**Polyunsaturated fatty acid status at birth and childhood allergy-related phenotypes: a pooled analysis of the MEFAB and RHEA birth cohorts**

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# Supplementary Table S1. Comparison of subject characteristics in MEFAB and RHEA between those included and not included in the main analyses

(Mean values and standard deviations; number of subjects and percentages)

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **MEFAB** | | | | | |  | | **RHEA** | | | | | | | |
|  | **Subjects not included (n 910)** | |  | **Subjects included (n 293)** | |  | |  | | **Subjects not included (n 1150)** | |  | **Subjects included (n 213)** | |  |
|  | **n** | **%** |  | **n** | **%** | **P\*** | |  | | **n** | **%** |  | **n** | **%** | **P\*** |
| **Parental characteristics** |  |  |  |  |  |  | |  | |  |  |  |  |  |  |
| Maternal age at birth (years) |  |  |  |  |  | 0·01 | |  | |  | |  |  |  | 0·56 |
| Mean | 29·1 | |  | 29·8 | |  | |  | | 29·6 | |  | 29·8 | |  |
| SD | 4·3 | |  | 4·2 | |  | |  | | 5·0 | |  | 4·8 | |  |
| Maternal BMI at study entry (kg/m2) |  |  |  |  |  | 0·06 | |  | |  |  |  |  |  | 0·28 |
| Mean | 24·2 | |  | 23·6 | |  | |  | | 25·2 | |  | 25·7 | |  |
| SD | 4·2 | |  | 3·9 | |  | |  | | 4·6 | |  | 5·8 | |  |
| Gestational weight gain (kg) |  |  |  |  |  | 0·06 | |  | |  |  |  |  |  | 0·68 |
| Mean | 11·6 | |  | 11·1 | |  | |  | | 14·1 | |  | 13·8 | |  |
| SD | 4·6 | |  | 4·0 | |  | |  | | 6·0 | |  | 6·1 | |  |
| Maternal smoking in pregnancy | 226 | 26·3 |  | 67 | 23·3 | 0·31 | |  | | 151 | 19·4 |  | 29 | 13·7 | 0·06 |
| Parity at birth, multiparous | 203 | 23·4 |  | 87 | 29·7 | 0·03 | |  | | 654 | 56·9 |  | 116 | 54·5 | 0·53 |
| Area-based socio-economic status at birth, high† | 253 | 38·2 |  | 94 | 36·3 | 0·10 | |  | |  | - |  | - | | - |
| Parental education, high | - | - |  | - | - | - | |  | | 282 | 36·4 |  | 91 | 42·9 | 0·12 |
| **Child characteristics** |  |  |  |  |  |  | |  | |  |  |  |  |  |  |
| Sex, female | 409 | 45·0 |  | 131 | 44·7 | 0·93 | |  | | 580 | 50·4 |  | 90 | 42·3 | 0·03 |
| Gestational age (weeks) |  |  |  |  |  | 0·92 | |  | |  |  |  |  |  | 0·46 |
| Mean | 39·9 | |  | 39·9 | |  | |  | | 38·3 | |  | 38·2 | |  |
| SD | 1·7 | |  | 1·6 | |  | |  | | 1·5 | |  | 1·5 | |  |
| Birth weight (grams) |  |  |  |  |  | 0·48 | |  | |  |  |  |  |  | 0·61 |
| Mean | 3271 | |  | 3296 | |  | |  | | 3208 | |  | 3191 | |  |
| SD | 511 | |  | 516 | |  | |  | | 454 | |  | 434 | |  |
| Breastfeeding, yes | - | - |  | - | - | - | |  | | 656 | 85·1 |  | 178 | 86·3 | 0·53 |
| Cord blood n-3 PUFAs (wt %) |  |  |  |  |  | 0·87 | |  | |  |  |  |  |  | 0·25 |
| Mean | 6·90 | |  | 6·89 | |  | |  | | 5·25 | |  | 5·38 | |  |
| SD | 1·58 | |  | 1·59 | |  | |  | | 1·29 | |  | 1·12 | |  |
| Cord blood n-6 PUFAs (wt %) |  |  |  |  |  | 0·52 | |  | |  |  |  |  |  | 0·11 |
| Mean | 32·08 | |  | 32·16 | |  | |  | | 32·44 | |  | 32·06 | |  |
| SD | 1·68 | |  | 1·69 | |  | |  | | 3·03 | |  | 2·35 | |  |

wt %, weight percentage of total fatty acids measured.

**\*** P values for difference were estimated using Student’s t test for continuous and Pearson's χ2 test for categorical variables.

† Area-based socio-economic status at birth was determined based on the postal code of the place of residence.

# Supplementary Table S2. Associations of primary PUFAs (polyunsaturated fatty acids) of interest with asthma ever at 6-7 years of age in MEFAB and RHEA cohorts, separately and in pooled analysis‡

(Relative risks (RRs) and 95% confidence intervals)

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Asthma ever§** | | | | | | | |
|  | **MEFAB (n 293)** | |  | **RHEA (n 213)** | |  | **Pooled (n 506)** | |
|  | **RR** | **95% CI** |  | **RR** | **95% CI** |  | **RR** | **95% CI** |
| ALA | 0·47 | 0·24, 0·93\* |  | 1·02 | 0·66, 1·58 |  | 0·79 | 0·57, 1·09†† |
| EPA+DHA | 0·67 | 0·46, 0·98\* |  | 0·59 | 0·39, 0·89\* |  | 0·65 | 0·49, 0·86\*\*† |
| Total n-3 PUFAs | 0·66 | 0·46, 0·96\* |  | 0·60 | 0·40, 0·92\* |  | 0·65 | 0·49, 0·86\*\*† |
| LA | 1·48 | 1·01, 2·17\* |  | 0·90 | 0·56, 1·44 |  | 1·17 | 0·90, 1·52† |
| AA | 1·28 | 0·85, 1·93 |  | 0·89 | 0·57, 1·41 |  | 1·06 | 0·78, 1·43† |
| Total n-6 PUFAs | 1·27 | 0·86, 1·87 |  | 0·79 | 0·52, 1·19 |  | 1·02 | 0·77, 1·36† |
| Total n-3:n-6 ratio | 0·64 | 0·44, 0·93\* |  | 0·70 | 0·44, 1·11 |  | 0·67 | 0·49, 0·90\*\*† |

AA, arachidonic acid (C20:4n-6); ALA, a-Linolenic acid (C18:3n-3); DHA, docosahexaenoic acid (C22:6n-3); EPA, eicosapentaenoic acid (C20:5n-3); LA, linoleic acid (C18:2n-6); total n-3 (n-6) PUFAs, the sum of n-3 (n-6) PUFAs present in the chromatogram.

*P* value for PUFA exposure-outcome association: \**P*<0·05; \*\**P*<0·01.

*P* value for PUFA exposure-cohort interaction: †*P* for interaction >0.10; ††*P* for interaction =0.07.

‡ Relative risks (RRs) and their 95% CIs were calculated using generalized linear models for binary outcomes (modified Poisson) adjusted for maternal age, maternal BMI at study entry, gestational weight gain, maternal smoking during pregnancy, parity, parental education, parent atopy, child age at outcome assessment, child sex, and for the pooled estimate, a cohort indicator. Effect estimates correspond to a standard deviation score (SDS) increase in PUFAs and to a unit increase in the total n-3:n-6 ratio.

§ Asthma ever was defined as ever-reported doctor diagnosis of asthma.

# Supplementary Table S3. Associations of primary PUFAs (polyunsaturated fatty acids) of interest with current rhinitis and eczema at 6-7 years of age in MEFAB and RHEA cohorts, separately and in pooled analysis‡

# (Relative risks (RRs) and 95% confidence intervals)

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Current rhinitis§** | | | | | | | |  | **Current eczema||** | | | | | | | |
|  | **MEFAB (n 293)** | |  | **RHEA (n 213)** | |  | **Pooled (n 506)** | |  | **MEFAB (n 293)** | |  | **RHEA (n 213)** | |  | **Pooled (n 506)** | |
|  | **RR** | **95% CI** |  | **RR** | **95% CI** |  | **RR** | **95% CI** |  | **RR** | **95% CI** |  | **RR** | **95% CI** |  | **RR** | **95% CI** |
| ALA | 1·02 | 0·86, 1·21 |  | 1·06 | 0·57, 1·96 |  | 1·02 | 0·87, 1·21† |  | 0·82 | 0·59, 1·13 |  | 1·21 | 0·67, 2·19 |  | 0·92 | 0·73, 1·17† |
| EPA+DHA | 0·88 | 0·68, 1·14 |  | 0·64 | 0·32, 1·30 |  | 0·84 | 0·66, 1·07† |  | 0·99 | 0·74, 1·33 |  | 0·82 | 0·46, 1·46 |  | 0·94 | 0·73, 1·22† |
| Total n-3 PUFAs | 0·87 | 0·68, 1·13 |  | 0·65 | 0·32, 1·31 |  | 0·84 | 0·65, 1·07† |  | 0·98 | 0·73, 1·33 |  | 0·83 | 0·46, 1·49 |  | 0·94 | 0·73, 1·22† |
| LA | 1·01 | 0·77, 1·33 |  | 1·28 | 0·79, 2·07 |  | 1·10 | 0·84, 1·44† |  | 1·00 | 0·74, 1·37 |  | 1·38 | 0·98, 1·96 |  | 1·16 | 0·91, 1·47† |
| AA | 1·07 | 0·80, 1·43 |  | 0·92 | 0·49, 1·74 |  | 1·05 | 0·80, 1·39† |  | 1·04 | 0·74, 1·47 |  | 0·73 | 0·49, 1·10 |  | 0·96 | 0·74, 1·26† |
| Total n-6 PUFAs | 1·10 | 0·83, 1·45 |  | 1·13 | 0·71, 1·80 |  | 1·11 | 0·87, 1·42† |  | 0·98 | 0·71, 1·36 |  | 1·20 | 0·74, 1·94 |  | 1·05 | 0·80, 1·38† |
| Total n-3:n-6 ratio | 0·88 | 0·67, 1·15 |  | 0·63 | 0·33, 1·21 |  | 0·82 | 0·64, 1·06† |  | 1·00 | 0·73, 1·36 |  | 0·77 | 0·45, 1·31 |  | 0·92 | 0·71, 1·20† |

AA, arachidonic acid (C20:4n-6); ALA, a-Linolenic acid (C18:3n-3); DHA, docosahexaenoic acid (C22:6n-3); EPA, eicosapentaenoic acid (C20:5n-3); LA, linoleic acid (C18:2n-6); PUFA, polyunsaturated fatty acid; total n-3 (n-6) PUFAs, the sum of n-3 (n-6) PUFAs present in the chromatogram.

*P* value for PUFA exposure-cohort interaction: †*P* for interaction >0·10.

‡ Relative risks (RRs) and their 95% CIs were calculated using generalized linear models for binary outcomes (modified Poisson) adjusted for maternal age, maternal BMI at study entry, gestational weight gain, maternal smoking during pregnancy, parity, parental education, parent atopy, child age at outcome assessment, child sex, and for the pooled estimate, a cohort indicator. Effect estimates correspond to a standard deviation score (SDS) increase in PUFAs and to a unit increase in the total n-3:n-6 ratio.

§ Current rhinitis was defined as presence of sneezing or a runny or blocked nose in the last 12 months without common cold or flu.

|| Current eczema was defined presence of an itchy rash in the last 12 months that affected any of the following places: the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears or eyes.

# Supplementary Table S4. Associations (pooled analysis; n 506) of primary PUFAs (polyunsaturated fatty acids) of interest with allergy-related phenotypes at 6-7 years of age after adjusting only for child sex and age‡

# (Relative risks (RRs) and 95% confidence intervals)

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Current wheeze§** | |  | **Current asthma||** | |  | **Current rhinitis¶** | |  | **Current eczema‡‡** | |
|  | **RR** | **95% CI** |  | **RR** | **95% CI** |  | **RR** | **95% CI** |  | **RR** | **95% CI** |
| ALA | 0·91 | 0·69, 1·20† |  | 0·61 | 0·32, 1·17† |  | 1·02 | 0·85, 1·22† |  | 0·92 | 0·73, 1·16† |
| EPA+DHA | 0·59 | 0·44, 0·79\*\*\*† | | 0·51 | 0·32, 0·79\*\*† | | 0·86 | 0·68, 1·09† |  | 0·98 | 0·77, 1·25† |
| Total n-3 PUFAs | 0·58 | 0·43, 0·78\*\*\*† | | 0·49 | 0·31, 0·76\*\*† | | 0·86 | 0·68, 1·09† |  | 0·98 | 0·76, 1·26† |
| LA | 1·32 | 1·08, 1·61\*\*† | | 1·40 | 1·01, 1·94\*† |  | 1·10 | 0·85, 1·42† |  | 1·10 | 0·87, 1·40† |
| AA | 1·33 | 0·99, 1·80†† |  | 1·27 | 0·84, 1·93† |  | 1·06 | 0·82, 1·38† |  | 0·95 | 0·73, 1·23† |
| Total n-6 PUFAs | 1·62 | 1·16, 2·27\*\*† | | 1·45 | 0·98, 2·16† |  | 1·12 | 0·88, 1·41† |  | 1·03 | 0·80, 1·34† |
| Total n-3:n-6 ratio | 0·52 | 0·38, 0·72\*\*\*† | | 0·44 | 0·27, 0·70\*\*† | | 0·85 | 0·66, 1·08† |  | 0·96 | 0·75, 1·24† |

AA, arachidonic acid (C20:4n-6); ALA, a-Linolenic acid (C18:3n-3); DHA, docosahexaenoic acid (C22:6n-3); EPA, eicosapentaenoic acid (C20:5n-3); LA, linoleic acid (C18:2n-6); total n-3 (n-6) PUFAs, the sum of n-3 (n-6) PUFAs present in the chromatogram.

*P* value for PUFA exposure-outcome association: \**P*<0·05; \*\**P*<0·01; \*\*\* *P*<0·001.

*P* value for PUFA exposure-cohort interaction: †*P* for interaction >0.10; ††*P* for interaction <0.05.

‡ Relative risks (RRs) and their 95% CIs were calculated using generalized linear models for binary outcomes (modified Poisson) adjusted for child age at outcome assessment, child sex, and a cohort indicator. Effect estimates correspond to a standard deviation score (SDS) increase in PUFAs and to a unit increase in the total n-3:n-6 ratio.

§ Current wheeze was defined as presence of wheezing or whistling in the chest in the last 12 months.

|| Current asthma was defined as a positive answer to both ever-reported doctor diagnosis of asthma and presence of wheezing or whistling in the chest in the last 12 months.

¶ Current rhinitis was defined as presence of sneezing or a runny or blocked nose in the last 12 months without common cold or flu.

‡‡ Current eczema was defined presence of an itchy rash in the last 12 months that affected any of the following places: the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears or eyes.

# Supplementary Table S5. Associations of secondary PUFAs (polyunsaturated fatty acids) of interest with current wheeze and asthma at 6-7 years of age in MEFAB and RHEA cohorts, separately and in pooled analysis‡

(Relative risks (RRs) and 95% confidence intervals)

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Current wheeze§** | | | | | | | |  | | | **Current asthma||** | | | | | | | | | | | | | | |
|  | **MEFAB (n 293)** | |  | **RHEA (n 213)** | |  | **Pooled (n 506)** | | |  | | | **MEFAB (n 293)** | | | |  | | **RHEA (n 213)** | | |  | | **Pooled (n 506)** | | |
|  | **RR** | **95% CI** |  | **RR** | **95% CI** |  | **RR** | **95% CI** | | |  | | | **RR** | **95% CI** |  | | **RR** | | **95% CI** |  | | **RR** | | **95% CI** |
| n-3 DPA | 0·68 | 0·41, 1·11 |  | - | - |  | - | - | | |  | | | 0·60 | 0·31, 1·16 |  | | - | | - |  | | - | | - |
| n-6 GLA | 1·12 | 0·70, 1·79 |  | 1·26 | 0·91, 1·74 |  | 1·11 | 0·84, 1·46† | | |  | | | 1·06 | 0·57, 1·96 |  | | 1·53 | | 0·86, 2·72 |  | | 1·11 | | 0·72, 1·72† |
| n-6 DGLA | 0·67 | 0·43, 1·04 |  | 1·23 | 0·87, 1·74 |  | 0·92 | 0·67, 1·27†† | | |  | | | 0·40 | 0·23, 0·72\*\* |  | | 1·67 | | 0·97, 2·90 |  | | 0·74 | | 0·43, 1·28†† |
| n-6 Osbond acid | 0·63 | 0·37, 1·09 |  | - | - |  | - | - | | |  | | | 0·79 | 0·41, 1·53 |  | | - | | - |  | | - | | - |

DGLA, dihomo-γ-linolenic acid (C20:3n-6); DPA, docosapentaenoic acid (C22:5n-3); GLA, γ-Linolenic acid (C18:3n-6); Osbond acid, C22:5n-6.

*P* value for PUFA exposure-outcome association: \*\**P*<0·01.

*P* value for PUFA exposure-cohort interaction: †*P* for interaction > 0·10; ††*P* for interaction <0·05.

‡ Relative risks (RRs) and their 95% CIs were calculated using generalized linear models for binary outcomes (modified Poisson) adjusted for maternal age, maternal BMI at study entry, gestational weight gain, maternal smoking during pregnancy, parity, parental education, parent atopy, child age at outcome assessment, child sex, and for the pooled estimate, a cohort indicator. Effect estimates correspond to a standard deviation score (SDS) increase in PUFAs and to a unit increase in the total n-3:n-6 ratio.

§ Current wheeze was defined as presence of wheezing or whistling in the chest in the last 12 months.

|| Current asthma was defined as a positive answer to both ever-reported doctor diagnosis of asthma and presence of wheezing or whistling in the chest in the last 12 months.

# Supplementary Table S6. Associations of secondary PUFAs (polyunsaturated fatty acids) of interest with current rhinitis and eczema at 6-7 years of age in MEFAB and RHEA cohorts, separately and in pooled analysis‡

(Relative risks (RRs) and 95% confidence intervals)

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Current rhinitis§** | | | | | | | |  | | | **Current eczema||** | | | | | | | | | | | | | |
|  | **MEFAB (n 293)** | |  | **RHEA (n 213)** | |  | **Pooled (n 506)** | | |  | | | **MEFAB (n 293)** | | | |  | | **RHEA (n 213)** | |  | **Pooled (n 506)** | | |
|  | **RR** | **95% CI** |  | **RR** | **95% CI** |  | **RR** | **95% CI** | | |  | | | **RR** | **95% CI** |  | | **RR** | | **95% CI** |  | **RR** | **95% CI** |
| n-3 DPA | 0·8 | 0·62, 1·03 |  | - | - |  | - | - | | |  | | | 1·04 | 0·74, 1·45 |  | | - | | - |  | - | - |
| n-6 GLA | 1·35 | 1·06, 1·72\* |  | 0·67 | 0·39, 1·17 |  | 1·10 | 0·89, 1·36††† | | |  | | | 0·93 | 0·69, 1·26 |  | | 1·46 | | 1·05, 2·04\* |  | 1·08 | 0·84, 1·4†† |
| n-6 DGLA | 1·09 | 0·82, 1·44 |  | 0·88 | 0·54, 1·43 |  | 1·01 | 0·79, 1·29† | | |  | | | 0·87 | 0·68, 1·13 |  | | 1·41 | | 0·99, 2·01 |  | 1·01 | 0·81, 1·26††† |
| n-6 Osbond acid | 0·86 | 0·64, 1·15 |  | - | - |  | - | - | | |  | | | 0·98 | 0·74, 1·31 |  | | - | | - |  | - | - |

DGLA, dihomo-γ-linolenic acid (C20:3n-6); DPA, docosapentaenoic acid (C22:5n-3); GLA, γ-Linolenic acid (C18:3n-6); Osbond acid, C22:5n-6.

*P* value for PUFA exposure-outcome association: \**P*<0·05.

*P* value for PUFA exposure-cohort interaction: †*P* for interaction > 0·10; ††*P* for interaction =0·07; †††*P* for interaction <0·05.

‡ Relative risks (RRs) and their 95% CIs were calculated using generalized linear models for binary outcomes (modified Poisson) adjusted for maternal age, maternal BMI at study entry, gestational weight gain, maternal smoking during pregnancy, parity, parental education, parent atopy, child age at outcome assessment, child sex, and for the pooled estimate, a cohort indicator. Effect estimates correspond to a standard deviation score (SDS) increase in PUFAs and to a unit increase in the total n-3:n-6 ratio.

§ Current rhinitis was defined as presence of sneezing or a runny or blocked nose in the last 12 months without common cold or flu.

|| Current eczema was defined presence of an itchy rash in the last 12 months that affected any of the following places: the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears or eyes.

# Supplementary Table S7. Associations of primary PUFAs (polyunsaturated fatty acids) of interest with current wheeze symptoms at 9 months and 4 years of age in the RHEA cohort (n 213)\*

(Relative risks (RRs) and 95% confidence intervals)

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Current wheeze**† | | |
|  | **9 months (n 228)** |  | **4 years (n 222)** |
|  | **RR (95% CI)** |  | **RR (95% CI)** |
| ALA | 0·99 (0·76, 1·28) |  | 1·57 (0·68, 3·60) |
| EPA+DHA | 1·00 (0·79, 1·28) |  | 0·74 (0·38, 1·44) |
| Total n-3 PUFAs | 1·02 (0·80, 1·29) |  | 0·75 (0·39, 1·44) |
| LA | 0·90 (0·71, 1·15) |  | 1·57 (0·98, 2·53) |
| AA | 1·08 (0·86, 1·35) |  | 0·54 (0·29, 1·01) |
| Total n-6 PUFAS | 1·03 (0·79, 1·34) |  | 0·86 (0·35, 2·11) |
| Total n-3:n-6 ratio | 1·02 (0·79, 1·31) |  | 0·84 (0·41, 1·72) |

AA, arachidonic acid (C20:4n-6); ALA, a-Linolenic acid (C18:3n-3); DHA, docosahexaenoic acid (C22:6n-3); EPA, eicosapentaenoic acid (C20:5n-3); LA, linoleic acid (C18:2n-6); total n-3 (n-6) PUFAs, the sum of n-3 (n-6) PUFAs present in the chromatogram.

**\*** Relative risks (RRs) and their 95% CIs were calculated using generalized linear models for binary outcomes (modified Poisson) adjusted for maternal age, maternal BMI at study entry, gestational weight gain, maternal smoking during pregnancy, parity, parental education, parent atopy, child age at outcome assessment, and sex. Effect estimates correspond to a standard deviation score (SDS) increase in PUFAs and to a unit increase in the total n-3:n-6 ratio.

† Current wheeze was defined as presence of wheezing or whistling in the chest since birth at 9 months of age (n/N= 62/228) and in the last 12 months at 4 years of age (n/N=10/222).

# Supplementary Table S8. Associations (pooled analysis; n 506) of primary PUFAs (polyunsaturated fatty acids) of interest with allergic disease-related phenotypes at 6-7 years of age after including birth weight and gestational age in multivariable models†

(Relative risks (RRs) and 95% confidence intervals)

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Current wheeze**‡ | |  | **Current asthma**§ | |  | **Current rhinitis**|| | |  | **Current eczema**¶ | |
|  | RR | 95% CI |  | RR | 95% CI |  | RR | 95% CI |  | RR | 95% CI |
| ALA | 0·91 | 0·70, 1·18 |  | 0·59 | 0·29, 1·17 |  | 1·03 | 0·87, 1·21 |  | 0·90 | 0·70, 1·15 |
| EPA+DHA | 0·61 | 0·44, 0·84\*\* |  | 0·47 | 0·27, 0·80\*\* |  | 0·83 | 0·64, 1·08 |  | 0·86 | 0·66, 1·13 |
| Total n-3 PUFAs | 0·60 | 0·44, 0·83\*\* |  | 0·44 | 0·26, 0·75\*\* |  | 0·82 | 0·63, 1·07 |  | 0·86 | 0·65, 1·13 |
| LA | 1·18 | 0·92, 1·51 |  | 1·28 | 0·83, 1·97 |  | 1·09 | 0·81, 1·45 |  | 1·20 | 0·94, 1·52 |
| AA | 1·34 | 0·98, 1·84 |  | 1·38 | 0·89, 2·15 |  | 1·06 | 0·80, 1·40 |  | 0·93 | 0·71, 1·21 |
| Total n-6 PUFAs | 1·49 | 1·05, 2·13\* |  | 1·42 | 0·94, 2·15 |  | 1·10 | 0·86, 1·41 |  | 1·07 | 0·81, 1·40 |
| Total n-3:n-6 ratio | 0·55 | 0·39, 0·78\*\* |  | 0·40 | 0·23, 0·70\*\* |  | 0·81 | 0·62, 1·07 |  | 0·84 | 0·63, 1·12 |

AA, arachidonic acid (C20:4n-6); ALA, a-Linolenic acid (C18:3n-3); DHA, docosahexaenoic acid (C22:6n-3); EPA, eicosapentaenoic acid (C20:5n-3); LA, linoleic acid (C18:2n-6); total n-3 (n-6) PUFAs, the sum of n-3 (n-6) PUFAs present in the chromatogram.

*P* value for PUFA exposure-outcome association: \**P*<0·05; \*\**P*<0·01.

†Relative risks (RRs) and their 95% CIs were calculated using generalized linear models for binary outcomes (modified Poisson) adjusted for maternal age, maternal BMI at study entry, gestational weight gain, maternal smoking during pregnancy, parity, birth weight, gestational age, parental education, parent atopy, child age at outcome assessment, sex, and a cohort indicator. Effect estimates correspond to a standard deviation score (SDS) increase in PUFAs and to a unit increase in the total n-3:n-6 ratio.

‡ Current wheeze was defined as presence of wheezing or whistling in the chest in the last 12 months.

§ Current asthma was defined as a positive answer to both ever-reported doctor diagnosis of asthma and presence of wheezing or whistling in the chest in the last 12 months.

|| Current rhinitis was defined as presence of sneezing or a runny or blocked nose in the last 12 months without common cold or flu.

¶ Current eczema was defined presence of an itchy rash in the last 12 months that affected any of the following places: the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears or eyes.

# Supplementary Table S9. Assessment of effect measure modification by parent atopy in the association of the total n-3:n-6 PUFA ratio with current symptoms of asthma, rhinitis, and eczema at 6-7 years in the pooled dataset

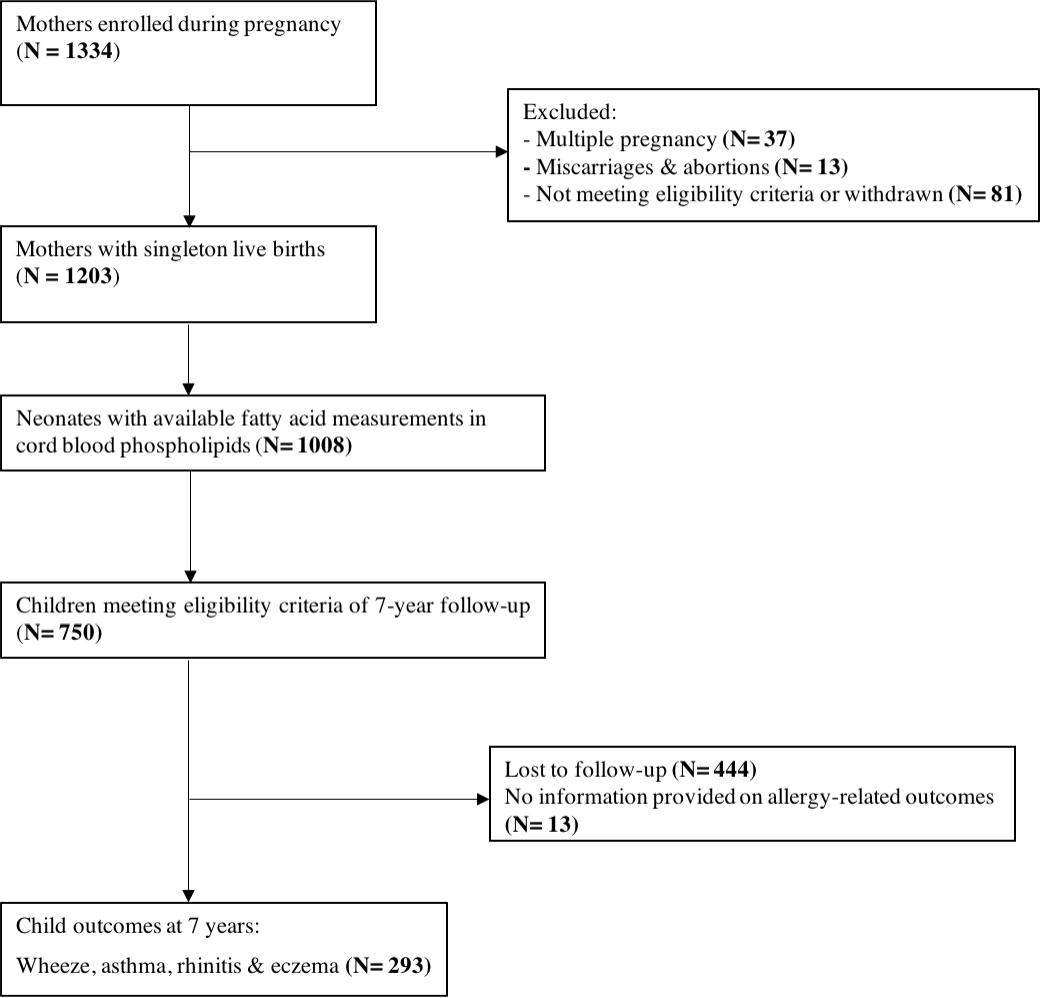
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | n | | Current asthma | | | | | |  | | Current rhinitis | | | | | |  | | Current eczema | | | | |
|  | RR | 95% CI | | | P value | |  | | RR | 95% CI | | P value | | |  | | RR | 95% CI | | | P value |
| Parent atopy, no | 283 | | 0·48 | 0·28, 0·82 | | | 0·01 | |  | | 0·84 | 0·59, 1·21 | | 0·36 | | |  | | 0·90 | 0·58, 1·38 | | | 0·63 |
| Parent atopy, yes | 223 | | 0·38 | 0·17, 0·83 | | | 0·02 | |  | | 0·82 | 0·59, 1·15 | | 0·25 | | |  | | 0·97 | 0·69, 1·36 | | | 0·86 |
| Measure of effect modification on multiplicative scale:  For current asthma: RR = 0·94 (95% CI 0·38, 2·34); P=0·90  For current rhinitis: RR = 0·92 (95% CI 0·56, 1·51); P=0·74  For current eczema: RR = 1·02 (95% CI 0·60, 1·73); P=0·94 | | | | | | | | | | | | | | | | | | | | | |
|  |  |  | | |  |  | |  | |  | | |  | |  |  | |  | | |  |
| Measure of effect modification on additive scale:  For current asthma: RERI = -0·31 (95% CI -1·87, 1·26); P= 0·70  For current rhinitis: RERI = 0·04 (95% CI -0·88, 0·95); P= 0·94  For current eczema: RERI = 0·21 (95% CI -1·04, 1·46); P= 0·74 | | | | | | | | | | | | | | | | | | | | | |

Effect estimates were adjusted for maternal age, maternal BMI at study entry, gestational weight gain, maternal smoking during pregnancy, parity, parental education, child age at outcome assessment, child sex, and a cohort indicator.

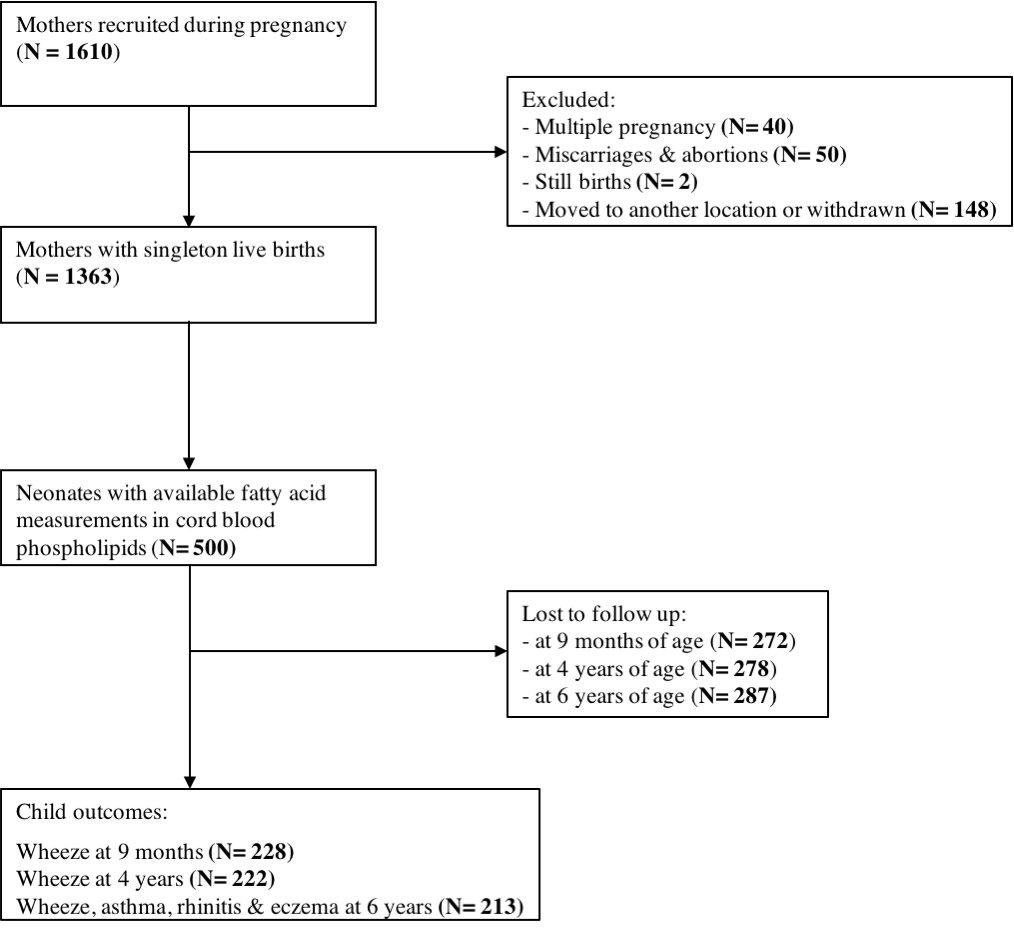
# Supplementary Table S10. Assessment of effect measure modification by sex in the association of the total n-3:n-6 PUFA ratio with current symptoms of asthma, rhinitis, and eczema at 6-7 years in the pooled dataset

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | n | | Current asthma | | | | | |  | | Current rhinitis | | | | | |  | | Current eczema | | | | |
|  | RR | 95% CI | | | P value | |  | | RR | 95% CI | | P value | | |  | | RR | 95% CI | | | P value |
| Child sex, male | 285 | | 0·47 | 0·27, 0·80 | | | <0·01 | |  | | 1·00 | 0·72, 1·39 | | 0·98 | | |  | | 0·95 | 0·67, 1·35 | | | 0·77 |
| Child sex, female | 221 | | 0·27 | 0·17, 0·43 | | | <0·001 | |  | | 0·61 | 0·37, 1·01 | | 0·06 | | |  | | 0·84 | 0·53, 1·34 | | | 0·47 |
| Measure of effect modification on multiplicative scale:  For current asthma: RR = 0·80 (95% CI 0·38, 1·69); P=0·56  For current rhinitis: RR = 0·57 (95% CI 0·32, 1·01); P=0·06  For current eczema: RR = 0·88 (95% CI 0·52, 1·49); P=0·63 | | | | | | | | | | | | | | | | | | | | | |
|  |  |  | | |  |  | |  | |  | | |  | |  |  | |  | | |  |
| Measure of effect modification on additive scale:  For current asthma: RERI = 0·58 (95% CI -0·09, 1·26); P= 0·09  For current rhinitis: RERI = -1·83 (95% CI -5·60, 1·94); P= 0·34  For current eczema: RERI = -0·41 (95% CI -1·89, 1·07); P= 0·59 | | | | | | | | | | | | | | | | | | | | | |

Effect estimates were adjusted for maternal age, maternal BMI at study entry, gestational weight gain, maternal smoking during pregnancy, parent atopy, parity, parental education, child age at outcome assessment, and a cohort indicator.



# Supplementary Fig. S1. Flow chart of the MEFAB participants



# Supplementary Fig. S2. Flow chart of the RHEA participants



# Supplementary Fig. S3. Directed acyclic graph for the association of fatty acid status at birth with allergic disease-related symptoms in childhood.

# Text accompanying Supplementary Fig. S3

In our models, selection of covariates for adjustment was based on a directed acyclic graph (DAG) approach. A DAG is a graphical tool that enables the visualization of the associations among the exposure of interest, the outcome(s) being studied, and all other related characteristics. It represents a conceptual model, and provides a structured way to present an overview of the research question and its context, therefore aiding in identifying the presence of confounding and ways to resolve it(1). Below, we use some illustrative examples for the reader who has not previous experience with DAGs. For those who are more interested in the concept of DAGs, there are some excellent reviews on this topic(1,2,3).

Arrows in DAGs represent direct effects of one factor on another, either protective or harmful. Moreover, arrows should follow time order, or else the diagram contradicts the basic principle that causes must precede their effects. The arrows and their direction are drawn based on prior knowledge. For instance, in Supplemental Figure 3, parental education, as a proxy of SES status, can affect both prenatal fatty acid exposure(4,5) and childhood allergic disease symptoms(6), so the arrows point away from parental education towards fatty acid status at birth as well as towards child allergic disease symptoms. The path from fatty acid status at birth towards child allergy via parental education is called a backdoor path because it starts with an arrowhead towards fatty acid status at birth, the exposure. The presence of a backdoor path in DAG identifies the presence of confounding. The path from prenatal fatty acids via gestational age to child allergy is not a backdoor path, as the first arrow points away from the exposure. There is good evidence to suggest that high exposure to n-3 long-chain PUFAs even in the last weeks of gestation increases the duration of gestation(7,8). Hence, gestational age is not a cause of prenatal PUFA exposure, but prenatal PUFAs can be regarded as a cause of gestational age. Additionally, the arrow from gestational age to child allergy indicates that gestational age predicts child allergy symptoms; an association that is supported by good epidemiological evidence(9). Gestational age is therefore in the causal pathway between fatty acid status and child allergy.

In our study, the DAG was drawn using DAGitty (www.dagitty.net)(10), a user friendly software that allows the instant identification of minimum but sufficient sets of factors to control for in the analysis in order to resolve confounding. The adjustment set in our study included the following covariates: maternal age, maternal BMI at study entry, gestational weight gain, maternal smoking during pregnancy, parental education, parity, parent atopy, child age and sex. Through this adjustment set, the backdoor paths from fatty acid status at birth towards child allergy are blocked.

Below, we present references for the assumed relationships in our DAG (Supplementary Fig. 3):

|  |  |
| --- | --- |
| **Parental education** | → prenatal PUFA status:(4,5)  → child allergy-related symptoms:(6)  → smoking during pregnancy:(11) |
|  | → child BMI:(12) |
| **Maternal age** | → smoking during pregnancy:(11) |
|  | → child BMI:(13) |
|  | → gestational age/birthweight:(14) |
|  |  |
| **Maternal BMI** | → prenatal PUFA status:(15) |
|  | → gestational age/birthweight:(16) |
|  | → child BMI:(17) |
|  | → child allergy-related symptoms:(18) |
| **Gestational weight gain** | → gestational age/birthweight:(16) |
|  | → child BMI:(19) |
|  | → child allergy-related symptoms:(18) |
| **Smoking during pregnancy** | → birthweight/gestational age:(20,21) |
|  | → child BMI:(22) |
|  | → child allergy-related symptoms:(23) |
| **Parity** | → prenatal PUFA status:(24) |
|  | → child allergy-related symptoms:(25,26) |
| **Parent atopy** | → prenatal PUFA status:(27) |
|  | → child allergy-related symptoms:(28) |
| **Prenatal PUFA status** | → birthweight/gestational age:(8,29) |
| **Child sex** | → prenatal PUFA status:(30) |
|  | → allergy-related symptoms:(31) |
| **Gestational age/birth weight** | → child allergy-related symptoms:(32,33) |
|  |  |
| **Child BMI** | → allergy-related symptoms:(34) |

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