## Supplementary on-line material for

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# Omega-3 fatty acid status enhances the prevention of cognitive decline by B vitamins in Mild Cognitive Impairment

***Supplementary tables***

**Table S1. Demographic and clinical variables at baseline according to combined omega-3 fatty acid tertiles.**

| Variables | Tertile1 | Tertile 2 | Tertile 3 | P Value |
|---|---|---|---|---|
| | Mean (SD)/% | Mean (SD)/% | Mean (SD)/% | |
| Age | 77.43 (5.31) | 76.39 (4.63) | 76.15 (4.47) | 0.224 |
| School Total | 14.11 (3.41) | 14.91 (3.35) | 14.74 (3.57) | 0.319 |
| Gender, n (%) | | | | |
| Male | 37 (49) | 53 (70) | 54 (74) | 0.0025 |
| Female | 39 (51) | 23 (30) | 19 (26) | |
| ApoE4+ | | | | |
| Yes | 57 (75) | 44 (58) | 53 (73) | 0.0495 |
| No | 19 (25) | 32 (42) | 20 (27) | |
| Ever Smokers , n (%) | | | | |
| Yes | 38 (51) | 35 (46) | 44 (60) | 0.209 |
| No | 37 (49) | 41 (54) | 29 (40) | |
| Systolic Blood Pressure | 147.95 (20.66) | 145.08 (20.28) | 146.68 (23.16) | 0.710 |
| Diastolic Blood Pressure | 80.07 (11.38) | 81.01 (10.49) | 79.23 (11.44) | 0.620 |
| Body Mass Index ( Kg/m2) | 25.18 (3.22) | 26.26 (3.72) | 26.20 (4.19) | 0.134 |
| tHcy | 12.71 (3.92) | 12.48 (3.81) | 10.85 (3.58) | 0.005 |
| Vitamin B12 | 359.38 (152.91) | 329.29 (100.41) | 365.25 (137.82) | 0.204 |
| Serum Folate | 25.05 (16.89) | 24.56 (16.67) | 33.17 (20.79) | 0.006 |
| Creatinine | 101.79 (16.44) | 95.28 (13.98) | 93.44 (18.86) | 0.006 |
| Vitamin B supplement use, n (%) | | | | |
| Yes | 65 (86) | 67 (88) | 56 (77) | 0.144 |
| No | 11 (14) | 9 (12) | 17 (23) | |

**Table S2. Results of the fit of the linear regression model for cognitive and clinical outcomes and concentrations of DHA**

| | **Treatment Effect[1]** | | | **Overall interaction[3]** | **Tertiles pairwise comparisons** | | |
|---|---|---|---|---|---|---|---|
| | Crude | Adjusted | P value[2] | P value[4] | [5]$P_{1st\ vs\ 2nd}$ | $P_{1st\ vs\ 3rd}$ | $P_{2nd\ vs\ 3rd}$ |
| **HVLT-DR** | | | | 0.003 | | | |
| Tertile 1 | -0.71 | -0.83 | 0.14 | | diff = 0.57 P = 0.47 | diff = 2.53 P = 0.001 | diff = 1.96 P = 0.015 |
| Tertile 2 | 0.98 | -0.25 | 0.65 | | | | |
| Tertile 3 | 1.32 | 1.70 | 0.002 | | | | |
| | | | | | | | |
| **TICS** | | | | 0.098 | | | |
| Tertile 1 | -1.08 | -0.84 | 0.37 | | diff = 0.97 P = 0.47 | diff = 2.78 P = 0.039 | diff = 1.81 P = 0.17 |
| Tertile 2 | 0.06 | 0.13 | 0.88 | | | | |
| Tertile 3 | 2.48 | 1.94 | 0.041 | | | | |
| | | | | | | | |
| **CDR (OR & 95% CI)** | | | | 0.097 | | | |
| Tertile 1 | 1.71 (0.55, 5.54) | 1.50 (0.48, 4.78) | 0.49 | | diff in log OR = - 1.05 P = 0.20 | diff in log OR = -1.76 P = 0.034 | diff in log OR = -0.70 P = 0.40 |
| Tertile 2 | 0.57 (0.18, 1.72) | 0.52 (0.17, 1.60) | 0.26 | | | | |
| Tertile 3 | 0.31 (0.09, 1.00) | 0.26 (0.08, 0.81) | 0.022 | | | | |
| | | | | | | | |
| **CDRsob** | | | | 0.17 | | | |
| Tertile 1 | 0.26 | 0.07 | 0.78 | | diff = - 0.51 P = 0.18 | diff = -0.65 P = 0.08 | diff = -0.14 P = 0.70 |
| Tertile 2 | -0.42 | -0.43 | 0.098 | | | | |
| Tertile 3 | -0.55 | -0.58 | 0.03 | | | | |

[1] Defined as the average score in treated minus the average score in placebo for HVLT-DR, TICS-M and CDRsob. For CDR it is the OR ratio for a worse outcome comparing treated to placebo. The crude estimate uses the raw data without any statistical modelling. The adjusted treatment effect was obtained by using statistical modelling and adjusting for baseline cognitive score, age, gender, apoe4 status, education and baseline tHcy.

[2] This is the P-value for testing the null hypothesis of no treatment effect within a fixed tertile. This applies to adjusted analysis only.

[3] Overall interaction tests the null hypothesis that treatment effects in 1st, 2nd and 3rd tertiles are all the same.

[4] This is the P-value for testing the null hypothesis of no overall interaction.

[5] $P_{1st\ vs\ 2nd}$ is the P-value for testing the null hypothesis that treatment effects in 1st and 2rd tertiles are the same. The same applies for $P_{1st\ vs\ 3rd}$ and $P_{2nd\ vs\ 3rd}$

**Table S3. Results of the fit of the linear regression model for cognitive and clinical outcomes and concentrations of EPA**

| | **Treatment Effect[1]** | | | **Overall interaction[3]** | **Tertiles pairwise comparisons** | | |
|---|---|---|---|---|---|---|---|
| | Crude | Adjusted | P value[2] | P value[4] | [5]$P_{1st\ vs\ 2nd}$ | $P_{1st\ vs\ 3rd}$ | $P_{2nd\ vs\ 3rd}$ |
| **HVLT-DR** | | | | 0.094 | | | |
| Tertile 1 | -0.23 | -0.76 | 0.18 | | diff = 1.14 P = 0.15 | diff = 1.71 P = 0.038 | diff = 0.56 P = 0.48 |
| Tertile 2 | 0.34 | 0.38 | 0.49 | | | | |
| Tertile 3 | 1.69 | 0.94 | 0.10 | | | | |
| | | | | | | | |
| **TICS** | | | | 0.40 | | | |
| Tertile 1 | -0.44 | 0.26 | 0.79 | | diff = -0.68 P = 0.61 | diff = 1.07 P = 0.44 | diff = 1.76 P = 0.19 |
| Tertile 2 | -0.31 | -0.43 | 0.65 | | | | |
| Tertile 3 | 2.30 | 1.33 | 0.17 | | | | |
| | | | | | | | |
| **CDR (OR & 95% CI)** | | | | 0.15 | | | |
| Tertile 1 | 1.40 (0.44, 4.50) | 1.51 (0.47, 4.98) | 0.49 | | diff in log OR = -1.29 P = 0.12 | diff in log OR = -1.55 P = 0.067 | diff in log OR = -0.26 P = 0.75 |
| Tertile 2 | 0.48(0.15, 1.50) | 0.41(0.13 1.28) | 0.13 | | | | |
| Tertile 3 | 0.43 (0.14, 1.31) | 0.32(0.10, 0.98) | 0.05 | | | | |
| | | | | | | | |
| **CDRsob** | | | | 0.35 | | | |
| Tertile 1 | 0.03 | -0.009 | 0.97 | | diff = -0.43 P = 0.25 | diff = -0.48 Pl = 0.20 | diff = -0.05 P = 0.89 |
| Tertile 2 | -0.2 | -0.44 | 0.10 | | | | |
| Tertile 3 | -0.55 | -0.49 | 0.058 | | | | |

For definitions, see legend to Table S2.

**Table S4. Cut points and numbers of subjects in each omega-3 tertile for the different analytes**

| **Analyte** | **Tertile 1** μmol/L | | **Tertile 2** μmol/L | | **Tertile 3** μmol/L | |
|---|---|---|---|---|---|---|
| | | | | | | |
| **Combined omega-3** | < 391 | | 391 - 579 | | > 579 | |
| | Placebo | B Vits | Placebo | B Vits | Placebo | B Vits |
| | n= 38 | n=37 | n= 38 | n=40 | n= 38 | n=34 |
| | | | | | | |
| **DHA** | < 255 | | 255 – 339 | | > 339 | |
| | Placebo | B Vits | Placebo | B Vits | Placebo | B Vits |
| | n=38 | n=38 | n=40 | n=36 | n=36 | n=37 |
| | | | | | | |
| **EPA** | < 135 | | 135 – 222 | | > 222 | |
| | Placebo | B Vits | Placebo | B Vits | Placebo | B Vits |
| | n=38 | n=37 | n=36 | n=41 | n=40 | n=33 |

**Table S5. Results for testing the cross-over interaction for overall omega-3 fatty acids**

| | Tertile 1 | | Tertile 2 | | Tertile 3 | | P value for cross-over interaction |
|---|---|---|---|---|---|---|---|
| | Effect | SE | Effect | SE | Effect | SE | |
| HVLT-DR | -0.94 | 0.56 | 0.42 | 0.55 | 1.14 | 0.57 | 0.093 |
| TICS-M | -1.07 | 0.94 | 0.55 | 0.94 | 1.78 | 0.95 | 0.235 |
| CDRsob | -0.025 | 0.26 | -0.384 | 0.27 | -0.529 | 0.256 | 0.787 |
| CDR Log odds ratio | 1.50 | 0.58 | 0.42 | 0.59 | 0.31 | 0.58 | 0.425 |

**Table S6. The change, per year, in the average HVLT-DR in placebo and treated across tertiles of each omega-3, using estimates from the linear mixed effect model**

| | **First tertile** | | **Second tertile** | | **Third tertile** | | **Tests of global interaction** | |
|---|---|---|---|---|---|---|---|---|
| **Type of omega** | Estimate (SD) | P | Estimate (SD) | P | Estimate (SD) | P | LRT[1] P | F-test[2] P |
| ***Total omega-3*** | | | | | | | **0.086** | **0.087** |
| Effect of time in placebo (slope)[3] | 0.16 (0.18) | 0.36 | 0.13(0.17) | 0.45 | -0.02(0.17) | 0.91 | | |
| Effect of time in B-vitamins (slope) | -0.13 (0.17) | 0.44 | 0.17(0.18) | 0.32 | 0.46(0.18) | **0.013** | | |
| | | | | | | | | |
| ***DHA*** | | | | | | | **0.025** | **0.025** |
| Effect of time in placebo (slope) | 0.22 (0.18) | 0.21 | 0.20(0.17) | 0.24 | -0.15(0.18) | 0.38 | | |
| Effect of time in B-vitamins (slope) | -0.05 (0.17) | 0.80 | 0.06(0.18) | 0.76 | 0.46 (0.17) | **0.009** | | |
| | | | | | | | | |
| ***EPA*** | | | | | | | 0.14 | 0.14 |
| Effect of time in placebo (slope) | 0.24 (0.18) | 0.17 | 0.04(0.18) | 0.82 | -0.01(0.17) | 0.93 | | |
| Effect of time in B-vitamins (slope) | -0.08 (0.18) | 0.66 | 0.22(0.17) | 0.20 | 0.33 (0.19) | 0.076 | | |

[1]LRT (Likelihood ratio test) comparing the model with interaction and the model without interaction. The maximum likelihood is used instead of restricted maximum likelihood

[2]F-test for testing linear combinations of parameters

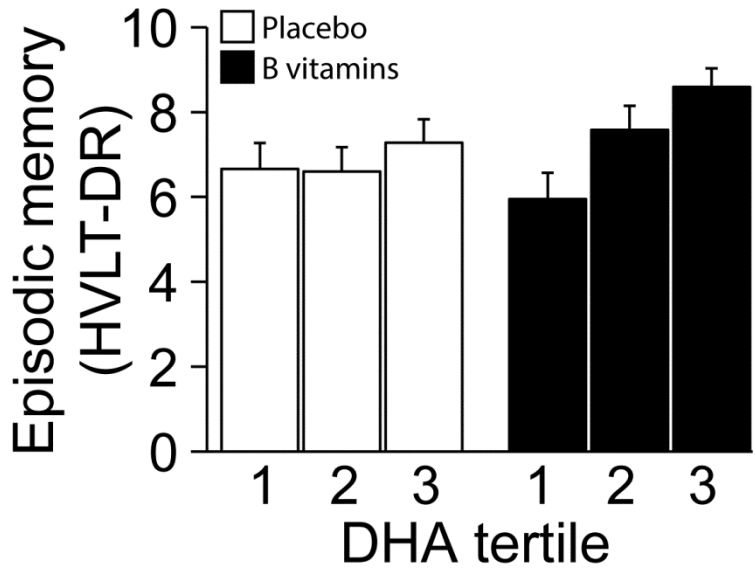
[3]The slope here is the change in the response (increase or decrease) per 1 year follow-up

P values for interaction between B vitamin treatment and tertiles of omega-3 were considered significant if < 0.1

*Supplementary figures*

**Fig S1. HVLT-DR for placebo vs. B vitamin-treated at 2 year follow-up for A) DHA and B) EPA tertiles**. The interactions were significant between B vitamin treatment and DHA tertiles (P = 0.003) and EPA tertiles (P = 0.094). In the third tertile of the DHA concentration, the memory score in the B vitamin group was higher than in placebo (P = 0.002). In the B vitamin group, memory score in the 3rd tertile of DHA was higher than in the 1st tertile (P = 0.001). See Table S2. In the B vitamin group, memory score in the 3rd tertile of EPA was higher than in the 1st tertile (P = 0.038). See Table S3. Columns show mean scores and error bars SEM.

**A**




**B**

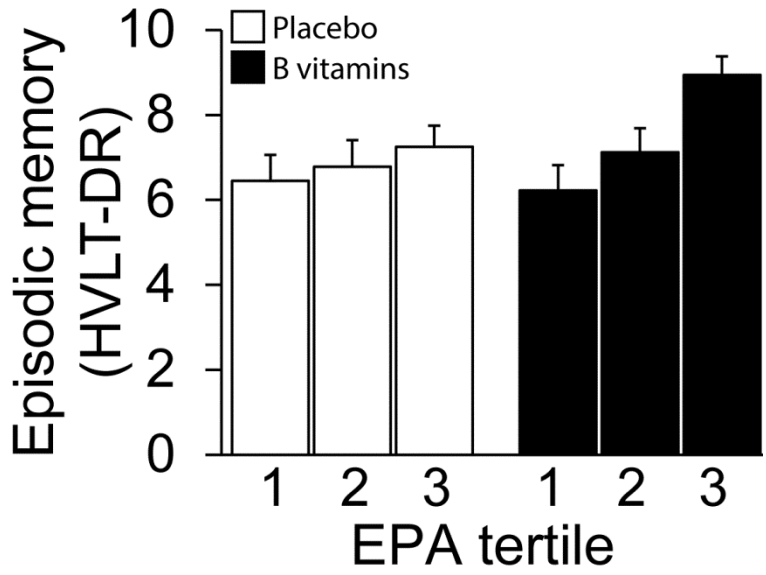
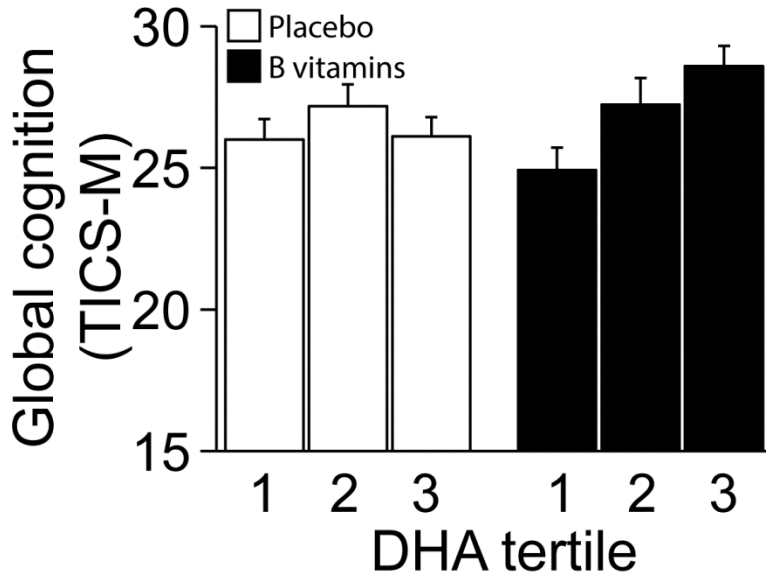


**Fig S2 TICS-M for placebo vs. B vitamin-treated at 2 year follow-up for A) DHA and B) EPA tertiles**. The interactions were significant between B vitamin treatment and DHA tertiles (P = 0.098), but not for EPA tertiles (P = 0.40). In the third tertile of the DHA concentration, the cognition score in the B vitamin group was higher than in placebo (P = 0.041). In the B vitamin group, memory score in the 3rd tertile of DHA was higher than in the 1st tertile (P = 0.039). See Tables S2 and S3. Columns show mean scores and error bars SEM.

**A**




**B**

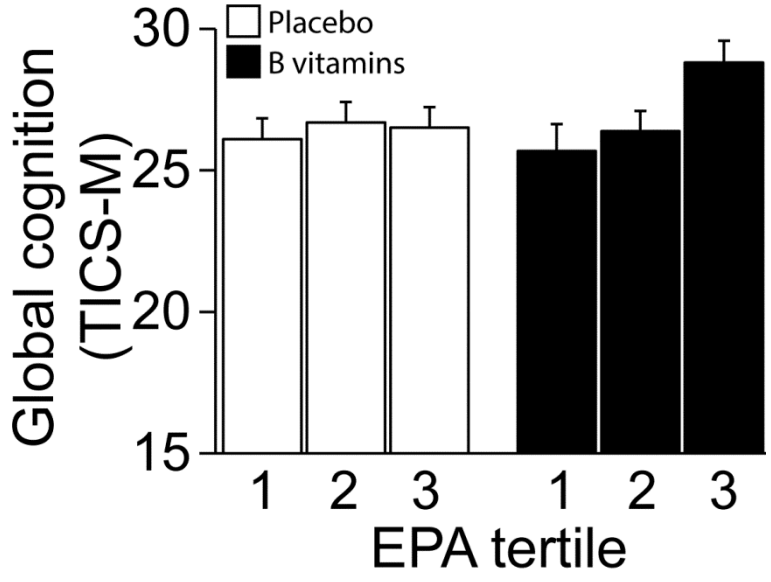
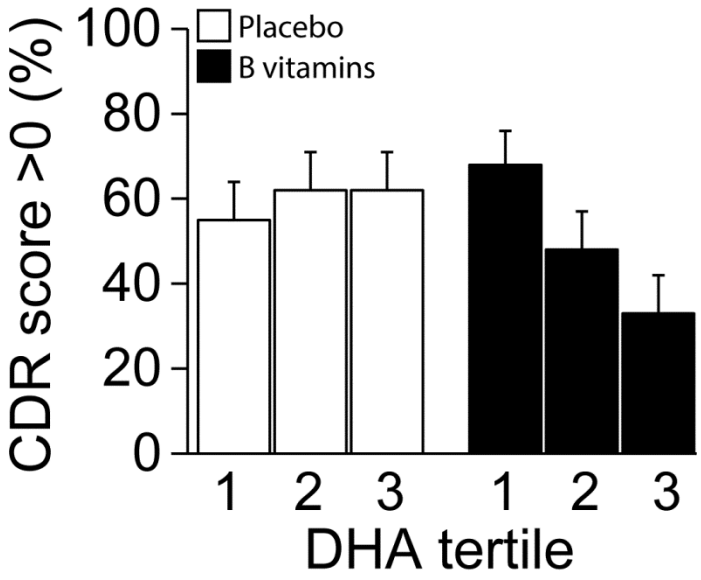


**Fig S3. CDR for placebo vs. B vitamin-treated at 2 year follow-up for A) DHA and B) EPA tertiles.** The interactions were significant between B vitamin treatment and DHA tertiles (P = 0.097), but not for EPA tertiles (P = 0.15). In the third tertile of the DHA concentration, the CDR score in the B vitamin group was lower than in placebo (P = 0.022). In the B vitamin group, CDR score in the 3rd tertile of DHA was lower than in the 1st tertile (P = 0.034). See Tables S2 and S3. Columns show mean scores and error bars SEM.

**A**




**B**

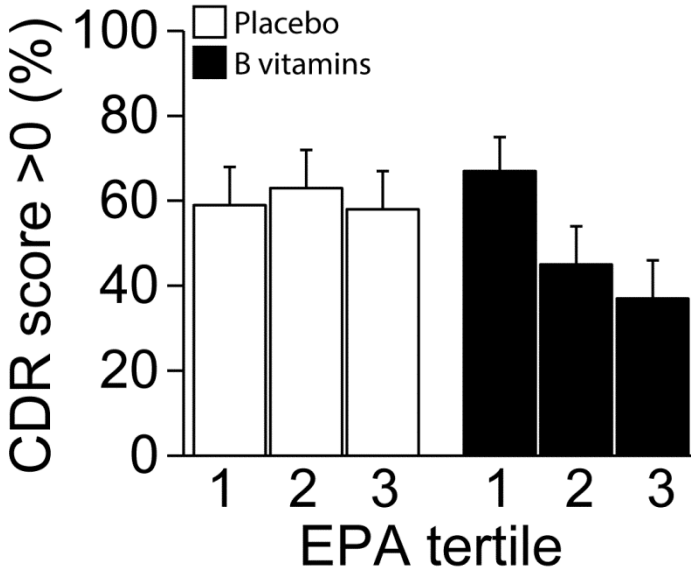
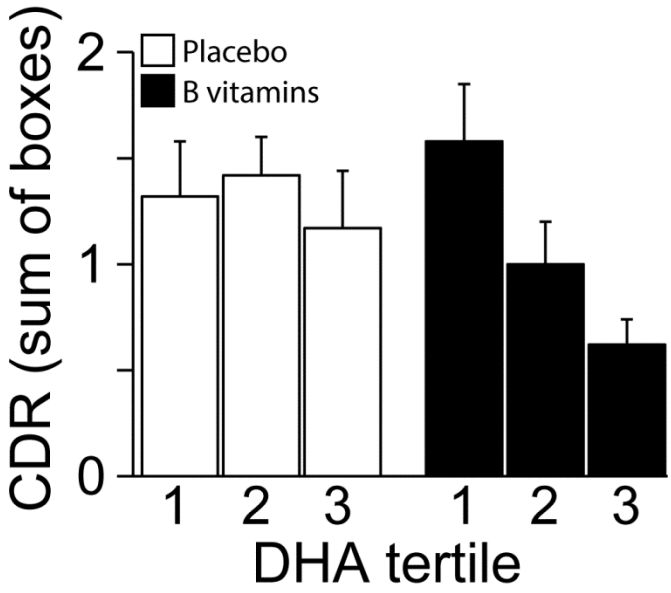


**Fig S4. CDRsob at 2 year at follow-up for A) DHA and B) EPA tertiles for placebo vs. B vitamin-treated.** The interactions were not significant between B vitamin treatment and DHA tertiles (P = 0.17) and EPA tertiles (P = 0.35). In the third tertile of the DHA concentration, the CDRsob score in the B vitamin group was lower than in placebo (P = 0.03). See Tables S2 and S3. Columns show mean scores and error bars SEM.

**A**




**B**

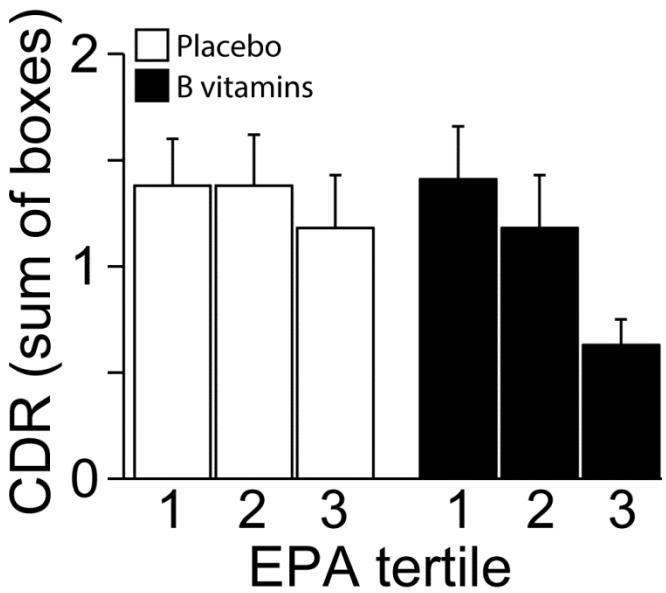
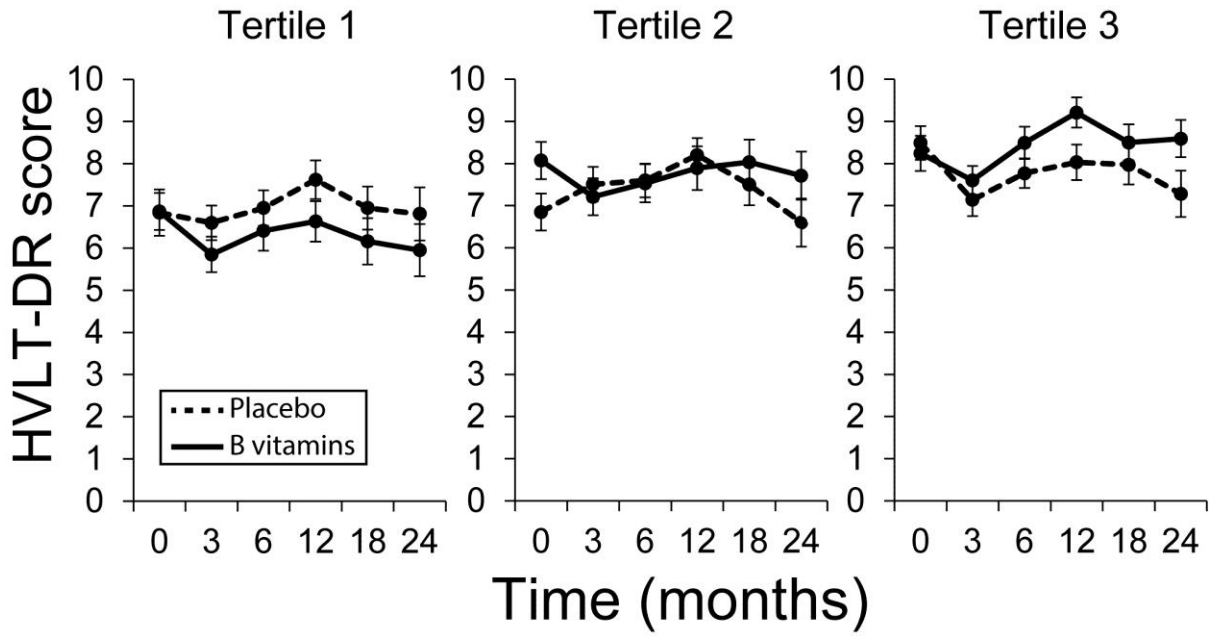


**Fig. S5. Longitudinal plots for HVLT-DR according to A) baseline DHA and B) EPA concentrations.** Ranges of the tertiles are given in Table S4. The likelihood ratio test for interaction between B vitamin treatment and DHA tertiles was significant (P = 0.025, Table S6). In the 3rd tertile of DHA, the average HVLT-DR significantly increased in the B vitamin group by 0.46 points per year of follow-up (P = 0.009) compared to no significant change in the placebo group (Table S6). The pattern for EPA was similar, but did not reach significance. Error bars indicate SEM.

**A**




**B**