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Randomised clinical trials of fish oil supplementation in high risk pregnancies

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Objective To test the postulated preventive effects of dietary n-3 fatty acids on pre-term delivery, intrauterine growth retardation, and pregnancy induced hypertension.

Design In six multicentre trials, women with high risk pregnancies were randomly assigned to receive fish oil (Pikasol) or olive oil in identically-looking capsules from around 20 weeks (prophylactic trials) or 33 weeks (therapeutic trials) until delivery.

Setting Nineteen hospitals in Europe.

Samples Four prophylactic trials enrolled 232, 280, and 386 women who had experienced previous pre-term delivery, intrauterine growth retardation, or pregnancy induced hypertension respectively, and 579 with twin pregnancies. Two therapeutic trials enrolled 79 women with threatening pre-eclampsia and 63 with suspected intrauterine growth retardation.

Interventions The fish oil provided 2·7 g and 6·1 g n-3 fatty acids/day in the prophylactic and therapeutic trials, respectively.

Main outcome measures Preterm delivery, intrauterine growth retardation, pregnancy induced hypertension.

Results Fish oil reduced recurrence risk of pre-term delivery from 33% to 21% (odds ratio 0·54 (95% CI 0·30 to 0·98)) but did not affect recurrence risks for the other outcomes (OR 1·26; 0·74 to 2·12 and 0·98; 0·63 to 1·53, respectively). In twin pregnancies, the risks for all three outcomes were similar in the two intervention arms (95% CI for the three odds ratios were 0·73 to 1·40, 0·90 to 1·52, and 0·83 to 2·32, respectively). The therapeutic trials detected no significant effects on pre-defined outcomes. In the combined trials, fish oil delayed spontaneous delivery (proportional hazards ratio 1·22; 1·07 to 1·39, $P = 0·002$).

Conclusions Fish oil supplementation reduced the recurrence risk of pre-term delivery, but had no effect on pre-term delivery in twin pregnancies. Fish oil had no effect on intrauterine growth retardation and pregnancy induced hypertension, affecting neither recurrence risk nor risk in twin pregnancies.

INTRODUCTION

Dyerberg *et al.* and Bang *et al.* proposed that high consumption of marine oils, rich in the long-chain n-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid, could explain the low incidence of cardiovascular diseases in Greenland Inuits[1–3]. This led to extensive research into the effects of fish oil on human health and physiology. It is now well established that the long-chain n-3 fatty acids interfere with eicosanoid production and lower plasma triglycerides, and that docosahexaenoic acid in particular is essential for optimal neural development[4–10].

It has been hypothesised that marine oils lower risks of certain complications of pregnancy, in particular pre-term delivery, intrauterine growth retardation, pre-eclampsia, and pregnancy induced hypertension. Epidemiological studies from the Faroe Islands suggested that marine diets increase birthweight[11–13], either by prolonging pregnancy[11] or by increasing fetal growth rate[14,15]. The suggested

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mechanisms included delayed timing of spontaneous delivery, resulting from altered balance between the prostaglandins involved in the initiation of labour[11], and increased fetal growth rate, resulting from improved placental blood flow due to lowered thromboxane/prostacyclin ratio[14] and blood viscosity[15]. Because some of their presumed mechanisms of action overlap with aspirin, it was thought that n-3 fatty acids could also protect against pre-eclampsia and pregnancy-induced hypertension[16–18]. Concerns have been raised, however, regarding the safety of fish oil supplementation in pregnancy, particularly regarding bleeding complications[13,19,20].

The present European multicentre study, in six simultaneously conducted randomised clinical trials, aimed at testing a number of hypotheses regarding safety aspects and possible prophylactic and therapeutic effects of fish oil supplementation in high risk pregnancies. The end points studied were recurrence risk of pre-term delivery, intrauterine growth retardation and pregnancy induced hypertension, risk of these outcomes in twin pregnancies (four 'prophylactic trials') and, furthermore, amelioration of threatening pre-eclampsia and suspected intrauterine growth retardation (two 'therapeutic trials').

METHODS

Subjects

The study was hospital-based with 19 centres (see Acknowledgements) in Denmark, Scotland, Sweden, England, Italy, The Netherlands, Norway, Belgium and Russia. Pregnant women seen at the centres were eligible if they belonged to one of the six trial groups defined below. The study was approved by the local ethical committees. All women received information, orally and written, about the study before they were asked for consent.

Design and entry criteria

The overall design was that of a multicentre randomised clinical intervention study, consisting of a series of prophylactic and therapeutic trials. The four prophylactic trials enrolled women after 16 weeks of gestation with an uncomplicated pregnancy, who in an earlier pregnancy had experienced (A) pre-term delivery (before 259 days of gestation), (B) intrauterine growth retardation (< 5th centile[21]) or (C) pregnancy induced hypertension (diastolic blood pressure > 100 mmHg), and (D) women with current twin pregnancies (trial D). The two therapeutic trials enrolled women with (E) threatening pre-eclampsia, i.e. women with signs or symptoms of pre-eclampsia in the current pregnancy (± intrauterine growth retardation), or (F) suspected intrauterine growth retardation (< 10th centile by ultrasonography[21]) in the current pregnancy. The six trials A-F will be referred to as Earl-PD, Earl-IUGR, Earl-PIH, Twins, Threat-PE, and Susp-IUGR, respectively. All six trials were mutually exclusive: Earl-PD did not contain any Earl-IUGR or Earl-PIH pregnancy and Earl-IUGR did not contain any Earl-PIH pregnancy; Twins, Treat-PE and Susp-IUGR were defined independently of experiences from any possible earlier pregnancies. In principle all clinical centres recruited for all six trials, although the relative recruitment rates varied between the centres (data not shown).

Exclusion criteria

The exclusion criteria in the trials were: diabetes mellitus in or before pregnancy; diagnosed severe fetal malformation or hydrops in current pregnancy; suspicion in current pregnancy, or occurrence in an earlier pregnancy, of placental abruption; drug or alcohol abuse; regular intake of fish oil or of nonsteroidal anti-inflammatory agents or other drugs with an effect on thrombocyte function or eicosanoid metabolism; allergy to fish products. In the therapeutic trials an additional exclusion criterion was high probability of delivering soon after randomisation (estimated within one week).

Intervention and blinding

The treatment was fish oil (Pikasol: 32% eicosapentaenoic acid, 23% docosahexaenoic acid, and 2 mg tocopherol/mL) whereas the controls received olive oil (oleic acid [18:1 n-9] 72%, linoleic acid [18:2: n-6] 12%) (percentages are w/w). In the four prophylactic trials four capsules of either oil were given per day, while in the two therapeutic trials nine capsules were given per day. For women randomised to fish oil, these amounts correspond to 2·7 g (1·3 g eicosapentaenoic acid and 0·9 g docosahexaenoic acid) and 6·1 g (2·9 g eicosapentaenoic acid and 2·1 g docosahexaenoic acid) of long-chain n-3 fatty acids per day in the prophylactic and therapeutic trials, respectively. Both oils were provided in 1 g identical-looking gelatine capsules, which were not identical in taste. Packages with capsules were identified by a cryptographed number, the code of which was known only by the Data Manager.

Assignment and allocation concealment

Restricted blockwise computer generated randomisation (1:1, individual-based) was employed within strata defined by cross tabulating clinical centres against the six trials. Within each stratum, the size of the first block was always four, after which block sizes of four, six, and eight occurred in random order. Randomisation identified a package number at the relevant centre, where packages were ordered in a random way as to oil type.

The packages contained enough capsules to cover the whole trial period for each woman.

Data management

Information regarding a woman's name, eligibility to study, and characteristics as to clinical group and centre, and regarding the result of randomisation (package number), was transferred between the main unit and the clinical units by fax and, later in the trial period, also by press button, voice-response telephone randomisation. A trial entry form with extensive base-line information was filled in at the time of randomisation, while a follow up form on the course of the pregnancy and the perinatal period was filled in within four weeks of delivery. These forms were subsequently mailed to the co-ordinating centre. A questionnaire, mailed after delivery to consecutive samples (n = 1150) of women at the Danish, Norwegian, Swedish and the English speaking centres, recorded self-experienced adverse effects, compliance, and blinding efficacy.

Sample size considerations, analytic strategy, and data monitoring

Sample size determinations were undertaken twice in this study. After four years of enrolment it was realised that the originally estimated sample sizes were unrealistically large. Therefore, a stopping strategy was developed which implied new, conditional sample size calculations[22]. A detailed account is given in appendix 1.

The stopping strategy was based on a number of *primary hypotheses*, defined *a priori*, for the prophylactic trials. They represented the clinical questions that the trial series was primarily meant to solve. For each primary hypothesis, a limited number of closely related *secondary hypotheses* was also pre-specified for the prophylactic trials by trialists unaware of the interim results (thus also *a priori*). These hypotheses represented additional, obvious questions to be answered. The primary and secondary hypotheses are stated in Tables 1 and 2.

During enrolment, a number of confidential interim analyses were undertaken by the Data Monitoring Committee, as described in appendix 1.

Outcome variables

Gestational age was assessed from early (pre-randomisation) ultrasound, if available, or else from data on the last menstrual period using Naegele's rule. Preterm delivery was defined as delivery at an estimated gestational age of less than 259 days (37 completed weeks), whereas early pre-term delivery was defined as delivery at an estimated gestational age of less than 238 days (34 completed weeks). Post-term delivery was defined as 294 days (42 completed weeks) or more. Intrauterine growth retardation < 10th centile, as well as deviation from birthweight expected from gestational age, were assessed from the infant's birthweight, gestational age and gender, on the basis of a Danish standard[21,23]. Pregnancy induced hypertension was defined as one or more recorded measurements of a diastolic blood pressure of above 90 mmHg at rest (thus different from entry criteria for the Earl-PIH trial). Pre-eclampsia was defined as the combination of pregnancy induced hypertension and proteinuria, which was defined as a urinary measurement of > 1+ in albustix, 0·3 g protein/L, 0·3 g protein/24 hours,

Table 1. Basic structure and primary hypotheses of the Fish Oil Trials In Pregnancy.

The prophylactic trials
Women were enrolled with uncomplicated pregnancies at higher than average risk because they had:
Had preterm delivery (before 259 days) in an earlier pregnancy (Earl-PD trial)
Had intrauterine growth retardation (below 5th centile) in an earlier pregnancy (Earl-IUGR trial)
Had pregnancy induced hypertension (diastolic blood pressure > 100 mmHg) in an earlier pregnancy (Earl-PIH trial)
Twins in the current pregnancy (Twins trial)
The primary hypotheses were that fish oil supplementation reduces the risk of:
Recurrence of preterm delivery (Earl-PD trial)
Recurrence of intrauterine growth retardation (Earl-IUGR trial)
Recurrence of pregnancy induced hypertension (Earl-PIH)
Preterm delivery in twin pregnancies (Twins trial)
Intrauterine growth retardation in twin pregnancies (Twins trial)
Pregnancy induced hypertension in twin pregnancies (Twins trial)
The therapeutic trials
Women were enrolled with a complication in the current pregnancy, i.e.:
Threatening pre-eclampsia (± intrauterine growth retardation) (Threat-PE trial)
Ultrasonically estimated fetal weight below the 10th centile[21] (Susp-IUGR trial)
The primary hypotheses were that fish oil supplementation increases:
Mean duration of time elapsed from enrolment to delivery (Threat-PE trial)
Mean birthweight adjusted for gestational age[21] (Susp-IUGR trial)

Table 2. Secondary (*a priori*) hypotheses of the Fish Oil Trials In Pregnancy.

Secondary hypotheses that relate to the prevention of preterm delivery*
Fish oil supplementation
Reduces the risk of early preterm delivery (< 238 days)
Reduces the risk of low birthweight (< 2500 g)
Increases mean pregnancy duration
Increases mean birthweight
Secondary hypotheses that relate to the prevention of intrauterine growth retardation†
Fish oil supplementation
Reduces the risk of low birthweight (< 2500 g)
Increases mean birthweight
Increases mean birthweight adjusted for pregnancy duration
Secondary hypotheses that relate to the prevention of pregnancy induced hypertension‡
Fish oil supplementation
Reduces the risk of pre-eclampsia
Reduces the frequency of antihypertensive therapy
Reduces mean diastolic blood pressure (highest recorded value)

No secondary hypotheses were pre-defined for the two therapeutic trials.
*Assessed in the Earl-PD trial and the Twins trial.
†Assessed in the Earl-IUGR trial and the Twins trial.
‡Assessed in the Earl-PIH trial and the Twins trial.

or 300 mmol protein/L. Antihypertensive therapy was recorded from the hospital records. Induced vaginal delivery and pre-labour caesarean section were regarded as elective deliveries. All definitions were decided upon *a priori*.

Statistical methods

χ^2 and Student's t tests were applied to test for differences in dichotomous and continuous variables, respectively. For dichotomous variables the odds ratio is used as a measure of effect. It designates the odds of the outcome in the fish oil group divided by the odds of the outcome in the olive oil group. This estimate is provided with 95% confidence intervals. Multiple linear regression was employed to adjust an observed difference in a continuous variable for possible imbalances in gestational age. In the Susp-IUGR trial, effect on fetal growth was assessed using birthweight for gestational age charts[21]. Effects on the timing of spontaneous delivery were assessed by Cox regression (proportional hazards model). To the extent the relevant information was available, all analyses were intention-to-treat.

RESULTS

Participant flow is shown in Fig. 1 for the aggregated trials. In the four prophylactic trials women were randomised around gestational week 20, whereas in the two therapeutic trials women were randomised around gestational week 33 (Table 3). In general, the randomisation produced comparable groups regarding proportion of smokers, mean gestational age, mean maternal age and mean diastolic blood pressure at randomisation (Table 3). No substantial differences in base-line variables were found, except in women with suspected intrauterine growth retardation, where gestational age at enrolment was higher in the olive oil group.

Tests of study hypotheses

Table 4 presents the results regarding the primary study hypotheses of the prophylactic trials. Tables 5, 6 and 7 present results regarding the corresponding secondary hypotheses. Below, the results are described for each clinical problem separately.

Recurrence of pre-term delivery was 33·3% in the olive oil group and 21·3% in the group receiving fish oil. This difference corresponded to a reduction in the odds of pre-term delivery of 0·54 (95% CI, 0·30 to 0·98) in the fish oil compared with the olive oil group (Table 4). When examining the effects on secondary end points related to pre-term delivery, the odds of early pre-term delivery were found to be reduced in the fish oil group by 0·32 (95% CI, 0·11 to 0·89), mean duration of pregnancy was increased by 8·5 days (95% CI, 1·9 to 15·2), and mean birthweight was increased by 209 g (95% CI 27 to 390) (Table 5). Occurrence of birthweight

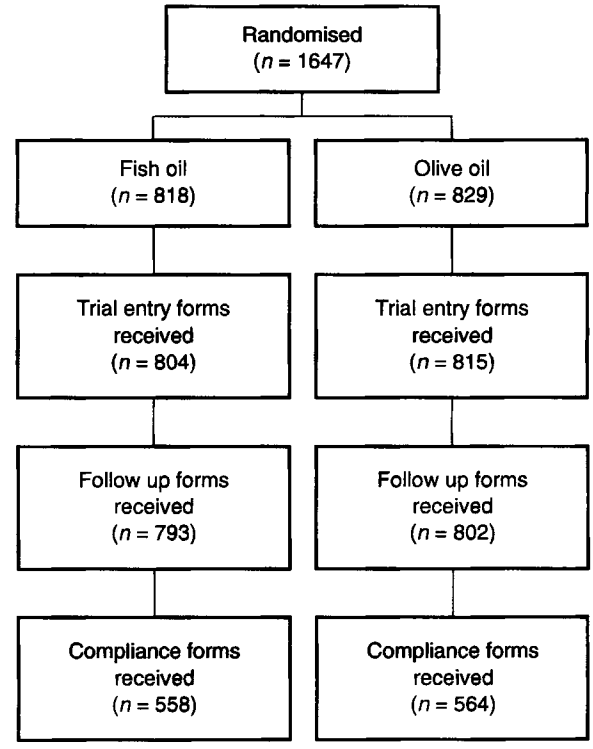

Fig. 1. Flow of subjects of the aggregated six trials of FOTIP.

Table 3. Baseline characteristics of the women in the six trials after randomisation. Values are given as *n* or mean (SD), unless otherwise indicated.

	Fish oil	Olive oil
Prophylactic trials		
Earl-PD trial		
No. randomised	110	122
Gestational age at randomisation (days)	131·8 (24·6)	130·5 (27·7)
Age (years)	29·3 (4·87)	30·0 (6·22)
Cigarette smokers (%)	45·2	41·2
Diastolic blood pressure (mmHg)	66·5 (9·06)	67·5 (8·71)
Earl-IUGR trial		
No. randomised	141	139
Gestational age at randomisation (days)	128·1 (24·2)	131·2 (24·1)
Age (years)	30·0 (4·64)	29·0 (3·93)
Cigarette smokers (%)	51·5	51·9
Diastolic blood pressure (mmHg)	66·4 (8·60)	65·9 (8·44)
Earl-PIH trial		
No. randomised	184	202
Gestational age at randomisation (days)	129·6 (21·4)	132·1 (26·6)
Age (years)	30·3 (7·01)	28·9 (5·32)
Cigarette smokers (%)	19·1	24·2
Diastolic blood pressure (mmHg)	73·7 (9·8)	74·0 (10·5)
Twins trial		
No. randomised	289	290
Gestational age at randomisation (days)	141·5 (21·1)	141·5 (21·3)
Age (years)	30·2 (6·18)	30·7 (6·35)
Nulliparous (%)	52·5	52·5
Cigarette smokers (%)	33·0	29·4
Diastolic blood pressure (mmHg)	68·6 (9·24)	68·4 (9·30)
Therapeutic trials		
Threat-PE trial		
No. randomised	44	35
Gestational age at randomisation (days)	231·9 (23·5)	219·6 (30·6)
Age (years)	32·1 (11·7) d	32·9 (14·6)
Nulliparous (%)	71·4	65·6
Cigarette smokers (%)	18·2	20·6
Diastolic blood pressure (mmHg)	94·3 (9·77)	92·2 (11·5)
Susp-IUGR trial		
No. randomised	36	27
Gestational age at randomisation (days)	227·1 (22·8)	231·2 (19·5)
Age (years)	29·3 (7·88)	29·8 (10·3)
Nulliparous (%)	52.0	51·9
Cigarette smokers (%)	30·6	29·6

Missing value proportions of randomised subjects were less than 18% (diastolic blood pressure), 10% (smoking), and 5% (age, gestational age, nulliparity).

< 2500 g was not significantly reduced (odds ratio 0·57 (95% CI 0·28 to 1·15)).

Occurrence of pre-term delivery was 44·9% in the twin olive oil group, which was similar to the 45·1% occurrence in the group receiving fish oil (Table 4). Occurrences of early pre-term delivery and of low birthweight, as well as mean pregnancy duration and birthweight, were also similar in the two groups (Table 5).

Recurrence of intrauterine growth retardation was 28·0% in the olive oil group, which was not significantly different from the recurrence of 32·8% in the group receiving fish oil (Table 4). Mean birthweight was, unexpectedly, significantly lower in the group receiving fish oil, with a difference of 151 g (95% CI, 15 to 286), and low birthweight tended to occur more frequently in the fish oil group, although the latter difference was nonsignificant (Table 6). The difference in mean birthweight was due to shorter gestations in the fish oil group, as the difference was abolished when adjustment was made for gestational age at delivery (Table 6). This, in turn, was related to a relatively larger proportion of early elective deliveries in the fish oil group. Among elective deliveries in the fish oil group (total n = 135), ten were pre-term, nineteen term and three post-term, whereas the figures for the olive oil group (total n = 132) were one, twenty nine, and zero,

Table 4. Tests of primary study hypotheses: prophylactic trials. PD = preterm delivery; IUGR = intrauterine growth retardation; PIH = pregnancy induced hypertension.

Study question	Trial	n*	Events/Women (%)		Odds ratio	
			Fish oil	Olive oil	Estimate (95% CI)	P†
Recurrence of PD	EARL-PD	228 (4)	23/108 (21·3)	40/120 (33·3)	0·54 (0·30; 0·98)	0·05
Recurrence of IUGR	EARL-IUGR	263 (17)	43/131 (32·8)	37/132 (28·0)	1·26 (0·74; 2·12)	0·42
Recurrence of PIH	EARL-PIH	350 (36)	55/167 (32·9)	61/183 (33·3)	0·98 (0·63; 1·53)	1·00
PD in twin pregnancies	Twins	569 (10)	129/286 (45·1)	127/283 (44·9)	1·01 (0·73; 1·40)	1·00
IUGR in twin pregnancies‡	Twins	1,111 (67)	165/554 (29·8)	148/557 (26·6)	1·17 (0·90; 1·52)	0·26
PIH in twin pregnancies	Twins	553 (26)	38/274 (13·9)	29/279 (10·4)	1·39 (0·83; 2·32)	0·24

*Total number in analysis (number of missing values of each end point in parentheses).
†Fisher's exact test.
‡Observation: an infant.

respectively. Among the ten women with pre-term birth in the fish oil group, the following indications for elective delivery were stated in the follow up forms: intrauterine growth retardation (4), intrauterine growth retardation and placental insufficiency (1), obstetric history (1), breech presentation (1), fetal distress and earlier intrauterine growth retardation (1), suspected intrauterine asphyxia (1). In one case, no indication was stated.

Occurrence of intrauterine growth retardation was 26·6% in the twin olive oil group, which was similar to the 29·8% of the group receiving fish oil (Table 4). Mean birthweight, as well as occurrence of low birthweight, was similar in the two groups (Table 6).

Recurrence of pregnancy induced hypertension occurred in 33·3% of the olive oil group, which was similar to the 32·9% recurrence among women receiving fish oil (Table 4). Recorded use of antihypertensive therapy, pre-eclampsia occurrence and mean diastolic and systolic blood pressures did not differ significantly between the two groups (Table 7).

Occurrence of pregnancy induced hypertension was 10·4% in the twin olive oil group, which was not significantly different from the occurrence of 13·9% among women with twins receiving fish oil (Table 4). Recorded use of antihypertensive therapy, pre-eclampsia occurrence and mean diastolic and systolic blood pressures were similar (Table 7).

In women with threatening pre-eclampsia in the current pregnancy, duration from randomisation until delivery tended to be longer ($P = 0·2$) in the group receiving olive oil (Table 8). This difference was attributable to the shorter gestational age at randomisation noted in the olive oil group (Table 4). Adjusting for gestational age at randomisation in a multiple linear regression model reduced the difference in gestational age at delivery from –8·8 days (95% CI –22·0 to 4·4) to +0·64 days (95% CI –9·6 to 10·9).

In women with suspected intrauterine growth retardation in current pregnancy, birthweight adjusted for gestational age at delivery[21] was similar in the two groups (Table 8).

Table 5. Secondary end points related to preterm delivery (PD). Values are given as events/women (%) or mean [SD, n], unless otherwise indicated.

	Fish oil	Olive oil	Estimate (95% CI)		P
			Odds ratio	Difference	
The Earl-PD trial					
Early PD	5/108 (4·6)	16/120 (13·3)	0·32 (0·11; 0·89)	—	0·04*
Low birthweight	15/108 (13·9)	26/118 (22·0)	0·57 (0·28; 1·15)	—	0·12*
Pregnancy duration (days)	269·2 [19·7, 108]	260·7 [29·5, 120]	—	8·5 (1·9; 15·2)	0·01
Mean birthweight (g)	3169 [674·0, 108]	2960 [707·1, 118]	—	208·7 (27·3; 390·2)	0·02
The Twins trial					
Early PD	37/286 (12·9)	44/283 (15·5)	0·81 (0·50; 1·29)	—	0·40*
Low birthweight	238/556 (42·8)	242/566 (42·8)	1·00 (0·79; 1·27)	—	1·00*
Pregnancy duration (days)	254·5 [24·0, 286]	254·4 [23·1, 283]	—	–0·1 (–3·98; 3·79)	0·96
Mean birthweight (g)	2512 [626·6, 556]	2498 [598·5, 566]	—	–13·4 (–85·2; 58·4)	0·71

*Fisher's exact test.

Table 6. Secondary end points related to intrauterine growth retardation (IUGR). Values are given as events/women (%) or mean [SD, *n*], unless otherwise stated.

			Estimate (95% CI)		
	Fish oil	Olive oil	Odds ratio	Difference	*P*
The Earl-IUGR trial					
Low birthweight	30/135 (22·2)	19/133 (14·3)	1·71 (0·91; 3·23)	—	0·11*
Mean birthweight (g)	2910 [604·5, 135]	3060 [514, 133]	—	−150·6 (−285·7; −15·4)	0·03
Difference adjusted for gestation**	—	—	—	1·31 (−98·9; 101·5)	0·98
The Twins trial					
Low birthweight (g)	238/556 (42·8)	242/566 (42·8)	1·00 (0·79; 1·27)	—	1·00*
Mean birthweight (g)	2512 [626·6, 556]	2498 [598·5, 566]	—	−13·4 (−85·2; 58·4)	0·71
Difference adjusted for gestation**	—	—	—	−8·2 (−52·8; 36·4)	0·72

*Fisher's exact test.
**Adjusted by including gestational age at delivery as explanatory variable in a multiple linear regression.

Timing of spontaneous onset of delivery

Accounting for elective delivery

The high proportion of elective deliveries (induced vaginal deliveries and pre-labour caesarean sections) hampered the ability to study effects on timing of spontaneous onset of delivery. As one way to account for this, survival analyses were employed which regarded women with elective deliveries as censored at the time they were delivered. Elective deliveries were distributed across the two randomisation groups as follows (fish oil *vs* olive oil): 14% *versus* 16% in women with earlier pre-term delivery, 24% *versus* 23% in women with earlier intrauterine growth retardation, 38% *versus* 38% in women with earlier pregnancy induced hypertension, 54% *versus* 54% in women with twin pregnancies, 79% *versus* 61% in women with threatening pre-eclampsia, and 35% *versus* 40% in women with suspected intrauterine growth retardation (all comparisons across oil groups were nonsignificant). In women with pre-term delivery, intrauterine growth retardation or pregnancy induced hypertension in an earlier pregnancy, timing of spontaneous delivery tended to be delayed in the fish oil group compared with the olive oil group (Fig. 2). The Cox proportional hazards ratios were 1·25 (95% CI 0·94 to 1·66, $P = 0·12$), 1·32 (95% CI 1·00 to 1·76; $P = 0·05$) and 1·24 (95% CI 0·95 to 1·61; $P = 0·11$). In twins, the proportional hazards ratio was 1·02 (95% CI 0·80 to 1·30; $P = 0·9$). In women with threatening pre-eclampsia or with suspected intrauterine growth retardation, the hazards ratios were 1·70 (95% CI, 0·72 to 4·02; $P = 0·22$) and 2·22 (95% CI 1·11 to 4·44; $P = 0·02$), respectively.

Table 7. Secondary end points related to pregnancy induced hypertension (PIH). Values are given as events/women (%) or mean [SD, *n*], unless otherwise stated.

			Estimate (95% CI)		
	Fish oil	Olive oil	Odds ratio	Difference	*P*
The Earl-PIH trial					
Pre-eclampsia**	11/152 (7·2)	17/169 (10·1)	0·70 (0·32; 1·54)	—	0·43*
Anti-hypertensive therapy	23/184 (12·5)	38/202 (18·8)	0·62 (0·35; 1·08)	—	0·10*
Blood pressure (mmHg)					
Diastolic	86·6 [16·9, 172]	88·2 [16·6, 186]	—	1·58 (−1·90; 5·06)	0·37
Systolic	135·6 [25·5, 172]	138·5 [26·9, 186]	—	2·86 (−2·61; 8·32)	0·31
The Twins trial					
Pre-eclampsia**	14/246 (5·7)	6/251 (2·4)	2·46 (0·93; 6·52)	—	0·07*
Anti-hypertensive therapy	19/289 (6·6)	9/290 (3·1)	2·20 (0·98; 4·94)	—	0·06*
Blood pressure (mmHg)					
Diastolic	80·4 [16·5, 276]	80·6 [13·2, 280]	—	0·14 (−2·32; 2·67)	0·89
Systolic	130·0 [25·0, 275]	129·3 [19·3, 280]	—	−0·62 (−4·34; 3·10)	0·74

*Fisher's exact test.
**Denominators were lower because of missing information on proteinuria.

Table 8. Tests of primary study hypotheses: therapeutic trials. Values are given as mean (SD, *n*), unless otherwise stated.

	Fish oil	Olive oil	Mean difference (95% CI)	*P*
The Threat-PE trial				
Duration until delivery (days)	29·2 (24·9, 42)	38·0 (32·8, 34)	−8·8 (−22·0; 4·4)	0·19
The Susp-IUGR trial				
Weight for gestational age (g)**	−582 (393, 34)	−554 (289, 26)	29 (−154; 212)	0·75

*Number of missing values for each of the two end points in question: 3 (Threat-PE trial) and 3 (Susp-IUGR trial).
**Adjusted according to birthweight for gestational age charts[21].

Aggregating trial groups

The trials could be naturally aggregated into three main groups: the prophylactic trials based on singleton pregnancies, the prophylactic trial with twin pregnancies, and the two therapeutic trials which enrolled women later and provided higher doses than the other trials. In the combined prophylactic singleton groups (Earl-PD, Earl-IUGR, and Earl-PIH), Cox regression showed delayed parturition in women receiving fish oil, with a hazards ratio of 1·27 (95% CI 1·08 to 1·49; $P = 0·004$). Twins (as reported above) exhibited no difference ($P = 0·9$). In the combined therapeutic trials (Threat-PE and Susp-IUGR), spontaneous delivery was delayed in the fish oil group with a hazard ratio of 2·00 (95% CI 1·16 to 3·45; $P = 0·01$).

After eliminating elective deliveries from these three data sets, occurrences of pre-term delivery and early pre-term delivery in the combined Earl-PD, Earl-IUGR, and Earl-PIH trials, were 10·6% (fish oil, $n = 301$) *versus* 15·2% (olive oil, $n = 323$) (OR 0·67, 95% CI 0·41 to 1·07) and 2·0% *versus* 5·0% (OR 0·39, 95% CI 0·15 to 1·01), respectively. The average duration of pregnancy was 4·8 days (95% CI 1·96 to 7·67) longer in the fish oil group. In twins, occurrences of pre-term delivery and early pre-term delivery were 64·1% (fish oil, $n = 128$) *versus* 65·1% (olive oil, $n = 129$) (OR 0·96; 95% CI 0·57 to 1·59) and 18·8% *versus* 25·8% (OR 0·70; 95% CI 0·39 to 1·27), respectively. The average duration of pregnancy was similar in the two groups (95% CI −5·53 to 6·88 days). In the combined therapeutic trials, occurrence of pre-term delivery was 6·5% (fish oil, $n = 31$) *versus* 7·1% (olive oil, $n = 28$) (OR 0·90; 95% CI 0·12 to 6·83) and average duration of pregnancy was 7·7 days (95% CI 1·70 to 13·65) longer in the fish oil group: no early pre-term delivery occurred at all in the two groups.

Aggregating all trials

When all six trials were combined, Cox regression analysis showed delayed parturition in women receiving fish oil with a proportional hazard ratio of 1·22 (95% CI 1·07 to 1·39; $P = 0·002$). Elective deliveries occurred in 40% of this data set. After they had been eliminated, occurrences of pre-term delivery and early pre-term delivery were 25·2% (fish oil, $n = 460$) *versus* 28·1% (olive oil, $n = 480$) (OR, 0·86; 95% CI 0·64 to 1·16) and 6·5% *versus* 10·0% (OR 0·63; 95% CI 0·39 to 1·01), respectively, while the average duration of pregnancy was 3·7 (95% CI 0·68 to 6·63) days longer in the fish oil group.

Possible adverse effects and rates of serious complications

Overall, more post-term deliveries occurred in the fish oil group, with a relative risk of 2·4 (95% CI 1·20 to 4·97, $P = 0·01$). Intracranial haemorrhage occurred in seven and three infants in the fish oil and olive oil group, respectively. This corresponded to a relative risk of 2·4 (95% CI 0·6 to 11·6, $P = 0·22$). No differences were observed between the fish oil and the olive oil group in any of the examined variables, which could reflect other bleeding complications or the occurrence of macrosomia (Table 9).

Stillbirths, early neonatal deaths, late neonatal deaths, transfer of the infant to neonatal care, and phototherapy of the baby all occurred at similar rates in the fish oil and olive oil group. The duration of stay in hospital after delivery was also similar, both for the infant and the mother (Table 10). No maternal death occurred.

Self-reported compliance, blinding efficacy and adverse effects

In the questionnaire mailed after delivery to the women, 80·3% (358/446) in the fish oil and 78·3% (353/451) in the olive oil group reported that they had taken the prescribed daily number of capsules (or more) until delivery. Among women allocated fish oil, 80·0% reported that they thought they had received fish oil, 5·0% thought they had received olive oil, while 15·0% did not know (385, 24, and 72 women, respectively). In the olive oil group, those figures were 16·9%, 43·5% and 39·5% (84, 216, and 196 women), respectively.

More women in the fish oil group answered with yes to the general question whether they had experienced any adverse effects of the capsules (32·0% (152/475) *vs* 17·0%

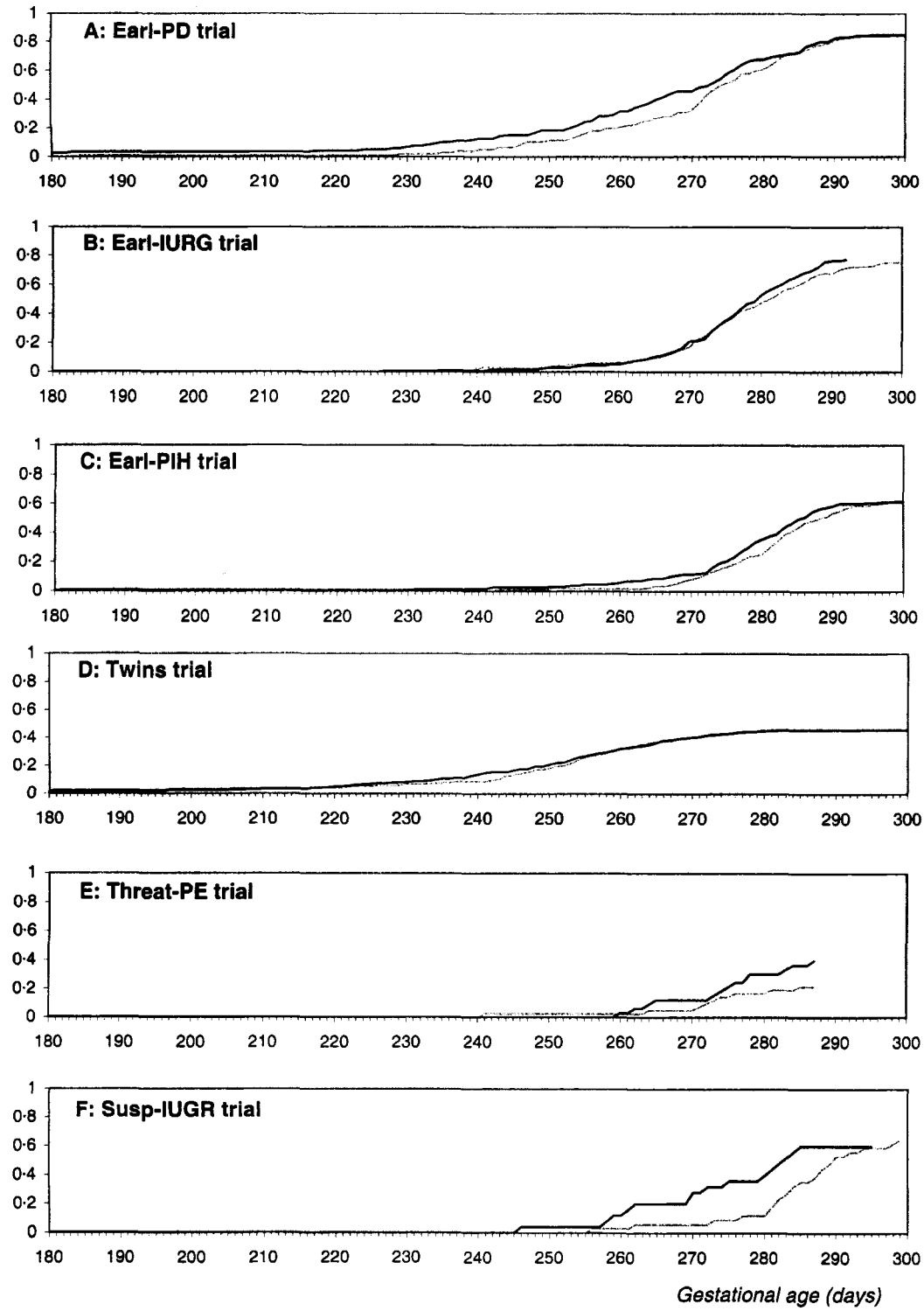

Fig. 2. Timing of spontaneous delivery in women receiving supplements with fish oil or olive oil. The y-axes represent proportions of women in each group having delivered spontaneously at a given point in time during pregnancy. The curves do not reach 100% because elective deliveries (induced vaginal deliveries and pre-labour caesarean sections) are not counted. The gray lines represent women having received fish oil, the black lines represent women having received olive oil. The six panels represent six different trials: A. Women with pre-term delivery in an earlier pregnancy (the Earl-PD trial); B. Women with intrauterine growth retardation in an earlier pregnancy (the Earl-IUGR trial); C. Women with pregnancy induced hypertension in an earlier pregnancy (the Earl-PIH trial); D. Women with twin pregnancy (the Twins trial); E. Women with threatening pre-eclampsia (the Threat-PE trial); F. Women with threatening intrauterine growth retardation (the Susp-IUGR trial). Women in the first four trials (A–D) were randomised around week 20 of gestation, and the fish oil group received 2·7 g/ day of n-3 fatty acids. Women in the last two trials (E–F) were randomised around week 33 of gestation, and the fish oil group received 6·1 g/ day of n-3 fatty acids.

Table 9. Potential adverse effects directly linked to hypothesised biological effects of fish oil. Values are given as events/women or infants (%) or mean [SD, *n*], unless otherwise stated.

			Estimate (95% CI)		
	Fish oil	Olive oil	Odds ratio	Difference	*P*
Postterm delivery	26/782 (3·3)	11/791 (1·4)	2·44 (1·20; 4·97)	—	0·01*
Macrosomia delivery	9/792 (1·1)	10/809 (1·2)	0·92 (0·37; 2·27)	—	1·00*
Vaginal bleeding after randomisation	36/802 (4·5)	39/816 (4·7)	0·96 (0·60; 1·53)	—	0·91*
Intracranial haemorrhage in infant	7/1107 (0·63)	3/1119 (0·27)	2·37 (0·55; 11·6)	—	0·22*
Exchange transfusion	0/792 (0)	3/802 (0·4)	0	—	0·25*
Maternal anaemia**	101/407 (24·8)	94/439 (21·4)	1·21 (0·88; 1·67)	—	0·25*
Estimated maternal blood loss (mL)	351·7 [282·7, 725]	344·7 [267·1, 751]	—	−7·0 (−35·1; 21·1)	0·63
Lowest measured maternal haemoglobin (g/L)**	122·8 [400·5, 407]	100·6 [45·4, 439]	—	−22·2 (−60·0; 15·6)	0·25

*Fisher's exact test.
**Denominators were lower because of missing information.

(84/495)). When asked about specific side effects, only belching (29·2% (133/455) *versus* 8·1% (37/454)) and unpleasant taste (17·0% (74/436) *versus* 2·3% (10/441)) differed, whereas other ailments (i.e. nausea, vomiting, diarrhoea, constipation, nose bleeding, and vaginal bleeding leading to hospital admittance (data not shown)) were equally distributed between the two oil groups.

DISCUSSION

The present study, consisting of six independent randomised clinical trials, addresses various possible effects of fish oil supplementation in high risk pregnancies.

A significant reduction was observed in the recurrence risk of pre-term delivery among women who had experienced pre-term delivery in an earlier pregnancy. The odds of such an event were reduced by 0·54 (95% CI 0·30 to 0·98) in the fish oil compared with the olive oil group. This was consistent with findings of a comparatively longer mean gestation, higher average birthweight, and lower odds of early pre-term delivery. No such effects were detected in women with twin pregnancies.

There was no convincing indication of any prophylactic effects of fish oil on intrauterine growth retardation or pregnancy induced hypertension, or on outcomes closely related to these end points. Among women with threatening pre-eclampsia, no effects were detected on duration from randomisation until delivery, and among women with suspected intrauterine growth retardation, no effects were detected on fetal growth.

Since artificial curtailments occurred in 40% of all pregnancies, survival statistics were employed to scrutinise for effects on the timing of spontaneous delivery, as they made better use of the information from the terminated pregnancies, by regarding women with elective delivery as being censored at the time they were delivered. These analyses were in general consistent with the

Table 10. Mortality and general morbidity data. Values are given as events/women or infants (%) or mean [SD, *n*], unless otherwise stated.

			Estimate (95% CI		
	Fish oil	Olive oil	Odds ratio	Difference	*P*
Spontaneous abortion	4/804 (0·50)	7/815 (0·86)	0·58 (0·17; 1·98)	—	0·54
Stillbirth	16/1056 (1·5)	19/1085 (1·8)	0·86 (0·44; 1·69)	—	0·74*
Neonatal deaths					
Early	3/1126 (0·4)	2/1144 (0·2)	2·04 (0·37; 11·14)	—	0·45*
Late	0/1125 (0)	2/1144 (0·1)	0·51 (0·05; 5·61)	—	1·00*
Child transferred to neonatal care	258/1062 (24·3)	283/1076 (26·3)	0·90 (0·74; 1·09)	—	0·30*
Phototherapy	111/1055 (10·5)	105/1070 (9·8)	1·08 (0·82; 1·43)	—	0·62*
Duration of stay in hospital after delivery					
Infant	7·74 [16·3, 1017]	7·63 [18·5, 1024]	—	−1·12 (−1·89; 1·66)	0·90
Mother	6·13 [18·8, 772]	6·46 [24·0, 782]	—	0·34 (−1·82; 2·49)	0·76

*Fisher's exact test.

above results, in as much as they exhibited delayed spontaneous delivery in the fish oil *versus* olive oil group in women with problems in earlier pregnancies, while no difference was detectable among twin pregnancies. Furthermore, among women with problems in the current pregnancy, delays in spontaneous delivery were also observed in the fish oil group. When all six trials were combined, a highly significant (P = 0·002) effect of fish oil was detected.

Among the three disease entities studied in this trial (i.e. pre-term delivery, intrauterine growth retardation, and pregnancy induced hypertension), the hypotheses relating to pre-term delivery were the best substantiated by *a priori* evidence. An earlier randomised trial found that healthy pregnant Danish women receiving fish oil from gestational week 30 onwards had a 4·0 days longer mean pregnancy, compared with women receiving olive oil. This finding could explain the observed population differences in pregnancy duration between Denmark and Faroes, where consumption of marine diets is highest, and led to the recommendation to test fish oil in women at high risk of pre-term delivery[13,24]. In a large quasi-randomised trial undertaken in London in the 1930s, halibut liver oil was given to pregnant women in parallel with minerals and vitamins, while the control group did not receive any supplement. In agreement with the hypothesis of an impact of n-3 fatty acids on the timing of delivery, fewer early deliveries were seen in the intervention group[25–27]. Observational epidemiological studies have been somewhat inconsistent, two being supportive[28,29], two negative[15,30,31] and one inconclusive[32].

Biologically, the pre-term hypothesis is based on the finding that prostaglandins are essential for the onset of labour in humans[33–36]. Thus, PGE_2 and $PGF_{2\alpha}$ bring about contraction of the myometrium and cervical ripening. Throughout pregnancy, labour can be initiated with these prostaglandins and just before the onset of parturition, a small increase in these prostaglandins is found in the amniotic fluid and plasma. On the other hand, prostacyclin (PGI_2) has, although not in all instances[37–39], been found to inhibit myometrial contractility[40,41]. Long-chain n-3 fatty acids may postpone parturition by down-regulating the formation of PGE_2 and $PGF_{2\alpha}$ involved in the triggering of parturition, and by increasing the formation of PGI_2 and PGI_3 leading to a more relaxed myometrium[11,42]. Two animal experiments support the notion that fish oil can prolong gestation[43,44]. Pregnant rats fed a diet mixed with fish oil had longer gestations than rats fed a diet mixed with arachis oil[43]. In pregnant ewes, in which premature labour had been induced by betametasone (an animal model employed to study tocolytics), intravenous infusion of a fish oil emulsion tended to postpone the onset of labour and delivery. Some animals even reverted from contractions to nonlabour[44].

The finding in the present study, overall and in particular subgroups, of delayed onset of spontaneous delivery in the fish oil group, is consistent with this hypothesis. The lack of effect observed in twin pregnancies is, on the other hand, not necessarily inconsistent with the hypothesis. The increased pre-term risk in twin pregnancies may be caused by such factors as a greater physical distension of the uterus or a greater (possibly nonnutritional) demand on the overall efficacy of the feto-maternal unit, which could overrule other determinants of the timing of spontaneous delivery. In the therapeutic trials, a larger dose was given per day (6·1 *vs* 2·7 g long-chain n-3 fatty acids), which may explain the apparently stronger effects in these women (see proportional hazard ratios and panels E-F in Fig. 2). These data agree with other evidence indicating a relatively acute effect of fish oil on timing of delivery[11,28–30,44].

Although well supported biologically[13,45], the fetal growth hypothesis was less well substantiated by *a priori* evidence. Thus, in the previously mentioned Danish trial, no effect of fish oil supplementation during pregnancy was detected on the fetal growth rate[24], which is consistent with the findings from the present trial. Several observational studies, however, have exhibited direct associations between various measures of intake of seafood and fetal growth rate[15,32,45–47]. If indeed these associations reflect causality, they are likely to be mediated by substances in seafood other than the n-3 fatty acids. Alternatively, n-3 fatty acids may exert these effects before 16–20 weeks of gestation, which is the earliest stage of intervention in the trials considered.

There is an extensive biologic basis for the hypothesis that fish oil may reduce the risk of pregnancy induced hypertension[16–18,48,49]. The *a priori* evidence from trials for a protective effect on pregnancy induced hypertension was, however, small. Although the London trial was compatible with an effect[25–27], no, or only marginal effects, were detected on blood pressure in pregnancy in the Danish trial[50]. Two other trials, undertaken in women at high risk of pre-eclampsia, were also negative with respect to pre-eclampsia or hypertension[51,52]. Two further trials were small and poorly documented[53,54]. The results of the present study are thus consistent with those of the three earlier trials[50–52]. There is one individual-based observational study in support of the pregnancy induced hypertension hypothesis[55], and some suggestive population comparisons[14,16,56].

Earlier concerns about serious adverse effects of fish oil in pregnancy[13,19,20] could not be substantiated. Belching and unpleasant taste occurred at higher rates in the fish oil group, confirming findings from the Danish

trial[24]. More cases of infant brain haemorrhage were seen in the fish oil than the olive oil group (7 *vs* 3 cases). Although this is compatible with a chance finding ($P=0{\cdot}22$), we recommend close monitoring of this end point in future trials of fish oil in pregnancy. The greater overall occurrence of post-term deliveries in the fish oil group suggests that recommendations to increase consumption of n-3 fatty acids in pregnancy should not involve the term and post-term period.

A fundamental assumption underlying the above interpretations is that olive oil is inert in relation to the end points studied. There is no direct evidence available from clinical trials to support or reject this notion. The main constituents of olive oil are oleic acid and linoleic acid, and the latter could, theoretically, interfere with prostanoid metabolism in a way opposite to the n-3 fatty acids. However, the amounts of linoleic acid provided by the supplements constituted a small fraction only of what Danish women consume on average (~ 3% and ~ 7% (w/w), respectively, in the prophylactic and therapeutic trials)[24].

In conclusion, the present study indicates that fish oil supplementation may be beneficial in women with a pre-term delivery in an earlier pregnancy, as it reduced the risk of pre-term delivery and of early pre-term delivery in such women. However, this conclusion cannot be generalised to other clinical groups. Particularly, no effects of fish oil were detected in twin pregnancies at all, and fish oil seemed to have no effect on recurrence risk of intrauterine growth retardation and pregnancy induced hypertension.

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The Data Monitoring Committee consisted of T. I. A. Sørensen (Chairman until February 1995), C. Gluud (Chairman from February 1995), H. O. Bang, J. Dyerberg (replacing H. O. Bang after his death), and K. Kristoffersen. C. Gluud was invited to join the Writing Group after the data collection had been terminated.

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Appendix 1.

Sample size considerations and analytic strategy

In the original protocol (dated 1991), sample size calculations were undertaken separately for women with previous intrauterine growth retardation, pregnancy induced hypertension or pre-eclampsia, and for women with previous pre-term delivery. The following conditions were assumed: recurrence risks among controls of 17·5%, a hypothesised relative risk reduction of 25%, and a 5% and 20% risk of type I and type II error, respectively. On these conditions, 2740 women would be needed with

previous intrauterine growth retardation, pregnancy induced hypertension or pre-eclampsia (aggregated), and 2740 women with previous pre-term delivery.

During enrolment it became clear that these numbers could not be reached. In mid-1995 a decision strategy for stopping the trial—which also involved a prioritised plan for the main statistical analyses—was worked out and decided upon by the trialists unaware of the interim results (thus *a priori)*. The strategy defined six primary hypotheses that the trials were aimed at testing, which are described below. Each of these hypotheses was considered to be potentially true, independently of the other hypotheses, and each was considered to be clinically important in its own right. Conditional sample size calculations were undertaken (based on data from 1065 enrolled women with completed follow up forms), again assuming hypothesised effects of 25 percent relative risk reductions for all endpoints and a risk of committing a type I error of 5 percent (2 alpha) [22]. The decision strategy worked with three dates for possible termination of trial, which were 1 July 1995, 1 January 1996, and 1 January 1997. According to this strategy, the trial would be stopped immediately (1 July 1995) if none of the tests had a predicted statistical power of 0·9 by 1 January 1996. Enrolment would be continued until 1 January 1996 if one or more of the six tests had a predicted statistical power of at least 0·9. Enrolment would be continued for one further year if more of the six tests by 1 January 1997 would have attained a predicted statistical power of at least 0·9.

The results from the confidential interim analysis (the code only known to the DMC) showed that one of the six end points had a predicted power of more than 0·9 by 1 January 1996, and that no further end points were predicted as having a power of more than 0·9 one year later. Therefore, enrolment continued until 1 January 1996.

The strategy referred to above defined six *primary hypotheses* which all related to the *prophylactic section* of the trials: fish oil reduces risks of:

1. Recurrence of pre-term delivery (assessed in trial Earl-PD).
2. Recurrence of intrauterine growth retardation (assessed in trial Earl-IUGR).
3. Recurrence of pregnancy induced hypertension (assessed in trial Earl-PIH).
4. Preterm delivery in twin pregnancies.
5. Intrauterine growth retardation in twin pregnancies and,
6. Pregnancy induced hypertension in twin pregnancies (the latest three hypotheses assessed in the Twins trial).

Later, further details in the analytic strategy were defined, still independently of trial results.

The following *primary hypotheses* were defined for the *therapeutic section* of the trials:

7. In women with diagnosed threatening pre-eclampsia, fish oil increases mean duration of time elapsed from enrolment to delivery, and
8. In women with suspected intrauterine growth retardation, fish oil increases mean birth weight adjusted for gestation age[21].

For each of the primary hypotheses of the prophylactic trials, which represented the clinical questions that the trial was primarily aimed at solving, a number of closely related *secondary hypotheses* were also prespecified by trialists unaware of interim results (thus also *a priori*). They represented additional obvious questions to be answered:.

Secondary hypotheses to primary hypothesis 1: In women with pre-term delivery in an earlier pregnancy, fish oil reduces the risk of early pre-term delivery (< 238 days) and of low birth weight (< 2500 g), and increases mean pregnancy duration and mean birth weight.

Secondary hypotheses to primary hypothesis 2: In women with intrauterine growth retardation in an earlier pregnancy, fish oil reduces the risk of low birth weight (< 2500 g), and increases mean birth weight and mean birth weight adjusted for pregnancy duration.

Secondary hypotheses to primary hypothesis 3: In women with pre-eclampsia or pregnancy induced hypertension in an earlier pregnancy, fish oil reduces the risk of pre-eclampsia, the risk of antihypertensive therapy use and mean diastolic blood pressure (highest recorded value).

Secondary hypotheses to primary hypotheses 4, 5 and 6: These were the same as those to primary hypothesis 1, 2, and 3, respectively, but they were now assessed in twin pregnancies rather than in women with the corresponding complication in an earlier pregnancy.

There were no prespecified secondary hypotheses to primary hypotheses 7 and 8.

Data monitoring committee

The independent Data Monitoring Committee (DMC) included three experts, one in the conduct of clinical trials (the chairman), one in fish oil research, and one in obstetrics. On five occasions the data manager reported interim results in strict confidence to the DMC. The DMC would advise the Steering Committee to stop the investigation before the scheduled time if there were strong evidence that fish-oil was clearly indicated or contraindicated, or if it were evident that no clear outcome could be obtained. The stopping criterion for the main end points was a statistical test exhibiting three standard deviations or more to either side from the null-value, while criteria for stopping due to adverse effects were a matter of judgement[57].