Association between maternal seafood consumption before pregnancy and fetal growth: evidence for an association in overweight women. The "EDEN mother-child" cohort (study of pre and early postnatal determinants of the child's development and health)

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SUMMARY

Studies, in countries with high seafood consumption, suggested its benefit on fetal growth and child development. The objective of our study was to determine the association between seafood consumption in French pregnant women and fetal growth. Pregnant women included in the EDEN mother-child cohort study completed two food frequency questionnaires on their usual diet in the year before and during the last three months of pregnancy (n=1805). Fetal circumferences were measured by ultrasound, and anthropometry at birth. Variables were compared across tertiles of the mother's seafood consumption by multiple linear regressions adjusted for confounding variables. Analyses were stratified according to maternal overweight because of interaction ($p<0.01$). As results, there was no association between seafood intake and fetal growth in the whole sample of women. For overweight women (n=464), a higher consumption before pregnancy was associated with higher fetal biparietal and abdominal circumferences and anthropometric measures. From the lowest to the highest tertiles, mean birthweight was 167g higher ($p=0.002$). No significant association was found with consumption at the end of pregnancy. In conclusion, high seafood consumption before pregnancy is positively associated with fetal growth in overweight women. Follow-up of the infants may help determine potential beneficial consequences for the child's health and development.

Keywords: Epidemiology, pregnancy, seafood, birthweight, fetal growth, overweight.

Abbreviations

(p)BMI: (pre-pregnancy) Body Mass Index

WA: Weeks of Amenorrhea

FFQ(s): Food Frequency Questionnaire(s)

FA(s): Fatty Acid(s)

INTRODUCTION

A high intake of marine fat rich in long-chain n-3 Fatty Acids (FAs) is associated with an increase in birthweight in humans.[1] In both epidemiologic[2-7] and intervention studies,[8-10] mainly performed in women from Denmark and the Faroe Islands, intake of fish or marine n-3 FAs by pregnant women increased birthweight by both prolonging duration of pregnancy and increasing fetal growth rate. Marine fat has been shown to reduce the risk of pre-term delivery[11, 12] and intrauterine growth retardation.[13] Several mechanisms have been suggested for this association. The first one is a delayed spontaneous delivery, resulting from altered balance between the prostaglandins involved in the initiation of labour.[1] Indeed n-3 FAs may decrease the endogenous production of arachidonic acid-derived eicosanoids such as prostaglandins $PGF_{2\alpha}$ and PGE_2.[14] The second one is an increased fetal growth rate, resulting from improved placental blood flow due to lowered thromboxane/prostacyclin ratio[15] and blood viscosity.[5]

Beside birthweight, neonatal head circumference has been positively related to fish intake. In a fishing community, Thorsdottir et al.[16] found that newborns of women consuming less fish and fish oil had a smaller head circumference than those of women consuming higher levels.

Studies from other parts of the world (USA, …) did not find such association between fish consumption and birth size,[17] or even found an inverse relationship between seafood intake during pregnancy and fetal growth.[18] A randomised controlled trial,[19] including women with a low dietary fish intake, did not find any differences in spontaneous delivery between fish oil supplemented and control groups. Thus, the role of maternal seafood consumption on fetal growth remains controversial.

Several studies found relations between maternal body mass index (BMI) and fetal growth[20-22] and maternal BMI may be associated with difference in food intake. Nevertheless, studies

interested in the association between seafood and/or marine n-3 FA and fetal growth did not take into account potential influence of overweight in this relation.

In the context of the well established association between smaller size at birth and adult metabolic and cardiovascular risk, the investigation of nutritional factors potentially affecting fetal growth in humans is warranted.[23-26]

The aim of the present study was therefore to explore the relationship between seafood consumption before and during pregnancy and fetal growth in a French population, with a particular care to the potential effect of maternal overweight on this relation.

METHODS

Population and study design

Pregnant women seen for a prenatal visit at the departments of Obstetrics and Gynaecology of the University Hospitals of Nancy and Poitiers before 24 weeks of amenorrhea (WA) were invited to participate. Enrolment started in 2003 in February in Poitiers and September in Nancy, lasted 27 months in each center and ended up in the inclusion of 2002 women (969 in Poitiers, 1033 in Nancy). Exclusion criteria were twin pregnancies, known diabetes before pregnancy, not being able to speak and read French, planned moving away from the region. Among women who fulfilled these inclusion criteria, 55% agreed to participate.

The study was approved by the Ethic Committee of the Bicêtre Hospital. Written consents were obtained from the mother for herself at inclusion and for her newborn child after delivery.

Standard ultrasound fetal measurements were recorded from routine examinations performed between 20-24 and 30-34 WA. Measurements included head, biparietal and abdominal circumferences and femur length. All ultrasound examinations were performed by a limited number of specialists who standardised procedures before the study.

At a visit performed between 24-28 WA by midwives research assistants, maternal height was measured with a wall Seca 206 stadiometer (Hamburg, Germany) to the nearest 0.2cm and maternal weight was measured using electronic Terraillon SL 351 scales (Hanson Ltd, UK) to the nearest 0.1kg. Skinfolds were measured using a commercial Harpenden caliper (Chasmor Ltd, London, UK) three times in the following order: tricipital (posterior aspect of the arm, at midpoint between the acromion and the olecranon), bicipital (anterior aspect of the arm, at midpoint between the acromion and the olecranon), subscapular (1cm below the lower angle at the scapula) and supra-iliac (1cm over the iliac crest, at the midaxillary line). After a five minute rest, three measures of systolic and diastolic blood pressures were performed at two-minute intervals with an Omron M4I device (Omron Healthcare Europe, Hoofddorp, The Netherlands). Women came to the examination in a fasting state and received a 50g glucose load. Glucose concentrations were measured on fasting and one hour after challenge. Weight before pregnancy, educational level and smoking habits during pregnancy were obtained by interview. Prepregnancy body mass index (pBMI) was computed as reported weight (kg) / measured height squared (m^2). According to references of the International Obesity Task Force, overweight was defined as a BMI of 25kg/m^2 and above and obesity as a BMI of 30kg/m^2 and above.

A second visit was performed by the same research assistants 1.8 days (range 0-16) after delivery. Mother's weight and skinfolds were obtained with the same protocol as above. Several anthropometric measurements were performed on the newborn. Circumferences were measured to the nearest 0.1cm in duplicate using a tape: left arm circumference measured at

midpoint between the acromion and the olecranon; left wrist circumference measured at the level of the styloid processes of the radius and ulna; head circumference measured at the largest occipitofrontal circumference. Skinfolds were measured in triplicate using a commercial Holtain caliper (Chasmor Ltd, London, UK)) in the following order: tricipital skinfold measured at the same level as the midarm circumference; subscapular skinfold measured at the lower angle of the scapula - It has been shown that in neonates, the sum of skinfolds is well correlated with fat mass as measured by Dual-Energy X-Ray.[27]

Gestational age at delivery (determined from the date of the last menstrual period and early ultrasound assessment), birthweight, recumbent length and weight on the day of the post-partum examination, placental weight (in Poitiers only) were extracted from clinical record. In the two obstetric departments, electronic Seca scales (Hamburg, Germany: Seca 737 in Nancy and Seca 335 in Poitiers) were used to measure infant weight and wooden somatometer (Testut, Béthune, France) to measure length.

Large- and Small-for-gestational age neonates were defined as babies with a birthweight respectively over the 90th percentile and below the 10th percentile of French gestational age and gender specific reference curves.[28]

Dietary assessment

Mothers completed two food frequency questionnaires (FFQs) similar to the questionnaire developed for the French population in the Fleurbaix-Laventie Ville Santé Study.[29] This FFQ has been validated against a series of 24 hour recalls.[30] The questionnaire used in the EDEN Study is very close to that of the FLVS Study with the addition of some questions for a more specific assessment of the intake of fish and trophallergic foods, foods rich in folates, n-3 FAs

and vitamin A. It inquires about the intake of 137 different foods or food groups with a 7-item scale ranking from never to more than once a day.

The first-trimester FFQ (completed at recruitment) concerned the usual diet during the year before pregnancy; the second FFQ (completed in the first few days following delivery) investigated food intake during the last three months of pregnancy. Six questions inquired about seafood consumption. They were "At which frequency did you eat": 1) fresh or frozen fish (bought unprocessed); 2) oily fish; 3) smoked or salted fish; 4) breaded fish; 5) dishes containing fish; 6) shellfish. We combined responses to the six questions and generated an average frequency of seafood servings per month for each woman, by weighing each frequency with the midpoint of the category (i.e. 2 for the category 1-3 servings/month).

To calculate energy and nutrient intakes, we multiplied, for each food, the intake frequency by the nutrient composition for a portion size. Portion sizes were determined using pictures for 12 food types (meat, French fries, pasta, vegetables, cakes, cheese) on a three level scale or were standard portions for the French adult population for other food types.[31] We then summed contributions across all foods to obtain total intake of energy and various macro- and micronutrients. Food composition was obtained from the SU.VI.MAX. nutrient composition database[32] which is based on a French nutrient composition database[33] and on US department of Agriculture publications; it is continually incremented by other published sources and personal communications from laboratories and manufacturers.[34-36] Energy and nutrient intakes were not estimated when more than 3 items of the FFQ were missing. Moreover, these estimations were not taken into account when total energy intake was under 1000 kcal/day or over 5000 kcal/day.

To study the hypothesis that other food frequently eaten by seafood eaters, could confound the studied relationship, dietary patterns were identified in a principal component analysis performed on the 33 food groups created from the 137 items of the FFQ.

Variable description and statistical analyses

The mean frequency of monthly seafood servings was compared between centers by Student's t test. Relationships between socio-demographic characteristics of women and seafood consumption were studied by linear regressions adjusted for center and mother's age. To study relationships between seafood consumption and fetal growth, we divided women into approximate tertiles according to their average seafood consumption (tertile 1 $\leq$ once a week / tertile 3 > twice a week) (the number of women reporting no consumption of seafood was too small to be analysed separately. However, analyses were run with and without including these women). Fetal growth was characterized by anthropometric measures at birth and ultrasound measures. Relationships between seafood consumption and these variables were studied by multiple linear regressions adjusted for center, mother's age, smoking habits, height, parity, gestational age, newborn's sex; variables were compared across tertiles of the mother's seafood consumption. Moreover risks of large- and small-for-gestational age newborns were studied according to seafood intake by logistic regression. Separate analyses were performed for seafood intake before pregnancy and during the last three months of pregnancy. Interaction terms between seafood consumption, before and in the last three months of pregnancy and gestational age (p=0.08 and 0.26 respectively), maternal BMI before pregnancy (p=0.003 and 0.38 respectively), average number of cigarettes smoked per day during pregnancy (p=0.47 and 0.31 respectively) and maternal educational level (p=0.11 and 0.67 respectively) were estimated for birthweight. Several adjustments for educational level, maternal food consumptions (energy and lipid intakes, alcohol intake, dietary pattern, vitamins and/or minerals supplementation), and maternal health (systolic/diastolic arterial pressure, fasting plasma glucose and triglycerides) were also performed.

All analyses were performed with SAS version 9.1 (Cary, N.C., USA).

RESULTS

Subjects characteristics

Data at birth were available for 1896 women out of 2002 included in the study because mainly of women who decided to stop the study, women lost for follow-up and miscarriages or fetal deaths. Analyses included 1805 women who completed the two FFQs and for whom seafood consumption was assessed (67 not included because of at least one missing FFQ, 13 for missing data on seafood consumption). Women for whom time between delivery and clinical exam were spaced from more than 7 days were not included also (n=11). The main characteristics of included women (and their newborns), compared with the 91 excluded ones, are shown in Table 1**.** Mean maternal age was 29 years (range 17-45), whereas excluded women were slightly younger. This latter group had less often reached a university level and were more often single. Among included women, mean pBMI was 23kg/m², 10.1% had a pBMI < 18.5kg/m², 17.5% were overweight and 8.3% were obese.

Mean birthweight of the offspring of included women was 3284g. The average frequency of seafood intake before pregnancy was 8.5 times/month. The most frequently consumed fish was white fish (60%). A reduction in the seafood consumption was observed over time falling to 8.2 times/month during the last three months of pregnancy (p=0.09). This was mainly due to a decrease in the intake of shellfish.

Sociodemographic characteristics of women and seafood consumption before pregnancy

Seafood consumption was higher with age by 13% per decade (p=0.0003). Overweight women ate less seafood than the others (7.9 vs. 8.7 times/month; p=0.05). Women with a

university level ate seafood more often than the others (9.0 vs. 7.9 times/month; p=0.001). Income level was also associated with seafood intake, with a higher consumption for women whose household's income was higher than 3,000 Euros/month compared to women with lower income (9.4 vs. 8.1 times/month; p=0.003). Women living alone ate seafood more often than the others (9.6 vs. 8.4 times/month; p=0.07). Seafood consumption was not significantly lower in women who had smoked at some point of the pregnancy than in women who had never smoked during their pregnancy (p=0.40), but decreased when the number of cigarettes smoked increased (p=0.13). Seafood intake did not differ according to the season when the dietary assessment was made (p=0.58). Seafood and alcohol consumptions were positively associated (p=0.003). Women who were supplemented with vitamins and/or minerals ate seafood more often (9.0 vs. 8.3 times/month; p=0.07).

All these relationships were also observed for seafood consumption during the last three months of pregnancy, except for those with vitamins and mineral supplementation and for alcohol consumption.

Seafood consumption and fetal growth

There was no relationship between seafood intake and newborn anthropometric, as well as ultrasound measures in the whole sample of women (Table 2). However, maternal overweight before pregnancy modified the relationship between seafood consumption and several outcomes (for birthweight and others newborn anthropometric variables: p for interaction $\leq$ 0.01).

Mean birthweight was significantly greater with higher seafood consumption in overweight women whereas this relation was not observed in non-overweight women (Figure 1). Because

maternal overweight was identified as a factor of interaction, results are given separately for women with a pBMI < and ≥ 25kg/m².

In non-overweight women, fetal growth was not associated with seafood consumption prior to pregnancy (Table 3A, Table 4, Table 5); whereas in overweight women, fetal growth was significantly associated with seafood consumption prior to pregnancy. In a first regression model adjusted for confounding variables, seafood consumption was considered with tertiles. In overweight women, a difference in pre-pregnancy seafood consumption from less than five times to more than nine times/month was associated with on average an increase in birthweight of 5.1%, height of 1.4%, head circumference of 1.3%, arm circumference of 4.4% and wrist circumference of 3.2% (Table 3B). Moreover, the sum of skinfolds was also greater, a mean difference of 8.0% was observed between the two extreme tertiles. After adjustment for maternal educational level or household's income, results remained unchanged: mean birthweight between low and high tertiles of seafood intake was 171 (5.3%) after adjustment for educational level.

A subsequent adjustment for total energy, lipid or alcohol intakes did not change the measures of association (Data not shown). One dietary pattern identified by principal component analysis was characterized by a high intake of fruit, vegetables, whole grain, and seafood and a low intake of food rich in fats and sugar. Spearman correlation between seafood consumption and the score for this pattern derived from the principal component analysis was 0.31 (p<0.0001). This pattern was associated with newborn anthropometry with the same trend as for seafood consumption (data not shown). When the model was adjusted for the pattern score, seafood consumption remained associated with newborn anthropometry whereas the pattern score was no longer significant. Mean birthweight difference between low and high tertiles of seafood intake was 163g (4.8%).

After further adjustments for maternal vitamins and/or minerals supplementation and for systolic or diastolic arterial pressure, fasting plasma glucose and triglycerides (at 6th month of pregnancy) or number of days between birth and measurements for those not performed at birth these associations remained unchanged: when all these variables were included in the model, the average birthweight difference between the lowest and highest tertiles of seafood consumption was 206g (6.3%), compared to 167g in the model without these additional adjustments (data not shown).

For ultrasound measures (Table 3B), between the first and the third tertiles of seafood consumption, abdominal circumference was 2.2% larger and head circumference 1.2% larger at 20-24 WA. At 30-34 WA, these observations followed the same trend but were not significant. However, similar observation was made for the biparietal diameter with a difference of 1.3% between the two extreme tertiles. No other significant association was found.

Furthermore, risks for large-or small-for-gestational age neonates did not vary according to seafood intake in all women or in non-overweight women (Table 5). In overweight women, the risk for small-for-gestational age neonates increased with decreasing seafood intake (OR=3.23 between tertile 1 and tertile 3, *p=0.04*), while the risk of large-for-gestational age neonates decreased (OR=0.29, *p=0.009*).

Results remained unchanged when shellfish were not taken into account, but relationships were less strong. Mean birthweight difference between low and high tertiles was 157g (4.8%; *p*=0.001). Moreover, when women who reported never consuming seafood were excluded

from the analyses, results were unchanged: the increase in BW between the two extreme tertiles of seafood consumption was 170g (5.2%; p=0.002).

No association was found between seafood intake and placental weight or length of gestation (data not shown). No statistically significant associations were found in non-overweight women.

DISCUSSION

In the French "EDEN mother-child" cohort, higher consumption of seafood before pregnancy was associated with greater fetal growth, in overweight women only. This relation included birthweight, height, head, arm and wrist circumferences and sum of skinfolds, with a similar trend for some ultrasound measures. Several other epidemiologic studies have shown that intake of fish or marine n-3 FAs by pregnant women is associated with an increase in birthweight occurring through both an increase in duration of pregnancy and fetal growth rate.[7, 37, 38] An increase in head circumference was also noted by Odent et al.[39] and Thorsdottir et al..[16] Several previously published studies demonstrated a heavier placenta[5] and a longer gestation[11, 12] among women who consumed more fish but we did not observe these associations.

However in these studies mainly performed in population with high all fish and shellfish intake, the observation was not limited to overweight women. This may be due to higher seafood consumption than that observed in our study. In the intervention study by Olsen et al. the level of n-3 FA intake was 6.1g/day in the intervention group vs. 2.7g/day in the control group, whereas in our study estimated mean intake was 1.2g/day.

Another difference lies in the fact that our study focused on seafood whereas many of the studies were often interested in fish or fish oil intake only.[7, 37, 38] In our study, results were unchanged when shellfish were not taken into account, but relationships were less strong.

Results found in subgroups analyses must be taken with caution because they are more prone to false positive errors. No study reported before an interaction between seafood intake and mother's overweight status for fetal growth. One study found an association between seafood

intake and birthweight only in non-smokers.[5] The interaction with smoking was tested and found non significant in our study. .

There may be however some biological explanation for an association stronger with higher mother's fat mass. A regular intake of fish before pregnancy may be associated with variations in the FAs composition stored in the adipose tissue. Overweight women have an enhanced ability to release FAs from adipose tissue to sustain fetal growth. The assumption of a role of stored FAs in the relationship seafood consumption and fetal growth is reinforced by the fact that seafood consumption before pregnancy is more strongly associated with fetal growth than consumption during the last three months of pregnancy. The storage of long chain polyunsaturated FAs and the balance of the n-3 and n-6 families in maternal adipose tissue is of great importance since it is a reserve of these FAs for the developing foetus;[40-43] they are among the materials required for fetal brain, nervous system and retinal growth.[44] All of the n-6 and n-3 FAs accumulated by the foetus are derived by transfer across the placenta, which is provided with a specific system to ensure this function. Placental FAs transfer involves diffusion as well as membrane and cytosolic FA binding proteins; membrane binding proteins that favour n-6 and n-3 FAs over non-essential FAs and arachidonic and docosahexaenoic acid over linoleic and α-linolenic acid may be important in facilitating placental transfer of the latter longer chain n-6 and n-3 FAs to the fetus. The substrate of the placenta is provided by the maternal diet and the high rate of mobilization from maternal adipose stores.

Our study is not based on a representative sample which may limit the applicability of the results to whole population. Compared to a national perinatal survey performed in 2003 on a representative sample of French women after delivery,[45] the 55% of eligible women who agreed to participate in the EDEN study had similar age, proportion of unmarried couples,

birthweight of the offspring, prematurity rate but had a higher frequency of university level (53 vs 43 %).

In conclusion, our study shows a relationship between maternal seafood intake and fetal growth in the French population, which seems to be specific to overweight women. We suggest that the enrichment in long chain n-3 FAs in the maternal adipose tissue stored before conception is a possible mediator of this relationship.

ACKNOWLEDGMENTS

We are indebted to the participating families, to the midwife research assistants (L Douhaud, S Bedel, B Lortholary, S Gabriel, M Rogeon, M Malinbaum) for data collection and to P Lavoine for checking, coding and data entry.

We acknowledge all the funding sources for the EDEN study: Fondation pour la Recherche Médicale (FRM), French Ministry of Research: IFR program, INSERM Nutrition Research Program, French Ministry of Health Perinatality Program, French Agency for Environment Security (AFFSET), French National Institute for Population Health Surveillance (INVS), Paris–Sud University, French National Institute for Health Education (INPES), Nestlé, Mutuelle Générale de l'Education Nationale (MGEN), French speaking association for the study of diabetes and metabolism (Alfediam), National Agency for Research (ANR non thematic program)

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Table 1 Maternal and neonatal characteristics of the cohort (n=1896)

	Mean (SE) or %		
	Included (n=1805)	Not included (n=91)	p (t test)
Mothers			
Age (years)	29.1 (4.9)	27.0 (5.0)	<0.0001
Height (m)	1.64 (0.06)	1.63 (0.06)	0.15
Prepregnant BMI (kg/m²)	23.2 (4.5)	23.6 (5.5)	0.42
Overweight (%)	26.2%	27.6%	0.78
Centre (% Poitiers)	49.4%	45.1%	0.42
University Level	53%	42%	0.03
Income >3000€/month (%)	27.4%	19.8%	0.11
Multipara	55%	55%	0.97
Single	7%	14%	0.02
Smoking during pregnancy	26%	32%	0.21
Intakes			
Seafood[(1)] (times/month)	8.5 (7.3)		
Seafood[(2)] (times/month)	8.2 (7.4)		
Total energy intake[(1)] (kcal/d) *	2303.6 (769.5)		
Total energy intake[(2)] (kcal/d) *	2376.5 (778.1)		
Total lipid intake[(1)] (g/d) *	97.7 (39.8)		
Total lipid intake[(2)] (g/d) *	104.1 (41.0)		
Alcohol intake[(1)] (g/d) *	7.6 (15.2)		
Alcohol intake[(2)] (g/d) *	1.4 (4.1)		
Newborn			
Males (%)	52.6%	51.1%	0.79
Gestational length (weeks)	39.3 (1.7)	38.8 (2.3)	0.03
Birthweight (g) *	3284.0 (507.6)	3167.0 (602.6)	0.04
Birth length (cm)	49.6 (2.3)	49.1 (2.7)	0.06
Placental weight (g) †	546.7 (122.0)	540.7 (151.6)	0.77

[(1)] Intakes before pregnancy, [(2)] Intakes in the last three months of pregnancy

** Only for women whom nutrient intakes has been estimated (n=1601)*

† only in Poitiers (n=832/39)

Table 2 Newborn anthropometric and ultrasound measures at 20-24 and 30-34 WA according to average seafood intake per month before pregnancy in the EDEN Study (n=1805) *

Seafood tertile †	Birthweight (g)				Birth height (cm)				Head circumference (cm)				Arm circumference (cm)				Wrist circumference (cm)				Sum of skinfolds (mm)			
	n=1802	Adjusted mean*	SE‡	p	n=1755	mean	SE	p	n=1712	mean	SE	p	n=1709	mean	SE	p	n=1707	mean	SE	p	n=1704	mean	SE	p
1	563	3269.70	16.53	0.56	555	49.54	0.08	0.54	545	34.53	0.05	0.30	545	10.36	0.04	0.56	543	7.89	0.02	0.32	542	8.67	0.07	0.49
2	642	3292.43	15.40		626	49.51	0.07		606	34.62	0.04		603	10.40	0.04		604	7.92	0.02		602	8.79	0.07	
3	597	3289.95	16.04		574	49.62	0.08		561	34.56	0.05		561	10.41	0.04		560	7.93	0.02		560	8.71	0.07	

Seafood tertile †		Biparietal diameter (mm)				Head circumference (mm)				Abdominal circumference (mm)				Femoral length (mm)			
			mean	SE	p		mean	SE	p		mean	SE	p		mean	SE	p
	20-24 WA	n=1780				n=1719				n=1729				n=1770			
1		555	54.66	0.12	0.79	528	199.37	0.50	0.40	532	178.19	0.45	0.09	550	38.89	0.09	0.56
2		637	54.75	0.11		614	200.30	0.46		616	178.65	0.41		634	38.77	0.08	
3		588	54.65	0.11		577	199.91	0.47		581	179.53	0.43		586	38.87	0.09	
	30-34 WA	n=1743				n=1706				n=1710				n=1733			
1		548	82.69	0.16	0.50	533	298.12	0.64	0.56	532	283.79	0.67	0.38	544	62.07	0.11	0.17
2		621	82.84	0.15		607	298.31	0.60		614	282.70	0.62		618	61.81	0.10	
3		574	82.95	0.15		566	297.42	0.62		564	283.79	0.65		571	62.04	0.11	

*** Model: adjusted for center, mother's age and height, smoking habits, parity, gestational age (at birth or at ultrasound assessment) and newborn's sex**

† Tertile 1: [0 - 5[times a month / Tertile 2: [5 - 9[times a month / Tertile 3: > 9 times a month

‡ Standard Error

Table 3A Newborn anthropometric and ultrasound measures at 20-24 and 30-34 WA according to average seafood intake per month before pregnancy in non-overweight women in the EDEN Study (n=1306) *

Seafood tertile †	Birthweight (g)				Birth height (cm)				Head circumference (cm)				Arm circumference (cm)				Wrist circumference (cm)				Sum of skinfolds (mm)			
	n=1304	mean	SE‡	p	n=1271	mean	SE	p	n=1243	mean	SE	p	n=1240	mean	SE	p	n=1239	Mean	SE	p	n=1238	mean	SE	p
1	380	3286.20	19.28	0.37	375	49.58	0.09	0.27	366	34.57	0.05	0.09	366	10.40	0.05	0.35	365	7.91	0.03	0.56	364	8.74	0.08	0.16
2	481	3266.32	17.08		468	49.39	0.08		455	34.57	0.05		452	10.35	0.04		453	7.92	0.02		453	8.64	0.07	
3	443	3248.67	17.94		428	49.42	0.09		422	34.43	0.05		422	10.30	0.04		421	7.88	0.03		421	8.52	0.08	

Seafood tertile †		Biparietal diameter (mm)				Head circumference (mm)				Abdominal circumference (mm)				Femoral length (mm)			
			mean	SE	p		mean	SE	p		mean	SE	p		mean	SE	p
	20-24 WA	n=1289				n=1246				n=1250				n=1285			
1		376	54.84	0.14	0.67	358	199.64	0.62	0.81	357	178.33	0.53	0.91	371	38.91	0.11	0.24
2		477	54.73	0.13		460	199.98	0.55		463	178.52	0.47		476	38.67	0.09	
3		436	54.67	0.13		428	199.48	0.57		430	178.65	0.49		438	38.76	0.10	
	30-34 WA	n=1265				n=1234				n=1237				n=1256			
1		368	82.94	0.18	0.85	355	298.50	0.75	0.35	354	283.58	0.80	0.18	364	62.07	0.13	0.15
2		469	82.81	0.16		457	298.15	0.66		463	281.61	0.70		467	61.73	0.11	
3		428	82.84	0.16		422	297.09	0.69		420	282.43	0.74		425	61.85	0.12	

*** Model: adjusted for center, mother's age and height, smoking habits, parity, gestational age (at birth or at ultrasound assessment) and newborn's sex**

† Tertile 1: [0 - 5[times a month / Tertile 2: [5 - 9[times a month / Tertile 3: > 9 times a month

‡ Standard Error

Table 3B Newborn anthropometric and ultrasound measures at 20-24 and 30-34 WA according to average seafood intake per month before pregnancy in overweight women in the EDEN Study (n=464) *

Seafood tertile †	Birthweight (g)				Birth height (cm)				Head circumference (cm)				Arm circumference (cm)				Wrist circumference (cm)				Sum of skinfolds (mm)			
	n=464	mean	SE‡	p	n=464	mean	SE	p	n=464	mean	SE	p	n=464	mean	SE	p	n=464	mean	SE	p	n=464	mean	SE	p
1	173	3250.20	31.87	0.002	170	49.53	0.15	0.009	169	34.51	0.09	0.004	169	10.30	0.07	0.0001	168	7.84	0.04	0.0002	168	8.62	0.13	0.001
2	149	3359.40	34.29		147	49.82	0.16		140	34.74	0.10		140	10.51	0.08		140	7.91	0.04		138	9.18	0.15	
3	142	3416.74	34.92		135	50.22	0.17		129	34.95	0.10		129	10.75	0.08		129	8.09	0.05		129	9.31	0.15	

Seafood tertile †		Biparietal diameter (mm)				Head circumference (mm)				Abdominal circumference (mm)				Femoral length (mm)			
			mean	SE	p		mean	SE	p		mean	SE	p		mean	SE	p
	20-24 WA	n=457				n=439				n=446				n=452			
1		169	54.23	0.21	0.17	160	198.93	0.77	0.06	165	178.07	0.84	0.004	170	38.90	0.16	0.44
2		148	54.81	0.22		142	201.20	0.81		142	178.98	0.90		146	39.04	0.17	
3		140	54.60	0.23		137	201.25	0.83		139	182.10	0.90		136	39.21	0.18	
	30-34 WA	n=444				n=444				n=444				n=444			
1		169	82.14	0.33	0.06	167	297.68	1.24	0.89	167	284.95	1.24	0.23	169	62.14	0.20	0.17
2		140	83.02	0.36		139	298.46	1.35		140	285.53	1.34		139	62.05	0.22	
3		135	83.24	0.36		133	298.00	1.37		133	288.02	1.37		135	62.59	0.22	

*** Model: adjusted for center, mother's age and height, smoking habits, parity, gestational age (at birth or at ultrasound assessment) and newborn's sex**

† Tertile 1: [0 - 5[times a month / Tertile 2: [5 - 9[times a month / Tertile 3: > 9 times a month

‡ Standard Error

Table 4 Mean birthweight according to average seafood intake per month before pregnancy separately in non-overweight and overweight women in the EDEN Study (n=1770)*

		BMI < 25 kg/m² (n=1306)			BMI > 25 kg/m² (n=464)		
		β†	95% CIs	Global p-value	β†	95% CIs	Global p-value
Seafood tertile †	**1**	37.53	[-14.51; 89.57]	0.37	-166.54	[-260.15; -72.93]	0.002
	2	17.66	[-31.03; 66.35]		-57.34	[-153.42; 38.74]	
	3	0			0		
Centre (Nancy/Poitiers)		-45.54	[-87.07; -4.01]	0.03	18,14	[-58.71; 94.99]	0.64
Mother's age (years)		-3.70	[-8.48; 1.08]	0.13	7.91	[-0,75; 16.57]	0.07
Mother's height (m)		13.81	[10.34; 17.28]	<0.0001	9.66	[3.90; 15.42]	0.001
Smoking habits (n)		-20.56	[-26.73; -14.39]	<0.0001	-8.08	[-18.62; 2.46]	0.13
Parity (n)		63.27	[38.52; 88.02]	<0.0001	52.64	[13.01; 92.27]	0.01
Gestational age (WG)		180.57	[168.38; 192.76]	<0.0001	167.56	[146.59; 188.53]	<0.0001
Newborn's sex (Female vs. Male)		-127.19	[-168.02; -86.36]	<0.0001	-161.56	[-237.61; -85.51]	<0.0001

*** Adjusted for center, mother's age and height, smoking habits (average number of cigarettes smoked during pregnancy), parity (number of previous pregnancies), gestational age (weeks of gestation) and newborn's sex**

† **Tertile 1: [0 - 5[times a month / Tertile 2: [5 - 9[times a month / Tertile 3: ≥ 9 times a month**

Table 5 Seafood intake before pregnancy in relation to risks of Large- and Small-for-Gestational Age neonates (LGA and SGA) in the EDEN Study (n=1731)

		Large for gestational age				Small for gestational age			
		% LGA	OR*	95% CI	Global p-value	%SGA	OR*	95% CI	Global p-value
All women (n=1731)									
Seafood intake	**Tertile 1**	6.5%	0.74	(0.46,1.17)	0.27	8.5%	0.99	(0.64,1.55)	0.92
	Tertile 2	9.3%	1.06	(0.70,1.59)		7.2%	0.92	(0.59,1.43)	
	Tertile 3	8.9%	1			7.8%	1		
BMI<25kg/m² (n=1280)									
Seafood intake	**Tertile 1**	6.7%	1.35	(0.75,2.45)	0.40	7.8%	0.71	(0.42,1.20)	0.44
	Tertile 2	8.0%	1.44	(0.84,2.47)		8.1%	0.87	(0.54,1.40)	
	Tertile 3	5.5%	1			9.2%	1		
BMI>25kg/m² (n=451)									
Seafood intake	**Tertile 1**	6.0%	0.29	(0.13,9.32)	0.009	10.1%	3.23	(1.12,9.32)	0.04
	Tertile 2	13.3%	0.63	(0.33,1.23)		4.2%	1.28	(0.37,4.43)	
	Tertile 3	19.3%	1			3.6%	1		

*** Odds Ratios adjusted for center, mother's age and height, smoking habits, parity, gestational age and newborn's sex**

Figure 1 Mean birthweight and average seafood consumption per week, according to maternal BMI. The EDEN study.

