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Maternal fish consumption, fatty acid levels and angiogenic factors: The Generation R Study

Paula K. Bautista Niño [a, 1], Myrte J. Tielemans [a, 1], Sarah Schalekamp-Timmermans [b], Jolien Steenweg-de Graaff [a, c], Albert Hofman [a], Henning Tiemeier [a, c], Vincent W. Jaddoe [a, d, e], Eric A.P. Steegers [b], Janine F. Felix [a, d, e], Oscar H. Franco [a, *]

[a] Department of Epidemiology, Erasmus MC, University Medical Center Rotterdam, The Netherlands
[b] Department of Obstetrics and Gynaecology, Erasmus MC, University Medical Center Rotterdam, The Netherlands
[c] Department of Child and Adolescent Psychiatry/Psychology, Erasmus MC, University Medical Center Rotterdam, The Netherlands
[d] The Generation R Study Group, Erasmus MC, University Medical Center Rotterdam, The Netherlands
[e] Department of Paediatrics, Erasmus MC, University Medical Center Rotterdam, The Netherlands

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ABSTRACT

Introduction: Angiogenic factors, such as placental growth factor (PlGF) and soluble Flt-1 (sFlt-1), are key regulators of placental vascular development. Evidence from *in vitro* studies indicates that fatty acids can affect angiogenesis. We investigated the associations of maternal fish consumption and fatty acids levels with angiogenic factors during pregnancy, and in cord blood in a large population-based prospective cohort.

Methods: First trimester fish consumption was assessed among 3134 pregnant women using a food-frequency questionnaire. Plasma fatty acid levels were measured in second trimester. Plasma PlGF and sFlt-1 were measured in first and second trimester and in cord blood. Associations of fish consumption or fatty acid levels with angiogenic factors were assessed by multivariable linear regression analyses.

Results: There were no consistent associations of total fish or lean fish consumption with levels of PlGF, sFlt-1, or sFlt-1/PlGF ratio. Neither fatty fish nor shellfish were associated with angiogenic factors. Plasma omega-3 polyunsaturated fatty acids, which are the main type of fatty acids in fish, were inconsistently associated with angiogenic factors in second trimester and cord blood. Yet, higher levels of arachidonic acid, an omega-6 polyunsaturated fatty acid, were associated with lower levels of PlGF and sFlt-1.

Discussion: We found no consistent associations of fish consumption or fatty acids levels with angiogenic factors in a population with low fish consumption. Studies including populations with higher fish consumption are required to fully grasp the potential effects of maternal fish consumption on placental angiogenesis.

1. Introduction

Important regulators of placental vascular development are angiogenic factors [1]. Well known proangiogenic proteins are Vascular Endothelial Growth Factor (VEGF) and Placental Growth Factor (PlGF). These proangiogenic factors might promote placental development by binding to its transmembrane receptor Flt-1, thereby inducing gene expression to promote proliferation and migration of trophoblasts [2,3]. A soluble variant of Flt-1, sFlt-1, binds to circulating proangiogenic factors with high affinity, thus diminishing PlGF and VEGF availability for the transmembrane Flt-1 receptor and inhibiting its proangiogenic signal [4,5]. Hence, sFlt-1 has an antiangiogenic effect. An imbalance between the levels of proangiogenic and antiangiogenic factors has been associated with pregnancy complications such as preeclampsia and intra-uterine growth restriction [5–7]. Additionally, plasma levels of PlGF and

Abbreviations: AA, arachidonic acid; ALA, alpha-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acid; FFQ, food-frequency questionnaire; LA, linoleic acid; PCB, polychlorinated biphenyls; PlGF, placental growth factor; PUFA, polyunsaturated fatty acid; sFlt-1, soluble Flt-1; VEGF, vascular endothelial growth factor.

* Corresponding author. Department of Epidemiology, Erasmus MC, University Medical Center Rotterdam, P.O. Box 2040, 3000 CA, Rotterdam, The Netherlands.
E-mail address: o.franco@erasmusmc.nl (O.H. Franco).

[1] Contributed equally.

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sFlt-1 have also been associated with offspring growth in our cohort [8]. As a measure of antiangiogenesis the sFlt-1/PlGF ratio could be used [9].

Multiple environmental factors are known to affect placental angiogenesis, including smoking, diabetes and maternal diet [10–12]. Maternal diet is also known to influence blood pressure in pregnancy and foetal outcomes as has been suggested in cohort and case–control studies [13–15]. Among other nutritional exposures, higher fish consumption before and during pregnancy has been associated with higher birth weight and lower risk of foetal growth retardation in two European observational studies [16,17], although this association was not found in our cohort [18]. Potential effects of fish may be mediated through polyunsaturated fatty acids (PUFAs), in particular omega-3 PUFAs. Omega-3 PUFA supplementation during pregnancy has been shown to increase pregnancy duration, head circumference and birth weight [19,20]. Evidence from *in vitro* studies indicates that some PUFAs stimulated angiogenesis in a cell line of first trimester human trophoblasts potentially by increasing the expression of angiogenic factors [21,22].

So far, no studies have evaluated the association between maternal dietary intake of foods high in PUFAs, such as fish, and human placental angiogenesis. Therefore, we investigated the association of maternal fish consumption with the angiogenic factors PlGF and sFlt-1 measured in the first and second trimester, and in cord blood. In addition, we evaluated the association of maternal plasma fatty acids levels in second trimester with PlGF and sFlt-1 levels in the second trimester and in cord blood in a large population-based birth cohort.

2. Methods

2.1. Study design and population

The present study was embedded in the Generation R Study, a population-based prospective cohort study from foetal life onwards in the city of Rotterdam, the Netherlands. Details of the study have been described before [23]. All participants provided written informed consent. The study was carried out in accordance with the World Medical Association Declaration of Helsinki and approved by the medical ethics committee of the Erasmus University Medical Center, Rotterdam, the Netherlands.

For this particular study, we included only Dutch women, because the food-frequency questionnaire (FFQ) was designed to evaluate a Dutch diet. Of the total of 4546 participating Dutch women, we excluded those enrolled after the 25th week of gestation (n = 515), with missing information on fish consumption (n = 846), multiple pregnancies (n = 1), unlikely total daily energy intake (n = 4) and those in whom no data on any of the angiogenic factors at any time point were available (n = 46), leaving 3134 women in our analyses (Fig. 1).

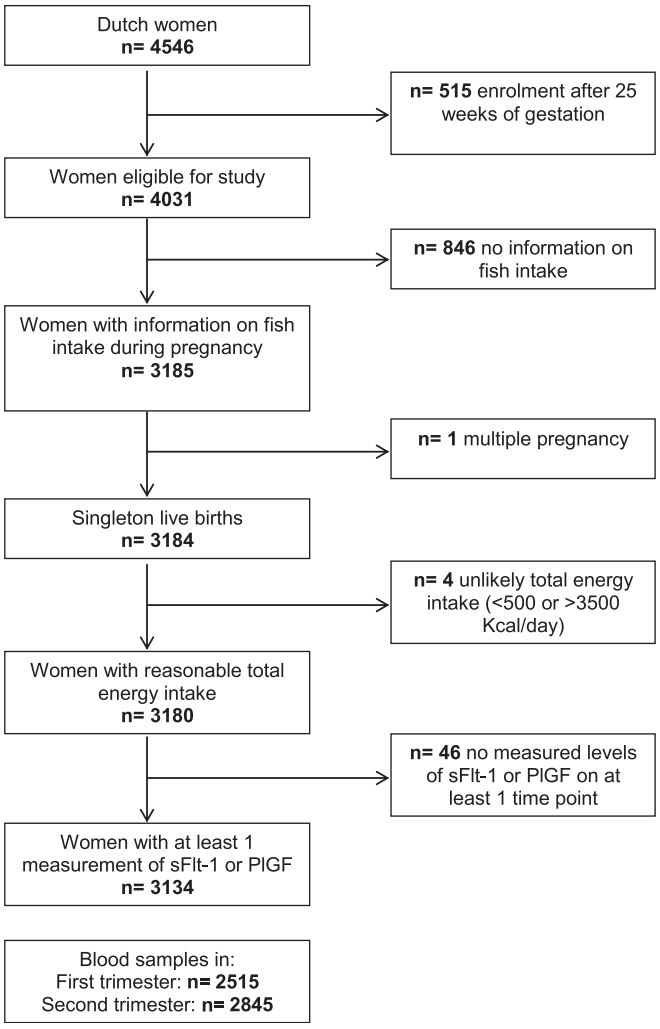

Fig. 1. Flow chart of participants included for analyses.

2.2. Fish intake

Maternal dietary intake, including fish consumption, was evaluated at enrolment (median 13.2 (95% range 9.9–21.6) weeks of gestation) using a self-administered semi-quantitative FFQ adapted from the validated FFQ of Klipstein-Grobusch et al. [24]. This FFQ measured food intake over the prior three months, and covered dietary intake during the first trimester of pregnancy. The FFQ considered consumption frequency, portion sizes [25], preparation method and additions. To calculate average daily nutritional values, the 2006 version of the Dutch food composition table was used [26].

Fish consumption (in grams per week) was assessed for total fish intake and for specific types of fish [18]. Fish consumption was grouped based on the nutrient content into: lean fish (codfish, plaice, catfish, sole fish, tuna, whiting and haddock), fatty fish (salmon, herring, mackerel, eel, sardines, halibut, bloater, trout, anchovy and gurnard) and shellfish (crab, lobster, shrimps and mussels). Processed fish, roe and fish derived from liver were not used in this analyses. Total fish includes all the types of fish consumed.

2.3. Blood sampling

Peripheral venous blood samples were drawn during the visits to the research centre in the first (median 12.9 weeks (95% range 9.8–17.3)) and second trimester (median 20.4 weeks (95% range 18.6–22.9)). Umbilical venous cord blood samples were obtained by midwives and obstetricians immediately after delivery (median gestational age at delivery 40.3 weeks (95% range 36.0–42.3)). All blood samples were transported to the regional laboratory, centrifuged and the plasma distributed into 250μL aliquots and stored at −80 °C [27].

2.4. Fatty acid levels

The analysis of plasma glycerophospholipid FA composition was performed on plasma samples derived during second trimester of pregnancy in the Division of Metabolic Diseases and Nutritional Medicine, Dr. von Hauner Children's Hospital, Ludwig-

Maximilians-University of Munich following the high-throughput method described by Glaser et al. [28]. The average coefficient of variation was 15.7%. Plasma FA concentration reflects only current nutritional status.

Plasma PUFA levels are a result of both dietary intake and metabolism. Plasma omega-3 and omega-6 PUFAs may compete in these metabolic processes because the same rate-limiting enzymes are needed [29]. Therefore, we did not only take into account omega-3 PUFAs but also evaluated the associations with omega-6 PUFAs which are derived from other dietary sources than fish.

For the analyses, we used relative concentrations of the individual FAs as weight percentage of the total sum of FAs (%) instead of absolute concentrations (mg/L), because fatty acid levels are not only a reflection of dietary intake but also of metabolism, the complex interplay between individual fatty acids, and multiple additional factors[30]. The analysed omega-3 PUFAs were docosahexaenoic acid (*DHA*), eicosapentaenoic acid (*EPA*) and alpha-linolenic acid (ALA). The analysed omega-6 PUFAs were linoleic acid (LA) and arachidonic acid (*AA*). Also omega-3/omega-6, EPA/AA and DHA/AA ratios were evaluated. The omega-3/omega-6 ratio was calculated by the sum of five omega-3 PUFAs (ALA, eicosatreionic acid, EPA, docasapentaenoic acid and DHA) divided by the sum the omega-6 PUFAs matching the carbon chain length of the omega-3 PUFAs (LA, dihomo-gamma-linolenic acid, AA, adrenic acid and osbond acid). The EPA/AA and DHA/AA ratios have been used as cardiovascular risk biomarkers [31].

2.5. Angiogenic factors

Levels of sFlt-1 and PlGF were measured in first and second trimester plasma and cord blood plasma at the Department of Clinical Chemistry of the Erasmus University Medical Center using a two-step chemi-luminescent microparticle immunoassay (CMIA) on the Architect System (Abbot Diagnostics B.V., Hoofddorp, the Netherlands). Cord blood plasma, originating from the foetal circulation, was evaluated in addition to maternal plasma because studies have suggested a discordant release of sFlt-1 into both circulations [32]. Plasma levels of sFlt-1 and PlGF were evaluated individually and additionally combined within the sFlt-1/PlGF ratio, which can be used as a measure of antiangiogenesis [9]. The between-run coefficients of variation for plasma sFlt-1 were 2.8% at 5.5 ng/mL and 2.3% at 34.0 ng/mL; and 4.7% at 24 pg/mL, and 3.8% at 113 pg/mL for plasma PlGF. The highest detected level was 150 ng/mL for sFlt-1 and 1500 pg/mL for PlGF [7].

2.6. Covariates

Information on maternal age, folic acid supplementation, parity and educational level was obtained from questionnaires completed by the mothers at enrolment. Information on smoking and alcohol consumption was obtained from questionnaires in each trimester. As season of conception might influence maternal fish consumption we back-calculated the month of conception from the gestational age and then categorized it into seasons: winter (December to February), spring (March to May), summer (June to August) and fall (September to November).

Maternal height and weight were measured at enrolment to calculate BMI (kg/m^2) (correlation coefficient with pre-pregnancy BMI 0.96). Gestational age at delivery, birth weight, foetal sex and pregnancy complications (pregnancy-induced hypertension and preeclampsia) were obtained from medical records. The occurrence of pregnancy complications was cross-validated using medical record review [7].

Table 1
Characteristics of participants (n = 3134)[a].

Maternal characteristics	Mean ± SD or median (95% range)
Age (years)	31.4 ± 4.3[b]
Gestational age at enrolment (weeks)	13.2 (9.9–21.6)[b]
BMI at enrolment (kg/m^2)	24.1 ± 3.9
Parity, *% (n)*	
Nullipara	60.1 (1883)
Multipara	39.9 (1251)
Highest education finished, *% (n)*	
Primary	3.2 (100)
Secondary	36.4 (1140)
Higher	60.5 (1894)
Smoking during pregnancy, *% (n)*	
Yes	28.5 (894)
No	71.5 (2240)
Alcohol use during pregnancy, *% (n)*	
Yes	67.2 (2105)
No	32.8 (1029)
Folic acid supplementation use, *% (n)*	
Yes	89.0 (2789)
No	11.0 (345)
Pregnancy-induced hypertension, *% (n)*	
Yes	5.3 (164)
No	94.7 (2914)
Preeclampsia, *% (n)*	
Yes	1.9 (56)
No	98.1 (2914)
Season of conception, *% (n)*	
Winter	28.3 (888)
Spring	23.6 (738)
Summer	21.8 (684)
Fall	26.3 (823)
Total daily energy intake (kJ/day)	8972 ± 2127[b,c]
Total fish intake (gram/week)[d]	75 (0–318)[b]
Lean fish intake (gram/week)[d]	24 (0–149)[b]
Fatty fish intake (gram/week)[d]	30 (0–150)[b]
Shellfish intake (gram/week)[d]	0 (0–34.5)[b]
Plasma fatty acids	
Docosahexaenoic acid (mg/L)	80.1 ± 20.0
Eicosapentaenoic acid (mg/L)	13.0 ± 4.2
Alpha-Linolenic acid (mg/L)	5.5 ± 1.8
Linoleic acid (mg/L)	352.3 ± 59.5
Arachidonic acid (mg/L)	154.5 ± 31.2
Omega-3/omega-6 ratio	0.2 ± 0.05
EPA/AA ratio	0.06 ± 0.04
DHA/AA ratio	0.5 ± 0.14
Angiogenic factors levels	
First trimester	
PlGF (pg/mL)	37.8 (14.4–154.7)
sFlt-1 (ng/mL)	4.9 (1.9–12.8)
sFlt-1/PlGF ratio	0.13 (0.02–0.43)
Second trimester	
PlGF (pg/mL)	185.9 (73.2–569.3)
sFlt-1 (ng/mL)	4.7 (1.5–16.0)
sFlt-1/PlGF ratio	0.02 (0.01–0.11)
Cord blood	
PlGF (pg/mL)	8.6 (0.0–20.7)
sFlt-1 (ng/mL)	0.45 (0.08–4.01)
sFlt-1/PlGF ratio	0.05 (0.01–0.42)
Child characteristics	
Sex, *% (n)*	
Boy	50.8 (1592)
Girl	49.2 (1542)
Gestational age at birth (weeks)	40.3 (36.0–42.4)
Birth weight (grams)	3497 ± 550

[a] Values are mean ± SD when normally distributed or median (95% range) when not normally distributed for continuous variables and percentages (n) for categorical outcomes. The number of missings were 0.4% (n = 14) for BMI at enrolment, 0.2% (n = 7) for parity, 0.6% (n = 19) for maternal education, 7.0% (n = 219) for smoking, 7.6% (n = 238) for alcohol use, 17.6% (n = 552) for folic acid supplementation use, 1.8% (n= 56) for pregnancy-induced hypertension, 5.2% (n = 164) for preeclampsia, 0.03% (n = 1) for season of conception, 13.9% (n = 435) for plasma fatty acids, 0.03% (n = 1) for child sex, 0.03% (n = 1) gestational age at birth and 0.2% (n = 5) for birth weight. AA: Arachidonic acid, DHA: Docosahexanoic acid, EPA: Eicosapentanoic acid, PlGF: Placental growth factor, sFlt-1: Soluble Flt-1.

[b] No missings.

[c] Value corresponds to a daily energy intake of 2143 ± 508 kcal/day.

[d] Absolute values of fish consumption.

2.7. Statistical analysis

We adjusted fish consumption for total energy intake to reduce measurement error, to control for confounding and to evaluate the effect of fish consumption independent of energy intake using the nutrient residual method [33]. Energy-adjusted fish consumption was derived by first regressing fish consumption on total energy intake, saving the residuals from this regression and then adding the median fish consumption to these residuals. This gave us an energy-adjusted fish intake for all women [33].

Subsequently, to reduce the influence of outlying data points, fish consumption was divided in categories. The energy-adjusted total fish consumption was grouped into five categories (0; 1–69; 70–139; 140–209; and >210 g per week), lean fish and fatty fish consumption into four categories each (0; 1–34; 35–69; and >70 g per week), and shellfish consumption into three categories (0; 1–13; >14 g per week) as previously described [18]. The category of "no fish consumption" was considered the reference category. The plasma levels of sFlt-1 and PlGF as well as the sFlt-1/PlGF ratio were natural logarithm (ln)-transformed to obtain a normal distribution.

We studied the association of maternal fish consumption with levels of angiogenic factors and the association of plasma FA levels with angiogenic factors using multivariable linear regression models.

Each of the following variables were evaluated as potential confounders in univariable models: age, parity, BMI, smoking, educational level, foetal sex, season of conception and energy intake. All these variables yielded a change of at least 5% in the effect estimate and were therefore included in the adjusted models. Tests for trend were done by including the fish consumption categories as a continuous variable in the linear regression models. A sensitivity analysis was performed in women without known diabetes mellitus or gestational diabetes (n = 2771) because these women may have received dietary advice which altered their dietary intake and additionally levels of angiogenic factors may differ [34].

Missing values for the covariates included in the regression models (0.4% for BMI, 0.2% for parity, 0.6% for educational level and 7% for smoking) were imputed using multiple imputation [35]. Five datasets were created and analysed independently and then the results were pooled. All analyses were performed using both the original data set and the imputed data sets. As the effect estimates were very similar, we present only the pooled effect estimates with their 95% confidence intervals.

All statistical analyses were performed using SPSS Statistics version 20 for Windows (PASW Inc., Chicago, IL, USA). *P*-values of <0.05 were considered statistically significant.

3. Results

Maternal and child characteristics are presented in Table 1 (Characteristics after imputations are presented in Supplementary table 1). Median total fish intake was 75 g/week (95% range 0–318), mainly of fatty fish and lean fish consumption.

3.1. Fish intake and angiogenic factors

Associations of energy-adjusted total fish intake and angiogenic factors are presented in Table 2. We found no association of maternal energy-adjusted total fish intake with PlGF levels at any of the three time points. The association of total fish intake with sFlt-1 levels was inconsistent. In the first trimester, women with a total fish intake of 1–69 g per week had 0.11 (95%CI 0.03, 0.20) ng/mL higher Ln-sFlt-1 levels than women who did not consume fish. However, the other categories of total fish intake were not associated with sFlt-1. In the second trimester, total fish intake was associated with higher sFlt-1 levels in all categories, although not statistically significant in the highest category which may be due to the lower number of women in this category (n = 196) than in the other categories (between 470 and 1063 women). We found no dose–response relation and no association was found in cord blood.

Table 2
Associations between weekly maternal total fish consumption and levels of angiogenic factors[a].

Total fish consumption (energy-adjusted g/week)	First trimester			Second trimester			Cord blood		
	n	Beta	95% CI	n	Beta	95% CI	n	Beta	95% CI
	Ln-PlGF pg/mL (n = 2515)			Ln-PlGF pg/mL (n = 2845)			Ln-PlGF pg/mL (n = 1552)		
0	155	Reference		181	Reference		108	Reference	
1–69	935	−0.028	−0.105, 0.049	1063	−0.017	−0.094, 0.060	560	−0.090	−0.464, 0.284
70–139	849	−0.008	−0.086, 0.069	934	−0.002	−0.080, 0.076	508	0.006	−0.195, 0.206
140–209	416	−0.007	−0.090, 0.077	470	−0.007	−0.091, 0.077	255	0.069	−0.349, 0.487
>210	160	−0.015	−0.113, 0.084	197	−0.044	−0.141, 0.053	121	−0.156	−0.402, 0.090
P for trend			*0.61*			*0.73*			*0.79*
	Ln-sFlt-1 ng/mL (n = 2513)			Ln-sFlt-1 ng/mL (n = 2844)			Ln-sFlt-1 ng/mL (n = 1695)		
0	155	Reference		181	Reference		111	Reference	
1–69	936	**0.114**†	**0.025, 0.203**	1063	**0.157***	**0.060, 0.255**	613	0.129	−0.095, 0.353
70–139	846	0.064	−0.025, 0.152	934	**0.106**†	**0.007, 0.204**	552	0.098	−0.128, 0.325
140–209	415	0.092	−0.004, 0.189	470	**0.141***	**0.035, 0.247**	294	0.124	−0.118, 0.365
>210	161	0.059	−0.055, 0.173	196	0.108	−0.015, 0.232	125	0.279	−0.002, 0.559
P for trend			*0.74*			*0.78*			*0.18*
	Ln-sFlt-1/PlGF ratio (n = 2505)			Ln-sFlt-1/PlGF ratio (n = 2844)			Ln-sFlt-1/PlGF ratio (n = 1469)		
0	155	Reference		181	Reference		102	Reference	
1–69	932	**0.142**†	**0.034, 0.250**	1063	**0.175***	**0.059, 0.290**	522	0.159	−0.055, 0.372
70–139	845	0.070	−0.039, 0.178	934	0.108	−0.009, 0.225	487	0.152	−0.063, 0.366
140–209	414	0.099	−0.018, 0.216	470	**0.148**†	**0.023, 0.273**	245	0.192	−0.039, 0.423
>210	159	0.063	−0.075, 0.201	196	**0.151**†	**0.006, 0.297**	113	**0.266**†	**0.001, 0.532**
P for trend			*0.44*			*0.65*			*0.10*

Results from multivariable linear regression model, based on imputed data. *P* for trend was conducted by including fish consumption categories in the multivariable linear regression model. The statistically significant values are presented in bold (P-value <0.05). The P for trend values are presented in italics.
†P < 0.05, *P < 0.01.
Abbreviations: CI: confidence interval, n: number of participants, PlGF: placental growth factor, sFlt-1: soluble Flt-1.
[a] Adjusted for total energy intake, maternal age, gestational age at measurement, BMI at enrolment, foetal sex, parity, maternal education, smoking, and season of conception.

Table 3
Associations between maternal fatty acid plasma levels and levels of angiogenic factors[a].

Fatty acids (weighed percentage)	Second trimester		Cord blood	
	Beta	95% CI	Beta	95% CI
	Ln-PlGF pg/mL (n = 2699)		Ln-PlGF pg/mL (n = 1311)	
DHA (22:6n-3)	−0.006	−0.024, 0.011	0.012	−0.089, 0.113
EPA (20:5n-3)	**−0.062†**	**−0.116, −0.007**	−0.077	−0.397, 0.243
ALA (18:3n-3)	0.158	−0.026, 0.342	0.853	−0.268, 1.973
LA (18:2n-6)	0.007	−0.001, 0.014	−0.015	−0.055, 0.025
AA (20:4n-6)	**−0.022***	**−0.035, −0.009**	−0.029	−0.093, 0.035
n-3/n-6 ratio	−0.157	−0.542, 0.227	1.003	−1.266, 3.271
EPA/AA ratio	−0.285	−0.754, 0.183	−0.146	−2.893, 2.601
DHA/AA ratio	0.087	−0.049, 0.223	0.341	−0.429, 1.112
	Ln-sFlt-1 ng/mL (n = 2699)		Ln-sFlt-1 ng/mL (n = 1444)	
DHA (22:6n-3)	−0.011	−0.032, 0.011	−0.051	−0.106, 0.005
EPA (20:5n-3)	−0.044	−0.112, 0.025	0.055	−0.127, 0.236
ALA (18:3n-3)	0.066	−0.166, 0.298	**0.676†**	**0.068, 1.284**
LA (18:2n-6)	−0.001	−0.010, 0.008	0.005	−0.017, 0.027
AA (20:4n-6)	**−0.022***	**−0.038, −0.007**	**−0.044†**	**−0.084, −0.005**
n-3/n-6 ratio	−0.021	−0.505, 0.463	−0.211	−1.466, 1.044
EPA/AA ratio	−0.129	−0.718, 0.461	1.001	−0.548, 2.550
DHA/AA ratio	0.074	−0.095, 0.243	0.029	−0.396, 0.455
	Ln-sFlt-1/PlGF ratio (n = 2699)		Ln-sFlt-1/PlGF ratio (n = 1238)	
DHA (22:6n-3)	−0.005	−0.031, 0.022	−0.023	−0.077, 0.032
EPA (20:5n-3)	0.018	−0.065, 0.101	0.086	−0.088, 0.261
ALA (18:3n-3)	−0.092	−0.372, 0.187	0.564	−0.031, 1.159
LA (18:2n-6)	−0.008	−0.019, 0.003	−0.008	−0.029, 0.014
AA (20:4n-6)	0.000	−0.020, 0.019	−0.032	−0.071, 0.007
n-3/n-6 ratio	0.136	−0.447, 0.719	0.324	−0.901, 1.549
EPA/AA ratio	0.157	−0.553, 0.867	1.043	−0.432, 2.518
DHA/AA ratio	−0.013	−0.219, 0.192	0.071	−0.342, 0.484

Results from multivariable linear regression model, based on imputed data. The statistically significant values are presented in bold (P-value <0.05).
†P < 0.05, *P < 0.01.
Abbreviations: AA: arachidonic acid, ALA: alpha-linolenic acid, CI: confidence interval, DHA: docosahexanoic acid, EPA: eicosapentanoic acid, LA: linoneic acid, n: number of participants, n-3/n-6: omega-3/omega-6 ratio, PlGF: placental growth factor, sFlt-1: soluble Flt-1.
[a] Adjusted for total energy intake, maternal age, gestational age at measurement, BMI at enrolment, foetal sex, parity, maternal education and smoking.

The associations of total fish intake with the sFlt-1/PlGF ratio were also inconsistent. In the first trimester, an energy-adjusted intake of 1–69 g per week of total fish intake, but none of the other categories, was associated with an increased ratio. In the second trimester total fish intake was associated with increased ratios in all categories except for 70–139 g per week, but there was no dose–response relation.

The association of lean fish consumption with levels of PlGF, sFlt-1 and the sFlt-1/PlGF ratio is presented in Supplementary table 2. There were some scattered significant associations for single categories of intake of lean fish, but no clear pattern could be distinguished. Neither fatty fish nor shellfish intake were associated with angiogenic factors (Supplementary tables 3 and 4). Restricting the analyses to women without diabetes resulted in a minor attenuation of the effect estimates (*data not shown*).

3.2. Plasma fatty acids and angiogenic factors

Of the omega-3 PUFAs, higher levels of EPA were associated with low PlGF levels in the second trimester and higher levels of ALA were associated with increased sFlt-1 levels in cord blood (Table 3). Higher levels of the omega-6 PUFA arachidonic acid were associated with significantly lower PlGF and sFlt-1 levels in the second trimester and with lower sFlt-1 levels in cord blood. We found no associations of any of the fatty acid ratio's with PlGF or sFlt-1.

4. Discussion

We found no consistent associations of first trimester maternal fish consumption with levels of the proangiogenic factor PlGF or the anti-angiogenic factor sFlt-1. Higher total fish intake increased sFlt-1 levels in some categories of intake, but there was not a dose–response effect. The association of fish consumption with the sFlt-1/PlGF ratio showed a similar pattern to that for sFlt-1. When we evaluated the effect of the plasma FA levels on angiogenesis, higher EPA was associated with lower PlGF levels in second trimester and ALA was associated with higher sFlt-1 levels in cord blood. Higher arachidonic acid was associated with lower sFlt-1 levels in both measurements and in second trimester PlGF levels.

Altered levels of pro-angiogenic and anti-angiogenic factors have been associated with adverse pregnancy outcomes, including preeclampsia and foetal growth restriction [6,7].

Maternal overall diet has been found to influence foetal growth [14], also consumption of food items such as fish has been associated with foetal growth in some studies [16,17,36]. Similarly, results from a systematic review have indicated that supplementation of omega-3 PUFAs resulted in lower prevalence of preterm birth and higher birthweight [20], although these results were not confirmed in another systematic review [19]. Results from several *in vitro* and animal studies, mostly in the field of cancer research, have suggested that food or food-derived compounds, such as components of cinnamon or grape seed, may influence angiogenesis [37,38]. However, whether maternal fish intake, or the intake of fish-derived compounds, is associated with markers of human placental angiogenesis has not yet been studied, to the best of our knowledge.

Our results suggest a potential anti-angiogenic effect of total fish intake in the second trimester reflected in the increased sFlt-1 levels and sFlt-1/PlGF ratio. In this association, the absence of a significant p-value for trend may not preclude the possibility of a threshold effect rather than a dose–response association [39]. Arachidonic acid (AA), an omega-6 PUFA, was significantly

associated with lower levels of both PlGF and sFlt-1, however fish is not a source of AA. Two omega-3 PUFAs EPA and ALA were associated with anti-angiogenesis, EPA through lower PlGF levels in second trimester and ALA through higher sFlt-1 levels in cord blood. These findings are in agreement with the effect of PUFAs on angiogenesis in cancer cell lines where omega-3 PUFAs, such as EPA, exert anti-inflammatory and antineoplastic effects, whereas omega-6 PUFAs, such as AA, promote inflammation and carcinogenesis [40,41]. In addition, omega-3 PUFAs were found to down-regulate the production of VEGF and thus inhibit angiogenesis in human tumours implanted in mice and rats. However, EPA, DHA and AA increased angiogenesis in first trimester trophoblast cells [22] and Basak et al. [21] reported that these PUFAs also increased the expression of COX-2 gene, which may be associated with induction of angiogenesis. However, EPA, DHA and AA increased angiogenesis in first trimester trophoblast cells [22] and Basak et al. [21] reported that these PUFAs also increased the expression of COX-2 gene, which may be associated with induction of angiogenesis.

Although the mechanisms underlying the different effects of PUFAs in cancer cells and in trophoblasts remain to be established, distinct fatty acids metabolism could be expected in different cell lines, because differences in the handling of fatty acids have been shown between first trimester trophoblasts and third trimester trophoblasts [21]. In our study, the increase in sFlt-1 levels, secreted by trophoblasts [42], may be explained by increased trophoblastic volume rather than anti-angiogenesis, which would be beneficial. Unfortunately, we were not able to evaluate the role of trophoblast or trophoblastic volume in the association of fish consumption with angiogenic factors, because this information was not collected in our participants. Our finding that AA was associated with both proangiogenic and antiangiogenic factors during pregnancy needs further study whether AA in pregnancy may indeed be involved in both processes.

Besides the intake of fatty acids, an important aspect of fish consumption is the bioaccumulation of polychlorinated biphenyls (PCBs) and dioxins. Foetal exposure to PCBs and dioxins increases the risk for intra-uterine growth retardation, leads to changes in thyroid hormone metabolism, immunosuppression and neurological deficits [17,36,43]. Also, plasma PCB levels have been associated with lower syncytiotrophoblast volume and increased PlGF levels in pregnant women [44]. It is possible that potential beneficial effects of PUFAs on placental angiogenesis might be counterbalanced by the effects of PCBs or other substances contained in fish. Also, interactions of the components of fish with other dietary and metabolic factors, as well as the effects of the preparation method of the fish are yet to be explored.

Our study has several strengths, including its large sample size, the population-based design, the in-depth information about fish intake and the extensive information on a large number of potential confounders. However, the study also has limitations. Our study population had a low fish consumption. The median total fish intake was 75 g (half a serving) per week and only 7% of pregnant women consumed more than 210 g of fish per week which was lower than the mean total fish intake in the general Dutch population of 88–104 g per week [45]. Also, total fish intake in the Netherlands was the lowest in a study across 10 European countries [45]. Therefore, we were likely limited in our ability to study the full effects of fish intake, mainly due to a low variation in fish consumption between the subjects especially in subgroups of specific types of fish. Also, the exact amount of fish intake measured by an FFQ might not be very precise. However, it does permit adequate ranking of participants by intake, as participants with a high intake will also report a higher intake of fish than those with a low intake [46]. Also, as mentioned above, there may be further aspects to fish intake beyond fatty acids that also influence levels of angiogenic factors and clinical outcomes that we were not able to take into account. Lastly, we got some scattered significant results. As we performed a large number of statistical tests, some significant results might arise by chance (multiple testing). However, our significant results were clustered within the second trimester and in two specific (and related) outcomes, so we feel that multiple testing is unlikely to explain all our findings.

In a population with low fish consumption, we found no consistent associations of total fish or specific groups of fish consumption with PlGF and sFlt-1 levels or the sFlt-1/PlGF ratio. These associations need further evaluation in studies including populations with a higher fish intake, and taking into account other components of fish intake, method of preparation and further dietary components to fully grasp the potential effects of maternal fish intake on placental angiogenesis.

Contribution to authorship

PKBN, JFF and OHF conceived the study. All authors (PKBN, MJT, SS-T, JS-G, AH, HT, VWJ, EAPS, JFF and OHF) were involved in planning and carrying out the study. PKBN and MJT analysed the data. PKBN, MJT and JFF wrote the initial drafts of the manuscript. All authors (PKBN, MJT, SS-T, JS-G, AH, HT, VWJ, EAPS, JFF and OHF) reviewed, edited and approved the final submitted version.

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Conflict of interest

All authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.placenta.2015.07.125.

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