

Evaluation of Sex-Specific Effects of Nutrition in Children born Early: An Individual Participant Data Meta-Analysis (ESSENCE-IPD)

STATISTICAL ANALYSIS PLAN



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2 ABBREVIATIONS AND DEFINITIONS

|  |  |
| --- | --- |
| ESSENCE-IPD | Evaluation of Sex-Specific Effects of Nutrition in Children born Early: An Individual Participant Data Meta-analysis |
| CI | Confidence interval |
| IPD-MA | Individual participant data meta-analysis |
| MD | Mean difference |
| RR | Risk ratio |
| UCI | Upper confidence interval |
| LCI | Lower confidence interval |

3 INTRODUCTION AND OVERVIEW

3.1 Study overview

Babies born preterm and small are at increased risk of poor growth, slow development and disability. As adults, they are at increased risk of obesity, diabetes, and heart disease. Providing small babies with enhanced nutrition soon after birth is associated with improved early growth and better cognitive development, but at the expense of increased risk for later adiposity, metabolic and cardiovascular disease. These effects appear to differ in girls and boys, with limited evidence from both experimental animal and human studies that boys may benefit, but girls may potentially be disadvantaged, by supplementation. Unfortunately, most clinical studies have not reported findings separately by sex and are not adequately powered to do so.

Aggregate data analyses identified in systematic reviews are limited by within trial variation in gestational age of the infants, co-morbidities of the infants, starting point and duration after birth of the intervention, macronutrient content of the intervention, comparisons used, and a range of outcomes and their definitions. Few trials describe multiple subgroups making aggregation of data almost impossible. Individual participant data meta-analysis (IPD-MA) can overcome some of these limitations.

Randomized trials that studied the effect of nutritional supplements on preterm or small babies and collected outcome data after discharge will be included in this IPD-MA. This IPD-MA will combine and re-analyse these data using consistent agreed outcome definitions and internationally standardised methods. The analysis will also explore subgroups including the effect of infant sex as well as the type and duration of the supplements. By undertaking this study, we hope to determine the benefits and risks of different supplement strategies for preterm and small girls and boys. This will inform the design of future randomised clinical trials to improve the growth, development and health of vulnerable babies.

3.2 Aims

To assess, using IPD-MA, the effects of macronutrient supplements in nutrition of preterm and small-for-gestational age infants on developmental, metabolic and growth outcomes after hospital discharge, according to pre-specified participant and treatment characteristics including:

* Infant sex
* Composition of the supplement
* Timing of the supplement
* Duration of the use of the supplement

3.3 Inclusion and exclusion criteria for studies

Published and unpublished randomised and quasi-randomised trials will be included if the intervention was intended to increase the intake of one or more macronutrients (protein, carbohydrate, fat, energy content or protein to energy ratio) with the primary aim of improving growth and development of the infant.

# 4 ANALYSIS

4.1 Analysis overview and general principles

This statistical analysis plan outlines the intended approach to analysis of the ESSENCE-IPD data and specifies the primary and secondary objectives and outlines intended exploratory analyses. It is anticipated that additional review of this approach will be required after the majority of the data are received and curated by the IPD Project Team. This review will be made blinded to outcome or any association analysis but will require inspection of the completeness of data from individual studies in major sub-groups. The final statistical analysis plan will be agreed by the Collaborative Group following these inspections.

All analyses will be based on the checked and updated individual participant data from all available trials. All randomised participants with outcome data available will be included in the analysis where possible (see section 4.1.1 Missing data). Analyses will be performed on an intention-to-treat basis with infants analysed in the treatment group to which they were randomised, regardless of the intervention received.

For each of the outcomes a one stage approach to analysis will be taken so that the individual participant data from all eligible trials are included in a single model. Fitting a single model for each outcome variable will enable the variation across trials to be accounted for within the model. A treatment by trial interaction term will be tested to assess heterogeneity of treatment effect across trials. If excessive statistical heterogeneity in treatment effect or inconsistency across trials is detected (i.e. if the trial by treatment interaction term is significant), then the rationale for combining trials will be questioned and the source of heterogeneity explored. Where the source of the heterogeneity can be determined those studies which are homogeneous will be pooled and the presented as sub-groups for all analyses.

Three separate models will be constructed to meet the aims of this IPD. All will include the nutritional intervention (control v supplemented groups) and sex and their interaction as effects. Data will be presented as relative risk (95% CI) for the overall intervention effect and separately for boys and girls and the significance level of the interaction compared against the appropriate critical value of P for a two sided test (see section 4.1.2 Multiplicity).

Additional models will be constructed which include (separately) supplement composition, supplement duration and supplement timing as main effects. Within each of these models the interactions will be sought with treatment and treatment by sex. If, in any model the main or interaction effects of sex fail to reach significance, reduced models will be fitted to efficiently explore non sex-specific effects of supplement composition, duration or timing on outcome.

Within each model significance will be formally tested against the appropriate critical value of P for a co-primary or secondary outcome (see section 4.1.2 Multiplicity).

In general, within each of the analysis approaches discussed above, binary outcomes will be analysed using log binomial regression models and results will be presented as risk ratios (RR) with 95% confidence intervals (CI) and associated two-sided p values. Continuous data will be analysed using general linear regression models and data will be reported as mean differences (MD) with 95% confidence Intervals (CI) and associated 2-sided p values. Study and infants from multiple births will be accounted for as a random effect in mixed-effect models. Categorical outcomes will be analysed using a proportional odds model approach (ordered logistic regression). The proportional odds/parallel regression assumption will be verified. Should the proportional odds assumption not be met different models will be fitted to describe the relationship between each pair of outcome groups. Data will be separated into the following subgroups; infancy, early childhood, later childhood, early adolescent, late adolescent and adulthood, and the outcomes inspected for heterogeneity across all studies.

The effects of the sex of the infant will be explored by presenting data separately for each sex as pre-specified subgroups, and by testing the treatment by sex interaction term within the model.

Analysis of raw growth measurements (head circumference, weight, and length) will be adjusted for corrected post-menstrual age and sex. Analysis of WHO calculated Z-scores of growth measurements will be unadjusted for age and sex.1

If data do not meet the assumptions of any model or if models fail to converge then standard alternative analyses will be explored (ie pooling subgroups, stratified analysis, alternative link functions).

4.1.1 Missing data

It is expected that the data available from separate studies will differ and therefore we could not expect to have an analysis dataset comprising all the requested components of the dependent variables nor all the independent variables. Data from infants will be included where possible if there are sufficient data to determine whether the outcome of interest is met or not and if there are complete data for adjustment for the pre-specified potential confounders. Should these criteria not be met that individual cannot be included in the analysis. Where possible, the reasons for missing data will be explored. It is not proposed to impute missing data since the assumption of “missing at random” is unlikely to be met.

### 4.1.2 Multiplicity

A large number of outcomes are being investigated in this study. This increases the chance of observing ’false positive’ results. However, the overall probability of a type 1 error is maintained at 5% for the two co-primary outcomes by testing each at 0.025 (i.e. splitting the critical P value evenly). No further adjustment for multiplicity is planned for comparisons made in secondary and exploratory analyses.

All outcomes are important in giving the full clinical picture of the likely benefits and risks of supplements for preterm and small infants. Results will be considered for groups of related outcomes, as well as individually. In this way no single result will be considered in isolation. Any result showing a significant effect, where related outcomes do not, will be interpreted with caution.

## 

## 4.2 Primary outcomes

## Outcomes have been selected to reflect the most clinically important measures of safety and effectiveness for this population group.

## Primary outcome:

## The co-primary outcomes will be cognitive impairment and metabolic risk.

## 1. Cognitive impairment:

* Below -1SD on standard tests of development (infancy) or cognition/intelligence quotient (later ages)

## 2. Metabolic risk: Any of the following (definitions to be confirmed)

* Metabolic outcomes
* Elevated plasma triglyceride concentrations
* Low high-density lipoprotein concentrations
* Elevated low-density lipoprotein concentrations
* Elevated fasting plasma glucose concentration
* Insulin resistance (investigator defined)
* Impaired glucose tolerance
* Diagnosis of type 2 diabetes
* Growth outcomes
* Overweight/obese
* Increased waist circumference
* Increased fat mass or fat mass percentage
* Cardiovascular risk outcomes
* High blood pressure
* Impaired flow-mediated vasodilatation (investigator defined)

## 4.3 Secondary outcomes

***All the secondary outcomes will be collected and categorized in the period of infancy, early childhood, later childhood, early adolescent, late adolescent and adulthood.***

* Cerebral palsy (any cerebral palsy, as defined by Collaborative Group),
* Severity of cerebral palsy (mild, moderate, severe – as defined by Collaborative Group),
* Developmental delay or intellectual impairment (none, mild (≤ 1 SD below test mean), moderate (≤ 2 SD below test mean, severe (≤ 3 SD below test mean), or as defined by Collaborative Group),
* Visual impairment (none, mild, moderate, severe; as defined by Collaborative Group),
* Deafness (none, mild, moderate, severe; as defined by Collaborative Group),
* Motor dysfunction (none, mild, moderate, severe; as defined by Collaborative Group or by the ABC or Gross Motor Classification System if available),
* School performance (as defined by Collaborative Group),
* Measures of psychological well-being,
* Growth assessments
* weight (raw data and z scores)
* length/height (raw data and z scores)
* head circumference (raw data and z scores)
* ponderal Index
* body mass index (BMI)
* waist circumference
* overweight/obese
* body composition (fat mass, fat free mass, lean fat mass, skinfold thickness, bioimpedance, DEXA)
* Cardiovascular risk outcomes
* blood pressure
* flow-mediated vasodilatation
* measures of sympathetic and parasympathetic tone e.g. heart rate variability
* cardiac size and structure,
* Metabolic outcomes
* type-2 diabetes
* blood lipid profiles (triglycerides, HDL, LDL, HDL:LDL)
* fasting blood glucose concentrations
* insulin concentrations
* insulin resistance
* glucose tolerance
* IGF-1 concentration,
* Bone development
* bone mineral content
* volumetric bone mineral density
* fractures,
* Brain development
* whole brain, white matter and grey matter volumes and volumes of individual brain regions
* brain maturation measured using MRI (white matter tracts, measures of diffusivity, myelination, surface folding)
* functional brain imaging,
* Health outcomes
* allergies (eczema, asthma, hayfever)
* respiratory function
* hospitalisation (duration)
* health care utilisation,
* Nutrition
* feeding tolerance
* intake (milk, energy)
* appetite
* breast feeding,
* Death – neonatal or later death up to the time of follow-up and cause of death,
* Quality of life,
* General health and use of healthcare resources,
* Adverse Events,
* Cost

## 4.4 Additional analyses

### 4.4.1 Subgroup analyses

Where data are available we will conduct subgroup analyses for the primary outcome and its components and for cardiovascular and metabolic risk factors. Differences between subgroups will be assessed and reported using the subgroup interaction term within the model.

Subgroups:

1. Sex of infant – male vs female. The subgroup of sex will be used for all outcomes, not just the primary outcome.
2. Size of infant at birth (≤ 1kg vs > 1kg at birth).
3. Size for gestation of the infant (≤ 10th centile vs > 10th centile or as defined by Collaborative Group).
4. Gestational age of infant at birth (≤ 28 completed weeks vs 28 to 32 completed weeks vs 33 to 36 completed weeks or as defined by the Collaborative Group).
5. Timing of supplement - from birth versus from start of enteral feeding versus from achievement of a specified minimum volume of enteral feeds versus post discharge (The feed volume is specified by the trialists).
6. Type of supplement (protein vs carbohydrate vs fat vs multicomponent and their interactions).
7. Breast milk vs formula as primary milk feed.
8. Duration of supplement (1-2 weeks vs 3-6 weeks vs more than 7 weeks or as defined by the Collaborate Group).
9. Different epochs (conducted up to the year of 2000 vs conducted in or after the year of 2001).

### 4.4.2 Adjusted analyses

#### Adjustment for covariates and potential confounders

Baseline trial entry variables known or suspected *a priori* to be moderately associated with the primary outcome will be adjusted for as covariates in a sensitivity analysis. The covariates adjusted for will include gestational age, birth weight z score and socioeconomic status.

### 4.4.3 Sensitivity analyses

The following sensitivity analyses will be conducted on the primary outcomes to assess the robustness of conclusions;

1. Exclusion of trials with an overall assessment of high risk of bias.
2. Exclusion of trials with high rates of participant exclusions during the trial, where losses were considered to have the potential to impact on the results.
3. Exclusion of trials with high rates of loss to follow-up (follow-up includes < 70% of those randomised)

Where eligible trials are unable to contribute data to the IPD we will assess the robustness of the inclusion or exclusion of these trials by combining their aggregate data with the IPD.

### 4.4.4 Additional analyses

Any subgroup analyses of variables which are continuous (and were split into multiple categories for the subgroup analyses) will also be investigated in their continuous form to determine the shape of the relationship if one appears to be indicated in the subgroup forest plot (e.g. U-shape, linear trend).

### 4.4.5 Multiple linear regression

Where data are available, multiple linear regression will be used to explore if growth trajectories predict the development of cognitive and metabolic outcomes. Growth trajectories will be presented using unadjusted mean z scores of estimates of growth from birth to age 2 years. The ANOVA test will be used to assess whether the independent variables collectively predicts the dependent variable. R-squared—the multiple correlation coefficient of determination—will be reported and used to determine how much variance in the dependent variable can be accounted for by the set of independent variables.  The t test will be used to determine the significance of each predictor and beta coefficients will be used to determine the magnitude of prediction for each independent variable.

### Reference

1. Computation of centiles and z-scores WHO Child Growth Standards: Length/Height-for-age, Weight-for-age, Weight-for-length, Weight-for-height and Body Mass Index-for age. Geneva: WHO; 2006:301-304.

5 SHELL TABLES

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## Table 1 Characteristic of the included trials

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Trial name | Author and year | Country | Population | Population  (N) | Intervention | Control | Intervention  (N) | Control  (N) | Boys in Intervention group (n) | Boys in Control group (n) | Duration of follow-up | Outcomes |
| Trial 1 |  |  |  |  |  |  |  |  |  |  |  |  |
| Trial 2 |  |  |  |  |  |  |  |  |  |  |  |  |
| … |  |  |  |  |  |  |  |  |  |  |  |  |
| Trial n |  |  |  |  |  |  |  |  |  |  |  |  |

## Table 2 Summary table: primary outcomes

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Outcome |  | Intervention  n/N | Control  n/N | RR | LCL | UCL | P value |
| Cognitive impairment  Metabolic risk | Overall |  |  |  |  |  |  |
| Girls |  |  |  |  |  |  |
| Boys  Overall  Girls  Boys |  |  |  |  |  |  |

## Table 2.1 Primary outcomes – results of individual trial

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Trial name |  | Intervention  n/N | Control  n/N | RR | LCL | UCL | P value |
| Trial 1 | Overall |  |  |  |  |  |  |
|  | Girls |  |  |  |  |  |  |
|  | Boys |  |  |  |  |  |  |
| Trial 2 | Overall |  |  |  |  |  |  |
|  | Girls |  |  |  |  |  |  |
|  | Boys |  |  |  |  |  |  |
| … |  |  |  |  |  |  |  |
| Trial n | Overall |  |  |  |  |  |  |
|  | Girls |  |  |  |  |  |  |
|  | Boys |  |  |  |  |  |  |

## Table 3 Summary table: binary secondary outcomes

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Outcome |  | Intervention  n/N | Control  n/N | RR | LCL | UCL | P value |
| Death | Overall |  |  |  |  |  |  |
| Girls |  |  |  |  |  |  |
| Boys |  |  |  |  |  |  |
| Cerebral palsy | Overall |  |  |  |  |  |  |
| Girls |  |  |  |  |  |  |
| Boys |  |  |  |  |  |  |
| … |  |  |  |  |  |  |  |

## Table 4 Summary table: continuous secondary outcomes

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Outcome |  | Intervention  [mean (SD)] | Control  [mean (SD)] | MD | LCL | UCL | P value |
| Weight z score at ?? | Boys |  |  |  |  |  |  |
| Girls |  |  |  |  |  |  |
| Overall |  |  |  |  |  |  |
| Weight at ?? | Boys |  |  |  |  |  |  |
| Girls |  |  |  |  |  |  |
| Overall |  |  |  |  |  |  |
| Length z score at ?? | Boys |  |  |  |  |  |  |
| Girls |  |  |  |  |  |  |
| Overall |  |  |  |  |  |  |
| Length at ?? | Boys |  |  |  |  |  |  |
| Girls |  |  |  |  |  |  |
| Overall |  |  |  |  |  |  |
| Head circumference z score at ?? | Boys |  |  |  |  |  |  |
| Girls |  |  |  |  |  |  |
| Overall |  |  |  |  |  |  |
| Head circumference at ?? | Boys |  |  |  |  |  |  |
| Girls |  |  |  |  |  |  |
| Overall |  |  |  |  |  |  |

## Table 5 Summary table: categorical secondary outcomes

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Outcome |  |  | Intervention  n/N | Control  n/N | RR | LCL | UCL | P value |
| Cerebral Palsy | Mild | Overall |  |  |  |  |  |  |
| Girls |  |  |  |  |  |  |
| Boys |  |  |  |  |  |  |
| Moderate | Overall |  |  |  |  |  |  |
| Girls |  |  |  |  |  |  |
| Boys |  |  |  |  |  |  |
| Severe | Overall |  |  |  |  |  |  |
| Girls |  |  |  |  |  |  |
| Boys |  |  |  |  |  |  |
| Developmental delay | Mild | Overall |  |  |  |  |  |  |
| Girls |  |  |  |  |  |  |
| Boys |  |  |  |  |  |  |
| Moderate | Overall |  |  |  |  |  |  |
| Girls |  |  |  |  |  |  |
| Boys |  |  |  |  |  |  |
| Severe | Overall |  |  |  |  |  |  |
| Girls |  |  |  |  |  |  |
| Boys |  |  |  |  |  |  |
| … |  |  |  |  |  |  |  |  |

## Table 6 Subgroup analyses (Primary outcomes)

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Subgroup |  |  | Intervention  n/N | Control  n/N | RR | LCL | UCL | heterogeneity p value |
| Size of infant at birth | ≤ 1kg | Overall |  |  |  |  |  |  |
| Girls |  |  |  |  |  |  |
| Boys |  |  |  |  |  |  |
| > 1kg | Overall |  |  |  |  |  |  |
| Girls |  |  |  |  |  |  |
| Boys |  |  |  |  |  |  |
| Size for gestation of the infant | ≤ 10th centile | Overall |  |  |  |  |  |  |
| Girls |  |  |  |  |  |  |
| Boys |  |  |  |  |  |  |
| > 10th centile | Overall |  |  |  |  |  |  |
| Girls |  |  |  |  |  |  |
| Boys |  |  |  |  |  |  |
| … |  |  |  |  |  |  |  |  |

### Table 6.1 Subgroup analyses (Death)

### Table 6.2 Subgroup analyses (Cerebral Palsy)

### …

### Table 6.n Subgroup analyses (Adverse Event)

## Table 7 Sensitivity analyses

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Analysis |  | Intervention  n/N | Control  n/N | RR | LCL | UCL | p value |
| Sensitivity analysis 1 | Overall |  |  |  |  |  |  |
| Girls |  |  |  |  |  |  |
| Boys |  |  |  |  |  |  |
| Sensitivity analysis 2 | Overall |  |  |  |  |  |  |
| Girls |  |  |  |  |  |  |
| Boys |  |  |  |  |  |  |