Supplemental Materials

Summary of Table S1 and S2: Issues with the Prior Omega-3 PTB Trials

Given the lack of baseline intake information and the complexities of omega-3 biochemistry and perinatal physiology, it is not surprising that many of the previous Omega3-PTB trials have been equivocal. In fact, many of the issues are only identifiable in hindsight and the limitations were not evident at the time of publication. Some of the key issues from these studies include: 1) use of a study population that had high omega-3 intakes at baseline (lack of an unexposed control group), 2) use of an oil supplement control that contained EPA/DHA precursors such as ALA, again resulting in the lack of an unexposed control group, 3) borderline failure of randomization with respect to a critical perinatal health factor (e.g. maternal smoking), 4) evaluating the effect of omega-3 prophylaxis only as an adjuvant in the presence of a pharmacological therapy (in studies of recurrent PTB this design may be necessary with respect to equipoise, but it does preclude the assessment an independent effect; these adjuvant trials should not be "averaged" with those that assess an independent effect), 5) reporting a strong but non-significant trend with PTB as evidence of no association/no effect (this is particularly problematic when the observed trend is corroborated by significant associations with continuous gestational length and a secondary PTB threshold [e.g.<34weeks]), and 6) reporting the results of study with high non-compliance as evidence of no association/no effect. Details are provided in Table S4 and Table S5 below. When these critical issues are considered, only a few of the studies from these two meta-analyses provide interpretable evidence, and the results of these studies are all consistent with the interpretation that increasing omega-3 intake can extend gestation. Thus, future trials that account for the issues discussed above should be able to characterize the utility of omega-3 interventions in different settings.

Table S1. Omega-3 Supplementation Trials for PTB Prevention from a Recent Meta-analysis [1]

Study	Study Location: Country and its baseline omega-3 PUFA intake level in 2010 [2, 3]	Description	Results and Interpretation	Evidence for the importance of omega-3 levels in PTB prevention
Olsen et al 1992 [4]	Denmark 1272.6 mg/day	533 pregnant women at week 30 of gestation were randomly assigned to fish oil (about 1280 mg EPA and 920mg DHA), olive oil, or no supplement.	This moderate size study in a high baseline intake country provides evidence for the importance of omega-3 in preventing PTB. Gestational length was increased in the fish oil group: vs. olive oil: +4.0 days (95%CI: 1.5, 6.4) vs. no oil: +1.6 days (95%CI: -0.8, 4.0). The association was larger when looking among those with low fish intake at baseline: fish oil vs. olive oil: 7.2 (95%CI: 2.2, 12.6), fish oil vs. no oil: 4.0 (95%CI: -0.9, 8.7) PTB rates were very low but the trend was consistent: fish oil: 9/266 (3.4%), no oil: 6/131 (4.6%), olive oil: 9/136 (6.6%); p for difference = 0.3 Interestingly, the linoleic acid in olive oil may reduce endogenous EPA/DHA synthesis from ALA [5, 6], and thus those who took olive oil may have had the lowest EPA/DHA levels. However, olive oil also contains ALA, and it is not an ideal control (it may simultaneously provide precursors and impair their conversion to EPA/DHA). Thus, the exact magnitude of the exposure difference between treatment and control is unclear but a significant association was observed.	Supportive
Bulstra-Ramakers et al 1994 [7]	Netherlands 310.2 mg/day	63 women with a history of IUGR in their previous pregnancy were randomly assigned to receive EPA (about 3g daily with an unknown amount of DHA) or placebo (coconut oil), starting at 12-14 weeks gestation.	This small study in high risk pregnancies cannot provide strong evidence either way about the importance of omega-3 in PTB prevention. The authors reported a pattern consistent with lower risk of PTB among those assigned to EPA. PTB rates: 8/32 (25.0%) in the EPA group, 10/31 (32.3%) in the placebo group. However, even with a high rate of PTB in both groups there were very few cases to analyze.	Unclear, slightly supportive
Onwude et al 1995 [8]	United Kingdom (England) 801.2 mg/day	An unclear number of women (160-233?) with a history of problems in prior pregnancies (i.e. delivered a small infant [<3rd%ile], had PIH, or had an unexplained still birth) were randomly assigned to take omega-3 capsules (about 1620 mg EPA and 1080 mg DHA daily) or air filled capsules starting at 19-26 weeks gestation.	This study among high risk pregnancies has very significant limitations for interpretation. The mean gestational length was slightly longer in the omega-3 group: 269 days vs. 268 days but the difference was not significant. In contrast the PTB rate (as presented) was slightly higher in the omega-3 group: 22/113 (19.5%) vs. 19/119 (16.0%). The exclusion criteria in the methods suggests that only 160 women were randomized for treatment, but the tables suggest that 232 (113+119) women were analyzed so it is not clear if the denominators used here are valid for determining PTB rates. Importantly, randomization nearly failed with respect to smoking (more smokers in the EPA group, p=0.08), and they had a large problem with noncompliance: more than 50% of participants in both groups failed to take at least 70% of their assigned pills. Additionally this study was conducted in a high omega-3 intake country so even those in the placebo group may have had moderate to high omega 3 intake from their diets.	Unclear
Malcolm et al 2003 [9]	United Kingdom (Scotland) 801.2 mg/day	100 pregnant women were randomized to receive either fish oil (200 mg DHA daily) or sunflower oil starting at 15 weeks gestation. After dropouts 63 women remained at delivery.	This is a small study that has very limited potential to provide evidence on this issue. There was only one case of PTB in the entire study. Mean gestational lengths were roughly similar. Fish oil: 279.7 days (standard deviation: 9.5 days), sunflower oil: 279.6 days (standard deviation: 8.5 days), and the PTB rates were similar and very low. Fish oil: 0/31 (0.0%), Sunflower oil: 1/32 (3.1%). Importantly, The United Kingdom is a high omega-3 intake country, and supplementation would not be expected to provide an advantage in this population even if omega-3 is crucial for reducing the risk of PTB (the participants, including controls, may have had high omega-3 intake at baseline).	Unclear

Tofail et al 2006 [10]	Bangladesh 136.0 mg/day	400 pregnant women were randomized to receive either fish oil (1800mg EPA and 1200 mg DHA daily) or soy oil starting at 25 weeks gestation. Characteristics such as preterm birth were presented for the 249 that remained, after dropouts, for assessment of their main outcome (neurodevelopmental status at 10 months).	This is a moderate size study conducted in a low omega-3 intake country that could have been more informative if the authors had used a different control supplement. The PTB rates were high and similar between the groups: Fish oil: 30/125 (24.0%), Soy oil: 27/124 (21.8%); p for difference =0.4 There was no significant difference in PTB rates but the reference group is problematic because they received soy oil, which is a source of ALA. ALA is a precursor for EPA and DHA. Thus both the control and intervention groups received omega 3 supplementation.	Unclear
Makrides et al 2010 [11]	5 centers in: Australia 463.0 mg/day	2399 women at 5 centers in Australia were randomized to receive fish oil (800 mg DHA plus 100 mg EPA daily) or control oil (a mix of rapeseed, sunflower, and palm oil) starting at 21 weeks gestation.	This is a large study conducted in an intermediate omega-3 intake country that could have been more informative if the authors had used a different control supplement. They observed a non-significant decrease in PTB rate in the fish oil group: fish oil 67/1197 (5.6%), control oil 88/1202 (7.3%) p=0.09, and the median gestational length was slightly longer in the fish oil group: 282 days (IQR: 275,288) vs. 281 days (IQR:275,287) p=0.05. Additionally, the fish oil group demonstrated a significant increase in post-term inductions/cesareans (p=0.01) and a significant decrease in extreme preterm births (<34 weeks; p=0.03). The fish oil group infants also had significantly higher birth weights (p=0.003) and were less likely be admitted to the NICU in the first 18 months (p=0.04). These results are consistent with the interpretation that EPA and DHA are needed to maintain gestation and yield healthy infants. Unfortunately, rapeseed oil is a component of the placebo and it contains ALA (LC omega3 PUFA precursor). Thus, this control oil may have diminished the magnitude and significance of the associations, since it also contained omega-3s.	Supportive
Escolano-Margit et al 2011 [12]	Germany 507.6 mg/day Spain 821.6 mg/day Hungary 186.2 mg/day	315 women were randomized 4 treatment groups starting at 20 weeks gestation. Initially there were 77 in the fish oil group and 80 in the placebo group. [13] The PTB data is presented in a follow-up publication that evaluated the infants at age 4, and with dropouts, there were 43 in the fish oil group (500 mg DHA 150 mg EPA daily) and 47 in the placebo group)[12].	This small study has several limitations that preclude it from providing strong evidence about the importance of omega-3 intake in PTB prevention. No direct statistical comparisons were made between the 2 groups but the mean gestational lengths were 38.9 weeks (standard deviation: 1.5) in the fish oil group and 39.0 weeks (standard deviation: 1.5) in the placebo group. There were only 7 cases of PTB between the 2 groups: 4/43 (9.3%) in the fish oil group and 3/47 (6.4%) in the placebo group. In this study PTB was defined as <35 weeks gestation which is not consistent with the other studies (<37 weeks gestation). Importantly, 84% of women in the study were already getting 200 mg DHA daily at the start of the study (week 20 of gestation). It is hard to interpret these results because the effect of supplementation among those with low baseline levels cannot be inferred by studying the effect of supplementation among those with high baseline levels. Another key point is that it is not clear what the placebo group received in terms of specific fatty acids. The placebo is not fully described but the authors note that it did not contain EPA or DHA. They do not mention if it contained EPA or DHA precursors such as ALA. [13] If the placebo contained EPA or DHA precursors then the results are not interpretable because the control group also received omega-3 supplementation.	Unclear

Table S2. Omega-3 Supplementation Trials for Recurrent PTB Prevention from a Recent Meta-analysis [14]

Study	Study Location: Country and its baseline omega-3 PUFA intake level in 2010 [2, 3]	Description	Results and Interpretation	Evidence for the importance of omega-3 levels in PTB prevention
Olsen et al 2000 [15]	19 centers in: Denmark 1272.6 mg/day UK (Scotland) 801.2 mg/day Sweden 673.0 mg/day UK (England) 801.2 mg/day Italy 419.6 mg/day Netherlands 310.2 mg/day Norway 809.6 mg/day Belgium 398.4 mg/day Russia 383.8 mg/day	This study presents an analysis of one trial for recurrent PTB prevention (n=232, only 228 were analyzed because 4 were missing outcome data) and a combined analysis of this trial with 5 similar trials. In the isolated recurrent PTB trial, women with a history of PTB in a prior pregnancy were randomized to receive fish oil pills (1300mg EPA and 900mg DHA daily) or olive oil pills starting around 20 weeks gestation. In the combined analysis, 3 trials had the same design but focused on women with a history of IUGR or PIH in a prior pregnancy or twins in the current pregnancy. These first 4 trials were considered prophylactic trials. In the 2 additional trials women with threatening preeclampsia or IUGR in the current pregnancy were randomized to receive fish oil (2900mg EPA and 2100mg DHA daily) or olive oil pills starting around 33 weeks gestation. These 2 trials were considered therapeutic trials.	This moderate size study provides evidence for the utility of omega-3 in preventing recurrent PTB. In the isolated recurrent PTB trial, PTB was less common in the fish oil group: PTB rates: fish oil 23/108 (21.3%), olive oil 40/120 (33.3%) p=0.05. In the combined analysis: Cox regression analyses revealed a significantly longer time to delivery in the fish oil treatment groups for the prophylactic trials combined (p=0.004) and the therapeutic trials combined (p=0.01). Additionally, 5 of the 9 countries involved in this study are high omega-3 intake countries (>600 mg/day) and the remaining 4 are intermediate intake countries. Thus the effect of supplementation was significant here, but this effect may be greater in countries with low omega-3 intake levels at baseline. However, as mentioned above, olive oil is not an ideal control in this context. The linoleic acid (LA) in olive oil may reduce endogenous EPA/DHA synthesis from ALA [5, 6], and thus those who took olive oil (the control group) may have had even lower EPA/DHA levels than the population at baseline. However, olive oil also contains ALA. If the conversion impairment effect predominated, then the "control" group here may have had omega-3 levels more similar to a low intake country, which could help explain why an association was observed. Alternatively it is possible that the association was driven by data points from the intermediate intake countries.	Supportive
Harper et al 2010 [16]	13 centers in: the United States 449.4 mg/day	852 women with history of prior PTB were randomized to receive omega-3 fish oil (1200 mg EPA and 800 mg DHA daily) or mineral oil capsules, starting around 16-22 weeks gestation. All participants also received 17 Alpha hydroxyprogesterone to prevent PTB.	This is a moderate size trial conducted in an intermediate omega-3 intake country and it may be informative for evaluating the additional therapeutic benefit of omega3 in high risk pregnancies already being treated with progesterone. However, it is not useful for assessing potential effect of omega-3 supplements in those that are not receiving progesterone. Although the associations are not significant, the trends presented here are consistent with the interpretation that there may be an added benefit of omega3 supplementation among those already receiving progesterone therapy. PTB rates were slightly lower in the fish oil group but this difference was not significant: fish oil group 164/434 (37.8%), mineral oil group 174/418 (41.6%) RR: 0.91 (95%CI 0.77-1.07). Gestational length (in weeks) was slightly higher in the fish oil group 37.7 (36.0-39.0) vs. 37.4 (35.7-38.7), but this difference was also not significant (p=0.26). All participants received 17 Alpha hydroxyprogesterone that is known to prevent PTB in high risk cases [17], but progesterone is not appropriate for population-level interventions or interventions in lower risk individuals.	Unclear, slightly supportive

Supplemental Materials

Table S3: The Full Analytic Dataset: Omega-3 PUFA intakes, Preterm Birth Rates, and Country Incomes for 184 countries in 2010

Countries are listed in from smallest to largest estimated total LC omega-3 PUFA intake (in mg/day). The countries in gold are more than 1 standard deviation (383mg/day) below the 600 mg/day threshold and the countries in green are above the 600 mg/day threshold. The countries in white have intermediate intakes between 217 and 600 mg/day. This corresponds to the color pattern used in Figure 2 of the paper.

Country[b]	Mean seafood based omega-3 intake among females >20 years old (mg/day) [a]	Mean plant based omega-3 intake among females >20years old (mg/day)[a]	Estimated total LC omega-3 PUFA (mg/day)[b]	National Preterm Birth Rate [c] (# preterm births per 100 live births)	Country income[d] (0=low 3=high)
Lesotho	10	173	44.6	11.9	1
Burundi	24	162	56.4	11.4	0
Honduras	29	158	60.6	12.2	1
Timor-Leste	25	209	66.8	12.1	1
Namibia	23	242	71.4	14.4	2
Pakistan	16	286	73.2	15.8	1
Swaziland	18	286	75.2	13.9	1
Rwanda	18	311	80.2	9.5	0
Kenya	41	225	86	12.3	0
Botswana	10	397	89.4	15.1	2
Eritrea	10	417	93.4	12.2	0
Ethiopia	49	246	98.2	10.1	0
Azerbaijan	36	342	104.4	8.5	2
Niger	28	392	106.4	9.4	0
Nepal	20	476	115.2	14	0

Somalia	47	343	115.6	12	0
Bhutan	23	474	117.8	10.2	1
Sudan	17	523	121.6	13.2	1
Burkina Faso	22	507	123.4	10.9	0
Kyrgyzstan	44	397	123.4	10.4	0
Yemen	52	363	124.6	13.2	1
Bolivia	16	547	125.4	9	1
Mongolia	15	563	127.6	13.5	1
Malawi	52	396	131.2	18.1	0
Montenegro	73	300	133	9.2	2
Mexico	39	471	133.2	7.3	2
Zimbabwe	5	641	133.2	16.6	0
Armenia	41	471	135.2	11	1
El Salvador	44	458	135.6	12.8	1
Bangladesh	46	450	136	14	0
Congo, Democratic Republic	60	389	137.8	11.9	0
Tanzania, United Republic	70	341	138.2	11.4	0
India	30	565	143	13	1
Djibouti	13	656	144.2	11.9	1
Liberia	51	474	145.8	13.9	0
Nicaragua	37	548	146.6	9.3	1
South Africa	14	696	153.2	8	2
Mozambique	25	679	160.8	16.4	0
Albania	46	587	163.4	9	2
Guatemala	21	716	164.2	7.7	1

Afghanistan	31	671	165.2	11.5	0
Belize	73	488	170.6	10.4	1
Uzbekistan	15	779	170.8	8.7	1
Costa Rica	52	627	177.4	13.6	2
Cote d'Ivoire (Ivory Coast)	139	205	180	14	1
Benin	74	536	181.2	10.6	0
Turkmenistan	66	588	183.6	9.8	1
Hungary	72	571	186.2	8.6	3
Georgia	111	389	188.8	8.8	1
Saudi Arabia	57	660	189	6	3
Madagascar	71	603	191.6	14.2	0
Zambia	68	620	192	12.9	1
Egypt	78	574	192.8	7.3	1
Colombia	94	512	196.4	8.8	2
Haiti	35	834	201.8	14.1	0
Togo	92	581	208.2	13.3	0
Mali	74	672	208.4	11.6	0
Ireland	95	633	221.6	6.4	3
Bosnia and Herzegovinia	66	783	222.6	7.9	2
Uganda	114	563	226.6	13.6	0
Guinea-Bissau	10	1086	227.2	11.2	0
Trinidad and Tobago	22	1026	227.2	8.1	3
Chad	70	791	228.2	13.1	0
Singapore	49	897	228.4	11.5	3
Nigeria	63	834	229.8	12.2	1

Cuba	61	845	230	6.4	2
Israel	231	2	231.4	8	3
Moldova	108	617	231.4	11.9	1
Kazakhstan	59	877	234.4	8.8	2
Iraq	44	956	235.2	6.5	1
Ecuador	35	1020	239	5.1	2
Cameroon	139	505	240	12.6	1
Syrian Arab Republic	17	1142	245.4	10.9	1
Tajikistan	19	1138	246.6	10.7	0
Bahamas	167	414	249.8	9.5	3
Dominica	153	487	250.4	11.9	2
Saint Vincent and the Grenadines	112	704	252.8	11.8	2
Serbia	56	1042	264.4	6.7	2
Panama	131	681	267.2	8.1	2
Comoros	243	126	268.2	16.7	0
United Arab Emirates	74	998	273.6	7.6	3
Guyana	225	254	275.8	13.2	1
Oman	45	1180	281	14.3	3
Iran, Islamic Republic	49	1201	289.2	12.9	2
Greece	210	397	289.4	6.6	3
Brunei Darussalam	233	284	289.8	12.1	3
Saint Lucia	264	132	290.4	11.1	2
Guinea	92	993	290.6	13.9	0
Sierra Leone	205	493	303.6	10	0
Argentina	48	1297	307.4	8	2
Peru	146	809	307.8	7.3	2

Kuwait	76	1169	309.8	10.6	3
Luxembourg	232	389	309.8	8.1	3
Netherlands	179	656	310.2	8	3
Mauritania	141	850	311	15.4	1
Belarus	118	969	311.8	4.1	2
Switzerland	211	507	312.4	7.4	3
Libyan Arab Jamahir	59	1279	314.8	8.3	2
Slovakia	59	1285	316	6.3	3
Morocco	62	1273	316.6	6.7	1
Venezuela, Bolivarian Republic	121	1003	321.6	8.1	2
Jordan	47	1403	327.6	14.4	2
Macedonia, former Yugoslav Republic	57	1353	327.6	6.8	2
Austria	181	735	328	10.9	3
Romania	61	1342	329.4	7.3	2
Slovenia	69	1367	342.4	7.5	3
Central African Republic	37	1536	344.2	12.6	0
Bahrain	53	1472	347.4	14	3
Uruguay	69	1406	350.2	10.1	2
Papua New Guinea	292	307	353.4	6.5	1
Qatar	49	1544	357.8	10.5	3
Malta	279	410	361	6.4	3
Poland	107	1270	361	6.7	3
Sao Tome and Principe	199	810	361	10.5	1
Cyprus	213	742	361.4	14.7	3

Congo	146	1109	367.8	16.7	1
Dominican Republic	54	1572	368.4	10.8	2
Cape Verde	120	1245	369	11.2	1
Paraguay	61	1576	376.2	7.8	1
Equatorial Guinea	92	1440	380	16.5	3
Ghana	268	574	382.8	14.5	1
Russian Federation	182	1009	383.8	7	2
Gabon	197	943	385.6	16.3	2
Czech Republic	111	1378	386.6	7.3	3
Croatia	144	1216	387.2	5.5	3
Ukraine	181	1053	391.6	6.5	1
Suriname	130	1312	392.4	8.8	2
Lebanon	8	1940	396	7.9	2
Belgium	281	587	398.4	7.9	3
Brazil	56	1750	406	9.2	2
Bulgaria	118	1447	407.4	7.5	2
Democratic People's Republic of Korea	23	1928	408.6	10.7	0
Italy	280	698	419.6	6.5	3
Vanuatu	354	336	421.2	12.9	1
Solomon Islands	408	103	428.6	12.4	1
Estonia	189	1224	433.8	5.7	3
Micronesia, Federated States	362	386	439.2	10.5	1
United States of America	140	1547	449.4	12	3
Gambia	191	1324	455.8	14	0
Australia	282	905	463	7.6	3

Marshall Islands	379	436	466.2	11.5	1
Grenada	252	1079	467.8	10.3	2
Algeria	42	2170	476	7.4	2
Lithuania	231	1245	480	5.7	2
Germany	226	1408	507.6	9.2	3
Latvia	182	1658	513.6	5.3	2
Canada	91	2117	514.4	7.8	3
Kiribati	490	185	527	9.6	1
Antigua and Barbuda	311	1092	529.4	5.8	2
Lao People's Democratic Republic	487	227	532.4	10.8	1
Tunisia	83	2260	535	8.9	2
Angola	86	2253	536.6	12.5	1
Tonga	444	470	538	7.5	1
Samoa	517	152	547.4	5.5	1
France	407	734	553.8	6.7	3
Fiji	319	1290	577	9.9	1
New Zealand	372	1036	579.2	7.6	3
Philippines	591	134	617.8	14.9	1
Viet Nam	583	283	639.6	9.4	1
Chile	407	1232	653.4	7.1	2
Mauritius	394	1334	660.8	12.6	2
Senegal	228	2199	667.8	9.7	1
Sweden	401	1360	673	5.9	3
Turkey	373	1519	676.8	12	2
China	36	3295	695	7.1	2
Portugal	582	598	701.6	7.7	3

Cambodia	678	217	721.4	10.5	0
Myanmar	616	598	735.6	12.4	0
Sri Lanka	725	107	746.4	10.7	1
United Kingdom	317	2421	801.2	7.8	3
Indonesia	755	259	806.8	15.5	1
Norway	594	1078	809.6	6	3
Spain	647	873	821.6	7.4	3
Republic of Korea	708	885	885	9.2	3
Finland	512	2015	915	5.5	3
Thailand	825	511	927.2	12	2
Japan	715	1218	958.6	5.9	3
Malaysia	977	384	1053.8	12.3	2
Jamaica	81	5624	1205.8	10.2	2
Denmark	1213	298	1272.6	6.7	3
Seychelles	1310	647	1439.4	11.6	2
Iceland	1226	1278	1481.6	6.5	3
Barbados	1979	167	2012.4	8.9	3
Maldives	3851	219	3894.8	7.9	2

[a] information from [2, 3] (note that reference 2 is a correction for the data supplement in reference 1)

[b] (plantomega3 x 0.2) + seafoodomega3 = total LC omega-3 PUFA

[c] information from [18]

[d] Gross National Income coded as a rank variable (0=low income, 3=high income) - information from [18]

Figure S1. Scatterplot of LC Omega-3 PUFA Intake Norms by Country Income Level. Country income is assessed as a 4 category ordinal rank variable (Gross National Income: 0 = low income and 3 = high income). One omega-3 outlier is omitted (Maldives: LC Omega-3 PUFA = 3895mg/day, GNI = 2). Spearman Rank Correlation = 0.44, and this correlation is unchanged when the outlier is included.

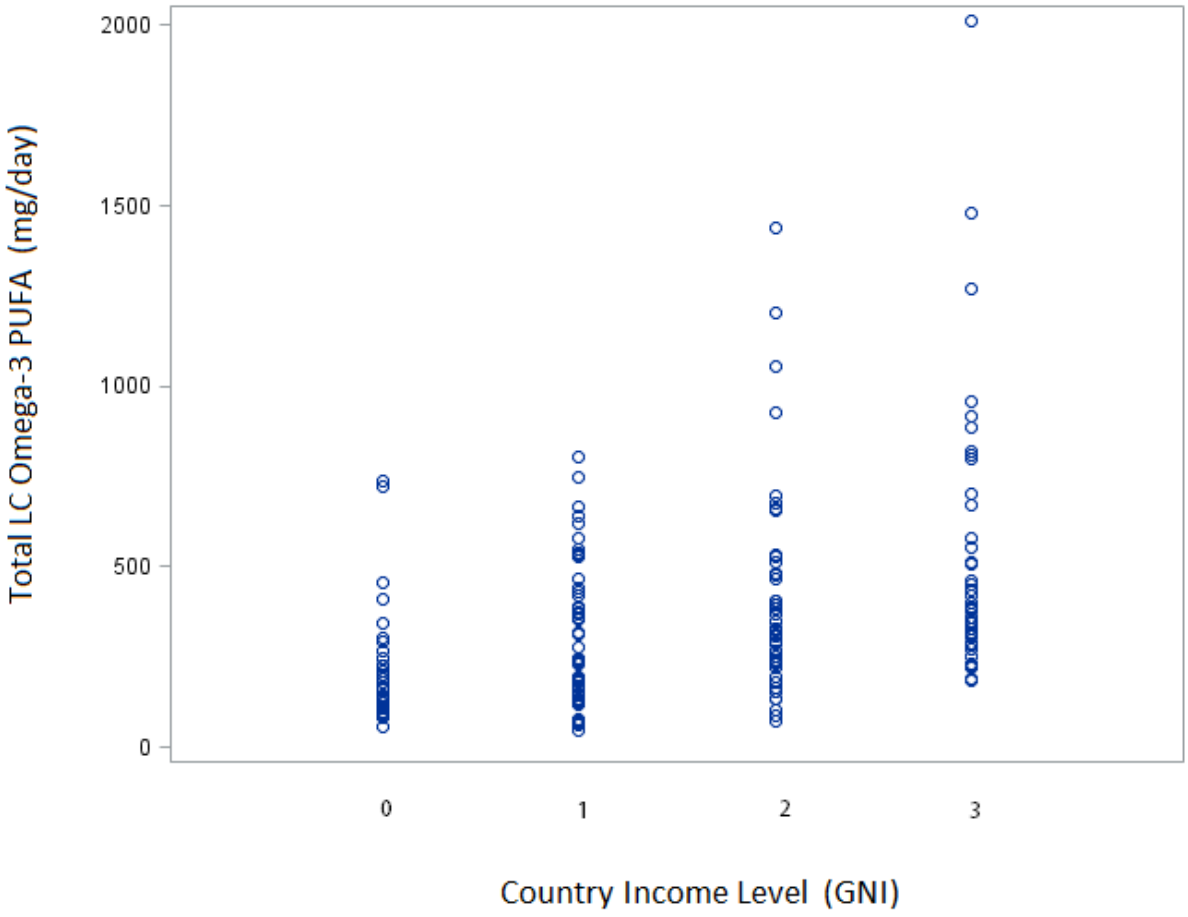


Table S4. Results from Regression Models within the Upper Linear Section of the Omega3-PTB Relationship: With and Without the Omega3 Intake Outlier

	Change in the # of preterm births (per 100 live births)	95% CI
≥600 mg/day LC omega-3 PUFA (n=27)		
Unadjusted Model		
LC Omega-3 PUFA [a]	-0.3	-0.9, 0.4
Adjusted Model		
LC Omega-3 PUFA [a]	-0.1	-0.6, 0.4
Country Income [b]	-2.0	-2.8, -1.1
≥600 mg/day LC omega-3 PUFA (n=26) [c]		
Unadjusted Model		
LC Omega-3 PUFA [a]	-0.4	-1.8, 0.9
Adjusted Model		
LC Omega-3 PUFA [a]	0.5	-0.6, 1.6
Country Income [b]	-2.2	-3.1, -1.2

[a] Change in the # of preterm births per 100 live births associated with a 1 standard deviation increase in LC Omega-3 PUFA (383 mg/day)

[b] Change in the number of preterm births per 100 live births associated with a 1 unit increase in GNI (a 4 category ordinal rank variable; higher rank corresponds to higher income)

[c] Maldives excluded as an omega-3 intake outlier

Table S5. Results from Regression Models within the Lower Linear Section of the Omega3-PTB Relationship. The exact point at which the relationship becomes nonlinear is unclear, and thus we considered a series of models with different cutoff points. A visual inspection of the penalized spline suggests the relationship becomes nonlinear between 500 and 700 mg/day LC Omega-3 PUFA.

Unadjusted Model			**Income Adjusted Model [a]**		
	Change in the # of preterm births per 100 live births [a]	**95% CI**		**Change in the # of preterm births per 100 live births [a]**	**95% CI**
0 to 400 mg/day (129 countries are in this range)					
LC Omega-3 PUFA	-3.3	-5.3, -1.3	LC Omega-3 PUFA	-0.9	-3.0, 1.3
			Country Income	-1.1	-1.7, -0.6
0 to 500 mg/day (144 countries are in this range)					
LC Omega-3 PUFA	-2.8	-4.5, -1.2	LC Omega-3 PUFA	-0.9	-2.6, 0.7
			Country Income	-1.2	-1.7, -0.7
0 to 600 mg/day[b] (157 countries are in this range)					
LC Omega-3 PUFA	-2.9	-4.2, -1.6	LC Omega-3 PUFA	-1.5	-2.8, -0.3
			Country Income	-1.1	-1.6, -0.7
0 to 700 mg/day[b] (165 countries are in this range)					
LC Omega-3 PUFA	-2.3	-3.4, -1.2	LC Omega-3 PUFA	-1.1	-2.2, 0.0
			Country Income	-1.2	-1.6, -0.8
0 to 800 mg/day[b] (169 countries are in this range)					
LC Omega-3 PUFA	-1.9	-2.9, -0.9	LC Omega-3 PUFA	-1.0	-2.0, -0.1
			Country Income	-1.2	-1.6, -0.8
0 to 900 mg/day (174 countries are in this range)					
LC Omega-3 PUFA	-1.7	-2.6, -0.8	LC Omega-3 PUFA	-0.7	-1.6, 0.1
			Country Income	-1.3	-1.7, -0.9

[a] associated with a 1 Standard Deviation increase in LC Omega-3 PUFA (383mg/day) or associated with a 1 unit increase in the 4 unit rank scale for country income (GNI - Gross National Income, higher rank corresponds to higher income)

[b] here the Omega3-PTB relationship is significant for both adjusted and unadjusted model

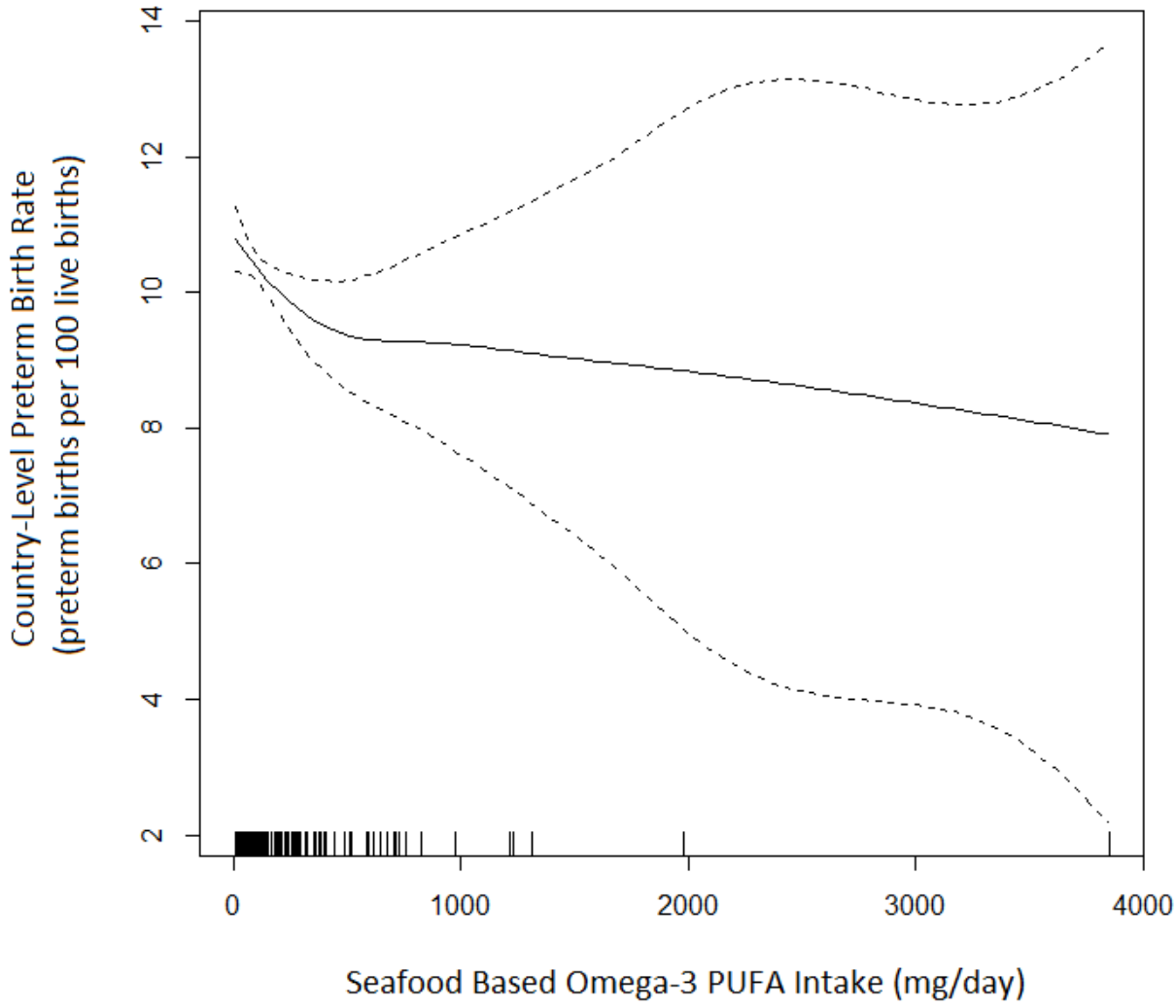
Figure S2: Penalized Splines Modeling the Seafood Based Omega3-PTB Relationship and Plant Based Omega3-PTB Relationship.

Panel A: Seafood based omega-3 intake norms among females are on the x axis and country-level preterm birth rates are on the y axis. Each vertical dash below the spline on the x axis represents a single country, allowing the figure to convey data density throughout the exposure distribution. The shape of the spline is most certain in the areas of greatest data density.

Panel B: Same as panel A except with plant based omega-3 intake norms on the x-axis.

Note: There appears to be more uncertainty (as expected) in the splines for the isolated constiuents of our exposure of interest (total LC Omega-3 PUFA). Having said this there appears to be a threshold at approximately 600 mg/day in the seafood based spline, and a threshold at approximately 3000 mg/day in the plant based spline. This pattern is consistent with the 20% conversion rate assumed in the main analysis.

A


Country-Level Preterm Birth Rate
(preterm births per 100 live births)
2
4
6
8
10
12
14
0
1000
2000
3000
4000
Seafood Based Omega-3 PUFA Intake (mg/day)

B

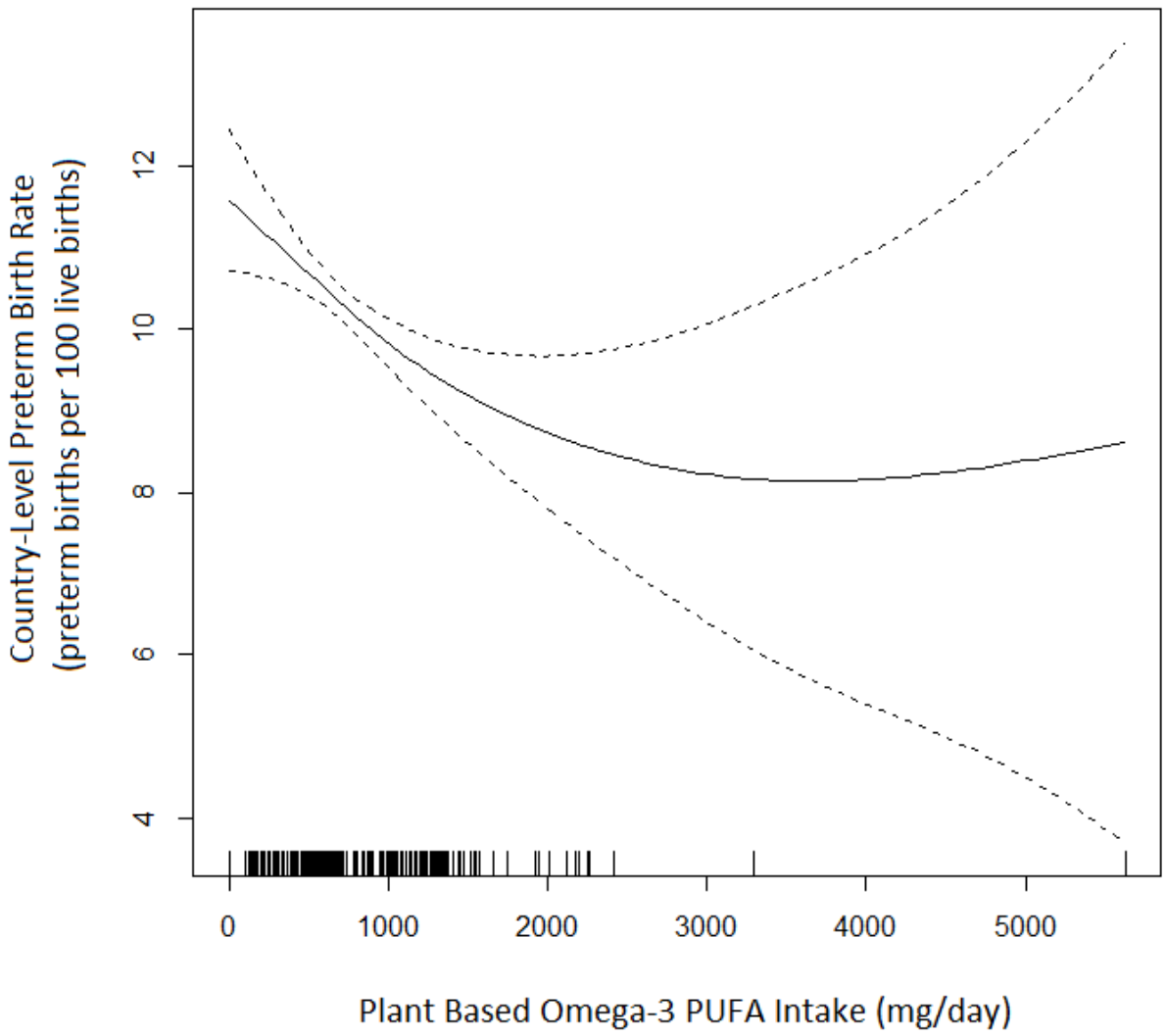

Country-Level Preterm Birth Rate
(preterm births per 100 live births)
4
6
8
10
12
0
1000
2000
3000
4000
5000
Plant Based Omega-3 PUFA Intake (mg/day)

Figure S3: Penalized Splines Modeling the Omega3-PTB Relationship with Alternative Conversion Rates for Plant Based Omega-3

Panels A-F: Country-level omega-3 intake norms among females are on the x axis and country-level preterm birth rates are on the y axis. The assumed conversion rate for ALA to EPA/DHA is listed below each panel. Each vertical dash below the spline on the x axis represents a single country, allowing the figures to convey data density throughout the exposure distribution. The shape of the splines are most certain in the areas of greatest data density.

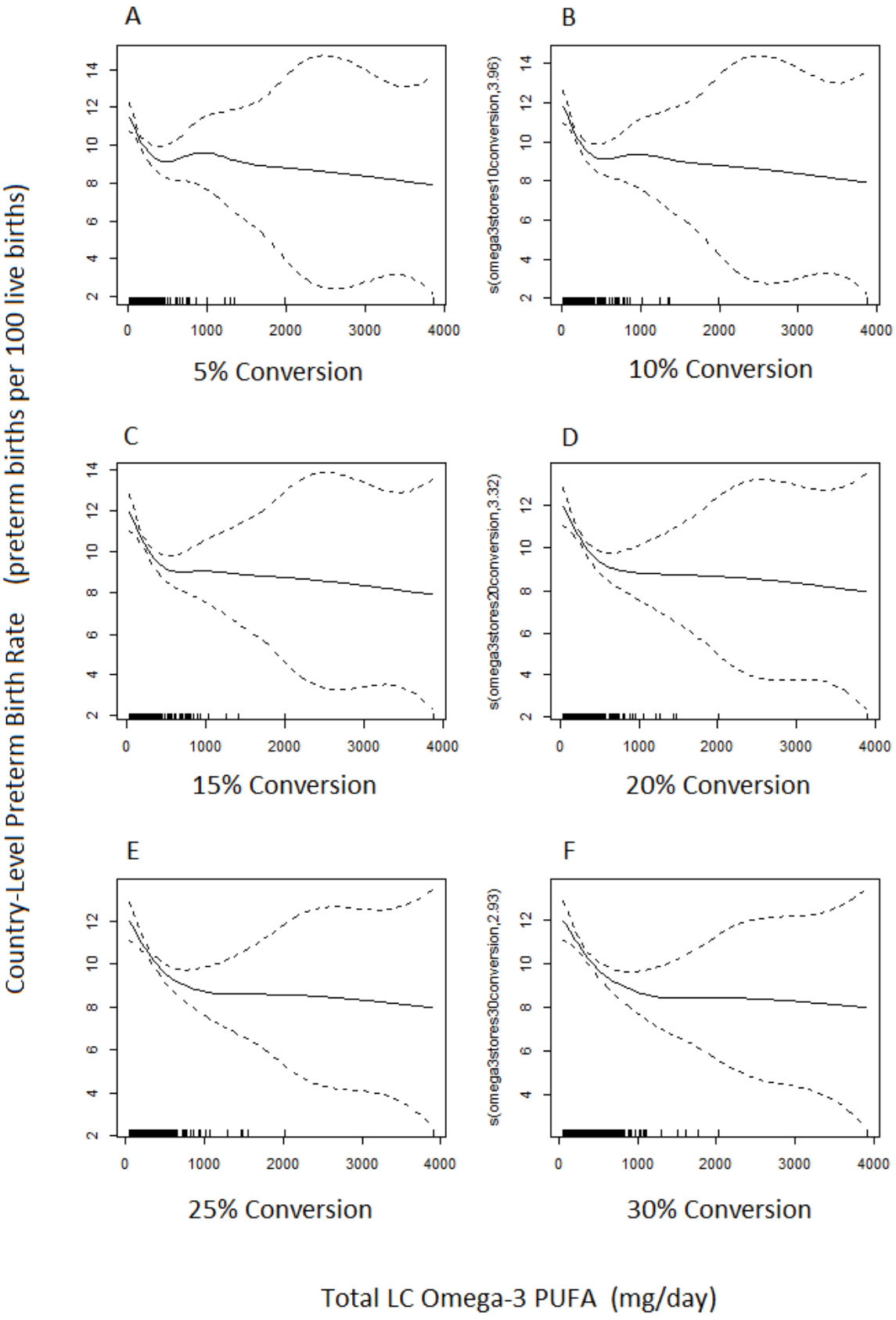

A
B
C
D
E
F
5% Conversion
10% Conversion
15% Conversion
20% Conversion
25% Conversion
30% Conversion
s(omega3stores10conversion,3.96)
s(omega3stores20conversion,3.32)
s(omega3stores30conversion,2.93)
Country-Level Preterm Birth Rate (preterm births per 100 live births)
Total LC Omega-3 PUFA (mg/day)

Table S6. Results from Regression Models Within the Lower Linear Section of the Omega3-PTB Relationship, When the ALA to EPA Conversion Rate is Allowed to Vary Away From the Published Estimate.

Unadjusted Model			Income Adjusted Model		
	Change in the # of preterm births per 100 live births [a]	**95% CI**		**Change in the # of preterm births per 100 live births [a]**	**95% CI**
Assumed ALA to EPA Conversion Rate: 0% **- an analysis considering only seafood based sources of omega-3 (EPA/DHA) [b]** (apparent threshold at 600 mg/day)					
LC Omega-3 PUFA	-1.8	-3.0, -0.6	LC Omega-3 PUFA	-0.8	-1.9, 0.3
			Country Income	-1.3	-1.7, -0.9
Assumed ALA to EPA Conversion Rate: 5% (apparent threshold at 400 mg/day)					
LC Omega-3 PUFA	-3.2	-5.2, -1.1	LC Omega-3 PUFA	-1.0	-3.0, 1.0
			Country Income	-1.3	-1.7, -0.8
Assumed ALA to EPA Conversion Rate: 10% (apparent threshold at 450 mg/day)					
LC Omega-3 PUFA	-3.1	-4.8, -1.3	LC Omega-3 PUFA	-1.2	-2.9, 0.6
			Country Income	-1.2	-1.7, -0.8
Assumed ALA to EPA Conversion Rate: 15% (apparent threshold at 500 mg/day)					
LC Omega-3 PUFA	-3.2	-4.8, -1.5	LC Omega-3 PUFA	-1.1	-2.8, 0.6
			Country Income	-1.2	-1.7, -0.8
Assumed ALA to EPA Conversion Rate: 20% [c] **- this is the rate assumed in the main analysis and it is based on a published estimate** (apparent threshold at 600 mg/day)					
LC Omega-3 PUFA	-2.9	-4.2, -1.6	LC Omega-3 PUFA	-1.5	-2.8, -0.3
			Country Income	-1.1	-1.6, -0.7
Assumed ALA to EPA Conversion Rate: 25% [c] (apparent threshold at 800 mg/day)					
LC Omega-3 PUFA	-1.8	-2.8, -0.9	LC Omega-3 PUFA	-0.9	-1.8, 0.0
			Country Income	-1.2	-1.6, -0.8
Assumed ALA to EPA Conversion Rate: 30% (apparent threshold at 900 mg/day)					
LC Omega-3 PUFA	-1.5	-2.4, -0.7	LC Omega-3 PUFA	-0.7	-1.5, 0.1
			Country Income	-1.2	-1.6, -0.8

[a] associated with a 1 Standard Deviation increase in LC Omega-3 PUFA (383mg/day) or associated with a 1 unit increase in the 4 unit rank scale for country income (GNI - Gross National Income, higher rank corresponds to higher income)

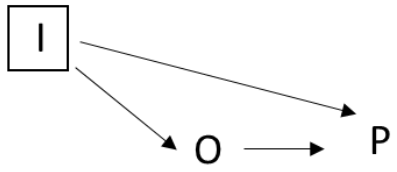
[b] because our exposure of interest is the total LComega-3 PUFA in vivo (EPA/DHA) and ALA is known to be converted to EPA/DHA in humans, exposure is known to be misclassified in this isolated analysis

[c] here the Omega3-PTB relationship is significant for both adjusted and unadjusted model

Figure S4: Directed Acyclic Graphs (DAGs) Representing Plausible Causal Relationships.

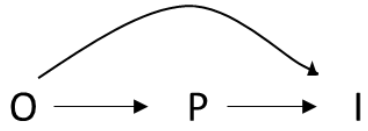
These DAGs allow us to explicitly characterize the assumptions which determine if adjusting for country income is appropriate. Panels a through g are examples of causal structures that may exist between the variables in this study. The causal relationships represented here are based on our *a priori* knowledge of the three variables in this study. Because country income is a composite variable reflecting multiple factors that could influence, or be influenced by, PTB rate, a variety of plausible causal structures can be drawn to relate country income, PTB rate, and omega3 intake norms. DAG theory [19] was used to identify the assumed causal structures in which adjustment for country income should reduce bias in the association between omega-3 intake norms and PTB rate. Panel a: Low country income serves primarily as a proxy for factors that can 1) cause low omega-3 intake and 2) cause high PTB rates through omega-3 independent causal pathways. Here we should adjust for country income as it serves as a confounder of the association between omega-3 intake and PTB rate. Panel b: A high PTB rate can have very large economic impacts [20-22], and here we assume that PTB rate influences country income. In this case we should not adjust for country income as it is a downstream consequence of our study outcome (PTB rate). Panel c: This is similar to panel b but we add the assumption that low omega3 intake norms can impact country income through mechanisms that do not involve PTB (perhaps through other pathologies such as cardiovascular disease). We should not adjust for country income here either, as this would condition on a collider and introduce bias. Panel d: Here we assume that reductions in country income mediate part of the effect of omega-3 intake norms on PTB. We should not adjust for country income as it is a causal intermediate. Panel e: In this DAG we assume that an unmeasured confounder causes both low omega-3 intake norms and low country income (e.g. chronic drought, poor crop yields, or collapsed fisheries). Under these assumptions adjusting for country income would reduce the confounding bias generated by the unmeasured confounder. Panel f: This DAG is the same as panel e, except that we now assume that the unmeasured confounder also causes PTB through mechanisms that do not involve county income. Here we should still adjust for country income, but some bias from the unmeasured confounder cannot be removed. Panel g is like panel f but the unmeasured confounder does not influence country income and thus no cofounding can be removed by adjusting for country income here. These causal models are not mutually exclusive, and it is not clear which best represents the true causal structure. Some of these DAGs suggest adjusting for country income and some do not, but whether adjusting or not, the direction and significance of the omega3-PTB relationship are consistent.

a

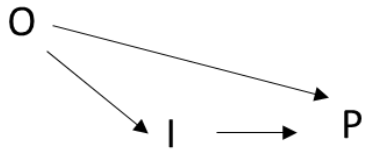


b

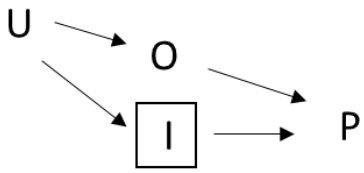
c



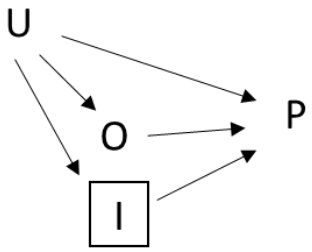
d



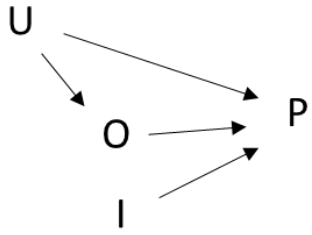
e



f



g



O = Omega-3 intake norms
P = PTB rate
I = Country income
U = Potential unmeasured confounder
□ = Adjustment should reduce bias

References for the Supplemental Materials

1. Saccone G, Berghella V. Omega-3 long chain polyunsaturated fatty acids to prevent preterm birth: a systematic review and meta-analysis. Obstetrics and gynecology. 2015 Mar;125(3):663-72.

2. Micha R, Khatibzadeh S, Shi P, Fahimi S, Lim S, Andrews KG, et al. Global, regional, and national consumption levels of dietary fats and oils in 1990 and 2010: a systematic analysis including 266 country-specific nutrition surveys. BMJ (Clinical research ed). 2014;348:g2272.

3. Global, regional, and national consumption levels of dietary fats and oils in 1990 and 2010: a systematic analysis including 266 country-specific nutrition surveys. BMJ (Clinical research ed). 2015 Mar 26;350:h1702.

4. Olsen SF, Sorensen JD, Secher NJ, Hedegaard M, Henriksen TB, Hansen HS, et al. Randomised controlled trial of effect of fish-oil supplementation on pregnancy duration. Lancet. 1992 Apr 25;339(8800):1003-7.

5. Innis SM. Omega-3 fatty acid biochemistry: perspectives from human nutrition. Military medicine. 2014 Nov;179(11 Suppl):82-7.

6. Schmitz G, Ecker J. The opposing effects of n-3 and n-6 fatty acids. Progress in lipid research. 2008 Mar;47(2):147-55.

7. Bulstra-Ramakers MT, Huisjes HJ, Visser GH. The effects of 3g eicosapentaenoic acid daily on recurrence of intrauterine growth retardation and pregnancy induced hypertension. British journal of obstetrics and gynaecology. 1995 Feb;102(2):123-6.

8. Onwude JL, Lilford RJ, Hjartardottir H, Staines A, Tuffnell D. A randomised double blind placebo controlled trial of fish oil in high risk pregnancy. British journal of obstetrics and gynaecology. 1995 Feb;102(2):95-100.

9. Malcolm CA, McCulloch DL, Montgomery C, Shepherd A, Weaver LT. Maternal docosahexaenoic acid supplementation during pregnancy and visual evoked potential development in term infants: a double blind, prospective, randomised trial. Archives of disease in childhood Fetal and neonatal edition. 2003 Sep;88(5):F383-90.

10. Tofail F, Kabir I, Hamadani JD, Chowdhury F, Yesmin S, Mehreen F, et al. Supplementation of fish-oil and soy-oil during pregnancy and psychomotor development of infants. Journal of health, population, and nutrition. 2006 Mar;24(1):48-56.

11. Makrides M, Gibson RA, McPhee AJ, Yelland L, Quinlivan J, Ryan P. Effect of DHA supplementation during pregnancy on maternal depression and neurodevelopment of young children: a randomized controlled trial. JAMA : the journal of the American Medical Association. 2010 Oct 20;304(15):1675-83.

12. Escolano-Margarit MV, Ramos R, Beyer J, Csabi G, Parrilla-Roure M, Cruz F, et al. Prenatal DHA status and neurological outcome in children at age 5.5 years are positively associated. The Journal of nutrition. 2011 Jun;141(6):1216-23.

13. Krauss-Etschmann S, Shadid R, Campoy C, Hoster E, Demmelmair H, Jimenez M, et al. Effects of fish-oil and folate supplementation of pregnant women on maternal and fetal plasma concentrations of docosahexaenoic acid and eicosapentaenoic acid: a European randomized multicenter trial. The American journal of clinical nutrition. 2007 May;85(5):1392-400.

14. Saccone G, Berghella V. Omega-3 supplementation to prevent recurrent preterm birth: a systematic review and metaanalysis of randomized controlled trials. American journal of obstetrics and gynecology. 2015 Mar 7.

15. 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 : an international journal of obstetrics and gynaecology. 2000 Mar;107(3):382-95.

16. Harper M, Thom E, Klebanoff MA, Thorp J, Jr., Sorokin Y, Varner MW, et al. Omega-3 fatty acid supplementation to prevent recurrent preterm birth: a randomized controlled trial. Obstetrics and gynecology. 2010 Feb;115(2 Pt 1):234-42.

17. Meis PJ, Klebanoff M, Thom E, Dombrowski MP, Sibai B, Moawad AH, et al. Prevention of recurrent preterm delivery by 17 alpha-hydroxyprogesterone caproate. The New England journal of medicine. 2003 Jun 12;348(24):2379-85.

18. Blencowe H, Cousens S, Oestergaard MZ, Chou D, Moller AB, Narwal R, et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. Lancet. 2012 Jun 9;379(9832):2162-72.

19. Howards PP. An overview of confounding. Part 2: how to identify it and special situations. Acta obstetricia et gynecologica Scandinavica. 2018 Apr;97(4):400-6.

20. Institute-of-Medicine, Committee-on-Understanding-Premature-Birth-and-Assuring-Healthy-Outcomes. Chapter 12: Societal Costs of Preterm Birth. In: Behrman RE, Butler AS, editors. Preterm Birth: Causes, Consequences, and Prevention. Washington (DC): National Academies Press (US); 2007.

21. Harrison MS, Goldenberg RL. Global burden of prematurity. Seminars in fetal & neonatal medicine. 2016 Apr;21(2):74-9.

22. Frey HA, Klebanoff MA. The epidemiology, etiology, and costs of preterm birth. Seminars in fetal & neonatal medicine. 2016;21:68-73.