**Supplemental Data**

**Supplemental Table 1. Characteristics of children and their parents at birth and randomisation between the N3RO trial and N3RO follow-up study**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Characteristics** | **N3RO trial \***  **(n=1196)** | **SD** | | **N3RO follow-up study (n=77)†** | **SD** | ***P*** |
| **Maternal characteristics** |  | |  |  |  |  |
| Maternal age (y)– mean ‡ | 30.4 | | 5.8 | 31.3 | 5.0 | 0.20 |
| Caucasian maternal race/ethnicity– no/total no (%) | 695/1172 (59.3%) | |  | 64/76 (84.2%) |  | <0.001 |
| Maternal Education |  | |  |  |  |  |
| *Completed secondary school* – no/total no (%) | 836/1084 (77.1%) | |  | 64/74 (86.5%) |  | 0.06 |
| *Completed tertiary education (postgraduate or undergraduate degree, diploma and/or certificate)* – no/total no (%) | 791/1075 (73.6%) | |  | 58/72 (80.6%) |  | 0.19 |
| *Full time education* § (y)– mean | 14.2 | | 2.9 | 14.6 | 2.4 | 0.16 |
| Consumed LCPUFA supplements during pregnancy– no/total no (%) | 226/1143 (19.8%) | |  | 24/73 (32.9%) |  | 0.007 |
| Smoked cigarettes during pregnancy– no/total no (%) | 205/1155 (17.8%) | |  | 6/72 (8.3%) |  | 0.04 |
| Birth mode – no/total no (%) |  | |  |  |  | 0.24 |
| *Vaginal* | 481/1195 (40.3%) | |  | 26/77 (33.8%) |  |  |
| *Caesarean* | 714/1195 (59.8%) | |  | 51/77 (66.2%) |  |  |
| Antenatal corticosteroids (Any given) – no/total no (%) | 1096/1178 (93.0%) | |  | 71/76 (93.4%) |  | 0.90 |
| Chorioamnionitis – no/total no (%) | 371/1072 (34.6%) | |  | 32/76 (42.1%) |  | 0.19 |
| **Paternal Education** |  | |  |  |  |  |
| *Completed secondary school* – no/total no (%) | 753/1026 (73.4%) | |  | 51/71 (71.8%) |  | 0.77 |
| *Completed tertiary education (postgraduate or undergraduate degree, diploma and/or certificate)* – no/total no (%) | 710/1006 (70.6%) | |  | 53/69 (76.8%) |  | 0.27 |
| *Full time education* ǁ (y)– mean | 14.0 | | 2.9 | 14.6 | 2.8 | 0.07 |
| **Infant at birth** |  | |  |  |  |  |
| Sex-Female – no/total no (%) | 560/1196 (46.8%) | |  | 33/77 (42.9%) |  | 0.50 |
| Gestational age < 27 weeks– no/total no (%) | 591/1196 (49.4%) | |  | 48/77 (62.3%) |  | 0.03 |
| Plurality- Singletons – no/total no (%) | 855/1196 (71.5%) | |  | 56/77 (72.7%) |  | 0.34 |
| Birth weight (g) – mean | 918.8 | | 239.2 | 916.1 | 212.3 | 0.92 |
| Birth length (cm) ¶– mean | 34.4 | | 3.2 | 34.6 | 2.6 | 0.43 |
| Birth head circumference (cm) \*\*– mean | 24.3 | | 2.0 | 24.4 | 1.8 | 0.56 |
| Apgar score ≥7 – no/total no (%) | 889/1188 (74.8%) | |  | 56/76 (74.7%) |  | 0.82 |
| CRIB score †† – median (IQR) | 10.0 (8.0-12.0) | |  | 10.0 (9.0-12.0) |  | 0.87 |
| **Infant at randomisation** |  | |  |  |  |  |
| Age (d) – median (IQR) | 3.0 (2.0-4.0) | |  | 3.0 (2.0-4.0) |  | 0.39 |
| First enteral feed (d) ‡‡– median (IQR) | 2.0 (1.0-3.0) | |  | 1.0 (1.0-2.0) |  | 0.15 |
| Type of first enteral feed-breastmilk– no (%) | 1113 (93.5%) | |  | 74 (96.1%) |  | 0.47 |
| α-linolenic acid (%) §§– mean | 0.7 | | 0.5 | 0.5 | 0.3 | 0.003 |
| Docosahexaenoic acid (%) §§– mean | 2.7 | | 0.9 | 3.1 | 0.9 | <0.001 |
| Eicosapentaenoic acid (%) §§– mean | 0.4 | | 0.3 | 0.4 | 0.3 | 0.01 |
| Linoleic acid (%) §§– mean | 10.9 | | 4.5 | 8.5 | 3.0 | <0.001 |
| Arachidonic acid (%) §§– mean | 10.2 | | 2.7 | 11.6 | 2.5 | <0.001 |
| Age first dose of study emulsion ‡‡(d)– median (IQR) | 4.0 (3.0-5.0) | |  | 4.0 (3.0-6.0) |  | 0.56 |
| Compliance of study emulsion (%) ǁǁ– median (IQR) | 93.5 (87.7-96.5) | |  | 91.7 (86.1-95.5) |  | 0.06 |
| Days to full enteral feeds ¶¶(d)– median (IQR) | 13.0 (10.0-19.3) | |  | 18.0 (12.0-26.5) |  | <0.001 |

N3RO, N-3 fatty acids for improvement of Respiratory Outcomes trial; SD, standard deviation; LCPUFA, long chain polyunsaturated fatty acids; Apgar, Appearance, Pulse, Grimace, Activity, and Respiration score; CRIB, clinical risk index for babies; IQR, interquartile range

\* N3RO cohort minus the participants (n=77) who completed the N3RO follow-up study

† N3RO follow-up study participants who completed the attention assessment3

‡ Data missing for 8 children in the N3RO trial cohort

§ Data missing for 217 children in the N3RO trial cohort and 5 in the N3RO follow-up study

ǁ Data missing for 318 children in the N3RO trial cohort and 13 in the N3RO follow-up study

¶ Data missing for 306 children in the N3RO trial cohort and 1 in the N3RO follow-up study

\*\* Data missing for 121 children in the N3RO trial cohort and 1 in the N3RO follow-up study

†† Data missing for 19 children in the N3RO trial cohort

‡‡ Data missing for 6 children in the N3RO trial cohort

§§ Data missing for 11 children in the N3RO trial cohort and 1 in the N3RO follow-up study

ǁǁ Data missing for 79 children in the N3RO trial cohort and 1 in the N3RO follow-up study

¶¶ Data missing for 46 children in the N3RO trial cohort

**Supplemental Table 2. Characteristics of children and their parents at birth and randomisation compared between N3RO follow-up study participants and non-participants**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Characteristics** | **N3RO follow-up study participants (n=77)** | **SD** | **N3RO follow-up non-participants \***  **(n=43)** | **SD** | ***P*** |
| **Maternal characteristics** |  |  |  |  |  |
| Maternal age(d)– mean | 31.3 | 5.0 | 28.8 | 5.5 | 0.01 |
| Caucasian maternal race/ethnicity– no/total no (%) | 64/76 (84.2%) |  | 18/43 (41.9%) |  | <0.001 |
| Maternal Education |  |  |  |  |  |
| *Completed secondary school* – no/total no (%) | 64/74 (86.5%) |  | 19/39 (48.7%) |  | <0.001 |
| *Completed tertiary education (postgraduate or undergraduate degree, diploma and/or certificate)* – no/total no (%) | 58/72 (80.6%) |  | 26/39 (66.7%) |  | 0.10 |
| *Full time education* † (y)– mean | 14.6 | 2.4 | 13.2 | 2.7 | 0.004 |
| Consumed LCPUFA supplements during pregnancy – no/total no (%) | 24/73 (32.9%) |  | 13/43 (30.2%) |  | 0.77 |
| Smoked cigarettes during pregnancy | 6/72 (8.3%) |  | 12/39 (30.8%) |  | 0.002 |
| Birth mode – no/total no (%) |  |  |  |  | 0.39 |
| *Vaginal* | 26 (33.8%) |  | 13 (30.2%) |  |  |
| *Caesarean* | 51 (66.2%) |  | 30 (69.8%) |  |  |
| Antenatal corticosteroids (Any given) – no/total no (%) | 71/76 (93.4%) |  | 39/42 (92.9%) |  | 0.91 |
| Chorioamnionitis – no/total no (%) | 32/76 (42.1%) |  | 18/43 (41.9%) |  | 0.98 |
| **Paternal Education** |  |  |  |  |  |
| *Completed secondary school* – no/total no (%) | 51/71 (71.8%) |  | 19/36 (52.8%) |  | 0.05 |
| *Completed tertiary education (postgraduate or undergraduate degree, diploma and/or certificate)* – no/total no (%) | 53/69 (76.8%) |  | 18/36 (50.0%) |  | 0.005 |
| *Full time education* ‡ (y)– mean | 14.64 | 2.8 | 12.52 | 2.9 | 0.001 |
| **Infant at birth** |  |  |  |  |  |
| Sex-Female – no (%) | 33 (42.9%) |  | 15 (34.9%) |  | 0.39 |
| Gestational age < 27 weeks – no (%) | 48 (62.3%) |  | 23 (53.5%) |  | 0.34 |
| Plurality- Singletons – no (%) | 56 (72.7%) |  | 22 (51.2%) |  | <0.001 |
| Birth weight (g) – mean | 916.1 | 212.3 | 889.1 | 211.1 | 0.50 |
| Birth length (cm) §– mean | 34.6 | 2.6 | 33.9 | 2.8 | 0.14 |
| Birth head circumference (cm) ǁ– mean | 24.4 | 1.8 | 24.4 | 1.7 | 0.95 |
| Apgar score ≥7 ǁ – no (%) | 56 (73.7%) |  | 29 (69.1%) |  | 0.59 |
| CRIB score ¶ – median (IQR) | 10.0 (9.0-12.0) |  | 10.0 (9.0-11.3) |  | 0.61 |
| **Infant at randomisation** |  |  |  |  |  |
| Age (d) – median (IQR) | 3.0 (2.0-4.0) |  | 4.0 (3.0-4.0) |  | 0.29 |
| First enteral feed (d) – median (IQR) | 1.0 (1.0-2.0) |  | 2.0 (1.0-3.0) |  | 0.01 |
| Type of first enteral feed-breastmilk – no (%) | 74 (96.1%) |  | 36 (83.7%) |  | 0.03 |
| α -linolenic acid ǁ– mean | 0.5 | 0.3 | 0.5 | 0.3 | 0.56 |
| Docosahexaenoic acid (%) ǁ– mean | 3.1 | 0.9 | 2.7 | 0.7 | 0.008 |
| Eicosapentaenoic acid (%) ǁ– mean | 0.4 | 0.3 | 0.5 | 0.3 | 0.72 |
| Linoleic acid (%) ǁ– mean | 8.5 | 3.0 | 9.1 | 2.6 | 0.25 |
| Arachidonic acid (%) ǁ– mean | 11.6 | 2.5 | 10.9 | 2.1 | 0.12 |
| Age first dose of study emulsion (d) – median (IQR) | 4.0 (3.0-6.0) |  | 4.0 (3.0-5.0) |  | 0.95 |
| Compliance of study emulsion \*\*(%) – median (IQR) | 91.7 (86.1-95.5) |  | 93.3 (87.2-96.4) |  | 0.23 |
| Days to full enteral feeds (d) – median (IQR) | 18.0 (12.0-26.5) |  | 13.0 (10.0-19.0) |  | 0.002 |

N3RO, N-3 fatty acids for improvement of Respiratory Outcomes trial; SD, standard deviation; LCPUFA, long chain polyunsaturated fatty acids; Apgar, Appearance, Pulse, Grimace, Activity, and Respiration score; CRIB, clinical risk index for babies; IQR, interquartile range

\* Children who were eligible to participate but did not participate in the follow-up study or who could not be contacted

† Data missing for 5 children in the N3RO follow-up study participants and 5 in the N3RO follow-up study non-participants

‡ Data missing for 13 children in the N3RO follow-up study participants and 10 in the N3RO follow-up study non-participants

§ Data missing for 1 child in the N3RO follow-up study participants and 2 in the N3RO follow-up study non-participants

ǁ Data missing for 1 child in each group

¶ Data missing for 1 child in the N3RO follow-up study non-participants

\*\* Data missing for 1 child in the N3RO follow-up study participants

**Supplemental Table 3. Sensitivity analysis of outcomes of assessments of attention (18 months’ corrected age) and Bayley-III scores (2-3 years’ corrected age) by treatment groups**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Outcome** | | | | **DHA**  **(n=37)**  **Mean** | | **SD** | | | **Control**  **(n=40)**  **Mean** | **SD** | **Adjusted** \*  **Mean difference (95% CI)** | ***P*** | **Adjusted** \*\*  **Mean difference (95% CI)** | ***P*** | |
| **Attention** | |  |  | |  | |  |
|  | ***Single object*** | | |  | |  | | |  |  |  |  |  | |  |
|  | Proportion of time spent looking at toy | | | 0.85 | | 0.15 | | | 0.87 | 0.12 | -0.02 (-0.09, 0.05) | 0.52 | -0.01 (-0.07, 0.04) | | 0.62 |
|  | Duration of time spent looking at toy (s) | | | 238.60 | | 61.72 | | | 251.42 | 39.40 | -11.51 (-36.25, 13.24) | 0.36 | -8.90 (-31.98, 14.17) | | 0.45 |
|  | Number of episodes of looking at toy | | | 9.70 | | 4.53 | | | 9.48 | 5.73 | -0.40 (-3.07, 2.27) | 0.77 | -0.26 (-2.35, 1.83) | | 0.81 |
|  | Average duration of looking at toy (s) | | | 38.36 | | 48.78 | | | 38.42 | 25.66 | -2.32 (-17.46, 12.82) | 0.76 | 1.75 (-13.74, 17.24) | | 0.82 |
|  | Longest look duration to toy (s) | | | 94.24 | | 62.47 | | | 91.79 | 41.90 | 5.35 (-18.14, 28.84) | 0.66 | 8.05 (-12.67, 28.77) | | 0.45 |
|  | ***Multiple object*** | | |  | |  | | |  |  |  |  |  | |  |
|  | Number of shifts in attention between toys | | | 41.14 | | 14.80 | | | 38.93 | 13.84 | 3.88 (-2.15, 9.91) | 0.21 | 2.96 (-2.26, 8.17) | | 0.27 |
|  | Duration of time spent looking at toys (s) | | | 229.98 | | 54.90 | | | 234.75 | 54.03 | -11.68 (-37.48, 14.12) | 0.37 | -2.19 (-25.00, 20.63) | | 0.85 |
|  | Number of episodes of looking away from toys | | | 10.49 | | 4.98 | | | 9.00 | 5.65 | 0.57 (-2.36, 3.51) | 0.70 | 0.99 (-1.60, 3.58) | | 0.45 |
|  | Average duration of looking at toys (s) | | | 4.86 | | 1.12 | | | 5.46 | 1.88 | -0.93 (-1.66, -0.19) | 0.01 | -0.56 (-1.24, 0.12) | | 0.10 |
|  | Proportion of time spent looking at toys | | | 0.87 | | 0.11 | | | 0.89 | 0.10 | -0.02 (-0.07, 0.03) | 0.46 | -0.02 (-0.06, 0.02) | | 0.38 |
|  | Longest look duration to the toys (s) | | | 32.67 | | 13.30 | | | 33.14 | 16.85 | -0.32 (-8.81, 8.17) | 0.94 | 0.35 (-6.73, 7.43) | | 0.92 |
|  | ***Distractibility*** | | |  | |  | | |  |  |  |  |  | |  |
|  | Latency to turn to a distractor when the child’s attention was focused (s) †, ‡ | | | 4.54 | | 2.03 | | | 4.41 | 1.83 | 0.13 (-0.83, 1.09) | 0.79 | **0.04 (-0.86, 0.94)** | | **0.93** |
|  | Proportion of turns to the distractor when the child’s attention was focused ‡ | | | 0.53 | | 0.35 | | | 0.54 | 0.32 | -0.01 (-0.17, 0.16) | 0.93 | 0.00 (-0.16, 0.16) | | 0.98 |
|  | Latency to turn to a distractor when the child’s attention was casual (s) | | | 2.06 | | 1.40 | | | 2.05 | 1.37 | -0.23 (-0.88, 0.43) | 0.50 | 0.06 (-0.58, 0.70) | | 0.85 |
|  | Proportion of turns to the distractor when the child’s attention was casual | | | 0.85 | | 0.19 | | | 0.85 | 0.21 | 0.03 (-0.06, 0.12) | 0.49 | -0.01 (-0.10, 0.08) | | 0.89 |
|  | Duration of looking to the distractor when ON (s) | | | 95.68 | | 43.83 | | | 83.15 | 48.36 | 17.76 (-3.54, 39.06) | 0.10 | 12.10 (-8.21, 32.42) | | 0.24 |
|  | Duration of looking to the distractor when OFF (s) | | | 42.54 | | 22.55 | | | 37.74 | 30.36 | 3.01 (-9.55, 15.57) | 0.64 | 1.91 (-10.09, 13.90) | | 0.76 |
| **Bayley-III** | | | |  | |  | | |  |  |  |  |  | |  |
|  | Cognitive score § | | | 100.30 | | 13.76 | | | 99.45 | 11.31 | -1.41 (-8.03, 5.21) | 0.68 | NA | | NA |
|  | Language score ǁ | | | 96.6 | | 12.97 | | | 93.24 | 14.89 | 1.23 (-5.65, 8.10) | 0.73 | NA | | NA |
|  | Motor score § | | | 98.85 | | 8.88 | | | 95.90 | 10.27 | 0.84 (-3.97, 5.66) | 0.73 | NA | | NA |

DHA, docosahexaenoic acid; SD, standard deviation; CI, confidence interval

\* Adjusted for stratification variables of the N3RO trial (sex, gestational age category (< 27 weeks or 27 to < 29 weeks) and centre (Flinders Medical Centre and Women’s and Children’s Hospital)), maternal smoking during pregnancy, maternal further education, concentration of eicosapentaenoic acid in infant whole blood at baseline.

\*\*Adjusted for stratification variables of the N3RO trial (sex, gestational age category (< 27 weeks or 27 to < 29 weeks) and centre (Flinders Medical Centre and Women’s and Children’s Hospital)) and corrected age of child at assessment

† Primary outcome of the attention assessment

‡ Data missing for 2 children in each group

§ Data missing for 10 children in the DHA group and 11 children in the control group

ǁ Data missing for 11 children in each group

**Supplemental Table 4. Sensitivity analysis using Wilcoxon Rank Sum Tests**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Outcome** | | | **DHA (n=37)** | | **Control (n=40)** | ***P*** |
|  | | | **Median (IQR)** | | |  |
| **Attention** | |
|  | ***Single object*** | | |  |  |  |
|  | Proportion of time spent looking at toy | | | 0.89 (0.82-0.95) | 0.93 (0.77-0.96) | 0.46 |
|  | Duration of time spent looking at toy (s) | | | 258.52 (210.90-285.20) | 267.94 (217.24-281.86) | 0.68 |
|  | Number of episodes of looking at toy | | | 10.00 (6.00-13.00) | 8.50 (5.00-12.00) | 0.48 |
|  | Average duration of looking at toy (s) | | | 24.50 (17.76-47.53) | 30.21 (18.47-55.97) | 0.31 |
|  | Longest look duration to toy (s) | | | 79.24 (48.56-113.60) | 79.86 (62.90-111.06) | 0.61 |
|  | ***Multiple object*** | | |  |  |  |
|  | Number of shifts in attention between toys | | | 42.00 (32.00-50.00) | 39.50 (31.50-47.00) | 0.59 |
|  | Duration of time spent looking at toys (s) | | | 236.72 (206.64-277.44) | 249.20 (222.19-270.16) | 0.83 |
|  | Number of episodes of looking away from toys | | | 10.00 (8.00-14.00) | 8.00 (5.50-12.00) | 0.10 |
|  | Average duration of looking at toys (s) | | | 4.58 (4.08-5.75) | 5.12 (4.12-6.38) | 0.21 |
|  | Proportion of time spent looking at toys | | | 0.89 (0.81-0.95) | 0.92 (0.87-0.96) | 0.28 |
|  | Longest look duration to the toys (s) | | | 29.60 (24.56-38.92) | 31.00 (22.42-38.68) | 1.0 |
|  | ***Distractibility*** | | |  |  |  |
|  | Latency to turn to a distractor when the child’s attention was focused (s) \*, † | | | 5.00 (3.24-6.31) | 4.85 (3.22-5.85) | 0.64 |
|  | Proportion of turns to the distractor when the child’s attention was focused † | | | 0.50 (0.20-0.82) | 0.50 (0.25-0.83) | 0.96 |
|  | Latency to turn to a distractor when the child’s attention was casual (s) | | | 1.89 (0.90-2.88) | 1.55 (1.03-2.86) | 0.89 |
|  | Proportion of turns to the distractor when the child’s attention was casual | | | 0.92 (0.79-1.00) | 0.93 (0.78-1.00) | 0.93 |
|  | Duration of looking to the distractor when ON (s) | | | 93.60 (63.96-126.22) | 65.50 (45.48-126.58) | 0.18 |
|  | Duration of looking to the distractor when OFF (s) | | | 37.04 (25.72-59.84) | 30.50 (17.50-45.68) | 0.11 |
| **Bayley-III** | | | |  |  |  |
|  | Cognitive score ‡ | | | 100.00 (90.00-105.00) | 100.00 (95.00-105.00) | 0.90 |
|  | Language score*5* | | | 97.00 (89.00-103.00) | 94.00 (86.00-100.00) | 0.46 |
|  | Motor score ‡ | | | 100.00 (94.00-103.00) | 97.00 (88.00-103.00) | 0.44 |

DHA, docosahexaenoic acid; IQR, Interquartile range

\* Primary outcome of the attention assessment

† Data missing for 2 children in each group

‡ Data missing for 10 children in the DHA group and 11 children in the control group

§ Data missing for 11 children in each group