



Corrigendum

Corrigendum to 'Plasma concentrations of long chain N-3 fatty acids in early and mid-pregnancy and risk of early preterm birth'



Olsen S.F.^{a,b,*}, Halldorsson T.I.^{a,c}, Thorne-Lyman A.L.^{b,d}, Strøm M.^{a,e}, Gøtz S.^a, Granstrøm C.^a, Nielsen P.H.^a, Wohlfahrt J.^a, Lykke J.A.^f, Langhoff-Roos J.^f, Cohen A.S.^g, Furtado J.D.^b, Giovannucci E.L.^{b,h}, Zhou W.^{i,j}

^a Centre for Fetal Programming, Department of Epidemiology Research, Statens Serum Institut, Copenhagen, Denmark

^b Department of Nutrition, Harvard TH Chan School of Public Health, Boston, USA

^c Faculty of Food Science and Nutrition, University of Iceland, Reykjavik, Iceland

^d Center for Human Nutrition, Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, USA

^e Faculty of Natural and Health Sciences, University of the Faroe Islands, Torshavn, Faroe Islands

^f Department of Obstetrics, Juliane Marie Centre, Rigshospitalet, University of Copenhagen, Denmark

^g Department of Congenital Diseases, Statens Serum Institut, Copenhagen, Denmark

^h Department of Epidemiology, Harvard TH Chan School of Public Health, Boston, USA

ⁱ Key Lab. of Reproduction Regulation of NPFPC, SIPPR, IRD, Fudan University, Shanghai, China

^j School of Public Health, Fudan University, Shanghai, China

In connection with a new ongoing project, it recently came to the knowledge of the author group that a substantial proportion of the plasma samples used in our study had already been thawed prior to the conduct of our study. Prior thawing of these samples was done in connection with an earlier unrelated study conducted by other researchers. This new knowledge prompted an examination of whether, and how, this previous thawing of part of the samples in our study might impact the results and conclusions that we had reported in the article.

As can be seen in (the new) Corrigendum Table 1, for the first trimester samples the proportion that had not been thawed was 89% (297/333) in the control group, whereas in the case group only 21% (77/367) had not been thawed before; for the second trimester samples the difference between controls and cases was even more pronounced, with 95% (326/342) of control samples and 16% (60/366) of case samples not having been thawed. As can be seen in the columns to the right in this table, plasma concentrations of EPA+DHA were substantially lower in samples that had been thawed. This applied to both first and second trimester samples, and patterns were similar for cases and controls.

Accounting for thawing by adjustment in a multivariable model

In Corrigendum Table 2, Panels A-i and B-i, we report associations between plasma fatty acids and early preterm risk using logistic regression to adjust for thawing and storing temperatures (see section 3.3 in original article). As we had reported in Table 2 Panel A of the original paper, for the 1st sample, before adjustment for thawing,

women in the lowest quintile (Q1) appeared to have 5.54 times (95% confidence interval 3.86 to 8.02 to 15.79, $p < 0.0001$) increased odds of early preterm birth, whereas women in the second to the lowest quintile (Q2) appeared to have 2.05 (95% CI 1.36 to 3.09, $p < 0.0006$) times increased odds, compared to women in the aggregated highest three quintiles (Q3+Q4+Q5). In this new analysis, after adjustment for differential thawing and storing temperatures, the odds ratio estimates for the lowest quintile for the 1st sample attenuated from 5.54 to 1.29 times (95% confidence interval 0.73 to 2.23, $p = 0.38$) and from 2.05 to 0.84 (95% CI 0.47 to 1.49, $p = 0.57$), respectively, meaning that the observed associations for the 1st sample disappeared after this adjustment (Corrigendum Table 2, Panels A-i). For the 2nd sample (Panel B-i), before any adjustment, women in the lowest quintile appeared to have 9.33 times (95% confidence interval 6.42 to 13.74, $p < 0.0001$) increased odds of early preterm birth, whereas women in the second to the lowest quintile appeared to have 2.73 (95% CI 1.78 to 4.20, $p < 0.0001$) times increased odds, compared to women in the aggregated highest three quintiles. After the additional adjustment, however, the odds ratio estimates were attenuated from 9.33 to 2.13 times (95% confidence interval 1.18 to 3.79, $p = 0.01$) and from 2.73 to 1.12 (95% CI 0.57 to 2.14, $p = 0.73$), respectively. In the article, we had also reported on results obtained from analyses where the EPA+DHA measurements had been based on the mean of the 1st and 2nd sample. In these analyses, after co-variate adjustment, women in Q1 appeared to have a 10.27 times (95% confidence interval 6.80 to 15.79, $p < 0.0001$) increased risk, whereas women in Q2 appeared to have a 2.86 (95% CI 1.79 to 4.59, $p < 0.0001$) times increased risk of early preterm birth, compared to women in Q3+Q4+Q5; these results were reported in the abstract. However, when further adjustment is made for thawing and freezer temperatures, these two estimates changed to 1.42 (0.65 to 3.03, $p = 0.4$) and 1.11 (0.50 to 2.41, $p = 0.8$), respectively.

DOI of original article: <http://dx.doi.org/10.1016/j.ebiom.2018.07.009>.

* Corresponding author at: Centre for Fetal Programming, Department of Epidemiology Research, Statens Serum Institut, Copenhagen, Denmark.

E-mail address: sfo@ssi.dk (O. S.F.).

<https://doi.org/10.1016/j.ebiom.2019.102619>

2352-3964/© 2019 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license. (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

Corrigendum Table 1

Sample treatment according of case and control status and in relation to EPA+DHA plasma concentrations.

Prior thawings	Controls (n = 333)		Preterm cases (n = 367)		Mean of EPA+DHA%			
	n	%	n	%	Controls		Preterm cases	
					Mean	SEM	Mean	SEM
Number of thawings, sample 1								
0	297	89	77	21	2.05	0.03	1.91	0.07
1	34	10	163	44	1.80	0.07	1.42	0.03
2+	2	1	127	35	1.22	0.18	1.32	0.03
Number of thawings, sample 2								
0	326	95	60	16	2.04	0.03	1.79	0.09
1	15	4	243	66	1.72	0.17	1.45	0.03
2+	1	0.3	63	17	1.69	–	1.39	0.07

Corrigendum Table 2Association between EPA+DHA concentrations and risk of early preterm birth. Shown are results for EPA+DHA measurements based on all 1st trimester samples (Panel A-i), based on 1st trimester samples but including only those samples that had never been thawed (Panel A-ii), based on all 2nd trimester samples (Panel B-i), and based on 2nd trimester samples but including only those samples that had never been thawed (Panel B-ii).

	Controls	Cases	Crude		Adjusted for thawing and temperature ¹		Controls	Cases	Further adjusted for covariates ²	
			OR (95% CI)	P-Value	OR (95% CI)	P-Value			OR (95% CI)	P-Value
A-i: 1st trimester samples										
All included (unselected)										
Test for association in spline regression	< 0.0001	..	0.90	0.98	
Test for linear v. spline association	0.0002	..	0.78	0.92	
EPA + DHA categorized into quintiles	< 0.0001	..	0.41	0.30	
Q1: 0.37 – 1.48	67	192	5.54 (3.86; 8.02)	< 0.0001	1.29 (0.73, 2.23)	0.38	67	192	1.24 (0.68, 2.25)	0.48
Q2: 1.48 – 1.81	67	71	2.05 (1.36; 3.09)	0.0006	0.84 (0.47, 1.49)	0.57	67	71	0.74 (0.40, 1.36)	0.34
Q3+4+5: 1.81 – 4.74	199	103	1	..	1	..	199	103	1	1
A-ii: 1st trimester samples										
Analysis restricted to never thawed										
Test for association in spline regression	0.36	..	0.69	
Test for linear v. spline association	0.56	..	0.53	
EPA + DHA categorized into quintiles ³	0.26	..	0.88	
Q1: 0.37 – 1.48	53	9	0.57 (0.25; 1.18)	0.13	0.87 (0.37, 1.91)	0.74	
Q2: 1.48 – 1.81	56	12	0.72 (0.35; 1.40)	0.34	0.85 (0.40, 1.72)	0.66	
Q3+4+5: 1.81 – 4.74	188	56	1	..	1	
B-i: 2nd trimester samples										
All included (unselected)										
Test for association in spline regression	< 0.0001	..	0.026	0.10	
Test for linear v. spline association	< 0.0001	..	0.033	0.069	
EPA + DHA categorized into quintiles	< 0.0001	..	0.035	0.064	
Q1: 0.47 – 1.42	68	226	9.33 (6.42; 13.74)	< 0.0001	2.13 (1.18, 3.79)	0.01	68	226	1.92 (1.02, 3.60)	0.045
Q2: 1.43 – 1.74	69	67	2.73 (1.78; 4.20)	< 0.0001	1.12 (0.57, 2.14)	0.73	69	67	0.90(0.45, 1.79)	0.78
Q3+4+5: 1.74 – 4.95	205	73	1	..	1	..	205	73	1	..
B-ii: 2nd trimester samples										
Analysis restricted to never thawed										
Test for association in spline regression	0.10	..	0.36	
Test for linear v. spline association	0.073	..	0.23	
EPA + DHA categorized into quintiles ³	0.18	..	0.16	
Q1: 0.47 – 1.42	61	17	1.87 (0.95; 3.58)	0.070	1.95 (0.98, 3.78)	0.056	
Q2: 1.43 – 1.74	64	13	1.36 (0.65; 2.72)	0.40	1.23 (0.58, 2.50)	0.58	
Q3+4+5: 1.74 – 4.95	201	30	1	..	1	

¹ Analyses were adjusted for differences in thawings (0, 1, 2 times) and temperatures (-20, -80, -196°C); for the analyses restricted to samples that had never been thawed (Panels A-ii and B-ii), analyses were adjusted for temperatures.

² Further adjusted for covariates: Maternal age, maternal height, parity, maternal pre-pregnancy BMI, SES, cohabitation, residence and smoking; coded as in the original article. For the analyses restricted to samples that had never been thawed (Panels A-ii and B-ii), entering all the covariates simultaneously into the model was not possible due lack of convergence; however, taking the covariates in one by one was possible, and this had only limited effect on the estimates of association (data not shown but available from the authors).

³ Cut points for defining quintiles of the samples that had never been thawed (Panel A-ii), are based on the 'unselected' controls (Panel A-i).

Accounting for thawing by means of restriction

Limiting the analysis to only comprise plasma samples that had not been thawed prior to our study reduced numbers available for analysis, particularly in the case groups. Restriction resulted in the following estimates (adjusted for temperature differences): For the 1st sample (Corrigendum Table 2, Panel A-ii), the odds ratio for

early preterm birth in the lowest quintile v. the aggregated highest three quintiles was 0.87 (95% CI 0.37 to 1.91, $p = 0.7$), whereas the odds ratio for early preterm birth in the second to the lowest quintile v. the aggregated highest three quintiles was 0.85 (95% CI 0.40 to 1.72, $p = 0.7$). For the 2nd sample (Panel B-ii), the corresponding figures were 1.95 (95% CI 0.98 to 3.78, $p = 0.06$) and 1.23 (95% CI 0.58 to 2.50, $p = 0.6$), respectively.

Conclusions

Clearly, accounting for thawing substantially affected all estimates. Notably, all associations for the early sample were attenuated to include the null, whereas an association – albeit severely attenuated compared to the initially reported association – seemed to remain for the mid-pregnancy sample with a doubling of the risk in the lowest quintile compared to the aggregated highest three quintiles. These patterns are still compatible with a preventive effect of EPA+DHA against preterm birth in women with a low intake, and with the possibility that the time

dynamic of this relationship may be a fast one as seen in a randomized controlled trial [1] (see Figures 1 and 2 and discussion on page 1948).

We recommend that the study be replicated based on other materials.

Reference

- [1] Olsen SF, Halldorsson TI, Li M, Strøm M, Mao Y, Che Y, Wang Y, Duan F, Olsen J, Zhou W. Examining the effect of fish oil supplementation in chinese pregnant women on gestation duration and risk of preterm delivery. *J Nutr* 2019 pii: nxz153. doi: [10.1093/jn/nxz153](https://doi.org/10.1093/jn/nxz153).