European Journal of Clinical Nutrition
https://doi.org/10.1038/s41430-018-0290-z

ARTICLE

Maternal and pediatric nutrition

Fat intake during pregnancy and risk of preeclampsia: a prospective cohort study in Denmark

Mariel Arvizu[1] · Myriam C. Afeiche[2] · Susanne Hansen[3] · Thorhallur F. Halldorsson[3,4] · Sjurdur F. Olsen[1,3] · Jorge E. Chavarro[1,5,6]

Received: 29 October 2017 / Revised: 23 April 2018 / Accepted: 1 August 2018

Abstract

Background Previous studies suggest that eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and alpha-linolenic acid (ALA), may decrease the risk of preeclampsia, but many suffer from important methodological limitations.

Methods We prospectively examined the association between fat intake during pregnancy and preeclampsia and among 65,220 singleton pregnancies in the Danish National Birth Cohort (1996–2002). Women were asked to report their diet around gestation week 20 with a food frequency questionnaire. Preeclampsia diagnosis was obtained via linkage with the Danish National Patient Registry. We estimated relative risks (RR) and 95% confidence intervals (95% CI) of preeclampsia and severe preeclampsia according to fat intake using logistic regression models with generalized estimating equations to account for repeated pregnancies per woman while adjusting for potential confounders.

Results We documented 1302 cases of preeclampsia, including 301 cases of severe preeclampsia. Intake of long-chain omega-3 fatty acids was associated to preeclampsia. Women in the top quintile of DHA intake had a lower risk of preeclampsia (RR 0.67 (0.51–0.89)) and severe preeclampsia (RR 0.46 (0.25–0.83)) than women in the bottom quintile. Women who met daily recommended intake of EPA+DHA according to the Dietary Guidelines for Americans (≥250 mg/day), had a lower risk of severe preeclampsia (RR 0.77 (0.60–0.99)), but not of preeclampsia (RR 0.93 (0.82–1.05)). Conversely, ALA intake was associated with higher risk of severe preeclampsia (RR 1.71 (1.07–2.75)).

Conclusions Higher intake of DHA is inversely related to preeclampsia and severe preeclampsia, whereas ALA increases the risk of severe preeclampsia among Danish women.

Introduction

Preeclampsia affects 4.6% of pregnancies worldwide and is a leading cause of life-threatening maternal morbidity and mortality [1, 2]. Preeclampsia is characterized by initial placental defective implantation (inappropriate dilatation of spiral arterioles) that results in endothelial dysfunction [3] marked by increased placental oxygen radical production [4] and diminished antioxidant capacity [5]. Despite its high relevance, relatively few risk factors have been identified to prevent this condition and its treatment has not dramatically changed in 50 years [3]. The best characterized risk factors for preeclampsia are fixed (e.g., age, parity, new paternity, prolonged birth intervals, history of preeclampsia, and

Electronic supplementary material The online version of this article (https://doi.org/10.1038/s41430-018-0290-z) contains supplementary material, which is available to authorized users.

✉ Jorge E. Chavarro
jchavarr@hsph.harvard.edu

[1] Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA, USA

[2] Department of Environmental Health, Harvard T.H. Chan School of Public Health, Boston, MA, USA

[3] Centre for Fetal Programming, Statens Serum Institut, Copenhagen, Denmark

[4] Unit for Nutrition Research, Faculty of Food Science and Nutrition, School of Health Sciences, University of Iceland, Reykjavík, Iceland

[5] Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA, USA

[6] Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA

Published online: 07 September 2018

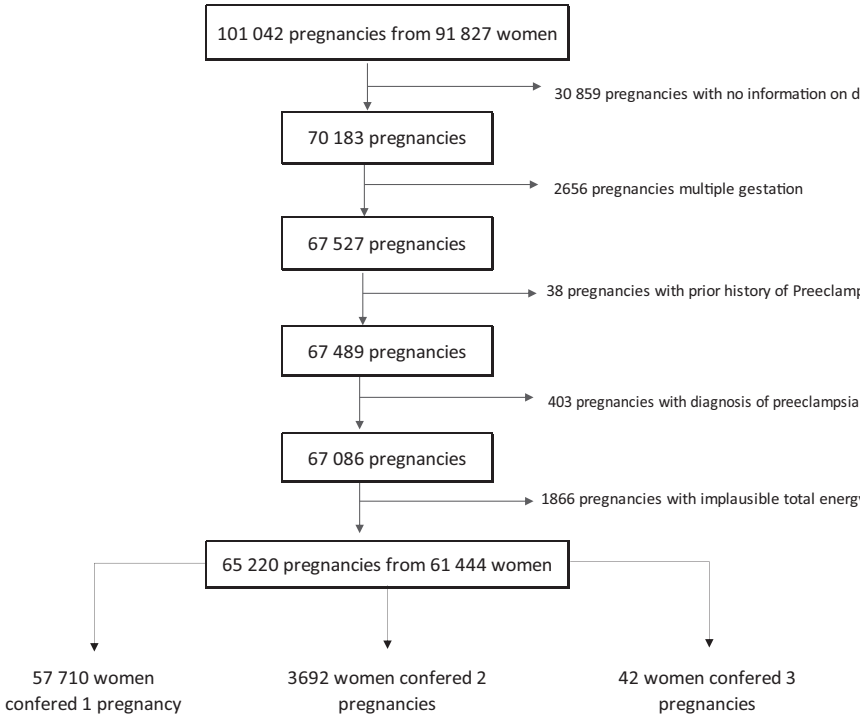

Fig. 1 The Danish National Birth Cohort participant's flowchart

genes) or cannot be modified once a pregnancy is established (e.g., obesity, multiple gestation, and chronic hypertension) [6]. Modifiable risk factors, such as diet, may be involved in the pathogenesis of this condition [7–10] representing an opportunity for prevention.

Long-chain omega-3 fatty acids (LCN-3), specifically eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and their precursor, alpha-linolenic acid (ALA) have anti-inflammatory properties and vascular benefits [5], whereas *trans* fatty acids have been found to trigger pro-inflammatory processes [11]. Previous studies have assessed the influence of fatty acid intake on the risk of developing preeclampsia. A report from this cohort, for example, did not find an association between intake of *trans* fats and risk of preeclampsia after *trans* fats were phased out of the Danish food supply [12]. Intake of LCN-3 fatty acids and preeclampsia has also been evaluated in various longitudinal studies [8, 13–16] and randomized clinical trials [17–20]. Most of these studies usually accrued small number of cases, which significantly compromised their statistical power to identify clinically relevant estimates. To address this issue, we investigated the relation between mid-pregnancy intake of different types of fatty acids and risk of preeclampsia among 65,220 singleton pregnancies in the Danish National Birth Cohort (DNBC). We hypothesized that intake of LCN-3 would be associated with a lower risk of preeclampsia.

Materials and methods

Study population

The DNBC is a prospective cohort study that recruited a total of 91,847 pregnant women throughout Denmark, between January 1996 and October 2002. Participants were enrolled during their first pre-natal visit, which was usually at 6–12 weeks gestation, and were followed during their entire pregnancy and post-partum. Women completed computer-assisted telephone interviews at study entry, gestational week 30 and at week 6 and 18 months post-delivery. Food frequency questionnaires (FFQ) were mailed to all study participants starting gestation week 25. The response rate within 1 week after mailing was 90%. Written consent to participate in the study was obtained from all participants. The DNBC was approved by the Danish Committee of Ethics and the Danish Data Protection Agency. Further details on the study design of the DNBC have been described elsewhere [21, 22].

Of the 101,042 pregnancies from the 91,827 women enrolled in the DNBC, we excluded pregnancies lacking dietary data ($n = 30{,}859$), and multiple gestations ($n = 2656$). From the remaining 67,527 singleton pregnancies with dietary data, we further excluded pregnancies from women with prior history of preeclampsia ($n = 38$), cases of preeclampsia that were diagnosed prior to dietary

assessment ($n = 403$) and 1866 pregnancies with implausible dietary intake (defined as <4200 kJ/day or >16,700 kJ/day). The final analytic dataset comprised of 65,220 pregnancies accrued among 61,444 women; 57,710 women contributed one pregnancy, 3692 women contributed two pregnancies and 42 women contributed three pregnancies to the study (Fig. 1).

Diet and supplement assessment

The FFQ displayed questions on 360 food items and dietary supplements. Women were asked to report how often they consumed each of the foods and beverages included in the questionnaire during last 4 weeks, to reflect food and nutrient intake around gestation week 20. Questions included 7–11 response categories for frequency of intake, which ranged from never to eight or more times per day. Nutrient intakes were estimated by summing the nutrient contribution of each food item in the questionnaire, taking into consideration the brand and type of fats used for cooking, as dressings or as a spread on bread. Nutrient contents of each food and standard portion size were obtained from a nutrient database based primarily on Danish Food Tables [21]. Intakes of multiple foods and nutrients assessed with the FFQ have been validated against 7-day weighed food diaries and blood biomarkers of intake [23, 24]. The main contributors of ALA intake were butter, oils, dressings, and margarines (roughly equal contribution) (38%), whole grain (mostly rye) (27%), and potatoes products including chips (6%). Seafood accounted for >90% of EPA and DHA intake. Participants were asked to report their fish intake in the FFQ in 5 distinct categories: 0–3, >3–10, >10–20, >20–30, and >30 servings/week. Fish species known to have low contents of methyl-mercury were the most commonly reported: cod, mackerel, herring, and salmon. Women also reported use of any supplements taken during pregnancy at their first interview (gestation weeks 6–12; 93% response rate), or if they ever took fish oil supplements before week 30 gestational age (87% response rate).

Preeclampsia assessment

Preeclampsia diagnosis was obtained via linkage to the Danish National Patient Registry. Preeclampsia was defined as elevated blood pressure (≥140/90 mmHg) and proteinuria (≥300 mg/24 h or ≥1+ urine dipstick) with onset after gestation week 20 that returned to normal before 8 weeks postpartum (ICD10 codes DO140, DO141, DO142, DO149, DO150, DO151, DO152, and DO159). More severe clinical presentations were defined as severe preeclampsia (blood pressure ≥160/110 mmHg; proteinuria ≥500 mg/24 h or dipstick 3+; or clinical manifestations of severity such as oliguria, cerebral or visual disturbance, persistent epigastric or right upper quadrant pain, impaired liver function, thrombocytopenia, etc.; diagnosis of HELLP syndrome or eclampsia; ICD10 diagnosis codes DO141, DO142, DO150, DO151, DO152, and DO159). In a validation study among DNBC participants was conducted to compare registry diagnosis to medical record review, we found that preeclampsia diagnosis had a specificity of 99% and a sensitivity of 69% while severe preeclampsia had a specificity of 100% and a sensitivity of 44% [25].

Assessment of covariates

Data regarding maternal age, pre-pregnancy body mass index (BMI), height, education, socio-economic status, cohabitation status, homeownership status, and prenatal vitamin use were obtained from the first telephone interview. Information on smoking during pregnancy and fish oil supplement use was obtained from the first and second telephone interviews.

Statistical analyses

Differences in demographic and nutritional characteristics across quintiles of total fat, ALA and DHA from diet and continuous baseline participant characteristics were evaluated using Kruskal–Wallis test, while chi-squared tests were used for categorical variables. We evaluated the intake of the following types of fatty acids: total, saturated, monounsaturated (MUFA), polyunsaturated (PUFA), linoleic acid (LA), arachidonic acid (AA), total omega-6 fatty acids (LA+AA), total omega-3 fatty acids (ALA+EPA +DHA), and LCN-3 (EPA+DHA). We then estimated the relative risk of preeclampsia and severe preeclampsia in relation to fat intake using logistic regression and employed generalized estimating equations (GEE) [26] with binomial logit link function and exchangeable working correlation structure, to account for within-woman correlations in outcome across pregnancies. Women were categorized according to increasing quintiles of intake of major types of fat modeled as nutrient densities (%calories/day) or as absolute intakes (mg/day). The relative risk of preeclampsia was computed as the risk in a specific quintile of fat intake relative to the risk in the lowest quintile of intake. Tests for linear trend [27] were conducted by using the median values of intake in each quintile. Robust estimators of the variance were used to compute 95% confidence intervals (CIs). We used directed acyclic graphs (DAG) and changes in point estimate methods [28] to select each covariate in the model considering their prior biological association to nutrient intake or if they were known predictors of preeclampsia.

To adjust for confounding, we fitted models including age at pregnancy (<20, <40, ≥40 years) and total energy intake (kJ/day). We also fitted multivariate models further

Table 1 Baseline participants' characteristics according to total fat and long-chain omega-3 fatty acids during 2nd trimester of pregnancy among 65,220 pregnancies from 61,444 women. The Danish National Birth Cohort, 1996–2002

Types of fats, % of total calories/day[a]	Total fat		Alpha-linolenic acid		Docosahexaenoic acid		Overall
Quintiles of intake	Q1	Q5	Q1	Q5	Q1	Q5	—
Median	20.2	35.0	0.55	1.02	0.01	0.10	
Number of pregnancies	13,044	13,044	13,044	13,044	13,044	13,044	65,220
Baseline characteristics							
Age at pregnancy (years)	29 ± 4	29 ± 5	28 ± 4	29 ± 4	28 ± 4	30 ± 4	29 ± 4
Pre-pregnancy BMI (kg/m)[b]	24 ± 4	23 ± 4	24 ± 4	23 ± 4	24 ± 5	23 ± 4	24 ± 4
Height (cm)	169 ± 6	168 ± 6	169 ± 6	169 ± 6	169 ± 6	169 ± 6	169 ± 6
High school graduate (%)	45	34	40	36	35	42	40
No spouse or partner (%)	2	2	2	2	2	2	2
Home owner (%)	65	70	65	71	68	68	69
Never smoker (%)	83	64	76	69	71	76	75
Nulliparous (%)	59	36	55	40	49	47	47
Supplement use during pregnancy							
Prenatal vitamins[b] (%)	40	39	40	39	39	39	39
Fish oil supplements[c] (%)	7	5	7	6	7	6	6
2nd Trimester diet characteristics							
Total calories/day	9357 ± 2197	10,750 ± 2564	9205 ± 2347	10,675 ± 2468	9920 ± 2464	9730 ± 2385	9925 ± 2387
Total fat (% calories/day)	20 ± 2	36 ± 3	24 ± 5	32 ± 6	28 ± 6	27 ± 5	27 ± 6
Saturated fat (% calories/day)	8 ± 1	17 ± 2	11 ± 3	14 ± 3	13 ± 4	12 ± 3	12 ± 3
MUFA (% calories/day)	7 ± 1	13 ± 1	8 ± 2	11 ± 2	10 ± 2	10 ± 2	10 ± 2
PUFA (% calories/day)	4 ± 0.8	5 ± 1	4 ± 0.6	5 ± 0.8	4 ± 1	5 ± 1	5 ± 1
Trans fat (% calories/day)	0.5 ± 0.1	1.1 ± 0.2	0.7 ± 0.2	0.8 ± 0.3	0.8 ± 0.3	0.7 ± 0.2	0.7 ± 0.2
Protein (% calories/day)	16 ± 3	15 ± 2	16 ± 3	15 ± 2	15 ± 3	17 ± 2	16 ± 2
Carbohydrate (% calories/day)	64 ± 4	48 ± 4	58 ± 6	52 ± 6	57 ± 7	55 ± 6	56 ± 6
Vitamin C (mg/day)	165 ± 103	111 ± 60	147 ± 100	120 ± 67	130 ± 89	142 ± 79	136 ± 60
Vitamin E (mg/day)	7 ± 2	9 ± 3	7 ± 2	9 ± 3	8 ± 3	8 ± 3	8 ± 3
Calcium (mg/day)	1493 ± 562	1326 ± 496	1540 ± 600	1264 ± 467	1407 ± 556	1381 ± 509	1409 ± 519

Data are presented as mean±SD for continuous variables and frequencies (%) for categorical variables

BMI body mass index, *Q1* lowest quintile of intake, *Q5* highest quintile of intake, *MUFA* monounsaturated fatty acid, *PUFA* polyunsaturated fatty acid

[a]Calories were estimated in kilojoules (kJ) per day

[b]Women that began prenatal vitamins before 6–12 weeks of gestation (baseline questionnaire)

[c]Women that took fish oil supplements before 30 weeks of gestation (second assessment questionnaire)

adjusted for known risk factors for preeclampsia in this population including pre-pregnancy body mass index (kg/m^2; ≤18, <25, <29, and ≥30), height in cm (<164, 165–168, 169–172, 173–198), parity (0, 1, 2, 3+ previous pregnancies), smoking status (non-smokers, daily smoker, daily smoker (15+ cigarettes and others), and education (high school, university). To obtain effect estimates that represent the isocaloric substitution of fat for carbohydrates, we included intakes of all sources of energy with the exception of carbohydrates to the models. Specifically, models included terms for intake of all major types of fat (saturated, mono-unsaturated, poly-unsaturated n6, polyunsaturated n3 and trans, all expressed as %calories/day) and for total protein intake (%calories/day) [29]. In addition, we included vitamin E intake, mg/day (continuous) and vitamin C intake, mg/day (continuous) intake because they are associated to preeclampsia in this population [7] and fish-oil supplement use before week 30 gestational age (yes/no), to differentiate relations from food and supplemental sources.

Finally, we investigated effect modification by age (<30 years or ≥30 years), BMI (<25 kg/m^2 or ≥25 kg/m^2), parity (nulliparous or parous), and fish oil supplementation before 30 week of gestational age (yes, no), using cross-product terms between fatty acid intake (continuous) and the effect of the potential modifier. All statistical analyses for this paper were generated by SAS software, Version 9.4 released in 2013 (Copyright SAS Institute Inc. Cary, NC, and USA).

Table 2 Risk of preeclampsia ((RR = 95% CI)) according to major type of fats from 2nd trimester of pregnancy ($n = 65{,}220$ pregnancies from 61,444 women). The Danish National Birth Cohort

Types of fat	Quintile of intake ranges (% calories/day)	Number of cases	Model 1: age and energy[a] RR (95% CI)	Model 2: multivariable[b] RR (95% CI)
Total fat	Q1 5.79–22.36	289	1.00 (REF.)	1.00 (REF.)
	Q2 22.37–25.46	291	1.01 (0.86–1.19)	1.06 (0.90–1.25)
	Q3 25.47–28.36	265	0.93 (0.79–1.10)	1.02 (0.85–1.21)
	Q4 28.37–32.02	238	0.85 (0.71–1.01)	0.97 (0.81–1.16)
	Q5 32.02–82.74	219	0.80 (0.67–0.97)[c]	0.97 (0.79–1.19)
			P trend = 0.004[d]	*P* trend = 0.56
Saturated fat	Q1 1.63–9.56	292	1.00 (REF.)	1.00 (REF.)
	Q2 9.57–11.32	281	0.92 (0.76–1.10)	0.94 (0.78–1.13)
	Q3 11.33–13.00	269	0.85 (0.68–1.06)	0.91 (0.72–1.14)
	Q4 13.01–15.16	238	0.72 (0.55–0.95)[c]	0.78 (0.60–1.03)
	Q5 15.16–29.74	222	0.63 (0.44–0.92)[c]	0.73 (0.50–1.06)
			P trend = 0.001	*P* trend = 0.07
Monounsaturated fat	Q1 1.47–7.81	295	1.00 (REF.)	1.00 (REF.)
	Q2 7.82–8.94	290	1.06 (0.88–1.28)	1.03 (0.85–1.24)
	Q3 8.95–9.99	240	0.92 (0.74–1.16)	0.86 (0.69–1.08)
	Q4 10.00–11.29	239	0.98 (0.75–1.29)	0.91 (0.69–1.19)
	Q5 11.30–38.00	238	1.10 (0.76–1.59)	0.95 (0.66–1.37)
			P trend = 0.81	*P* trend = 0.60
Polyunsaturated fat	Q1 0.91–3.84	289	1.00 (REF.)	1.00 (REF.)
	Q2 3.85–4.26	288	1.02 (0.86–1.21)	1.11 (0.93–1.32)
	Q3 4.27–4.64	239	0.86 (0.71–1.03)	0.99 (0.82–1.20)
	Q4 4.65–5.11	240	0.87 (0.72–1.06)	1.05 (0.85–1.28)
	Q5 5.12–19.07	246	0.92 (0.73–1.16)	1.17 (0.92–1.50)
			P trend = 0.25	*P* trend = 0.35
Total omega-6[e]	Q1 0.71–3.02	276	1.00 (REF.)	1.00 (REF.)
	Q2 3.03–3.35	285	1.06 (0.89–1.27)	1.15 (0.96–1.37)
	Q3 3.36–3.65	252	0.96 (0.79–1.16)	1.06 (0.87–1.28)
	Q4 3.66–4.04	258	1.00 (0.82–1.22)	1.14 (0.93–1.40)
	Q5 4.05–19.72	231	0.91 (0.72–1.16)	1.07 (0.83–1.38)
			P trend = 0.36	*P* trend = 0.67
Total omega-3[f]	Q1 0.12–0.75	279	1.00 (REF.)	1.00 (REF.)
	Q2 0.76–0.87	280	1.04 (0.87–1.23)	1.08 (0.91–1.29)
	Q3 0.88–0.98	257	0.97 (0.81–1.16)	1.05 (0.87–1.26)
	Q4 0.99–1.12	249	0.96 (0.80–1.16)	1.06 (0.87–1.28)
	Q5 1.13–4.27	237	0.95 (0.76–1.18)	1.08 (0.86–1.34)
			P trend = 0.50	*P* trend = 0.45

Preeclampsia was defined as elevated blood pressure (≥140/90 mmHg) and proteinuria (≥300 mg/24 h or ≥1+ urine dipstick) with onset after gestation week 20, that returned to normal before 8 weeks postpartum

R relative risk, *CI* confidence interval, *Q* Quintile, *Q1* lowest quintile of intake, *Q5* highest quintile of intake

[a]Model was adjusted for total energy intake, kJ/day (continuous) and age at pregnancy (<20, <40, ≥40 years)

[b]Model adjusted for total energy intake, kJ/day (continuous) protein intake, % calories/day (continuous), and intakes of all the remaining types of fat, % calories/day (continuous), age at pregnancy categories in years (<20, <40, ≥40 years), pre-pregnancy body mass index, kg/m^2 (≤18, <25, <29, ≥30), height in cm (<164, 165–168, 169–172, 173–198), parity (previous pregnancies 1, 2, 3, and nulliparous), smoking status (non-smokers, daily smoker, daily smoker (15+ cigarettes and others), education (high school, university) vitamin E intake, mg/day (continuous), vitamin C intake, mg/day (continuous) and use of fish oil supplements before week 30 gestational age (yes/no)

[c]$P \le 0.05$ for comparing the reference quintile (Q1) to each quintile of intake

[d]Linear trends represent the median intake as nutrient densities of each quintile as a continuous variable in a separate model

[e]Sum of Linoleic acid (LA)+arachidonic acid (AA)

[f]Sum of Alpha-Linolenic Acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA)

Results

A total of 61,444 women with information on mid-pregnancy diet, contributed 65,220 corresponding pregnancies included for this analysis. The mean±SD age at pregnancy was 29 ± 4 years, pre-pregnancy BMI was 24 ± 4 kg/m^2 and height±SD 169 ± 6 cm. We documented 1302 cases of preeclampsia (2.0%) including 301 cases of severe preeclampsia (0.5%) during the study period. The median for total fat intake (% of energy) in the lowest (Q1) and highest (Q5) quintile of intake was 20.2% and 35.0%, 0.55% and 1.02% for ALA and 0.01% and 0.10% for DHA, respectively (Table 1). Women in Q5 of dietary total fat and ALA intake, were more likely to have higher consumption of total calories, all major types of fat and vitamin E. Conversely, women in Q5 were less likely to have higher dietary calcium, carbohydrate, vitamin C intake, compared to Q1. Intake of DHA was positively related to dietary polyunsaturated fatty acids and vitamin C but inversely related to total energy intake, total fat intake, saturated and trans fat intake. Women in Q5 of total fat and ALA intake, were less likely to have ever smoked, being nulliparous and to have graduated from high school, compared to women in Q1. Pre-pregnancy BMI was inversely related to total fat, ALA and DHA intake whereas DHA intake alone was inversely related to ever smoking, nulliparous, and positively related to age at pregnancy and high school graduate. Use of prenatal vitamins was unrelated to intake of DHA, ALA or total fat. Finally, fish oil supplement use was inversely related to ALA, DHA and total fat intake among women in Q5 (Table 1). A total of 39% of women reported use of prenatal vitamin supplements and 6% reported use of fish oil supplements (Table 1).

Total fat intake was inversely associated with risk of preeclampsia in age and energy adjusted models (Table 2). In these models, women in the highest quintile of total fat intake had a 20% (95% CI 33–3%) lower risk of preeclampsia than women in the bottom quintile of total fat intake. This relation disappeared, however, after adjustment for potential confounders. Furthermore, intakes of saturated, MUFA, PUFA, total n-6 PUFAs, and total omega-3 PUFA were not associated with preeclampsia (Table 2) or severe preeclampsia.

When intakes of specific LCN-3 were separately evaluated, we observed a tendency towards a positive association between ALA intake and preeclampsia risk (Fig. 2a, b). Conversely, intake of DHA was inversely related to preeclampsia risk (Fig. 2e, f). These associations were stronger for severe preeclampsia. Specifically, women in the highest quintile of ALA intake had 71% (95% CI 7–175%) higher risk of severe preeclampsia than women in the lowest quintile (Fig. 2b). On the other hand, women with the highest quintile of DHA intake had 33% (95% CI 0.51–0.80) lower risk of preeclampsia and 46% (95 CI 25–83%) lower risk of severe preeclampsia than women in the bottom quintile of intake (Fig. 2e, f).

We evaluated the robustness of our results by performing several sensitivity analyses. First, we modeled the association of absolute intakes with risks of preeclampsia and severe preeclampsia to allow comparison with guidelines for daily intake recommendations during pregnancy. The association of LCN-3 intake (EPA+DHA) with preeclampsia and severe preeclampsia was similar when intake was modeled as absolute intake (mg/day) rather than as energy densities (Supplementary Table 1). Among all pregnancies, 68% met the recommended daily intake (RDI) of at least 250 mg/day of LCN-3 from the Dietary Guidelines for Americans 2015 (DGA) [30] and 13% met The Official Dietary Guidelines from Denmark for 2015 [31], of consuming at least 200 mg/day of DHA. Meeting DGA recommended intake of LCN-3 during pregnancy was related to a lower risk of severe preeclampsia RR 0.77 (95% CI 0.60–0.99) but not of preeclampsia overall RR 0.93 (95% CI 0.82–1.05). Meeting the Danish recommendations of DHA intake during pregnancy, (≥200 mg/day) was not associated with preeclampsia or severe preeclampsia (Supplementary Table 2). Fish oil supplement use was not related to risk of preeclampsia RR 0.90 (95% CI 0.71–1.14)) or severe preeclampsia RR 0.61 (95% CI 0.34–1.11). Lastly, we found no evidence of significant heterogeneity in the associations of total omega-3, ALA and LNC-3 intake with preeclampsia or severe preeclampsia across subgroups of women defined by pre-pregnancy BMI (<25 kg/m^2/≥25 kg/m^2) age at pregnancy (<30 years/≥30 years), parity (nulliparous/parous) and fish oil supplement use (yes/no) (Supplementary table 3).

Discussion

We examined women's second trimester intake of different types of fat in relation to risk of preeclampsia and severe preeclampsia among participants of the Danish National Birth Cohort. We found that women with higher intakes of LCN-3 had a lower risk of preeclampsia, particularly of severe presentations of this disease. In addition, we found that women with high intakes of ALA experienced higher rates of severe preeclampsia. Finally, meeting US dietary guideline recommendations of EPA+DHA intake was related to a lower risk of severe preeclampsia.

Our findings agree with previous studies. For example, in a cohort of 1718 US women, higher intake of fish (DHA +EPA) during the 1st trimester of pregnancy, was inversely related to preeclampsia (Odds ratio (OR) 0.84 95% CI 0.69–1.03 per 100 mg/day) [8]. Also, case–control studies have reported inverse associations from intake and umbilical cord

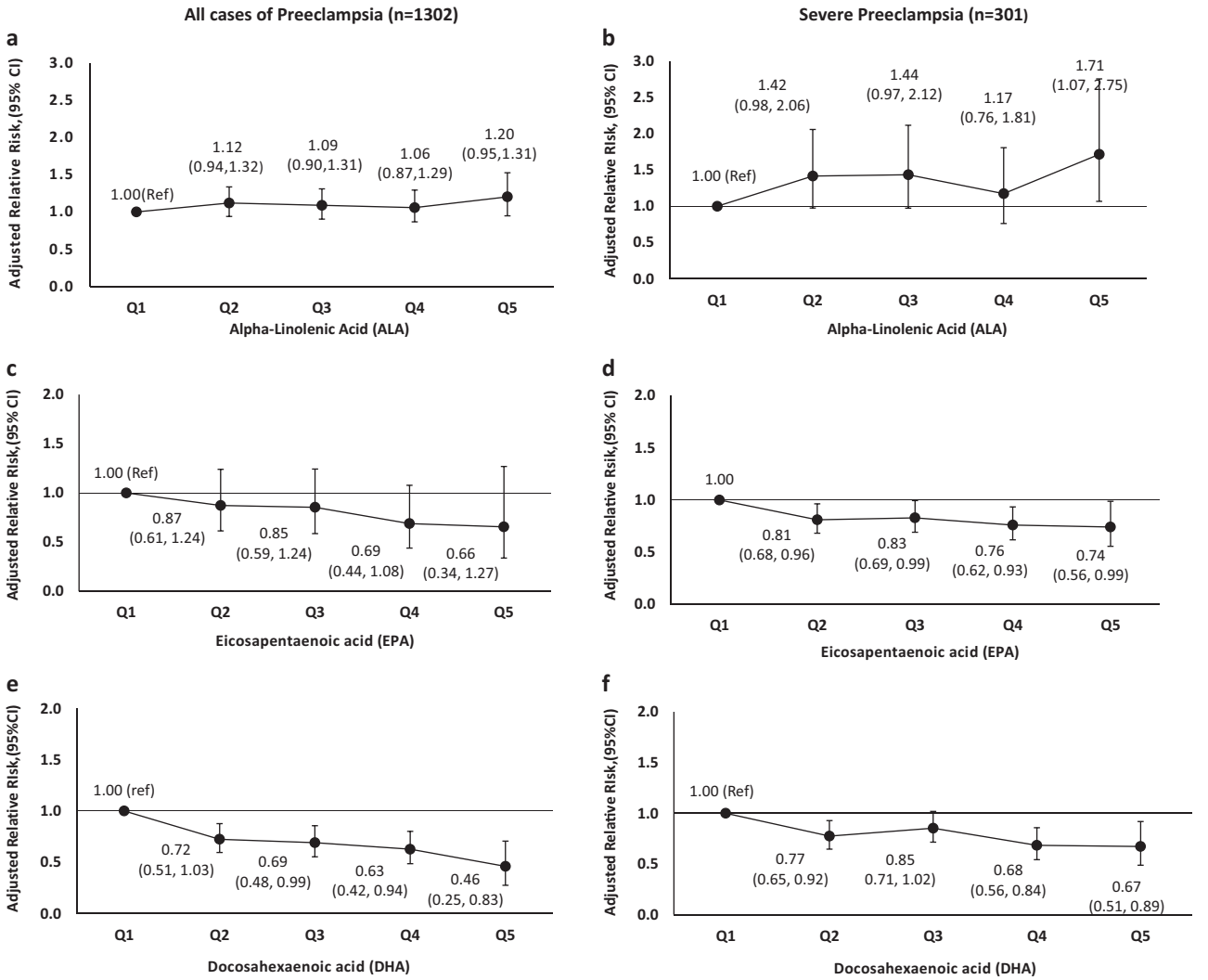

Fig. 2 Diet from 2nd trimester: omega-3 fatty acids in relation to risk of preeclampsia and severe preeclampsia. **a, c, e** Relative risk of Preeclampsia and 95% confidence intervals (RR (95% CI)), quintiles of intake, % total calories/day (quintile 1, Q1; quintile 2, Q2; quintile 3, Q3; quintile 4, Q4; quintile 5, Q5). Median intake (nutrient densities, %) in each quintile for Alpha-linolenic acid (ALA) were 0.55, 0.67, 0.76, 0.85, and 1.02, respectively; Eicosapentaenoic acid (EPA) intake was 0.03, 0.07, 0.10, 0.14, and 0.23, respectively; Docosahexaenoic acid (DHA) intake was 0.01, 0.03, 0.04, 0.06, and 0.10, respectively. **b, d, f** Relative risk of severe PE (RR [95% CI]) by quintiles of ALA, EPA, and DHA from diet, *P* for trend = 0.09, 0.17, 0.01, respectively. All models were adjusted for total energy intake, kJ/day (continuous) protein intake, % total calories/day (continuous), and intakes of all the remaining types of fat, age at pregnancy categorized in years (<20, <40, ≥40 years), pre-pregnancy body mass index, kg/m^2 (≤18, 25–29, ≥30), height in cm (<164, 165–168, 169–172, 173–198), parity (previous pregnancies categories 1, 2, and 3, nulliparous), smoking status (non-smokers, daily smoker, daily smoker (15+ cigarettes and others), education (high school, university) vitamin E intake, mg/day (continuous), vitamin C intake, mg/day (continuous) and use of fish oil supplements before week 30 gestational age (yes/no)

levels [15], placenta [14], and maternal erythrocyte total omega-3 fatty acids [16]. Similarly, a meta-analysis of RCT evaluating the effects of fish oil supplements during pregnancy, reported a non-significant 14% lower risk of preeclampsia (pooled RR 0.86, 95% CI 0.59–1.27) [32]. The main source of discrepancies between our findings and past literature is the limited number of cases of preeclampsia. Between all RCT a total of 1683 women were recruited accruing 42 preeclampsia cases randomized to fish oil supplements. This is in sharp contrast with the present study which accrued 1302 cases of preeclampsia and 301 cases of severe preeclampsia among 65,522 pregnancies. A clinical trial with 80% statistical power to identify effects of fish oil supplementation on preeclampsia, would need to recruit at least 31,027 women if the true magnitude of the effect is that reported by the meta-analysis of RCT. These results not only add evidence in support of the of LCN-3 in the prevention of preeclampsia but also highlight the need to interpret the findings of RCT bearing in mind their

limitations and consider the value of large prospective cohort studies as an important source of information.

The anti-inflammatory and vascular benefits of LCN-3 in pregnancies with preeclampsia are well documented. Animal models reveal that omega-3 fatty acids reduce levels of oxidative stress markers such as F_2 isoprostanes, a risk marker for developing preeclampsia [33]. LCN-3 in humans, have also been related to reductions in placental oxidative damage and increases in placental levels of pro-resolving mediators [5]. Additionally, DHA may decrease the expression of vascular cell adhesion protein, thereby reducing vascular damage, thus the risk of preeclampsia [34]. LCN-3 also reduce inflammation by disturbing proinflammatory eicosanoid synthesis [32].

We found an unexpected positive association between ALA and preeclampsia risk. While this was contrary to our hypothesis, this finding is consistent with the results reported by Oken et al. [8] (OR 1.35, 95% CI (0.66–2.74) in a smaller prospective cohort study. More broadly, however, the association between ALA and preeclampsia has been inconsistent throughout the literature. Wang et al. [14] found that placental ALA concentrations of women with preeclampsia were not different compared to normotensive women. Similarly, Menhendale and colleagues [35] found that red blood cell and cord blood ALA concentrations had a tendency to be lower in preeclamptic women compared to control women. Qiu et al. [13], also found that the risk of preeclampsia was lower in increasing quartiles of ALA erythrocyte fatty acids concentrations. However, reverse causation could explain these findings since blood samples were taken after the diagnosis of preeclampsia. Physiological adaptations of the disease itself or lifestyle changes triggered by the diagnosis may be reflected in blood fatty acid levels resulting in a spurious association. In our study, ALA intake was mainly in the form of processed fats and oils such as butter or margarine, thus ALA intake may be reflective of an unhealthy dietary pattern rather than be causally related to preeclampsia in this population. Careful investigation of alternative explanations of these findings are warranted to clarify the interpretation of ALA and risk of preeclampsia.

Our study has several limitations inherent in collecting longitudinal data such as confounding and measurement error. First, diet was assessed around gestational week 25 and participants were asked to report on their intake during the last 4 weeks, thus timing of the questionnaire may not adequately capture the relevant exposure window for preeclampsia. However, excluding diagnoses of preeclampsia prior to completion of the FFQ as well as excluding women with prior preeclampsia diagnosis and adjusting in the multivariable model for parity and pre-pregnancy BMI, allowed us to limit the chance of reverse causation. It is also not possible to know to what extent our findings may be generalizable to non-European women or settings with differences in patterns of fish consumption or sources of omega-3 intake. For example, at the time the study was conducted (1996–2002), dietary recommendations in Denmark did not advise pregnant women to avoid fish intake as a strategy to limit exposure to mercury and other environmental chemicals. Strengths of our study include the prospective design, the use of a previously validated FFQ, the high specificity of preeclampsia assessment through linkage with a National Disease Registry, and the large sample size that allowed accrual of cases necessary for the estimation of relative risks with significant statistical power and to examine different effects by disease severity, relative to the existing literature.

In summary, we found that DHA intake during pregnancy reduces the risk of preeclampsia by 33% and severe preeclampsia by 54%. We also found that current US dietary guideline recommendations on daily intake of omega-3 fatty acids during pregnancy are related to a lower risk of preeclampsia. Prior to considering a randomized trial with the size necessary to identify associations of the magnitude reported here, additional confirmation of this question in other prospective cohort studies is needed to strengthen the existing knowledge base and justify the need to conduct such trial.

Author contributions SFO and JEC designed the research study. MA and SH analyzed data. SFO and TIH contributed to the acquisition of data. The manuscript was written by MCA and MA. MA, SH, MCA, TIH, SFO, and JEC critically revised the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Khan KS, Wojdyla D, Say L, Gülmezoglu AM, Van Look PFA. WHO analysis of causes of maternal death: a systematic review. Lancet (Lond, Engl) [Internet]. 2006;367:1066–74. http://www.ncbi.nlm.nih.gov/pubmed/16581405
2. Abalos E, Cuesta C, Grosso AL, Chou D, Say L. Global and regional estimates of preeclampsia and eclampsia: A systematic review. Eur J Obstet Gynecol Reprod Biol [Internet]. 2013;170:1–7. https://doi.org/10.1016/j.ejogrb.2013.05.005. Available from
3. Noris M, Perico N, Remuzzi G. Mechanisms of Disease: pre-eclampsia. Nat Clin Pract Nephrol [Internet]. 2005;1:98–114. http://www.nature.com/nrneph/journal/v1/n2/full/ncpneph0035.html
4. Jones ML, Mark PJ, Mori TA, Keelan JA, Waddell BJ. Maternal dietary omega-3 fatty acid supplementation reduces placental oxidative stress and increases fetal and placental growth in the rat. Biol Reprod [Internet]. 2013;88:37. http://www.ncbi.nlm.nih.gov/pubmed/23269667

5. Jones ML, Mark PJ, Waddell BJ. Maternal dietary omega-3 fatty acids and placental function. Reprod [Internet]. 2014;147:R143–52.http://www.ncbi.nlm.nih.gov/pubmed/24451224
6. Lain KY, Roberts JM. Contemporary concepts of the pathogenesis and management of preeclampsia. JAMA [Internet]. 2002;287:3183–6.http://www.ncbi.nlm.nih.gov/pubmed/12076198
7. Klemmensen A, Tabor A, Østerdal ML, Knudsen VK, Halldorsson TI, Mikkelsen TB. et al. Intake of vitamin C and E in pregnancy and risk of pre-eclampsia: prospective study among 57 346 women. BJOG [Internet]. 2009;116:964–74. http://www.ncbi.nlm.nih.gov/pubmed/19522799
8. Oken E, Ning Y, Rifas-Shiman SL, Rich-Edwards JW, Olsen SF, Gillman MW. Diet during pregnancy and risk of preeclampsia or gestational hypertension. Ann Epidemiol [Internet]. 2007;17:663–8. http://www.ncbi.nlm.nih.gov/pubmed/17521921
9. Hofmeyr GJ, Lawrie TA, Atallah AN, Duley L, Torloni MR. Calcium supplementation during pregnancy for preventing hypertensive disorders and related problems. Cochrane Database Syst Rev [Internet]. 2014;6:CD001059. http://www.ncbi.nlm.nih.gov/pubmed/24960615
10. Xu H, Shatenstein B, Luo Z-C, Wei S, Fraser W. Role of nutrition in the risk of preeclampsia. Nutr Rev [Internet]. 2009;67:639–57. http://www.ncbi.nlm.nih.gov/pubmed/19906251
11. Mozaffarian D, Aro A, Willett WC. Health effects of trans-fatty acids: experimental and observational evidence. Eur J Clin Nutr [Internet]. 2009;63(Suppl 2):S5–21. http://www.ncbi.nlm.nih.gov/pubmed/19424218
12. Chavarro JE, Halldorsson TI, Leth T, Bysted A, Olsen SF. A prospective study of trans fat intake and risk of preeclampsia in Denmark. Eur J Clin Nutr [Internet]. 2011;65:944–51. http://www.nature.com/doifinder/10.1038/ejcn.2011.66
13. Qiu C, Sanchez SE, Larrabure G, David R, Bralley JA, Williams MA. Erythrocyte omega-3 and omega-6 polyunsaturated fatty acids and preeclampsia risk in Peruvian women. Arch Gynecol Obstet [Internet]. 2006;274:97–103. http://www.ncbi.nlm.nih.gov/pubmed/16520922
14. Wang Y, Walsh SW, Kay HH. Placental tissue levels of non-esterified polyunsaturated fatty acids in normal and preeclamptic pregnancies. Hypertens Pregnancy [Internet]. 2005;24:235–45. http://www.ncbi.nlm.nih.gov/pubmed/16263596
15. Velzing-Aarts FV, van der Klis FR, van der Dijs FP, Muskiet FA. Umbilical vessels of preeclamptic women have low contents of both n-3 and n-6 long-chain polyunsaturated fatty acids. Am J Clin Nutr [Internet]. 1999;69:293–8. http://www.ncbi.nlm.nih.gov/pubmed/9989695
16. Williams MA, Zingheim RW, King IB, Zebelman AM. Omega-3 fatty acids in maternal erythrocytes and risk of preeclampsia. Epidemiol [Internet]. 1995;6:232–7. http://www.ncbi.nlm.nih.gov/pubmed/7619928
17. Olsen SF, Sørensen JD, Secher NJ, Hedegaard M, Henriksen TB, Hansen HS. et al. Randomised controlled trial of effect of fish-oil supplementation on pregnancy duration. Lancet (Lond, Engl) [Internet]. 1992;339:1003–7. http://www.ncbi.nlm.nih.gov/pubmed/1349049
18. Onwude JL, Lilford RJ, Hjartardottir H, Staines A, Tuffnell D. A randomised double blind placebo controlled trial of fish oil in high risk pregnancy. Br J Obstet Gynaecol [Internet]. 1995;102:95–100. http://www.ncbi.nlm.nih.gov/pubmed/7756226
19. Olsen SF, Secher NJ, Tabor A, Weber T, Walker JJ, Gluud C. Randomised clinical trials of fish oil supplementation in high risk pregnancies. Fish Oil Trials In Pregnancy (FOTIP) Team. BJOG [Internet]. 2000;107:382–95. http://www.ncbi.nlm.nih.gov/pubmed/10740336
20. D'Almeida A, Carter JP, Anatol A, Prost C. Effects of a combination of evening primrose oil (gamma linolenic acid) and fish oil (eicosapentaenoic+docahexaenoic acid) versus magnesium, and versus placebo in preventing pre-eclampsia. Women Health [Internet]. 1992;19:117–31. http://www.ncbi.nlm.nih.gov/pubmed/1492408
21. Olsen J, Melbye M, Olsen SF, Sørensen TI, Aaby P, Andersen AM. et al. The Danish National Birth Cohort--its background, structure and aim. Scand J Public Health [Internet]. 2001;29:300–7. http://www.ncbi.nlm.nih.gov/pubmed/11775787
22. Olsen SF, Mikkelsen TB, Knudsen VK, Orozova-Bekkevold I, Halldórsson TI, Strøm M. et al. Data collected on maternal dietary exposures in the Danish National Birth Cohort. Paediatr Perinat Epidemiol [Internet]. 2007;21:76–86. http://www.ncbi.nlm.nih.gov/pubmed/17239183
23. Mikkelsen TB, Olsen SF, Rasmussen SE, Osler M. Relative validity of fruit and vegetable intake estimated by the food frequency questionnaire used in the Danish National Birth Cohort. Scand J Public Health [Internet]. 2007;35:172–9. http://www.ncbi.nlm.nih.gov/pubmed/17454921
24. Mikkelsen TB, Osler M, Olsen SF. Validity of protein, retinol, folic acid and n-3 fatty acid intakes estimated from the food-frequency questionnaire used in the Danish National Birth Cohort. Public Health Nutr [Internet]. 2006;9:771–8. http://www.ncbi.nlm.nih.gov/pubmed/16925883
25. Klemmensen AK, Olsen SF, Osterdal ML, Tabor A. Validity of preeclampsia-related diagnoses recorded in a national hospital registry and in a postpartum interview of the women. Am J Epidemiol [Internet]. 2007;166:117–24. http://www.ncbi.nlm.nih.gov/pubmed/17556761
26. Fitzmaurice, G., Laird, N., & Ware, J. (2011). Applied longitudinal analysis (2nd ed., Wiley Series in Probability and Statistics). Hoboken, New Jersey: Wiley. Chapter 13 Marginal Models: Generalized estimating equations (GEE): pp 221–243.
27. Rosner B Fundamentals of Biostatistics. 5th ed. Pacific Grove, California: Doxbury Press; 2005.
28. Weng H-Y, Hsueh Y-H, Messam LLM, Hertz-Picciotto I. Methods of covariate selection: directed acyclic graphs and the change-in-estimate procedure. Am J Epidemiol. 2009;169:1182–90.
29. Willett W Nutritional epidemiology. Oxford University Press; 1998. 514 p.
30. Department of Health and Human Services US. Dietary Guidelines for Americans 2015-2020 Eight Edition [Internet]. Available from: http://health.gov/dietaryguidelines/2015/guidelines/
31. Denmark M of E and F of. The Official Dietary Guidelines [Internet]. 2015 [cited 2017 Oct 4]. Available from: http://altomkost.dk/deofficielleanbefalingertilensundlivsstil/de-officielle-kostraad/
32. Makrides M, Duley L, Olsen SF. Marine oil, and other prostaglandin precursor, supplementation for pregnancy uncomplicated by pre-eclampsia or intrauterine growth restriction. Cochrane Database Syst Rev [Internet]. 2006;3:CD003402. http://www.ncbi.nlm.nih.gov/pubmed/16856006
33. Erlebacher A. Immunology of the maternal-fetal interface. Annu Rev Immunol [Internet]. 2013;31:387–411. http://www.ncbi.nlm.nih.gov/pubmed/23298207
34. Sørensen JD, Olsen SF, Pedersen AK, Boris J, Secher NJ, Fitz-Gerald GA. Effects of fish oil supplementation in the third trimester of pregnancy on prostacyclin and thromboxane production. Am J Obstet Gynecol [Internet]. 1993;168(3 Pt 1):915–22. http://www.ncbi.nlm.nih.gov/pubmed/8456902
35. Mehendale S, Kilari A, Dangat K, Taralekar V, Mahadik S, Joshi S. Fatty acids, antioxidants, and oxidative stress in pre-eclampsia. Int J Gynaecol Obstet [Internet]. 2008;100:234–8. http://www.ncbi.nlm.nih.gov/pubmed/17977540