Omega-3 Supplementation and Prevention of Preterm Delivery

-a systematic review

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Bachelor’s Thesis in Clinical Nutrition 15 ECTS
Programme in Dietetics 180/240 ECTS
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Examiner: Mette Axelsen
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Abstract

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Background: To be born prematurely is associated with higher mortality and morbidity and preterm birth (PTB) is a problem world wide. It is defined by parturition before 37 completed weeks of gestation. There is a suggested association between omega-3 (n-3) fatty acids and gestational length and previous studies including women with low risk of preterm delivery (PD) have shown a decreased risk when supplemented with n-3 capsules. Whether this is applicable to pregnant women with high risk of PD is not clear since the scientific evidence regarding these women is limited.

Objective: The aim of this systematic review was to evaluate the evidence whether n-3 supplementation may reduce the risk of PD in women with high risk pregnancies compared to women receiving placebo.

Search strategy: The literature search was carried out in the databases PubMed and Scopus. MeSH-terms and free-text search words of relevance for the research question were used.

Selection criteria: The inclusion criteria were: randomised controlled trials, human studies, articles written in English and stating PD, PTB or gestational age as a primary or secondary outcome. The interventions were required to include only women with high risk pregnancies receiving long chain n-3 supplements. Studies including women with multiple pregnancies were excluded. Moreover, “fortification of foodstuffs” and “intervention starting after onset of third trimester” were also selected as exclusion criteria.

Data collection and analysis: Three articles that matched the inclusion criteria were identified and reviewed by three authors individually using a template from The Swedish Council on Health Technology Assessment. The combined strength of evidence for the three articles was set using the GRADE system.

Main results: The main finding of this systematic review was that there is moderate evidence (+ + +) that n-3 supplementation does not have an effect on reducing the risk of PTB in women with high risk pregnancies.

Conclusions: Supplementation of n-3 can at present not be recommended to women with high risk pregnancies as a tool to prevent PTB in the western world. However, it can not be ruled out that there may be other reasons for recommending n-3 supplementation during pregnancy.
Sammanfattning

Titel: Omega-3 Supplementering och Prevention av Prematur Födsel - en systematisk översiktsartikel

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Syfte: Syftet med denna systematiska översiktsartikel var att undersöka huruvida n-3 supplementering kan minska risken för prematur födsel bland kvinnor med hög-riskgraviditeter jämfört med kvinnor som får placebo.


Urvalskriterier: Inklusionskriterierna var: randomiserade kontrollerade studier, human studier och artiklar skrivna på engelska som angivit förtidsbörd, prematur födsel eller graviditetslängd som primärt eller sekundärt utfallsmått. Att interventionerna utfördes på kvinnor med hög risk för prematur födsel som fick långkedjiga n-3 supplement var också ett krav. Studier gjorda på kvinnor med flerbarnsbörd exkluderades, liksom studier där interventionen bestod av n-3 berikade livsmedel eller startade efter att tredje trimestern hade påbörjats.


Resultat: Denna systematiska översiktsartikels huvudsakliga resultat var att det finns måttlig evidens (+ + +) för att n-3 supplementering inte minskar risken för prematur födsel hos kvinnor med hög-riskgraviditeter.

Slutsats: I nuläget finns det inte belägg att rekommendera n-3 supplementering till kvinnor med hög-riskgraviditeter för att minska risken för prematur födsel i västvärlden. Det går dock inte att utesluta andra skäl till rekommendation av n-3 supplementering under graviditet.
Abbreviations and explanations

17P = 17α-hydroxyprogesterone caproate
BMI = Body Mass Index
CI = Confidence Intervals
DHA = Docosahexaenoic acid
E% = Percent of total energy intake
EFSA = European Food Safety Authority
EPA = Eicosapentaenoic acid
IUGR= Intra Uterine Growth Retardation
kcal= kilocalories
LC = Long Chain
n-3 = Omega-3
n-6 = Omega-6
OR = Odds Ratio
PD = Preterm Delivery
PG = Prostaglandins
PIH = Pregnancy-Induced Hypertension
PTB = Preterm Birth
PUFA = Polyunsaturated Fatty Acids
RCT = Randomised Controlled Trial
RR = Risk Ratio
SBU = Statens Beredning för medicinsk Utvärdering
(The Swedish Council on Health Technology Assessment)
WHO = World Health Organisation

Eicosanoides: Biologically active signalling molecules derived from 20-carbon fatty acids
Preterm birth: Being born before 37 completed weeks of gestation
Preterm delivery: Giving birth before 37 completed weeks of gestation
Primigravida: A woman pregnant for the first time
Multigravida: A pregnant woman who has experienced one or more previous pregnancies
Multiple pregnancy: A women carrying more than one foetus
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Introduction

Preterm birth (PTB) is defined as parturition before 37 completed weeks of gestation. Globally, it is the most common cause of death amongst infants and it is estimated that 10% of all births are preterm (1). In Sweden it is estimated that almost 5% of all singleton births and 42% of multiple births are preterm (2). Aside from higher mortality, infants born prematurely also suffer higher morbidity both in the short and longer term. Being born prematurely may cause issues with underdeveloped organs such as the lungs, eyes and gastro-intestinal tract. Cognitive impairment is also a major problem for these children (3).

There are several risk factors for giving birth before reaching full-term. A previous preterm delivery (PD) is associated with an increased risk of recurrence of PD, and other risk factors include: hypertension, maternal age, multiple pregnancies, high body mass index (BMI), smoking and socioeconomic factors (4, 5). Still, the mechanisms behind PD are not yet fully understood. One factor that deserves further investigation is the suggested association between omega-3 (n-3) and length of gestation. For example, an epidemiologic study comparing birth data from the Faroe islands and Denmark showed a longer pregnancy duration in the Faroese population which correlated with a higher fish intake (6).

Long chain (LC) omega-3 and omega-6 (n-6) fatty acids are polyunsaturated and essential to human beings as we cannot synthesize them ourselves. They are therefore needed as a part of a balanced diet. Sources of n-3 fatty acids include oily fish, some types of algae, walnuts and rapeseed oil, although only the marine sources i.e. oily fish and algae contain LC n-3 (eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)). Fatty acids from the n-6 family can be found in vegetable oils and to some extent in meat, dairy products and eggs (7).

In Sweden the recommended dietary intake of n-3 fatty acids is >1E% (8), which is equal to 2.2 g for a person with a daily intake of 2,000 kcal. Moreover, it is stated in the Nordic Nutrition Recommendations that an intake of 200-250 mg EPA and DHA is associated with positive health effects (8). Both the World Health Organisation (WHO) and the European Food Safety Authority (EFSA) also recommend an intake of 250 mg EPA and DHA per day (9). The average Swedish intake of n-3 fatty acids is estimated to be 2.7 g/day (10), similar between the genders, which exceeds the recommendations for both men and women.

In a systematic analysis by Micha et al (11) it was found that 45 out of 187 countries had an adequate average intake of marine sources of omega-3, i.e. >250 mg, whereas 2/3 of the countries had a very low mean consumption: <100 mg/day. The lowest intakes were generally found in countries in Sub-Saharan Africa, North Africa, the Middle East and some Asian regions. The same systematic analysis concluded that the global mean intake of DHA and EPA is 163 mg/day although the national variation ranges from 5 mg - 3886 mg/day (11).

LC n-3 fatty acids promote normal growth and overall health and also play a vital role in the development of the neurological system and vision (12). In the beginning of the third trimester the foetus' brain starts to grow rapidly and 30-45 mg of DHA is accumulated in the central nervous system daily (13). Omega-3 and omega-6 share the same metabolic pathway and compete for the same enzymes. Historically the dietary intake ratio between n-6 and n-3 fatty acids has been as low as 1-2:1 in western diets, whereas the current western diet has a ratio of approximately 20-30:1 (14). LC polyunsaturated fatty acids (PUFA) of the n-3 and n-6 families are precursors to prostaglandins
(PG), which are a type of eicosanoides. Prostaglandins are hormone-like substances with many physiological functions, e.g. blood pressure regulation, muscle tonus, and they also play a role in the onset of parturition (15). It is believed that an increased intake of n-3 fatty acids reduces the PGs derived from n-6 fatty acids which are vasoconstricting and may induce labour (16).

As previously mentioned, a history of PD increases the recurrence risk. Today different pharmacological prophylactic treatments such as 17α-hydroxyprogesterone caproate (17P) can be given to women with high risk pregnancies (16). 17P is a synthetic version of the hormone progesterone and is believed to reduce the risk of PD by contributing to a lower activity of the uterine smooth muscles (17). As it stands, n-3 is not currently used as a prophylactic treatment for PD. However, it is a topic that is under research. A systematic review including women with low risk pregnancies has shown a positive effect of n-3 supplementation or fortification on pregnancy duration and a decreased risk of PD (18). The doses used in the systematic review ranged from 133 mg of DHA to 2.7 g EPA + DHA. One of the included studies was conducted using fortification of eggs, and showed a significant reduction in PD (19). Another study, not included in the systematic review, administering fortified milk powder, containing DHA amongst other nutrients, did not show any significant results for the outcome measure gestational age. A tendency was seen towards longer gestation but the power was estimated to be too low as this was a secondary outcome (20). Most studies that are conducted include only women with low risk of PD and the clinical relevance of further lowering the risk in this population can be discussed.

Problem

PTB is associated with higher mortality and morbidity and is a problem worldwide. A previous PD is the greatest risk factor for parturition before 37 weeks. The scientific evidence regarding women with high risk pregnancies and n-3 supplementation is limited and not up to date, although it could be argued that these women would potentially benefit the most from such intervention. Since a positive effect has been found in women with low risk pregnancies, it is likely that women with high risk pregnancies could also benefit from this prophylactic treatment and therefore an updated systematic review is needed.

Aim

The aim of this systematic review was to evaluate the evidence whether n-3 supplementation could reduce the risk of PD in women with high risk pregnancies.

Research question

Does omega-3 supplementation during pregnancy, compared with placebo, reduce the risk of preterm delivery in women with high risk pregnancies?
Method

This systematic review summarises the literature published until January 2015 regarding omega-3 supplementation in high risk pregnancies and the reduction of risk of PD.

Inclusion and exclusion criteria

When searching for scientific articles the following criteria were used during the data collection:

Inclusion criteria
- Randomized controlled trial (RCT)
- Articles written in English
- Preterm birth, preterm delivery or gestational age stated as a primary or secondary outcome
- LC n-3 (EPA and DHA) supplementation during pregnancy
- Women with high risk pregnancies
- Human studies

Exclusion criteria
- Women with multiple pregnancy
- Fortification of foodstuffs
- Intervention starting after onset of third trimester (gestation week 29)

Collection of data

A systematic literature search in two databases (PubMed and Scopus) was conducted by all three authors. MeSH-terms and free-text search words in several combinations were used during the search in PubMed to ensure that no relevant articles were missed. The search strategy in PubMed included the use of filters to exclude articles that were not written in English, of a different study design than RCT and to include only human studies. The search function was different in Scopus which made it more difficult to limit and define the searches. Text words relevant to the research question were used. Search functions “AND”, “OR” and “AND NOT” were used in both databases to extract relevant articles. Titles and, if necessary, abstracts were read and articles were either excluded or included for further analysis depending on the relevance for the research question. In total 32 articles were identified by this method. Dates and search words are shown in Table 1.
<table>
<thead>
<tr>
<th>Search</th>
<th>Database</th>
<th>Date</th>
<th>Search words</th>
<th>Limitations</th>
<th>Matches found</th>
<th>Chosen articles</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pubmed</td>
<td>27/01/15</td>
<td>Fatty acids, omega-3 OR fish oils AND obstetric labor, premature</td>
<td>English, RCT, human</td>
<td>13</td>
<td>9</td>
<td>(19, 21-28)</td>
</tr>
<tr>
<td>2</td>
<td>Pubmed</td>
<td>27/01/15</td>
<td>Fatty acids, omega-3 OR fish oils AND pregnancy duration</td>
<td>English, RCT, human</td>
<td>8</td>
<td>6[3]</td>
<td>(19, 22, 27, 29-31)</td>
</tr>
<tr>
<td>3</td>
<td>Pubmed</td>
<td>27/01/15</td>
<td>Fatty acids, omega-3 OR fish oils AND gestational duration</td>
<td>English, RCT, human</td>
<td>5</td>
<td>3[3]</td>
<td>(19, 22, 27)</td>
</tr>
<tr>
<td>4</td>
<td>Pubmed</td>
<td>27/01/15</td>
<td>Fatty acids, omega-3 OR fish oils AND gestational length</td>
<td>English, RCT, human</td>
<td>17</td>
<td>7[3]</td>
<td>(19, 22, 28, 32-35)</td>
</tr>
<tr>
<td>5</td>
<td>Pubmed</td>
<td>27/01/15</td>
<td>Fatty acids, omega-3 OR fish oils AND pregnancy length</td>
<td>English, RCT, human</td>
<td>13</td>
<td>10[8]</td>
<td>(19, 22, 28, 30, 32-37)</td>
</tr>
<tr>
<td>6</td>
<td>Scopus</td>
<td>28/01/15</td>
<td>Omega-3 AND supplement AND preterm birth OR premature birth OR pregnancy length OR pregnancy duration OR gestational length OR gestational duration OR preterm labor OR premature labor AND NOT breastfeeding AND NOT nursing AND NOT Rat</td>
<td>English, article</td>
<td>17</td>
<td>6 [6]</td>
<td>(22, 27-30, 33)</td>
</tr>
</tbody>
</table>

[ ] = duplicates

A review of the methods of the 32 articles was performed to evaluate the correspondence between the articles and the inclusion criteria. This resulted in three included articles by Harper et al (2010), Olsen et al (2000), and Onwude et al (1995) (24, 26, 31). Snowballing was performed on the three included articles and a systematic review on the effect of PUFA intake during pregnancy (42), which led to the identification of a fourth article (52). However it was excluded due to poor accordance with the inclusion criteria. In the excluded article, gestational length was measured, although the main focus was pregnancy induced hypertension (PIH) and intra-uterine growth retardation (IUGR) and neither PTB or gestational age were stated as a primary or secondary outcome. See flowchart (Figure 1). Reasons for the exclusion of articles are presented in Appendix 1.
Processing of data

The three included articles were read, analysed and graded separately by the three authors. The outcome measure reviewed was PD i.e. parturition before 37 completed weeks of gestation. Using a template from The Swedish Council on Health Technology Assessment (SBU) (53), the authors individually evaluated the risk of selection bias, performance bias, detection bias, attrition, reporting bias and other considerations such as conflicts of interest, resulting in a combined score for each article: low, moderate or high risk of bias. Subsequently the grading was compared and consensus was reached regarding a score for each article. Grading of evidence for the three articles was performed by the three authors together by using a modified GRADE-template from the University of Gothenburg.

GRADE is an acronym for “the Grading of Recommendations Assessment, Development and Evaluation” and is a system for grading quality and strength of evidence in health care (54). The score system within GRADE rates evidence and quality as High (+++), Moderate (++), Low (+) or Very Low (+).
Results

The included articles are summarised in Table 2 and subsequently presented individually in text. A compiled judgment of the strength of evidence can be found under “Grading of evidence”.

Table 2. Description of studies.

<table>
<thead>
<tr>
<th>Author, Year Country</th>
<th>Study design</th>
<th>Population (n/drop-outs)</th>
<th>Intervention Control</th>
<th>Primary</th>
<th>Delivery &lt; 37 weeks</th>
<th>Compliance Measurement of compliance</th>
<th>Quality of study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harper, M., et al. 2010 United States of America</td>
<td>RCT 13 multicentre trials</td>
<td>Pregnant women with Previous PD on 17α-hydroxyprogesterone treatment. n = 852 (no drop outs)</td>
<td>Intervention: 4 capsules daily containing a total of 2 g n-3 fatty acids, of which 1.2 g EPA and 0.8 g DHA. Control: 4 matching capsules containing inert mineral oil</td>
<td>Primary</td>
<td>Intervention group: 37.8% Control group: 41.6% (RR 0.91, 95% CI 0.77 to 1.07)</td>
<td>The compliance rate was 85.1% in the fish oil group and 84.8% in the placebo group Measurement method: Capsule counting</td>
<td>High</td>
</tr>
<tr>
<td>Olsen, S. F., et al. 2000. Denmark</td>
<td>RCT 6 multicentre trials</td>
<td>Pregnant women with previous PD or PIH or IUGR. n = 898 (57 drop outs)</td>
<td>Intervention: 4 capsules (Pikasol) daily containing a total of 2.7 g n-3 fatty acids, of which 1.3 g EPA and 0.9 g DHA. Control: 4 capsules containing olive oil</td>
<td>Primary</td>
<td>Intervention group: 10.6% Control group: 15.2% * (OR 0.67, 95% CI 0.41 to 1.07).</td>
<td>80.3% in the fish oil group and 78.3% in the olive oil group took their prescribed capsules Measurement method: Compliance forms. 98% answering frequency.</td>
<td>Moderate-high</td>
</tr>
<tr>
<td>Onwude, J. L., et al. 1995. United Kingdom.</td>
<td>RCT</td>
<td>Pregnant women with a history of at least one small baby, a history of PIH, a history of unexplained stillbirth or abnormal uterine artery Doppler blood flow. n = 233 (1 drop out)</td>
<td>Intervention: 9 capsules (MaxEpa) daily containing a total of 2.7 g n-3 fatty acids of which 1.62 g EPA and 1.08 g DHA. Control: 9 matching air-filled capsules daily</td>
<td>Secondary</td>
<td>Intervention group: 18.6% Control group: 16%**</td>
<td>50% in the fish oil group and 57% in the placebo group took &gt;70% of prescribed capsules Measurement method: Capsule counting</td>
<td>Low-moderate</td>
</tr>
</tbody>
</table>

*Data presented after eliminating elective deliveries.
**Confidence intervals not shown.
Description of studies

Omega-3 fatty acid supplementation to prevent recurrent preterm birth: A randomized controlled trial (24).

This double-blinded RCT was carried out in 13 centres in the USA. Overall 852 pregnant women with a previous singleton spontaneous PD were included and none was lost to follow up. The women were randomised to either the intervention group or the placebo group. Both groups received a weekly intramuscular injection of 17P caproate (250 mg). Additionally the intervention group was supplemented with n-3 capsules, containing 1.2g EPA and 0.8g DHA, whilst the control group received matching capsules filled with inert mineral oil, to be taken daily in both cases.

All women were included between 16-22 weeks of gestation and capsules were given until parturition or 37 completed weeks of gestation, whichever occurred first. Gestational age was determined using ultrasound examination and information on the last menstrual period. The study objective was to evaluate the effect of n-3 supplementation on the recurrence risk for PD in women already receiving 17P caproate. The primary outcome stated was delivery before 37 completed weeks of gestation. Compliance was measured by capsule count and the mean compliance rate for the n-3 group was 85.1% and 84.8% in the placebo group.

Results: The gestational length was on average two days longer in the intervention group. However, the difference was not significant (P=0.26). Nor was there a significant difference in the risk of PD (relative risk [RR] 0.91, 95% confidence interval [CI] 0.77 to 1.07).

Quality assessment: The study quality was rated as high. The participants were randomised through the simple urn method and the baseline characteristics for the two groups were similar. Considering that the study was double-blinded, none was lost to follow up and compliance was deemed to be satisfactory, the risk of bias was judged to be low.

Randomised clinical trials of fish oil supplementation in high risk pregnancies (26).

This study, consisting of six separate multicentre double-blinded RCTs, was carried out in 19 centres in Europe. The study in whole included 1619 women, distributed over six trials based on the women's medical and obstetric history. In all six trials the women were randomised to an intervention group or a control group. Trial A included 232 women with a previous PD. Trial B included 280 women with IUGR in a prior pregnancy. Trial C included 386 women who in an earlier pregnancy had experienced PIH. Trial D included 579 women carrying twins. These four trials were labelled as prophylactic. The two remaining trials, trial E and trial F, were labelled therapeutic. Trial E included 79 women with threatening pre-eclampsia and trial F included 63 women with suspected IUGR in their current pregnancies. Trial D, E and F did not match the inclusion criteria for this systematic review and therefore only the 898 women from trial A, B and C are included in this systematic review.
The intervention groups in trials A, B and C were supplemented with a daily dose of n-3 capsules containing 1.3g EPA and 0.9g DHA. The control groups were given matching capsules of olive oil. Supplementation started around gestational week 20 for all women. Gestational age was determined using ultrasound examination where possible and information on the last menstrual period when no ultrasound examination was performed. The study objective was to investigate the presupposed positive effect of n-3 intake on PD, PIH and IUGR, which were also the main outcome measures. Compliance was measured through questionnaires mailed to the women in Scandinavian and English-speaking countries. In total 57 women dropped out (6%).

Results: When elective deliveries were excluded the gestational length was on average 4.8 days (95% CI 1.96 to 7.67) longer in the group who received fish oil, but there was no significant reduction in risk of PD in the combined trials (odds ratio [OR] 0.67, CI 0.41 to 1.07). However, in trial A (women with a previous PD) the recurrence risk was significantly reduced (OR 0.54, 95% CI 0.30 to 0.98).

Quality assessment: The study quality was rated as moderate/high. The participants were randomised through restricted block-wise computer generated randomisation and the baseline characteristics were similar for the intervention and control groups in all trials. The overall risk of bias was low/moderate considering the incomplete compliance measuring method, the absence of analysis of drop-out data and inconsistency in the method used to determine gestational age.

A randomised double blind placebo controlled trial of fish oil in high risk pregnancy (31).

This double-blinded RCT was carried out in the UK. A total of 232 pregnant women were included in the study, of whom 160 were multigravida with either a history of one or more small babies, a history of proteinuric or nonproteinuric PIH or a history of unexplained stillbirth. The remaining 72 were primigravida with abnormal uterine arcuate artery Doppler flow at 24 weeks of gestation. One woman from the group of multigravida was lost to follow up. The women were randomised to either the intervention group or the control group. The intervention group received n-3 capsules containing 1.62 g EPA and 1.08 g DHA and the control group was given matching air-filled capsules to be taken daily.

Supplementation started around gestational week 24 and was continued until gestational week 38. Gestational age was determined using ultrasound examination. The objective of the study was to examine the potential beneficial effect of n-3 fatty acid supplementation during pregnancy. Main outcome measures were occurrence of proteinuric or nonproteinuric PIH or birth weight < 3rd centile. Gestational age at delivery was a secondary outcome. Compliance was measured through capsule counting, 50% of the participants in the intervention group and 43% in the control group took > 70% of their supplied capsules.

Results: There was no significant difference in any of the outcome measures. Mean gestation in weeks was 38.1 in both the intervention and control group (95% CI between the groups was -0.07 to 0.06). Delivery before 37 completed weeks of gestation occurred in 18.6% of the pregnancies in the intervention group and 16% of the pregnancies in the control group. However, no CI or P-values were presented for this outcome.
Quality assessment: The study quality was rated as low/moderate. The randomisation was computer-generated and carried out through the principle of third-party randomisation and the baseline characteristics were sufficiently similar. The principal issue of the study was the poor compliance. Moreover, the absence of an appropriate statistical presentation of the outcome measure of interest for this systematic review contributed to the overall risk of bias being judged to be moderate/high.

Grading of evidence

The GRADE analysis of the outcome measure PD is presented in Table 3. The aggregated strength of evidence was determined to be moderate (+++). Some limitations concerning the internal validity were agreed upon due to the unsatisfactory compliance in Onwude et al (31), and the unsuitable method of measuring compliance in Olsen et al (26). Additionally, the difficulty in blinding the marine taste of the n-3 capsules contributed to the assessment of the internal validity. No limitations were found regarding homogeneity: none of the studies found a significant positive effect of the intervention although two of the studies showed a positive trend in the reduction of risk of PD. Moreover, Olsen et al (26) found a difference (p=0.05) when Trial A, including the women with a previous PD and thus being at the highest risk, was analysed separately. Some limitations were also seen in terms of precision due to the absence of confidence intervals or P-values in Onwude et al (31) for the outcome measures of interest in this systematic review. There were no limitations observed as to the external validity or risk of publication bias.

Table 3. Strength of evidence.

<table>
<thead>
<tr>
<th>Outcome measure</th>
<th>Preterm delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of studies</td>
<td>3</td>
</tr>
<tr>
<td>Study design – internal validity</td>
<td>Some limitations</td>
</tr>
<tr>
<td>Homogeneity</td>
<td>No limitations</td>
</tr>
<tr>
<td>Study population – external validity</td>
<td>No limitations</td>
</tr>
<tr>
<td>Precision</td>
<td>Some limitations</td>
</tr>
<tr>
<td>Publication bias</td>
<td>No limitations</td>
</tr>
<tr>
<td>Strength of evidence</td>
<td>Moderate (+ + +)</td>
</tr>
</tbody>
</table>
Discussion

The main finding of this systematic review is that there is moderate evidence (+ + +) that n-3 supplementation does not have an effect on reducing the risk of PD in women with high risk pregnancies. There was no difference in parturition before 37 weeks of gestation amongst the women who received n-3 capsules and those who did not. However, a trend of a small increase in gestational length was observed.

Method discussion

The literature search was conducted in the databases PubMed and Scopus. Performing a search in these databases requires some skill due to the difficulty of limiting the search without missing relevant articles. The matter is further complicated by the fact that the databases are not designed in the same way and therefore function differently. To minimise the risk of not identifying all articles matching the inclusion criteria, several searches with different search words were carried out which can be seen as a strength of this article. Another strength is that three authors independently reviewed the three included articles, using standardised templates (53).

Criteria for inclusion and exclusion were determined to enable an answer to the research question. PTB, PD or gestational age stated as a primary or secondary outcome were decided upon to ensure that the study in question was designed to investigate the outcome of interest for this systematic review. If an outcome is presented without being stated as primary or secondary, there may be a risk of type II error due to the sample size not being calculated for observing a significant difference in the outcome of interest. Trials including n-3 fortified foodstuffs instead of supplementation through capsules were excluded due to the possible confounding effects of other components in the food.

To keep the intervention and study populations homogenous, exclusion criteria were set regarding interventions including multiple pregnancies and onset of supplementation. Trials including women with multiple pregnancies were excluded since women carrying more than one foetus run a considerably higher risk of PD than other women (55). Whether n-3 supplementation reduces the risk of PD in women with multiple pregnancies is an interesting and important question. However, this population requires a separate systematic review. Furthermore, it was decided that supplementation of n-3 should commence before the women entered the third trimester considering the hypothetical increased requirement during this period (56).

The relevance of choosing “intervention starting after onset of third trimester (gestation week 29)” as an exclusion criteria can be discussed. A rapid effect of n-3 supplementation on gestational length has been proposed (18), but the scientific evidence supporting this theory is limited and most studies commence supplementation when the women are in the second trimester. Therefore, only trials with this design were included to keep the interventions comparable.
Result discussion

'High risk pregnancies' is a term used in the three included articles describing the participating women, although there is no consistent definition. All trials in this systematic review included women with an increased risk of PD, but the studies all had different inclusion criteria and the underlying risk factors differed in the women. One of the studies only included women with a previous PD (24), whereas the other two included women with various risk factors (26, 31). A significant difference between intervention and control women was observed in the study by Olsen et al when Trial A (including only women with a previous PD) was analysed independently, which may be an indication for the importance of separating the different risk factors in future studies. The absence of significant results in the study conducted by Harper et al, which also only included women with a previous PD, can possibly be explained by the fact that they were already undergoing treatment to prevent recurrence. An additional risk reduction of n-3 supplementation on PD might not be possible in women already receiving 17P. Since a slight tendency towards a reduction in risk of PD was seen in the studies by Harper et al and Olsen et al, another possible reason for the absence of significant results is that the sample sizes might have been too small to reach power.

A major difficulty in conducting RCTs involving supplementation with n-3 capsules is the complexity of blinding. All studies in this systematic review were designed to be double-blinded, however, in two of the studies it was reported that many of the women in the intervention groups correctly guessed that they were allocated to the fish-oil group. In the article by Olsen et al, the authors even state that the capsules were not identical in taste. Some studies attempt to flavour both the placebo and fish-oil capsules with other flavours e.g. orange (22), although a potentially better solution would be to not attempt to mask the fish taste, but instead add fish flavour to the placebo capsules. That way, participants in the control groups would be more likely to believe that they are allocated to the intervention group.

A prerequisite for obtaining a possible effect of the intervention in a RCT is that the participants follow given instructions. It is very difficult to draw conclusions from studies where compliance is low due to the fact that the fundamental idea of an RCT is that there is one intervention group and one control group and that they follow two different regimes. In the study by Onwude et al compliance was poor, which is the main reason for its assessed low/moderate quality. There is a possibility that an effect of the n-3 supplementation could have been observed if the percentage of capsules taken had been higher in the intervention group. It may also be possible that a significant difference could have been observed if the doses n-3 in the included studies had been higher. Similar doses has shown a reduction of PD in low risk pregnancies (18) and it could be argued that for women with high risk pregnancies those doses are not sufficient.

The content of the placebo capsules is a possible confounding factor. As discussed in the article by Olsen et al, it is not possible to know for certain that olive oil is completely inert, as it contains n-6 fatty acids. If n-6 in fact has an adverse effect in preventing PD, the n-3 supplementation would appear more beneficial in studies where the placebo capsules contain n-6 fatty acids. Since the placebo consisted of olive oil capsules in the only trial with a significant difference (Trial A, Olsen et al), there is a slight possibility that this difference is due to an increased risk of PD in the placebo group, rather than a decreased risk in the intervention group.

Another potential confounder is the women's food intake in general and particularly their fish intake. Regardless if a significant difference is obtained or not, this might be explained by variation
between the groups in food intake. Only one of the included studies in this systematic review measured fish intake at baseline to ensure similarity between the groups (24). In the studies that did not measure fish intake, a non-significant difference might be caused by a higher intake of fish in the control group. As a participant in a study investigating positive effects of n-3, it is conceivable that, consciously or subconsciously, one might be inclined to eat more fish than usual.

Omega-3 in a local and global perspective

Since all three of the studies included in this systematic review were carried out in the developed world where n-3 intake is generally sufficient, and none of the articles reported the women's n-3 intake, it is possible that n-3 supplementation has no effect on women with an already adequate n-3 intake. As previously mentioned the average Swedish intake of n-3 fatty acids exceeds the recommendation of >1E%. However, it is important to keep in mind that it is in fact an average, therefore being an inadequate measure of individual intake. It is probable that there is a great difference between the individuals with the lowest and highest intakes, and n-3 supplementation may be beneficial for women who do not meet the recommendations.

Globally, the range of n-3 intake is very wide and many people do not meet the recommendations. Hypothetically, if the whole world's population were to eat oily fish, or n-3 supplements derived from fish, in amounts meeting the recommendations, the already threatened fish population would be in even greater danger than it is today. According to Nichols et al (57) the global fish stock is so vulnerable that it might collapse as soon as 2048. It can be argued that the solution to the problem of providing the whole world with adequate amounts of LC n-3 fatty acids does not lie solely in increased fish consumption, but in alternative sources such as algae and possibly even genetically modified foods.

Conclusion

In conclusion, there is moderate evidence that n-3 supplementation has no effect on preventing PD in women with high risk pregnancies in the western world. At present, n-3 supplementation cannot be recommended as a tool to prevent PD for women with a high risk. However, it is plausible that supplementation has other beneficial effects since n-3 is important in foetal development. Other reasons for recommending n-3 capsules in pregnancy cannot therefore not be ruled out.

If further research is carried out on this topic, it would be advisable to separate women with different risk factors since women with certain risk factors, such as a previous PD as shown in the article by Olsen et al, may benefit more than women with other risk factors from supplementation with n-3. Since the three studies included in this systematic review were all carried out in the western world where n-3 intake is generally sufficient, it could be relevant to investigate if n-3 supplementation has an effect in women with high risk pregnancies in less developed parts of the world, where n-3 intake is lower (11). Measuring n-3 status and fish intake at baseline and throughout the study to identify a possible difference in outcome between women with high and low levels of n-3 would be interesting. If such a difference were to be observed, this could be helpful in setting nutritional recommendations for pregnant women in the future.
References


45. Mulder KA, King DJ, Innis SM. Omega-3 fatty acid deficiency in infants before birth identified using a


# Appendix 1

## Included and excluded articles

<table>
<thead>
<tr>
<th>Article</th>
<th>Reason for exclusion or inclusion</th>
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<tbody>
<tr>
<td>Reference</td>
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- Not high-risk pregnancies
- Study excluded preterm births
- Part of previous study (Helland 2001)
- Systematic review
- Not high-risk pregnancies
<table>
<thead>
<tr>
<th>Reference</th>
<th>Note</th>
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<tbody>
<tr>
<td>Mulder KA, King DJ, Innis SM. Omega-3 fatty acid deficiency in infants before birth identified using a randomized trial of maternal DHA supplementation in pregnancy. PLoS ONE. 2014;9(1).</td>
<td>Not high-risk pregnancies</td>
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