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# Effect of one year krill oil supplementation on depressive symptoms and self-esteem of Dutch adolescents: A randomized controlled trial

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ABSTRACT

*Introduction:* Observational studies have shown a relationship between omega-3 long-chain polyunsaturated fatty acids (n-3 LCPUFA) and depression in adolescents. However, n-3 LCPUFA supplementation studies investigating the potential improvement in depressive feelings in adolescents from the general population are missing.
*Methods:* A one-year double-blind, randomized, placebo controlled krill oil supplementation trial was conducted in two cohorts. Cohort I started with 400 mg eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) or placebo, after three months this increased to 800 mg EPA and DHA per day, whilst cohort II started with this higher dose. Omega-3 Index (O3I) was monitored via finger-prick blood measurements. At baseline, six and 12 months participants completed the Centre for Epidemiologic Studies Depression Scale (CES-D) and the Rosenberg Self Esteem questionnaire (RSE). Adjusted mixed models were run with treatment allocation/O3I as predictor of CES-D and RSE scores.
*Results:* Both intention-to-treat and assessing the change in O3I analyses did not show significant effects on CES-D or RSE scores.
*Conclusion:* There is no evidence for less depressive feelings, or higher self-esteem after one year of krill oil supplementation. However, due to a lack of adherence and drop-out issues, these results should be interpreted with caution.

*Abbreviations*

| | |
|---|---|
| AA | arachidonic acid, 20:4n-6; |
| CES-D | Centre for Epidemiologic Studies Depression Scale |
| DHA | Docosahexaenoic acid, C22:6n-3 |
| DPA | Docosapentaenoic acid, 22:5n-3 |
| EPA | Eicosapentaenoic acid, C20:5n-3 |
| LCPUFA | long-chain polyunsaturated fatty acid |
| LGSE | Lower general secondary education |
| O3I | Omega 3-Index |
| ObA | Osbond acid, n-6 docosapentaenoic acid, 22:5n-6 |
| RSE | Rosenberg Self Esteem questionnaire |

## 1. Introduction

According to the World Health Organisation approximately 322 million people (4.4% of the world population) are affected by depression [1]. Whilst relatively uncommon in childhood (prevalence in the 6–12 months prior to assessment of 0.4–2.5%) [2], depression prevalence increases sharply during adolescence [3], approximately 14–25% of adolescents experiences at least one episode of depression before the age of 18 years [4]. Adolescent depression has serious social, mental and

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physical consequences, there is an increased risk of reoccurrence of depression, increased risk of anxiety, and increased risk of suicide compared to adolescents without a depression [5]. However, adolescent depression has also been associated with lower educational attainment, lower wages, poorer social relationships, and poorer self-rated health [6, 7]. Moreover, sub-threshold depression (i.e., depressive symptoms present, but not enough to reach a diagnosis of major depression) has also been associated with negative outcomes, such as the development of major depression and reduced quality of life [8, 9]. Sub-threshold depression is common in adolescence with point prevalence between 0.24 and 14%, and life-time prevalence (i.e., life-time being up to age of measurement during adolescences) between 1 and 22.9% [8, 9]. The prevalence numbers vary in different populations (ages, gender, country), but also due to the manner in which depression is assessed and according to criteria for (subthreshold) depression used.

Depression is a complex and incompletely understood multi-factorial disorder, and probably has heterogeneous aetiologies [10]. Depression has been characterized by biological changes such as increased levels of pro-inflammatory cytokines [11, 12], alterations in immune function [13], elevation of plasma homocysteine levels [14], changes in brain structure [15, 16], blood flow abnormalities [17], decreased glucose metabolism [18], and a low Omega-3 Index (O3I) [19, 20].

Omega-3 long chain polyunsaturated fatty acids (n-3 LCPUFA) such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) have long attracted attention for their possible positive effect on depression. The biological plausibility of n-3 LCPUFA intake for the prevention or treatment of depression can be explained by the numerous important functions of n-3 LCPUFA in the body. For example, n-3 LCPUFA can modulate neuroendocrine factors, have anti-inflammatory properties, play a role in neurogenesis and in neuroplasticity [21, 22], all factors which could reduce or counteract biological changes associated with depression. Observational studies, mostly show that blood and brain concentrations of n-3 LCPUFAs are lower in people with depression compared to people without depression [20, 23, 24]. N-3 LCPUFA supplementation intervention trials in adults with a diagnosed depression mostly show positive results (for meta-analyse see amongst others [25-27]).

Depression mostly has its onset during adolescence and adolescents' depression can have profound long lasting effects [5] and it is therefore an important area of research. There are a number of observational studies investigating the relationship between n-3 LCPUFA and depression in adolescents [20, 28-31]. Most, but not all, of these observational studies show a relationship between n-3 LCPUFA and depression (i.e. higher intake or higher concentrations in blood, less depression [20, 28, 29, 31]). Additionally, a number of small scale (i.e., all <70 participants) n-3 LCPUFA supplementation studies in children and/or adolescents with a diagnosed depressive disorder have shown positive effects of supplementation on depression scores [32-35], but not all have shown positive effects [36]. Considering the potential positive effect of n-3 LCPUFA supplementation on depression, the high prevalence of adolescent (sub-threshold) depression and the fact that adolescents depression is notoriously underdiagnosed [37, 38], it is important to investigate whether n-3 LCPUFA supplementation can be effective for depressive feelings in adolescents from the general population.

The main aim of this study was to assess the effect of one year of krill oil supplementation, a source of the n-3 LCPUFAs DHA and EPA, on depressive feelings in adolescents of lower general secondary education. We hypothesised that one year krill oil supplementation would result in lower depressive feelings.

Furthermore, we explored the effect of the krill oil supplementation on self-esteem. Self-esteem is a core construct in mental health and refers to the subjective evaluation of ones worth as a person [39]. Low self-esteem has been suggested to be a risk factor for the development of many mental illnesses including depression [39]. However to our knowledge, studies exploring the effect of n-3 LCPUFA supplementation on self-esteem are missing.

## 2. Methods

This study was approved by the Medical Ethical Committee of Atrium-Orbis-Zuyd Hospital Heerlen, the Netherlands (NL45803.096.13). The study is registered at the Netherlands Trial Register (NTR4082) and at Clinicaltrials.gov (NCT02240264). Full details about the designs and methods of the study have been reported previously [40]. Below a shortened version of the design and methods is presented. Note that in the current manuscript only the data with regard to depressive feelings and self-esteem are presented.

### 2.1. Design

The current study was a double-blind, randomized, placebo controlled intervention trial with repeated measurements (baseline (T0), 3 months (T1), 6 months (T2), and 12 months (T3)) to study the effect of one year of krill oil supplementation on amongst others depressive feelings and self-esteem of second year high school students attending lower general secondary education (LGSE) in the Netherlands. Participants were recruited in two cohorts: Cohort I from November 2013 to February 2014 and Cohort II from November 2014 to February 2015. Data were collected from February 2014 to April 2015 and from February 2015 to April 2016, respectively. After informed consent was received, a finger prick blood sample was collected to determine the Omega-3 Index (O3I). The only inclusion criterion for the study was that students had an O3I < 5%. Exclusion criteria were having an O3I > 5%, having an allergy to fish or shellfish or haemophilia. Aim of the study was to increase the Omega-3 Index (O3I) of participants in the krill oil group to 8–11%. This target O3I was based on the O3I associated with the lowest mortality risk in coronary heart disease [41]. The study participants participated in four test sessions (T0, T1, T2, T3) where they completed various tests and questionnaires and provided a finger-prick blood sample for the analysis of the O3I, as described below.

### 2.2. Intervention

The intervention started after baseline test moment (T0). In Cohort I participants were asked to take four placebo or krill oil capsules, containing in total 260 mg EPA and 140 mg DHA per day with their dinner, as this is the fattest meal of the day and the presence of fat helps with the absorption of EPA and DHA [42]. After three months of supplementation a personalized dose adjustment was planned to account for interpersonal differences in metabolism. However, at T1 only 3 participants achieved the target O3I of 8–11%, therefore all participants (both krill and placebo) were asked to increase the daily dosage to eight capsules per day, containing 520 mg EPA and 280 mg DHA per day. Furthermore, it was decided that Cohort II would start with eight capsules containing 520 mg EPA and 280 mg DHA per day. The placebo contained a mix of olive oil, corn oil, palm oil, and medium chain triglycerides the following ratio 4:4:9:3. The fatty acid composition of the placebo reflected the fatty acid composition of the average European diet.

### 2.3. Blood analyses

Whole blood was obtained from a finger prick at T0, T1, T2, and T3. Whole blood fatty acid compositions were analysed according to the HS-Omega-3 Index methodology [41, 43]. Results are given as EPA plus DHA expressed as a percentage of total identified fatty acids after response factor correction. Since the O3I is defined as EPA + DHA in erythrocytes, the O3I in the current study was calculated using a sliding correction factor [43].

### 2.4. Questionnaires

#### 2.4.1. Centre for Epidemiologic studies depression scale

Depressive feelings were assessed with the Dutch version of the Centre for Epidemiologic Studies Depression Scale (CES-D) [44]. Although developed for adults, the CES-D has been shown to have a high reliability in both clinical and non-clinical samples of children and adolescents ($\alpha = 0.88$) and also reasonable sensitivity ($\alpha = 0.76$) [45]. The questionnaire consists of 20 questions which assess six symptom areas of depression. The score on the questionnaire can vary between 0 and 60, with higher scores indicating more depressive feelings. Originally a cut-off score of $\geq$16 was suggested as indication for depression, although some have suggested a higher cut-off score of $\geq$22 for adolescents [46]. Moreover, it has also been suggested that the CES-D score should be considered as a continuum of increasing severity [9]. For the current study both the standard cut-off score of $\geq$16 and the adolescents cut-off score of $\geq$22 were used for descriptive purposes, whilst the CES-D continuous score was used for the main analyses.

#### 2.4.2. Rosenberg self-esteem questionnaire

Self-esteem was assessed with the Dutch version of the Rosenberg self-esteem questionnaire (RSE) [47]. The reliability of the RSE has been shown to be high ($\alpha = 0.72$–$0.88$) [48]. The RSE consists of 10 questions about the participants' evaluation of themselves. The total score of the RSE can range from 0 to 30 with higher scores indicating higher self-esteem. For the current study the RSE score was used as a continuous score.

#### 2.4.3. Additional measures

At T0, T2, and T3 a number of variables were measured as they are known to be associated with depression: BMI (weight/length$^{2,}$ self-reported at baseline) [49, 50], sex [51, 52], age in years [53], alcohol consumption (number of days that alcohol was consumed times number of standardized units of alcohol that were consumed per moment) [54], and number of cigarettes smoked per week (if student indicated to smoke 0.5 cigarette or more per week this was coded a smoking yes, otherwise smoking no) [55]. Moreover, parental level of education (subdivided in 8 levels ranging from primary school to university level) as a proxy for socioeconomic status [56], pubertal status as assessed by the Pubertal Development Scale (subdivided in 5 levels from prepubertal to postpubertal) [57], and diagnosis which might influence learning (yes/no, e.g. autism or ADHD) [58] were assessed. At T3 only, participants were asked to guess their group allocation (placebo or krill oil group).

### 2.5. Statistical analyses

#### 2.5.1. Sample size calculation

Sample size calculation for multilevel analyses were executed in RMass, and showed that a sample of 183–285 participants would be sufficient [59]. This sample size is based on an effect size of 0.25 on the letter digit substitution task at 6 months and an effect size equal or 10% larger at 12 months, a drop-out rate of 25% per measurement moment (thus 43% in total), an error variation between 0.4 and 0.5 and an intercept variation of 0.3 to 0.5 with fixed effects.

#### 2.5.2. Imputing and recoding covariates

Data on drinking and smoking were only collected at T0 and T3, and the average score between 0 and 12 months was imputed for drinking at T2. A cut-off score of 0.5 cigarette per week was used to code yes/no for smoking at T2 (i.e., if the participant indicate to smoke 0.5 cigarette or more per week, smoking was coded as 'yes'). Level of parental education was recoded to low (vocational education and training and below) and high (university of applied sciences and higher).

Some participants had a maximum of two missing items on the CES-D (11, 15, and 7 participants at T0, T2, and T3 respectively) or the RSE (12, 6, and 6 participants at T0, T2, and T3 respectively). The missing items were imputed by the person average score on the other items on that time point as suggested by Bono et al.[60]..

#### 2.5.3. Group comparison, treatment guess and adherence

Group comparisons and treatment guess analyses were conducted in SPSS statistics version 24 (IBM).

The following baseline comparisons were conducted: krill oil group versus the placebo group, participants who completed the study versus participants who dropped-out, participants who completed the study actively (i.e., taking capsules) versus those who quit taking capsules (i.e., both those who dropped out completely and those who still participated in testing).

Baseline comparisons were conducted using ANOVA for continuous variables and chi-squared test for categorical variables. Fatty acid concentrations, CES-D score, RSE score, and participants' characteristics (BMI, age, sex, alcohol consumption, smoking, level of parental education, pubertal status, school and cohort) were compared between the groups to determine if the groups differed at baseline. Blood fatty acid concentrations were measured on all time points and compared between the krill oil and placebo groups.

Treatment guess was compared between participants in the krill oil group and participants in placebo group with a chi-squared test.

Adherence was assessed by counting the returned remaining capsules. As an additional measure for adherence the average increase in O3I between T0 and T2, and between T0 and T3 were noted. Lastly, we documented how many participants had a decrease in their O3I, had an increase up to 2.5%, and had an increase of more than 2.5%.

#### 2.5.4. Effect on depressive feelings and self-esteem

Intention-to-treat analyses were conducted using linear mixed models that accounted for repeated measurements in subjects. These analyses were executed in R statistical environment (R studio version 3.3.2) with the package nlme (version 3.1–131) using the standard settings [61].

Models allowed a comparison between groups (krill oil versus placebo condition) and within groups (T0 data compared to intervention at T2 and T3). Besides time trends (baseline as a reference) and treatment X test moment interactions, all estimates were adjusted for drinking behaviour, smoking behaviour, level of parental education, age at baseline, pubertal status at baseline, sex, BMI at baseline, diagnosis related to learning, and cohort number. Furthermore, moderation analyses for sex were executed and if necessary sub group analyses (boys and girls separate) were conducted. Secondary analyses were conducted with O3I instead of group allocation. Moreover, sensitivity analyses with only participants who were present at all-time points and for participants from the two cohorts separately was conducted. A $p < 0.05$ was considered to be statistically significant.

## 3. Results

A total of 288 students provided informed consent, of whom 267 were randomized into the study. 19 participants did not meet inclusion criteria, one retracted consent, one had other reasons. After randomization, one participant withdrew consent before starting supplementation and one participant quit during the first test moment, both were excluded from the analyses. Additionally, due to logistic issues, 9 participants with an O3I > 5% were not excluded before the start of the study, they were excluded from the analyses. So, for 256 participants data for at least one time moment was available. Do note that due to missing data CES-D score was available for 251 participants, and RSE score for 254 participants.

During the study 53 (20.6%) students withdrew completely from the study and 82 (31.9%) stopped active participation (i.e., they were tested, but did not take capsules). Main reported reasons for discontinuing the study were lack of motivation, difficulty swallowing

capsules, and difficulties remembering to take capsules (see Fig. 1) .

Baseline characteristics for the placebo and the krill oil group can be found in Table 1, Table 2 and Table 3. There were more girls in the krill oil group than boys (54 girls, 72 boys) compared to the placebo group (79 girls, 51 boys; $\chi^2 = 8.224$, $p = .004$). There were no other significant differences between the placebo and krill oil group in participant's characteristics (all $p > .185$), blood values (all $p > .055$) or baseline depressive feelings or self-esteem scores ($p = .259$ and $p = .237$, respectively)

At baseline 74 students (29.5%) had a score ≥16 on the CES-D (i.e., indicative for depression), at T2 48 (21.3%), and at T3 54 (26.5%). Using a stricter cut-of criteria of ≥22, 17.1%, 12% and 18.6% reached the cut off point for depression at T0, T2, and T3, respectively.

### 3.1. Blood fatty acids

Concentrations of blood fatty acids at T0, T1, T2, and T3, are shown in Table 2. Compared to the placebo group, the krill oil group had significantly higher EPA, DPA, DHA concentrations, a higher O3I (all $p < 0.001$), and significantly lower concentrations of AA and ObA (all $p < .038$) at T1, T2, and T3. Given that participants in cohort I started with a

**Table. 1**
Participants' characteristics at baseline.

| | Placebo | N | Krill | N | P[3] |
|---|---|---|---|---|---|
| Age (years) | 14.07 ± 0.48 | 130 | 14.15 ± 0.51 | 126 | 0.185 |
| Male/female N (%) | 51/79 (39/61) | 130 | 72/54 (57/43) | 126 | **0.004** |
| Smoking[1] (no/yes) N (%) | 117/12 (91/9) | 129 | 113/12 (90/10) | 126 | 0.935 |
| BMI | 20.13 ± 3.05 | 123 | 19.84 ± 2.96 | 116 | 0.455 |
| Alcohol units per week[2] | 0.34 ± 1.16 | 129 | 0.61 ± 2.29 | 126 | 0.234 |
| Level of parental education (low/high) N (%) | 54/69 (44/56) | 123 | 49/66 (43/57) | 115 | 0.840 |

[1] Smoking was defined as anybody who indicated to smoke more than 0 cigarettes per week;
[2] Alcohol units per week was operationalized as number of days per week that alcohol is consumed times units per consumption moment
[3] ANOVA was used for age, BMI, and alcohol units per week, Chi Square for smoking, level of parental education, and sex. Significant differences ($p < .05$) are noted in bold.

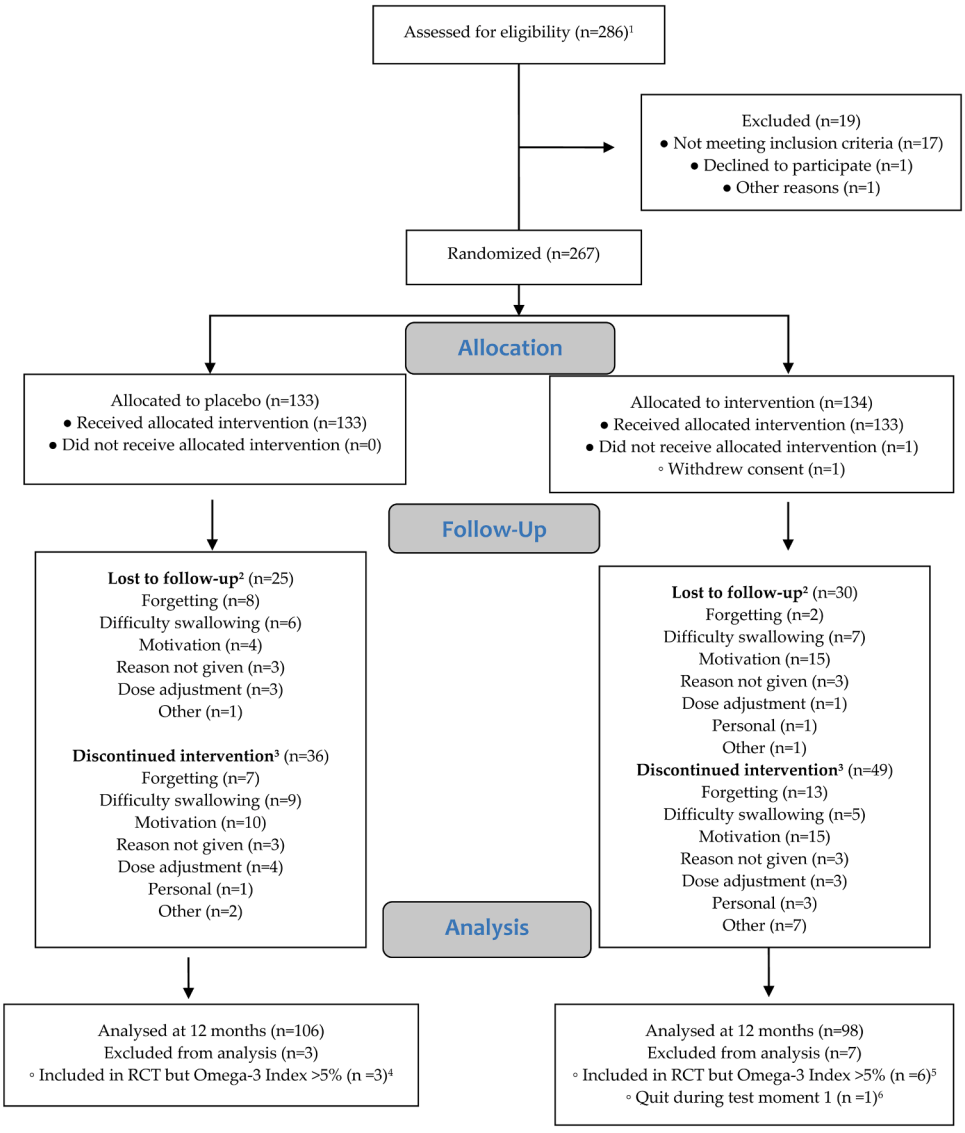


**Fig.. 1.** Flow chart. Flow chart adapted from Consort Guidelines [32] [1] Two additional participants provided consent, but then withdrew their consent again before blood sampling, these two participants are not included in this flowchart [2] Participants whom quite taking capsules and quite participation in testing.[3] Participants whom quite taking capsules but did participate in testing. [4] Three participants with an O3I >5% were included in the placebo group of these one was lost to follow up (reason forgetting), one discontinued the intervention (reason dose adjustment) and one finished the intervention, they were all excluded from the analyses. [5] Six participants with an O3I >5% were included in the intervention group of these one was lost to follow up (reason motivation), two discontinued the intervention (reason motivation/ personal) and three finished the intervention, they were all excluded from the analyses.[6] This person is noted under lost to follow-up (reason other).

**Table 2**
Fatty acids in blood at different time points in intention to treat analysis.

| Fatty acid (%wt/wt of total FA) | Baseline placebo (n 130) | krill (n 126) | p | 3 months placebo (n 124) | krill1[3](n 118) | p | 6 months[1] placebo (n 116) | krill (n 108) | P | 12 months[2] placebo (n 104) | krill (n 95) | p |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AA 20:4n-6 | 11.19 ± 1.36 | 11.12 ± 1.18 | 0.657 | 10.97 ± 1.18 | 10.26 ± 1.13 | <0.001 | 11.02 ± 1.49 | 10.28 ± 1.41 | <0.001 | 11.15 ± 1.30 | 10.72 ± 1.49 | 0.029 |
| EPA 20:5n-3 | 0.38 ± 0.14 | 0.38 ± 0.15 | 0.860 | 0.43 ± 0.15 | 0.93 ± 0.58 | <0.001 | 0.41 ± 0.14 | 0.95 ± 0.69 | <0.001 | 0.40 ± 0.12 | 0.75 ± 0.58 | <0.001 |
| ObA 22:5n-6 | 0.43 ± 0.11 | 0.45 ± 0.10 | 0.154 | 0.41 ± 0.17 | 0.32 ± 0.12 | <0.001 | 0.42 ± 0.09 | 0.32 ± 0.11 | <0.001 | 0.38 ± 0.12 | 0.34 ± 0.13 | 0.038 |
| DPA 22:5n-3 | 1.22 ± 0.20 | 1.22 ± 0.17 | 0.858 | 1.29 ± 0.23 | 1.58 ± 0.34 | <0.001 | 1.30 ± 0.20 | 1.54 ± 0.35 | <0.001 | 1.30 ± 0.19 | 1.47 ± 0.31 | <0.001 |
| DHA 22:6n-3 | 2.60 ± 0.44 | 2.49 ± 0.46 | 0.055 | 2.61 ± 0.52 | 3.25 ± 0.73 | <0.001 | 2.69 ± 0.53 | 3.40 ± 0.90 | <0.001 | 2.72 ± 0.54 | 3.20 ± 0.84 | <0.001 |
| O3I | 3.83 ± 0.54 | 3.72 ± 0.55 | 0.106 | 3.88 ± 0.64 | 5.10 ± 1.29 | <0.001 | 3.95 ± 0.64 | 5.29 ± 1.61 | <0.001 | 3.98 ± 0.63 | 4.86 ± 1.43 | <0.001 |

AA = arachidonic acid, DHA = docosahexaenoic acid, DPA= docosapentaenoic acid, EPA = eicosapentaenoic acid, O3I = Omega-3 Index, ObA = Osbond acid.
[1] 2 participants did participate in testing but did not have a blood sample available [2] 5 participants did participate in testing but did not have a blood sample available. [3] This includes both the participants from Cohort 1 who took 400 mg EPA + DHA per day and participants from Cohort 2, who took 800 mg EPA + DHA per day. Supplemental Table 1 show the fatty acids for the separate cohort.

**Table 3**
**Comparison of krill oil and placebo groups on** scores of the Centre for Epidemiologic Studies Depression Scale and the Rosenberg's Self-Esteem Scale.

| | CES-D | | P | RSE | | P |
|---|---|---|---|---|---|---|
| | Krill *M* ± SD (min-max) | Placebo *M* ± SD (min-max) | | Krill *M* ± SD (min-max) | Placebo *M* ± SD (min-max) | |
| Baseline | 11.52 ± 9.00 (0–43) | 12.91 ± 10.51 (1–53) | 0.259 | 21.89 ± 5.61 (4–30) | 21.01 ± 6.22 (1–30) | 0.237 |
| 6 months | 9.98 ± 8.72 (0–41) | 11.21 ± 9.71 (0–48) | 0.319 | 22.69 ± 5.34 (8–30) | 21.93 ± 5.48 (0–30) | 0.298 |
| 12 months | 10.86 ± 8.92 (0–49) | 13.60 ± 11.95 (0–49) | 0.066 | 23.00 ± 4.96 (2–30) | 21.74 ± 6.36 (2–30) | 0.120 |

different dose than cohort 2, the detailed fatty acid concentrations for both cohorts can be found in the supplementary files (supplementary Table S1).

### 3.2. Depressive feelings

The random intercept model with time point (T0 as reference), condition (krill or placebo), the interaction term (test moment x condition) and covariates showed that group allocation did not predict score on the CES-D (see Table 4). Interaction analysis for sex X condition did not shown a significant interaction effect ($p = .162$). Separate analyses for the cohorts, and with only participants who participated in all test moments showed similar results to the analysis with all participants (Supplementary Table 2, 3 and 4).

The random intercept model with time point (T0 as reference), O3I, and covariates showed that O3I did not predict depression score. Separate cohort analyses, and with only participants who participated in all test moments showed similar results to the analysis with all participants (Supplementary Table 2, 3 and 4).

Interaction analysis for sex and O3I did show a significant interaction effect ($p = .03$). Subgroup analyses showed that for girls there was a possible significant relationship between higher O3I and higher depression score ($b = 1.08$, SE= 0.56, $p = .053$, 95% CI [0.01; 2.15]), indicating that girls with a higher O3I might have more depressive feelings. For boys no relationship between O3I and depression score was shown ($b = -0.37$, SE= 0.36, $p = .310$, 95% CI [−1.06; 0.33]). Closer inspection of the data showed that there were 5 participants whom had a Z-score on the CES-D of >3.29, which is considered to be an extreme outliers and not a reliable value (Field, 2013). These 5 participants were all girls, when these girls were excluded from the analyses, the interaction term was non-significant ($p = .139$).

### 3.3. Self-esteem

The random intercept model with time point (T0 as reference), condition (krill or placebo), the interaction term (time moment x condition) and covariates showed that group allocation did not predict score on the RSE. The interaction analysis for sex X condition did not show an interaction effect ($p = .651$). Separate cohort analyses, and with only participants who participated in all test moments showed similar results to the analysis with all participants (see Supplementary Table 5, 6 and 7).

The random intercept model with time point (T0 as reference), O3I, and covariate showed that O3I did not predict self-esteem score. The interaction analysis for sex X O3I did not show an interaction effect either ($p = .652$) Analyses separate for cohort 1 and 2, and with only participants who participated in all test moments showed similar results to the analysis with all participants (see Supplementary Table 5, 6 and 7).

### 3.4. Treatment guess

After the one year supplementation period 63 participants (65.6%) in the placebo group and 45 participants (50.6%) in the krill oil group correctly guessed their original treatment allocation. The percentage that correctly guessed treatment allocation did differ significantly with more participants in the placebo group correctly guessing their group allocation ($\chi^2 = 4.313$; $p = .038$).

### 3.5. Drop-out and compliance

There were no baseline differences between those who finished the study completely with supplementation and those who quit taking capsules for age, BMI, drinking behaviour, sex, smoking behaviour, level

**Table 4**
Multilevel analyses of score on the Centre for Epidemiologic Studies Depression Scale by condition (intention to treat) and according to Omega-3 Index.

| Condition | | | Omega-3 Index | | |
|---|---|---|---|---|---|
| | b (SE) | 95%CI | | b (SE) | 95%CI |
| T2 | −1.50 (0.82) | [−3.09; 0.08] | T2 | −1.64 (0.66) | **[−2.93; −0.36]** |
| T3 | 0.95 (0.86) | [−0.71; 2.61] | T3 | 0.18 (0.67) | [−1.12; 1.49] |
| Krill | −0.65 (1.32) | [−3.20; 1.91] | Omega-3 Index | 0.38 (0.34) | [−0.28; 1.03] |
| T2 x krill | 0.37 (1.18) | [−1.92; 2.66] | | | |
| T3 x krill | −1.04 (1.22) | [−3.41; 1.32] | | | |
| Alcohol | −0.07 (0.19) | [−0.44; 0.30] | Alcohol | −0.06 (0.19) | [−0.44; 0.31] |
| Smoking[1] | 2.66 (1.22) | **[0.29; 5.03]** | Smoking[1] | 2.72 (1.24) | **[0.31; 5.13]** |
| Age | 0.47 (1.28) | [−2.02; 2.96] | Age | 0.31 (1.28) | [−2.18; 2.80] |
| Pubertal status[2] | | | Pubertal status[2] | | |
| Beginning | −5.42 (4.46) | [−14.07; 3.23] | Beginning | −6.04 (4.44) | [−14.67; 2.60] |
| Mid | −1.78 (4.32) | [−10.17; 6.61] | Mid | −2.26 (4.31) | [−10.63; 6.12] |
| Advanced | −0.82 (4.43) | [−9.41; 7.78] | Advanced | −1.34 (4.42) | [−9.93; 7.26] |
| Post | 8.83 (6.23) | [−3.25; 20.92] | Post | 8.03 (6.21) | [−4.04; 20.10] |
| BMI | 0.49 (0.20) | **[0.11; 0.87]** | BMI | 0.51 (0.20) | **[0.12; 0.89]** |
| LPE [3] | 0.46 (1.17) | [−1.81; 2.73] | LPE [3] | 0.39 (1.17) | [−1.88; 2.67] |
| Sex [4] | 4.83 (1.60) | **[1.72; 7.94]** | Sex [4] | 4.97 (1.60) | **[1.86; 8.07]** |
| Cohort [5] | 1.47 (1.17) | [−0.80; 3.75] | Cohort [5] | 1.45 (1.16) | [−0.80; 3.70] |
| Diagnosis [6] | 2.43 (1.17) | **[0.16; 4.69]** | Diagnosis [6] | 2.63 (1.16) | **[0.37; 4.89]** |

[1] No smoking as reference.
[2] Pre-pubertal as reference.
[3] Low level of parental education as reference.
[4] Boy as reference.
[5] Cohort 1 as reference.
[6] No diagnosis as reference. T2 = test-point 6 months, T3 = time-point 12 months.

**Table. 5**
Multilevel analyses of score on the Rosenberg's Self-Esteem Scale by condition (intention to treat) and according to Omega-3 Index.

| | Condition | | | Omega-3 Index | |
|---|---|---|---|---|---|
| | b (SE) | 95%CI | | b (SE) | 95%CI |
| T2 | 0.64 (0.42) | [−0.19; 1.46] | T2 | 0.55 (0.35) | [−0.12; 1.22] |
| T3 | 0.47 (0.45) | [−0.40; 1.34] | T3 | 0.62 (0.35) | [−0.06; 1.30] |
| Krill | 0.25 (0.77) | [−1.25; 1.75] | Omega-3 Index | −0.03 (0.18) | [−0.38; 0.32] |
| T2 x krill | −0.18 (0.62) | [−1.38; 1.01] | | | |
| T3 x krill | 0.23 (0.64) | [−1.00; 1.47] | | | |
| Alcohol | 0.11 (0.10) | [−0.09; 0.31] | Alcohol | 0.12 (0.10) | [−0.08; 0.32] |
| Smoking[1] | −1.58 (0.67) | **[−2.87; −0.29]** | Smoking[1] | −1.75 (0.67) | **[−3.05; −0.45]** |
| Age | −0.91 (0.78) | [−2.42; 0.61] | Age | −0.90 (0.78) | [−2.41; 0.62] |
| Pubertal status[2] | | | Pubertal status[2] | | |
| Beginning | 4.71 (2.71) | [−0.54; 9.97] | Beginning | 4.89 (2.70) | [−0.35; 10.14] |
| Mid | 3.34 (2.63) | [−1.76; 8.44] | Mid | 3.42 (2.62) | [−1.67; 8.51] |
| Advanced | 3.13 (2.69) | [−2.09; 8.34] | Advanced | 3.25 (2.68) | [−1.97; 8.47] |
| Post | 2.13 (3.79) | [−5.23; 9.49] | Post | 2.38 (3.78) | [−4.98; 9.74] |
| BMI | −0.23 (0.12) | [−0.46; 0.005] | BMI | −0.24 (0.12) | **[−0.47; −0.005]** |
| LPE [3] | 0.17 (0.71) | [−1.21; 1.54] | LPE [3] | 0.13 (0.71) | [−1.25; 1.51] |
| Sex [4] | −3.23 (0.97) | **[−5.22; −1.44]** | Sex [4] | −3.41 (0.97) | **[−5.30; −1.52]** |
| Cohort [5] | 0.31 (0.71) | [−1.07; 1.69] | Cohort [5] | 0.33 (0.70) | [−1.04; 1.70] |
| Diagnosis [6] | −0.88 (0.68) | [−2.20; 0.44] | Diagnosis [6] | −0.91 (0.68) | [−2.22; 0.40] |

[1] No smoking as reference.
[2] Pre-pubertal as reference.
[3] Low level of parental education as reference.
[4] Boy as reference.
[5] Cohort 1 as reference.
[6] No diagnosis as reference. T2 = test moment 6 months, T3 = test moment 12 months.

of parental education, pubertal status, fatty acids, depression score or self-esteem score. There was a difference in drop-out between cohorts, more students stopped taking capsules in cohort 2 compared to cohort 1 ($\chi^2 = 11.329$, $p = .001$). Moreover, in some schools more students dropped out than in other schools ($\chi^2 = 28.299$, $p = .029$).

Comparing those that actively participated (with taking capsules), with those who only participated in testing (without taking capsules), and those that quit completely, similar patterns were seen. No difference for age, BMI, drinking behaviour, sex, smoking behaviour, school or, level of parental education, pubertal status, fatty acids, depression score, or self-esteem score. But there was a difference between cohorts ($\chi^2 = 13.139$, $p = .001$).

Looking at those students that were active participants at T2, the increase in O3I, compared to T0 was on average 2.02% (SD 1.58%). At T2, compared to T0, 5 (6.8%) of the active participants had a decrease in their O3I, 44 (60.3%) had an increase between 0 and 2.5% and 24 (32.9%) had an increase of >2.5%. At T3, compared to T2, there was an average decrease in the O3I of 0.70% in active krill oil participants. When comparing T3 to T2, 35 (70%) of active participants in the krill oil group had a decrease in their O3I and were thus most likely not adherent with the protocol, the other 15 (30%) had an increase varying from 0.14 to 1.96%.

Participants were asked to return leftover capsules at the last test moment (T3). Of the active participants at T3 (i.e., those taking capsules), 56 handed in leftover capsules and 37 counted the leftover capsules at home. On average participants had 629 ± 395 leftover capsules. This means that participants did not take the capsules on 79 days of the approximately 180 days between T2 and T3.

## 4. Discussion

The current krill oil supplementation trial in adolescents attending LGSE who had a low baseline O3I did not show improvements in depressive feelings in the krill oil supplementation condition compared to placebo, nor did supplementation lead to a higher self-esteem score. Sensitivity analyses did also not show an association of higher O3I with a lower depression score or better self-esteem score. Note that our analyses of neurocognitive test scores also did not shown an effect of supplementation on cognitive measures [62]. However, the aim of the study was that participants in the krill oil group would achieve a predetermined O3I of 8–11% and that two distinct groups with regard to O3I would be achieved (thus no or minimal overlap in O3I between krill and placebo group); neither of which was achieved. The average O3I in the krill oil group was 5.29% at T2 and 4.86% at T3, which indicates an

average increase in O3I of 1.57% and 1.14% compared to T0, respectively. However, between T2 and T3 there was actually an average decrease in O3I in participant in the krill oil group who said to take the capsules. Moreover, only three (2.5% of those in krill oil group of whom blood values were available), 10 (9.3%), and two (2.1%) participants achieved the target O3I of >8% at T1, T2, and T3, respectively. Thus, the average O3I in the krill oil group was at all time-points well below the intended range of 8–11% and the participants were non adherent with the protocol. It has been argued that positive effects of O3I only exist at higher levels of O3I. For example, in the study of Markhus and colleagues, there was only a linear relation between O3I and pregnancy depressive feelings when the O3I was above 5.1% [63]. Moreover, in cardiovascular health an O3I>8% has been associated with the greatest risk reduction [64].

Depressive feelings were very common in this sample of adolescents attending LGSE. When using the cut-off score of ≥16 on the CES-D, 29.5%, 21.3% and 26.5% had scores indicative of depression at T0, T2, and T3, respectively. When using the higher cut-off score of ≥22, still 17.1%, 12% and 18.6% of participants had a score indicative of depression at T0, T2, and T3, respectively. Surprisingly, there were only 6 students who indicated to have a diagnosed depression. These numbers of students with a possible depression might seem high, but other studies in adolescents have shown similar rates. For example, Oddy et al. showed that 21.2% of the sample had scores on the Beck Depression Inventory for Youth indiciative of mild to severe depressive symptoms [30]. In the study of Grant and colleagues 21.7% of boys and 34.4% of girls had scores indicative of mild to extremely severe depression [65]. Mamalakis et al. showed in their sample an average CES-D score of 14.9 [28], which is very comparable to the average scores in the current study.

To our knowledge there are no previously published n-3 LCPUFA supplementation trials that assessed the effect of N-3 LCPUFA on depressive feelings in adolescents who were recruited from the general population. LCPUFA supplementation studies in adults, children and adolescents with a diagnosed depressive disorder mostly showed positive effects of supplementation (for meta-analyses in adults see amongst others [25, 66, 67]). Furthermore, it has been suggested that the positive effects of n-3 LCPUFA supplementation are only apparent in adults with diagnosed depression and when supplementation contains >50% EPA [68]. It is however uncertain if and how the results from the studies on depression in adults can be compared to studies in adolescents. For example, it has been suggested that juvenile-onset and adult-onset depression are distinct with different causes and different outcomes [69, 70]. Moreover, in adolescence the brain is still in development and this is accompanied by profound physical, social, emotional and cognitive development [71]. All these factors could influence depression, but depression in itself also disrupts these developmental processes which can have severe long term negative effects, and which are possibly more profound than the long-term effects of depression in adulthood. Moreover, the use of the LCPUFA in the body might also be different in adolescents compared to adults. For example, up to 18 years old the amount of DHA in the brain rises and then it plateaus until the age of 88 year [72]. The few studies that have been conducted in children or adolescents with a depressive disorder mostly showed a positive effect of n-3 LCPUFA supplementation on depression score [32-35], although not all showed positive effects [36]. However, the majority of these studies were rather small and of limited duration (i.e., all 16 weeks or less); and therefore larger, adequately powered and longer duration studies are warranted.

As mentioned previously the increase in O3I in the krill oil group was lower than anticipated and lower than the target of 8–11%. Only three (2.5% of those in krill oil group of whom blood values were available), 10 (9.3%) and two (2.1%) participants achieved that at T1, T2, and T3, respectively. The reason for this low number of participants that achieved the target O3I can be largely explained by the large number of participants that quit supplementation and the non-adherence of the active participants. Despite utilizing a multitude of techniques to improve adherence including: sending participants a daily text message reminder; motivational telephone talks; as well as provision of a tip sheet with tips on how to remember to take the capsules (i.e. example of tips were: put the capsules on the kitchen table, put a reminder alarm in your phone, or but a sticky note with a reminder somewhere). The study did last for one year, which might simply be too long, this is echoed in the fact that most participants quit because of lack of motivation/inability to remember to take the capsules. Moreover, the students had to take eight capsules per day, which many students indicated to be too much and was another reason why many students dropped-out (inability to take capsules). Long study duration and high number of capsules have been suggested to influence drop-out and adherence [73]. It is however important to realize that if n-3 LCPUFA supplementation is found to be beneficial for depressive feelings, a high dose of capsules might need to be taken for a prolonged period of time (i.e., months or years). Adherence with medication, and thus most likely supplements as well, has already been found to be problematic in those with depression [74], but might even be more challenging in adolescents.

The limitation of the current study is the fact that self-rated questionnaires to assess depression were used instead of clinician interviews. Self-rated questionnaires to assess depression could lead to false positives, or false negatives. However, the CES-D has been validated and in the main analyses we did not use cut-off scores but used the continuous score on the CES-D. Another limitation of the current study is the fact, as discussed, that there was a rather high dropout rate and there were difficulties with achieving adherence to the protocol. However, blood samples and test scores were available of the majority of participants that quit taking capsules, so they could be included in analyses. Moreover, participants that dropped-out did not differ significantly from those that continued the study actively on relevant baseline characteristics.

To summarize, the current study did not show an effect of one year of krill oil supplementation on depression score and on self-esteem score in adolescents with a low baseline O3I that attended LGSE. However, due to the lack of compliance and high drop-out rate, we feel we cannot preclude that a relationship between krill oil supplementation and depression score or self-esteem score in adolescents does not exist. Therefore, more adequately powered studies in this specific age group with high rates of depressive symptoms are warranted. Moreover specific attention should be paid to achieve adherence, decrease drop-out rates and achieve a set target O3I.

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## Authors' contributions

All authors read and approved the final version of the manuscript.

## Supplementary files

Supplementary Table S1: Fatty acids in blood at different time points intention to treat separate for cohort 1 and 2; Supplementary Table S2: Multilevel analyses of score on the Centre for Epidemiologic Studies Depression Scale by condition (intention to treat) and according to Omega-3 Index for Cohort 1 participants only; Supplementary Table S3: Multilevel analyses of score on the Centre for Epidemiologic Studies Depression Scale by condition (intention to treat) and according to Omega-3 Index for Cohort 2 participants only; Supplementary Table S4: Multilevel analyses of score on the Centre for Epidemiologic Studies Depression Scale by condition (intention to treat) and according to

Omega-3 Index for participant with data available for all time-points; Supplementary Table S5: Multilevel analyses of score on the Rosenberg self-esteem scale by condition (intention to treat) and according to Omega-3 Index for Cohort 1 participants only; Supplementary Table S6: Multilevel analyses of score on the Rosenberg self-esteem scale by condition (intention to treat) and according to Omega-3 Index for Cohort 2 participants only; Supplementary Table S7: Multilevel analyses of score on the Rosenberg Self-esteem Scale by condition (intention to treat) and according to Omega-3 Index for participant with data available for all time-points.

## CRediT authorship contribution statement

**I.S.M. van der Wurff:** Methodology, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization, Project administration. **C. von Schacky:** Conceptualization, Methodology, Investigation, Resources, Writing - review & editing, Funding acquisition. **T. Bergeland:** Resources, Writing - review & editing, Project administration. **R. Leontjevas:** Methodology, Formal analysis, Data curation, Writing - review & editing. **M.P. Zeegers:** Writing - review & editing, Supervision. **P.A. Kirschner:** Writing - review & editing. **R.H.M. de Groot:** Conceptualization, Methodology, Investigation, Writing - review & editing, Supervision, Project administration, Funding acquisition.

## Declaration of Competing Interest

CVS is owner of Omegametrix, who is responsible for the blood analyses in this study. TB is a former employee of Aker Biomarine, who is partly funding the study. All other authors declare no conflict of interest. The sponsor had no role in the analyses, or interpretation of data.

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## Supplementary materials

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## References

[1] O. World Health, Depression and other common mental disorders: global health estimates, in, 2017, pp. 1–24.
[2] J.E. Fleming, D.R. Offord, Epidemiology of childhood depressive disorders: a critical review, J. Am. Acad. Child Adolesc. Psychiatry 29 (1990) 571–580.
[3] G. Saluja, R. Iachan, P.C. Scheidt, M.D. Overpeck, W. Sun, J.N. Giedd, Prevalence of and risk factors for depressive symptoms among young adolescents, Arch. Pediatr. Adolesc. Med. 158 (2004) 760–765.
[4] R.C. Kessler, E.E. Walters, Epidemiology of DSM-III-R major depression and minor depression among adolescents and young adults in the National Comorbidity Survey, Depress. Anxiety 7 (1998) 3–14.
[5] D.M. Fergusson, J.M. Boden, L.J. Horwood, Recurrence of major depression in adolescence and early adulthood, and later mental health, educational and economic outcomes, Brit. J. Psychiatry 191 (2007) 335–342.
[6] M. Johar, J. Truong, Direct and indirect effect of depression in adolescence on adult wages, Appl. Econ. 46 (2014) 4431–4444.
[7] K. Naicker, N.L. Galambos, Y. Zeng, A. Senthilselvan, I. Colman, demographic Social, and health outcomes in the 10 years following adolescent Depression, J. Adoles. Health 52 (2013) 533–538.
[8] E.A. Bertha, J. Balázs, Subthreshold depression in adolescence: a systematic review, Eur. Child Adolesc. Psychiatry 22 (2013) 589–603.
[9] R. Wesselhoeft, M.J. Sørensen, E.R. Heiervang, N. Bilenberg, Subthreshold depression in children and adolescents - a systematic review, J. Affect. Disord. 151 (2013) 7–22.
[10] F. Rice, Genetics of childhood and adolescent depression: insights into etiological heterogeneity and challenges for future genomic research, Genome Med. 2 (2010) 68.
[11] M. Maes, R. Yirmyia, J. Noraberg, S. Brene, J. Hibbeln, G. Perini, M. Kubera, P. Bob, B. Lerer, M. Maj, The inflammatory & neurodegenerative (I&ND) hypothesis of depression: leads for future research and new drug developments in depression, Metab. Brain Dis. 24 (2009) 27–53.
[12] O.J.G. Schiepers, M.C. Wichers, M Maes, Cytokines and major depression, Progr. Neuro-Psychopharmacol. Biol. Psychiatry 29 (2005) 201–217.
[13] L. Capuron, A.H Miller, Immune system to brain signaling: neuropsychopharmacological implications, Pharmacol. Therapeut. 130 (2011) 226–238.
[14] M. Folstein, T. Liu, I. Peter, J. Buel, L. Arsenault, T. Scott, W.W. Qiu, The homocysteine hypothesis of depression, Am. J. Psychiatry 164 (2007) 861–867.
[15] S. Campbell, G. MacQueen, An update on regional brain volume differences associated with mood disorders, Curr. Opin. Psychiatry 19 (2006) 25–33.
[16] W.C. Drevets, Neuroimaging studies of mood disorders, Biol. Psychiatry 48 (2000) 813–829.
[17] M. Liotti, H.S. Mayberg, S. McGinnis, S.L. Brannan, P. Jerabek, Unmasking disease-specific cerebral blood flow abnormalities: mood challenge in patients with remitted unipolar depression, Am. J. Psychiatry 159 (2002) 1830–1840.
[18] T.A. Kimbrell, T.A. Ketter, M.S. George, J.T. Little, B.E. Benson, M.W. Willis, P. Herscovitch, R.M. Post, Regional cerebral glucose utilization in patients with a range of severities of unipolar depression, Biol. Psychiatry 51 (2002) 237–252.
[19] T.C. Baghai, G. Varallo-Bedarida, C. Born, S. Häfner, C. Schüle, D. Eser, R. Rupprecht, B. Bondy, C. von Schacky, Major depressive disorder is associated with cardiovascular risk factors and low Omega-3 index, J. Clin. Psychiatry 72 (2011) 1242–1247.
[20] J.V. Pottala, J.A. Talley, S.W. Churchill, D.A. Lynch, C. von Schacky, W.S. Harris, Red blood cell fatty acids are associated with depression in a case-control study of adolescents, Prostaglandins Leukot. Essent. Fatty Acids 86 (2012) 161–165.
[21] R.P. Bazinet, S. Layé, Polyunsaturated fatty acids and their metabolites in brain function and disease, Nat. Rev. Neurosci. 15 (2014) 771–785.
[22] N. Parletta, C.M. Milte, B.J. Meyer, Nutritional modulation of cognitive function and mental health, J. Nutr. Biochem. 24 (2013) 725–743.
[23] R.K. McNamara, C.-.G. Hahn, R. Jandacek, T. Rider, P. Tso, K.E. Stanford, N. M. Richtand, Selective deficits in the omega-3 fatty acid docosahexaenoic acid in the postmortem orbitofrontal cortex of patients with major depressive disorder, Biol. Psychiatry 62 (2007) 17–24.
[24] R.K. McNamara, R. Jandacek, T. Rider, P. Tso, Y. Dwivedi, G.N. Pandey, Selective deficits in erythrocyte docosahexaenoic acid composition in adult patients with bipolar disorder and major depressive disorder, J. Affect. Disord. 126 (2010) 303–311.
[25] K.M. Appleton, P.J. Rogers, A.R. Ness, Updated systematic review and meta-analysis of the effects of n-3 long-chain polyunsaturated fatty acids on depressed mood, Am. J. Clin. Nutr. 91 (2010) 757–770.
[26] M.H. Bloch, J. Hannestad, Omega-3 fatty acids for the treatment of depression: systematic review and meta-analysis, Mol. Psychiatry 17 (2012) 1272–1282.
[27] G. Grosso, A. Pajak, S. Marventano, S. Castellano, F. Galvano, C. Bucolo, F. Drago, F. Caraci, Role of omega-3 fatty acids in the treatment of depressive disorders: a comprehensive meta-analysis of randomized clinical trials, PLoS ONE 9 (2014).
[28] G. Mamalakis, M. Kiriakakis, G. Tsibinos, C. Hatzis, S. Flouri, C. Mantzoros, A. Kafatos, Depression and serum adiponectin and adipose omega-3 and omega-6 fatty acids in adolescents, Pharmacol. Bioch. Behav. 85 (2006) 474–479.
[29] K. Murakami, Y. Miyake, S. Sasaki, K. Tanaka, M Arakawa, Fish and n-3 polyunsaturated fatty acid intake and depressive symptoms: ryukyus Child Health Study, Pediatrics 126 (2010) e623–e630.
[30] W.H. Oddy, S. Hickling, M.A. Smith, T.A. O'Sullivan, M. Robinson, N.H. De Klerk, L.J. Beilin, T.A. Mori, J. Syrette, S.R. Zubrick, S.R. Silburn, Dietary intake of omega-3 fatty acids and risk of depressive symptoms in adolescents, Depress. Anxiety 28 (2011) 582–588.
[31] S. Tsuchimine, M. Saito, S. Kaneko, N. Yasui-Furukori, Decreased serum levels of polyunsaturated fatty acids and folate, but not brain-derived neurotrophic factor, in childhood and adolescent females with depression, Psychiatry Res. 225 (2015) 187–190.
[32] T. Jana, H. Zuzana, S. Anna, K. Barbora, G. Irina, W. Iveta, S. Katarína, G. Iveta, Š. Ján, Ď. Zdeňka, Omega-3 fatty-acids modulate symptoms of depressive disorder, serum levels of omega-3 fatty acids and omega-6/omega-3 ratio in children. A randomized, double-blind and controlled trial, Psychiatry Res. (2020), 112911.
[33] H. Nemets, B. Nemets, A. Apter, Z. Bracha, R. Belmaker, Omega-3 treatment of childhood depression: a controlled, double-blind pilot study, Am. J. Psychiatry 163 (2006) 1098–1100.
[34] A. Fristad, A. Vesco, A. Young, M. Fristad, Pilot RCT of omega-3 and individual-family psychoeducational psychotherapy for children and adolescents with depression, J. Clin. Child Adolesc. Psychol. 45 (2017) 1025–1037.
[35] R.K. McNamara, J. Strimpfel, R. Jandacek, T. Rider, P. Tso, J.A. Welge, J. R. Strawn, M.P. DelBello, Detection and treatment of long-chain omega-3 fatty acid deficiency in adolescents with SSRI-resistant major depressive disorder, PharmaNutrition 2 (2014) 38–46.
[36] V. Gabbay, R.D. Freed, C.M. Alonso, S. Senger, J. Stadterman, B.A. Davison, R. G. Klein, A double-blind placebo-controlled trial of omega-3 fatty acids as a monotherapy for adolescent depression, J. Clin. Psychiatry (2018) 79.
[37] R.C. Kessler, S. Avenevoli, K.R. Merikangas, Mood disorders in children and adolescents: an epidemiologic perspective, Biol. Psychiatry 49 (2001) 1002–1014.
[38] P.J. Leaf, M. Alegria, P. Cohen, S.H. Goodman, S.M. Horwitz, C.W. Hoven, W. E. Narrow, M. Vaden-Kiernan, D.A. Regier, Mental health service use in the community and schools: results from the four-community MECA study, J. Am. Acad. Child Adolesc. Psychiatry 35 (1996) 889–897.

[39] J.F. Sowislo, U. Orth, Does low self-esteem predict depression and anxiety? A meta-analysis of longitudinal studies, Psychol. Bull. 139 (2013) 213–240.
[40] I.S. van der Wurff, C. von Schacky, K. Berge, P.A. Kirschner, R.H. de Groot, A protocol for a randomised controlled trial investigating the effect of increasing Omega-3 index with krill oil supplementation on learning, cognition, behaviour and visual processing in typically developing adolescents, BMJ Open 6 (2016), e011790.
[41] W.S. Harris, The omega-3 index as a risk factor for coronary heart disease, Am. J. Clin. Nutr. 87 (2008) 1997S–2002S.
[42] C.T.M. Van Rossum, H.P. Fransen, J. Verkaik-Kloosterman, E.J.M. Buurma-Rethans, M.C. Ocké, C.T.M.v. Rossum, H.P. Fransen, J. Verkaik-Kloosterman, E.J. M. Buurma-Rethans, M.C. Ocké, Dutch National Food Consumption Survey 2007-2010 - Diet of children and adults aged 7 to 69 years, in, Bilthoven, the Netherlands, 2011, pp. 59–59.
[43] W.S. Harris, C. von Schacky, Y. Park, Standardizing methods for assessing omega-3 fatty acid biostatus, in: R.K. McNamara (Ed.), Nova Science Publishers, Inc, 2013, pp. 385–398.
[44] L.S. Radloff, The CES-D Scale: a Self-Report Depression Scale for Research in the General Population, Appl. Psychol. Meas. 1 (1977) 385–401.
[45] E. Stockings, L. Degenhardt, Y.Y. Lee, C. Mihalopoulos, A. Liu, M. Hobbs, G. Patton, Symptom screening scales for detecting major depressive disorder in children and adolescents: a systematic review and meta-analysis of reliability, validity and diagnostic utility, J. Affect. Disord. 174 (2015) 447–463.
[46] P. Cuijpers, P. Boluijt, A. Van Straten, Screening of depression in adolescents through the Internet: sensitivity and specificity of two screening questionnaires, Eur. Child Adolesc. Psychiatry 17 (2008) 32–38.
[47] B.H. Stoodley, M. Rosenberg, Society and the Adolescent Self-Image, in, 1966, pp. 125–125.
[48] R.W. Robins, H.M. Hendin, K.H. Trzesniewski, Measuring Global Self-Esteem, Construct validation of a single-item measure and the rosenberg self-esteem scale, Pers. Soc. Psychol. Bull. 27 (2001) 151–161.
[49] E. Atlantis, M. Baker, Obesity effects on depression: systematic review of epidemiological studies, Int. J. Obes. 32 (2008) 881–891.
[50] S. Cortese, B. Falissard, M. Angriman, Y. Pigaiani, C. Banzato, G. Bogoni, M. Pellegrino, S. Cook, F. Pajno-Ferrara, B.D. Bernardina, M.-.C. Mouren, C. Maffeis, The relationship between body size and depression symptoms in adolescents, J. Pediatr. 154 (2009) 86–90.
[51] B. Allgood-Merten, P.M. Lewinsohn, H Hops, Sex differences and adolescent depression, J. Abnorm. Psychol. 99 (1990) 55–63.
[52] L. Wichstrøm, The emergence of gender difference in depressed mood during adolescence: the role of intensified gender socialization, Dev. Psychol. 35 (1999) 232–245.
[53] B.L. Hankin, L.Y. Abramson, T.E. Moffitt, P.a Silva, R McGee, K.E Angell, Development of depression from preadolescence to young adulthood: emerging gender differences in a 10-year longitudinal study, J. Abnorm. Psychol. 107 (1998) 128–140.
[54] J.C. Skogen, A.K. Knudsen, M. Hysing, B. Wold, B. Sivertsen, Trajectories of alcohol use and association with symptoms of depression from early to late adolescence: the Norwegian Longitudinal Health Behaviour Study, Drug. Alcohol. Rev. 35 (2016) 307–316.
[55] M.R. Munafò, B. Hitsman, R. Rende, C. Metcalfe, R. Niaura, Effects of progression to cigarette smoking on depressed mood in adolescents: evidence from the National Longitudinal Study of Adolescent Health, Addiction 103 (2008) 162–171.
[56] E. Goodman, G.B. Slap, B. Huang, The public health impact of socioeconomic status on adolescent depression and obesity, Am. J. Public Health 93 (2003) 1844–1850.
[57] A.C. Petersen, L. Crockett, M. Richards, A. Boxer, A self-report measure of pubertal status: reliability, validity, and initial norms, J. Youth Adolesc 17 (1988) 117–133.
[58] V. Zakopoulou, V. Mavreas, P. Christodoulides, A. Lavidas, E. Fili, G. Georgiou, G. Dimakopoulos, M. Vergou, Specific learning difficulties: a retrospective study of their co morbidity and continuity as early indicators of mental disorders, Res. Dev. Disabil. 35 (2014) 3496–3507.
[59] C.f.H. Statistics, RMass in.
[60] C. Bono, L.D. Ried, C. Kimberlin, B. Vogel, Missing data on the Center for Epidemiologic Studies Depression Scale: a comparison of 4 imputation techniques, Res. Soc. Admin. Pharmacy 3 (2007) 1–27.
[61] J. Pinheiro, D. Bates, S. DebRoy, D. Sarkar, nlme: linear and nonlinear mixed effects models, (2007) 1–97.
[62] I.S.M. van der Wurff, C. von Schacky, T. Bergeland, R. Leontjevas, M.P. Zeegers, J. Jolles, P.A. Kirschner, R.H.M. de Groot, Effect of 1 year krill oil supplementation on cognitive achievement of dutch adolescents: a double-blind randomized controlled trial, Nutrients 11 (2019) 1230.
[63] M.W. Markhus, S. Skotheim, I.E. Graff, L. Frøyland, H.C. Braarud, K.M. Stormark, M.K. Malde, Low omega-3 index in pregnancy is a possible biological risk factor for postpartum depression, PLoS ONE 8 (2013) e67617.
[64] W.S. Harris, C. Von Schacky, The Omega-3 Index: a new risk factor for death from coronary heart disease? Prev. Med. 39 (2004) 212–220.
[65] R. Grant, A. Bilgin, J. Guest, M.J. Morris, M. Garg, R. Pearce, The relative value of measures of omega-3 index, perceived stress, cortisol and sleep time in identifying depression among a cohort of Australian adolescents, Int. J. CHild Health Nutr. 4 (2015) 40–49.
[66] G. Grosso, F. Galvano, S. Marventano, M. Malaguarnera, C. Bucolo, F. Drago, F Caraci, Omega-3 fatty acids and depression: scientific evidence and biological mechanisms, Oxidat. Med. Cell. Longevity, 2014 (2014).
[67] P.-.Y. Lin, K.-.P. Su, A meta-analytic review of double-blind, placebo-controlled trials of antidepressant efficacy of omega-3 fatty acids, J. Clin. Psychiatry 68 (2007) 1056–1061.
[68] Y. Liao, B. Xie, H. Zhang, Q. He, L. Guo, M. Subramaniapillai, B. Fan, C. Lu, R. Mclntyer, Efficacy of omega-3 PUFAs in depression: a meta-analysis, Transl Psychiatry 9 (2019) 1–9.
[69] J. Kaufman, A. Martin, R.A. King, D. Charney, Are child-, adolescent-, and adult-onset depression one and the same disorder? Biol. Psychiatry 49 (2001) 980–1001.
[70] A. Ramirez, L. Ekselius, M. Ramklint, Depression in young adult psychiatric outpatients: delimiting early onset, Early Interv. Psychiatry 9 (2015) 108–117.
[71] E.A. Crone, R.E. Dahl, Understanding adolescence as a period of social–affective engagement and goal flexibility, Nature Rev. Neurosci. 13 (2012) 636–650.
[72] J.T. Brenna, Arachidonic acid needed in infant formula when docosahexaenoic acid is present, Nutr. Rev. 74 (2016) 329–336.
[73] I.S.M. van der Wurff, B.J. Meyer, R.H.M. de Groot, A review of recruitment, adherence and drop-out rates in Omega-3 polyunsaturated fatty acid supplementation trials in children and adolescents, Nutrients 9 (2017).
[74] M.R. DiMatteo, H.S. Lepper, T.W. Croghan, Depression is a risk factor for noncompliance with medical treatment: meta-analysis of the effects of anxiety and depression on patient adherence, Arch. Intern. Med. 160 (2000) 2101–2107.