PCR primers at the Benaroya Research Institute at Virginia Mason in Seattle. The HLA-DR4 subtypes considered as SE positive included DRB1*0401, *0404, *0405, *0408, *0409, *0410, *0413, *0416, *0419 and *0421. The HLA-DR1 subtypes considered as SE positive included DR1*0101, *0102, *0104, *0105, *0107, *0108 and *0111. A participant who is positive for at least one DR4 or DR1 subtype was considered SE positive.

Anti-CCP2 was measured in serum using ELISA kits (Diastat; Axis-Shield, Dundee, UK), where positivity was defined as >5 units. RF was measured by nephelometry (Dade Behring,

Newark, Delaware, USA) and reported in IU/mL. Positivity for RF was based on 1987 ACR recommendations,³⁴ using a cut-off level higher than that observed in 95% of 490 randomly selected blood donor controls from the Denver area.

Nested case–control study

Selection of cases and controls—Our original nested case–control study consisted of 30 anti-CCP2-positive cases and 47 controls negative for RF and anti-CCP2, detected either at baseline or during follow-up of the SERA cohort.³³ We selected an additional 27 SERA participants who were positive *only* for high titre RF by nephelometry (ie, a titre of >50 IU/mL), which was more than two times the standard SERA cut-off, and which has been shown to be a strong predictor for future RA.³⁶ We also selected an additional 10 anti-CCP2-positive cases for a total of 40 anti-CCP2-positive cases; cases could be positive for RF as well. To our original controls, we added 22 RF and anti-CCP2-negative controls from the SERA at-risk population who were frequency matched to the new cases on age at visit, sex, race/ethnicity and study site, for a total of 69 controls.

Measurement of n-3 FA biomarker—Measurements of n-3 FAs in RBC membranes (n-3 FA% in RBCs) were obtained for all participants in the nested case–control study as a biomarker of fatty acid status/exposure that captures both dietary intake and physiological processes (ie, conversion to longer-chain FAs) and serves as a longer-term measure of n-3 FAs as RBCs exhibit an in vivo life span of ~120 days.³⁷ RBCs were separated within 30 min of blood draw, flash frozen in liquid nitrogen and stored at –70°C. In anti-CCP2 and RF cases, the RBC sample from the first visit where autoantibody positivity was detected was selected. In controls, the RBCs sample collected from the frequency-matched age at visit sample was selected. Samples were sent to the University of Florida Analytical Toxicology Core Laboratory, where lipids were extracted for measures of the FAs present. Samples were methylated and analysed by gas chromatography (Hewlett-Packard 6890; Agilent, Santa Clara, California, USA) with mass spectral detection (Hewlett-Packard 5973), with performance monitored using an internal standard. Triplicate analysis of a composite control RBC sample yielded coefficients of variation <15% for all analytes. Fatty acids were measured as a percentage of the total lipid weight in the RBC sample (($\mu\text{g FA}/\mu\text{g total lipid}$) $\times 100$). The n-3 FAs analysed included 18:3n-3 alpha-linolenic acid (ALA), 20:5n-3 (EPA), 22:5n-3 docosapentaenoic acid (DPA) and 22:6n-3 (DHA), and a summed total of the aforementioned n-3 FAs (total n-3 FA% in RBCs). In addition, we created an n-3 FA% in RBCs variable that summed EPA and DHA, which are the two most common types of n-3 FAs found in fatty fish and n-3 FA supplements.

Statistical analyses of nested case–control study—To assess the association between n-3 FA% in RBCs and the autoantibody outcomes, we used multinomial logistic regression, allowing us to estimate the association between total n-3 FA% in RBCs and autoantibody status in one model, while treating RF and anti-CCP2 positivity as separate outcomes relative to the control group. Subsequent analyses were performed on individual FA to determine whether the effect on autoantibody positivity was stronger for certain FAs. The multinomial models were adjusted for the frequency-matched variables of age, sex, race/ethnicity and site to account for the frequency-matched design.^{38,39} Adjustment for

current smoking status was done due to its well-known association with RA.²¹ Income and education were included in the model as precision variables (ie, decreasing the standard error of the primary association) and are both associated with risk of developing RA.⁴⁰ All n-3 FA% in RBCs were standardised for comparisons across FAs, where the OR is calculated for a 1 SD increase in FA.

In order to test for effect modification by SE status, we included interaction terms between SE and n-3 FA% in RBCs. As we detected a significant interaction between SE and n-3 FA % in RBCs for RF, results were stratified by SE status for both RF and anti-CCP2 for comparison.

Analysis of n-3 FA supplement use in SERA cohort at baseline visit

Description of the SERA cohort at baseline—To address the association between n-3 FA supplement use and prevalence of autoantibody positivity, we limited the SERA cohort to the baseline study visit to reduce bias that may be introduced by changes in behaviour resulting from participants' knowledge of autoantibody status. In total, 2159 participants (592 DAISY parents, 1567 FDRs) had complete information on n-3 FA supplement use, SE, smoking, race/ethnicity, education and income at baseline.

Definition of prevalent autoantibody positivity—Participants were considered prevalent for anti-CCP2 and/or RF if they tested positive for either autoantibody at their baseline visit. Positivity for anti-CCP2 was defined as >5 units; participants could be positive for RF as well. Positivity for RF by nephelometry was defined using the standard >95% cut-off established in general population controls; participants could only be positive for RF.

n-3 FA supplement use measurement in the cohort—Participants were asked at baseline if they took dietary supplements within the past year, with specific questions about use of 'fish oil, fish liver oil or n-3 FAs' or use of multivitamins that contained n-3 FAs in the past year. Our variable of interest was n-3 FA supplement use in the past year (yes vs no).

Statistical analyses for SERA cohort at baseline—To analyse the outcomes of RF and anti-CCP2 positivity, we used multinomial logistic regression analysis, where we included an interaction term for n-3 FA supplement use and SE status, adjusting for age at visit, sex, race/ethnicity, site, current smoking status, education and income. As in the nested case-control analysis, we detected a significant interaction between SE and n-3 FA supplement use for prevalence of RF positivity; therefore, results were stratified by SE status for both prevalent RF and anti-CCP2 positivity for comparison. All statistical analyses were performed using SAS software, V.9.3.

RESULTS

Analysis of a biomarker of n-3 FA status in a nested case-control study

No significant differences in descriptive characteristics were found between groups in the nested case-control study (table 1). Interaction analysis indicated that SE was a significant

effect modifier of the association between increasing total n-3 FA% in RBCs and high titre RF positivity (interaction $p=0.04$) (table 2). In SE-positive participants, RF cases were more likely to have lower levels of total n-3 FA% in RBCs compared with controls (OR 0.26, 95% CI 0.09 to 0.77, $p=0.02$), while in SE-negative participants, no association was observed between total n-3 FA% in RBCs and RF (OR 0.92, 95% CI 0.48 to 1.77, $p=0.81$) (table 2). Similar to our previous report,³³ anti-CCP2 positivity was inversely associated with increasing total n-3 FA% in RBCs (OR 0.59, 95% CI 0.36 to 0.96) and EPA+DHA% in RBCs (OR 0.56, 95% CI 0.34 to 0.92) overall. However, when we examined effect modification by SE, we observed increasing total n-3 FA% in RBCs to be inversely associated with anti-CCP2 positivity in SE-positive participants (OR 0.44, 95% CI 0.21 to 0.93, $p=0.03$), but not in SE-negative participants (OR 0.77, 95% CI 0.41 to 1.45, $p=0.42$) (table 2).

Mean levels of total n-3 FA% in RBCs stratified by autoantibody and SE groups supported these findings, where the lowest levels were observed in participants who were anti-CCP2 and SE positive, as well as participants who were RF positive and SE positive (see online supplementary figure S1). Furthermore, while not statistically significant, mean total n-3 FA% in RBCs were lowest in participants positive for both anti-CCP2 and RF (see online supplementary figure S2).

Analysis of n-3 FA supplement use in SERA cohort at baseline

There were significant differences in race/ethnicity, education and income by autoantibody positivity status at baseline (table 3).

The association between reported n-3 FA supplement use and prevalence of RF was modified by SE positivity (interaction $p=0.02$), where supplement use was associated with a lower prevalence of RF positivity in SE-positive participants (OR 0.32, 95% CI 0.12 to 0.82, $p=0.02$), but not in SE-negative participants (OR 1.21, 95% CI 0.63 to 2.31, $p=0.56$) (table 4).

The effect of n-3 FA supplement use on prevalence of anti-CCP2 positivity was not significantly modified by SE positivity (interaction $p=0.13$); however, reported n-3 FA supplement use was marginally associated with a lower prevalence of anti-CCP2 positivity in SE-positive participants (OR 0.26, 95% CI 0.06 to 1.13, $p=0.07$), but not in SE-negative participants (OR 1.11, 95% CI 0.35 to 3.51, $p=0.86$) (table 4).

DISCUSSION

Building upon our previous findings demonstrating an inverse association between n-3 FA and anti-CCP2 positivity in a population at risk for RA,³³ our current results show that n-3 FAs are also inversely associated with RF positivity, suggesting n-3 FAs may have a broader impact on RA-related autoimmunity in general. Furthermore, the association between n-3 FAs and RA-related autoimmunity was strongest in SE-positive participants.

RF positivity was inversely associated with both total n-3 FA% in RBCs in the nested case-control study and n-3 FA supplement use in the SERA cohort at baseline, and SE was a

significant effect modifier in both studies. Results were consistent across studies even with different definitions of RF positivity. For the nested case-control study, we used a definition for RF positivity (>50 UI/mL) that is more predictive of future RA.³⁶ For the SERA cohort baseline analysis, we used the standard cut-off for RF positivity, which is also predictive of future RA and reflects what would be used in standard clinical practice.

As for n-3 FA% in RBCs and anti-CCP2, we observed an outright inverse association with anti-CCP2 positivity and n-3 FA, similar to our previous findings.³³ However, the observed interaction between n-3 FAs and SE on RF positivity prompted evaluation of a similar interaction with anti-CCP2. While the interaction term was not significant, we observed increasing n-3 FA% in RBCs were associated with a lower prevalence of anti-CCP2 positivity in SE-positive, but not SE-negative participants. The likely explanation for a non-significant interaction term between n-3 FA% in RBCs and SE on anti-CCP2 positivity was that the difference in the point estimates in the SE strata was not as pronounced as with RF positivity.

Likewise, while we did not observe significant associations with anti-CCP2 in the SERA cohort, trends were consistent overall, where n-3 FA supplement use was associated with a lower prevalence of anti-CCP2 in SE-positive, but not SE-negative participants. The likely explanation for marginally significant findings between n-3 FA supplement use and anti-CCP2 positivity is the small number of participants who were anti-CCP2 positive, as over twice as many participants were RF positive.

Similar associations observed between n-3 FA and both anti-CCP2 positivity and RF positivity suggest a protective role of n-3 FAs against RA-related autoimmunity in general. We were unable to determine whether the association between n-3 FAs and autoantibodies might be driven by participants positive for both anti-CCP2 and RF (see online supplementary figure S2); larger studies of at-risk subjects will be necessary in order to tease apart this relationship. Given the numerous ways n-3 FAs affect inflammation and the immune system,^{24–26} it is not surprising to see similar associations between anti-CCP and RF in participants without RA; whether or not these effects persist in progression to clinically apparent RA remains to be seen.

Similar to the interaction between SE and smoking on development of seropositive RA,¹⁹²¹ our findings of an interaction between n-3 FA and RA-related autoimmunity suggest that RA pathogenesis is a complex process that depends on both genetic risk and environmental exposures, including the timing thereof. A possible mechanistic explanation of our observed interaction could be the ability of DHA to alter lipid rafts, which change the conformation and surface expression of major histocompatibility complex class II molecules, thereby altering antigen presentation and antibody generation.⁴¹⁴² The ability of long-chain n-3 FAs to affect lipid microdomains has also been linked to regulatory functions of CD4+ T cells,⁴³ which could play an important role in RA-related autoimmunity.⁴⁴ These mechanisms should be explored further.

Assessment of n-3 FA status and autoantibody status occurred at the same visit, which limits the inference regarding the temporal relationship of the association. However, previous

prospective studies in RA where dietary n-3 FAs appear protective against RA development,^{29–31} and prospective studies in type 1 diabetes, where both increasing dietary n-3 FA intake and increasing n-3 FA % in RBCs were associated with a decreased risk of diabetes-related autoimmunity,⁴⁵ suggest that n-3 FA exposure is likely affecting RA-related autoantibody status. Furthermore, supplemental n-3 FA use is not a likely consequence of RA-related autoimmunity as participants were asked about n-3 FA supplement use before they were aware of their autoantibody status. Nevertheless, prospective studies and continued follow-up of at-risk populations such as SERA are required to confirm the temporality of this relationship.

Previous studies suggest n-3 FAs could possibly protect against the development of RA,^{29–31} where our results suggest that the mechanism behind this might be through the prevention of autoantibody development. More importantly, our results suggest the effect of longer-chain n-3 FAs on RA-related autoimmunity could depend on SE status. Further elucidation of the interaction between n-3 FAs and SE on the initial generation of autoimmunity, as well as the progression to clinically apparent RA, could provide insight on the population most likely to benefit from prevention strategies and the timing of such strategies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Nested case-control study population characteristics by autoantibody status (RF and/or anti-CCP2 positive)

Descriptive variable	Anti-CCP2(+) (n=40)	High titre RF(+) (n=27)	RF(−) and anti-CCP2(−) (n=69)	p Values[*]
Age at study visit (mean±SD)	43.7±15.4	48.1±13.2	46.5±13.9	0.45
Non-Hispanic whites, n (%)	25 (62.5)	23 (85.2)	47 (68.1)	0.12
Sex, n (% female)	30 (75.0)	17 (63.0)	52 (75.4)	0.44
Education, n (% >high school)	29 (72.5)	23 (85.2)	54 (78.3)	0.47
Income, n (% \$40 000 annually)	28 (70.0)	23 (85.2)	46 (66.7)	0.19
Shared epitope, n (% positive)	24 (60.0)	11 (40.7)	44 (63.8)	0.11
Ever smoker, n (% yes)	11 (27.5)	11 (40.7)	30 (43.5)	0.24
Current smoker, n (% yes)	2 (5.0)	3 (11.1)	6 (8.7)	0.66

^{*} p Value calculated is an omnibus test of difference; analysis of variance for continuous variables, and χ^2 test for categorical variables.

anti-CCP, anti-cyclic citrullinated peptide; RF, rheumatoid factor.

Table 2

Nested case-control study associations between increasing n-3 FA% in RBCs and RF and anti-CCP2 positivity, in those with and without the SE (27 high titre RF cases, 40 anti-CCP2 cases and 69 controls)

<u>High titre RF(+)</u>						
<u>SE positive (n=11 cases)</u>			<u>SE negative (n=16 cases)</u>			
n-3 FA% in RBC	OR*	95% CI	p Value	OR*	95% CI	p Value $\ddagger$
Total n-3 FA	0.26	0.09 to 0.77	0.02	0.92	0.48 to 1.77	0.81
ALA (18:3n-3)	1.02	0.54 to 1.95	0.95	1.25	0.60 to 2.61	0.55
EPA (20:5n-3)	0.42	0.10 to 1.82	0.25	0.98	0.55 to 1.76	0.95
DPA (22:5n-3)	0.38	0.16 to 0.86	0.02	0.72	0.33 to 1.54	0.39
DHA (22:6n-3)	0.33	0.12 to 0.90	0.03	1.00	0.51 to 1.97	0.99
EPA+DHA	0.29	0.10 to 0.90	0.03	0.98	0.52 to 1.86	0.95
<u>Anti-CCP2(+)</u>						
<u>SE positive (n=24 cases)</u>			<u>SE negative (n=16 cases)</u>			
n-3 FA% in RBC	OR*	95% CI	p Value	OR*	95% CI	p Value $\ddagger$
Total n-3 FA	0.44	0.21 to 0.93	0.03	0.77	0.41 to 1.45	0.42
ALA (18:3n-3)	0.95	0.57 to 1.60	0.85	0.67	0.27 to 1.73	0.43
EPA (20:5n-3)	0.29	0.09 to 0.89	0.03	0.64	0.33 to 1.23	0.18
DPA (22:5n-3)	0.82	0.45 to 1.48	0.52	0.94	0.47 to 1.88	0.86
DHA (22:6n-3)	0.49	0.25 to 0.95	0.03	0.82	0.43 to 1.56	0.54
EPA+DHA	0.41	0.19 to 0.88	0.02	0.76	0.40 to 1.42	0.39

* Adjusted for age at visit, sex, race/ethnicity, site, current smoker, income and education. The ORs reported are for an SD difference in n-3 FA%. SDs: total n-3 FA=1.8; ALA=0.3; EPA=0.6; DPA=2.2; DHA=3.5; EPA+DHA=4.1.

$\ddagger$ p Value for interaction, testing a difference in the effect of n-3 FA% in RBCs between SE-positive and SE-negative strata.

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anti-CCP, anti-cyclic citrullinated peptide; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; n-3 FA, omega-3 fatty acids; RBC, red blood cell; RF, rheumatoid factor; SE, shared epitope.

Table 3

Study population characteristics by autoantibody status (RF and anti-CCP2 positive) of the SERA cohort at their baseline visit with complete records

Descriptive variable	Anti-CCP2(+) (n=44)	RF(+) (n=106)	RF(-) and anti-CCP2(-) (n=2009)	p Value*
Age at baseline visit (mean±SD)	44.0±15.8	46.8±14.5	44.1±14.0	0.16
Non-Hispanic whites (%)	63.6	69.8	81.1	<0.01
Sex (% female)	72.7	72.6	68.6	0.58
Education (% >high school)	68.2	84.9	81.8	0.04
Income (% \$40 000 annually)	63.6	84.0	75.7	0.02
Shared epitope (% positive)	61.4	50.0	53.2	0.45
Ever smoker (% yes)	31.8	40.6	38.9	0.59
Current smoker (% yes)	6.8	10.4	12.2	0.49

* p Value calculated is an omnibus test of difference; analysis of variance for continuous variables, and χ^2 test for categorical variables.

anti-CCP, anti-cyclic citrullinated peptide; RF, rheumatoid factor; SERA, Studies of the Etiology of RA.

SERA cohort (n=2159) associations between self-reported n-3 FA supplement use and prevalence of RF (n=106 participants) and anti-CCP2 (n=44 participants) at baseline in those with and without SE

Table 4

<u>RF(+)</u>						
<u>SE positive (n=53 RF(+))(+)</u>				<u>SE negative (n=53 RF(+))(+)</u>		
n-3 FA supplement	OR *	95% CI	p Value	OR *	95% CI	p Value
Yes vs no	0.32	0.12 to 0.82	0.02	1.21	0.63 to 2.31	0.56
<u>Anti-CCP2</u>						
<u>SE positive (n=27 anti-CCP2(+))</u>				<u>SE negative (n=17 anti-CCP2(+))</u>		
n-3 FA supplement	OR *	95% CI	p Value	OR *	95% CI	p Value
Yes vs no	0.26	0.06 to 1.13	0.07	1.10	0.35 to 3.49	0.87
<u>P_{inter}[†]</u>						
						0.13

* Model includes interaction term between n-3 FA supplement use and SE, adjusted for age, sex, race/ethnicity, site, current smoking, education and income.

[†] p Value for interaction, testing a difference in the effect of n-3 FA supplement use between SE-positive and SE-negative strata.

anti-CCP, anti-cyclic citrullinated peptide; n-3 FA, omega-3 fatty acids; RF, rheumatoid factor; SE, shared epitope; SERA, Studies of the Etiology of RA.