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Complete List of Authors:	Lin, Luling; The University of Auckland Liggins Institute, Crowther, Caroline; The University of Auckland Liggins Institute Gamble, Greg; The University of Auckland Liggins Institute Bloomfield, Frank; The University of Auckland Liggins Institute Harding, Jane; University of Auckland Liggins Institute
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Sex-specific effects of nutritional supplements in infants born early or small: Protocol for an individual participant data meta-analysis (ESSENCE IPD-MA)

Luling Lin (0000-0002-8448-1504)[1], <luling.lin@auckland.ac.nz>

Caroline A. Crowther[1], <c.crowther@auckland.ac.nz>

Gregory D. Gamble (0000-0003-0412-3203)[1], <gd.gamble@auckland.ac.nz>

Frank H. Bloomfield[1], <f.bloomfield@auckland.ac.nz>

Jane E. Harding (0000-0003-2697-1422) [1], <j.harding@auckland.ac.nz>

for **the ESSENCE IPD-MA Group[2]**

[1] Liggins Institute, University of Auckland, Auckland, New Zealand

[2] Membership of the ESSENCE IPD-MA Group is listed in the Contribution section

Corresponding author

Jane E. Harding

Liggins Institute, University of Auckland

Building 503, Level 2, 85 Park Road, Auckland P Private Bag 92019, Auckland 1142, New Zealand

E-mail: j.harding@auckland.ac.nz

Telephone: +6499236439

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ABSTRACT

Introduction

Preterm and small for gestational age (SGA) infants are at increased risk of poor growth, disability and delayed development. While growing up they are also at increased risk of obesity, diabetes and later heart disease. The risk of such adverse outcomes may be altered by how preterm and SGA infants are fed after birth. Faltering postnatal growth is common due to failure to achieve recommended high energy and protein intakes, and thus preterm and SGA infants are often provided with supplemental nutrition soon after birth. Enhanced nutrition has been associated with improved early growth and better cognitive development. However, limited evidence suggests that faster growth may increase the risk for later adiposity, metabolic and cardiovascular disease, and that such risks may differ between girls and boys.

Methods and analysis

We will search OvidMedline, Embase, Cochrane CENTRAL, Cochrane Database of Systematic Reviews, controlled-trials.com, clinicaltrials.gov and anzctr.org.au for randomised trials that studied the effects of macronutrient supplements for preterm and SGA infants on developmental, metabolic and growth outcomes after hospital discharge. The co-primary outcomes will be cognitive impairment and metabolic risk. Individual participant data (IPD) from all available trials will be included using an intention-to-treat approach. A one-stage procedure for IPD meta-analysis (MA) will be used, accounting for clustering of participants within studies. Exploratory subgroup analyses will further investigate sources of heterogeneity, including sex and size of infants, different timing, duration and type of supplements.

Ethics and dissemination: This IPD-MA is approved by the University of Auckland Human Participants Ethics Committee (reference number: 019874). Individual studies have approval from relevant local ethics committees. Results will be disseminated in a peer-reviewed journal and presented at international conferences.

PROSPERO registration number: CRD42017072683

Strengths and limitations of this study

- This IPD-MA will synthesise data from multiple randomised clinical trials using uniformly defined relevant outcomes for all available trials.
- Subgroup analyses may allow detection of potential sex differences that could not be achieved through aggregate analyses.
- This IPD-MA will explore the interactions between treatment and participant-level characteristics.
- A potential limitation is that the analyses planned will depend on obtaining the relevant individual participant data.
- There may be considerable heterogeneity as interventions are likely to be very varied and the trials performed over several decades.

INTRODUCTION

Infants born preterm or small for gestational age (SGA) are at increased risk of poor growth, disability and delayed development. [1-3] As adults, they are at increased risk of obesity, diabetes and later heart disease.[4] How infants born small are fed after birth may alter the risk of these adverse outcomes. Providing preterm and SGA infants with enhanced nutrition soon after birth is associated with improved early growth and better cognitive development,[5-7] but observational data suggest that early faster growth may increase the risk for later adiposity, metabolic and cardiovascular disease.[8]

It has been recognised for centuries that girls and boys grow differently, experience different metabolic and endocrine milieux, and have different cognitive and health outcomes. Little attention has been paid to improving outcomes following preterm birth by treating girls and boys differently, although it is well recognised that preterm boys compared to girls have higher mortality and morbidity,[9] and are more likely to have adverse developmental and educational outcomes.[10] There is substantial evidence that perinatal insults can result in different adult phenotypes in males and females.[11] For example, animal studies across many different species after a wide variety of prenatal insults show that males are more likely than females to exhibit adverse effects such as impaired renal function, hypertension, insulin resistance, altered hypothalamic-pituitary-adrenal (HPA) axis function and altered growth in later life.[12] The reasons for this sex difference in vulnerability to early environmental perturbations are not well understood, but may include faster growth and hence greater substrate demands in males, altered tempo of maturation, different exposure to sex steroids and sex-specific epigenetic mechanisms.[12] Unfortunately, most clinical studies have not reported findings separately by sex and are not adequately powered to do so. Further, because the majority of animal experiments are done in polytocous species, prenatal and postnatal sex effects cannot be separated in mixed-sex litters. There is little reliable evidence about how best to feed preterm babies to optimise both short- and long-term health outcomes, and almost none about targeting nutrition by sex.

Hypotheses

The effects of early nutritional supplements on post-discharge development, markers of metabolic risk and growth are different in girls and boys.

Need for individual participant data meta-analysis

Systematic reviews using aggregate data meta-analyses are limited due to within-trial variation in gestational age of the infants at birth, co-morbidities of the infant, starting time and duration of the intervention, macronutrient content of the intervention and control groups. Outcomes within aggregate meta-analyses such as cerebral palsy, motor dysfunction or hearing loss also include a range of severity of disability. Aggregate meta-analyses tend to vary in completeness and in the definitions used for the outcomes. Not all trials combine the same outcomes in composite outcomes or use the same measures of neuro-developmental outcome. Few trials describe multiple subgroups, making meta-analysis of data almost impossible. Importantly, few trials to date have provided the sex of the infant as a subgroup variable.

One method to ameliorate some of the limitations of aggregate data meta-analysis is to combine the large volume of individual trial data available to perform an individual participant data (IPD) meta-analysis (MA). The estimates of treatment effects with an IPD-MA often differ from aggregate meta-analyses.[13] IPD-MA allows the inclusion of additional unpublished data provided by the trialists, and allows consistent re-categorisation of definitions of outcomes and populations in order to answer the clinical questions of interest. Further, IPD-MA may allow more detailed meta-analysis of key outcomes, taking into account both subject-level and study-level sources of heterogeneity in treatment effects. Thus, IPD-MA offers the potential to help clarify the sex-specific effects of early macronutrient supplements provided to preterm and SGA infants.

METHODS AND ANALYSIS

This study will use an IPD-MA approach and follow the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[14]

Aims

To assess, using IPD-MA, the effects of macronutrient supplements in nutrition of preterm and SGA infants on developmental, metabolic and growth outcomes after hospital discharge, and in particular, whether these effects differ in girls and boys.

Criteria for inclusion and exclusion

We will include published and unpublished randomised and quasi-randomised trials without restrictions on date of publication or language. Trials that studied infants born preterm (< 37 weeks' gestation) or born small (birthweight <2.5 kg or < 10th centile for gestational age) and in which 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 will be included.

Interventions can be enteral or parenteral or a combination, commence at any time during the hospitalisation or after discharge from hospital, and must be provided for a minimum duration of one week. Trials that report on comparisons between un-supplemented nutrition and supplemented nutrition with parenteral supplements, human breast milk supplements, formula milk or other macronutrients will be eligible for inclusion, for example: 1) Parenteral formulation A versus parenteral formulation B with different macronutrient composition; 2) Human milk (mother's own or donor) versus supplemented human milk (mother's own or donor); 3) Human milk (mother's own or donor) versus formula milk (term or preterm); 4) Supplemented human milk (mother's own or donor) versus formula (term or preterm); 5) Supplemented human milk A (mother's own or donor) versus supplemented human milk B (mother's own or donor); 6) Formula A versus formula B with different macronutrient composition (including preterm versus term formula, brand A versus brand B). We will exclude trials that examine the timing of the introduction of nutrition (early versus delayed feeding); that compare macronutrients of different composition (e.g. different types of lipids or proteins); studies whose outcomes focus on gastrointestinal development rather than growth and development; and studies reporting on variations in composition of

micronutrients (including sodium, potassium, calcium, phosphorus, vitamins, other minerals, amino acids, fatty acids).

Outcome data must be reported beyond term equivalent age (≥ 37 weeks' post-menstrual age) or following discharge from hospital after birth. Where the data are available, the outcomes will be categorised and evaluated in toddlers (less than 3 years), childhood (3 to 8 years), adolescence (9 to 18 years) and adulthood (more than 18 years).

The co-primary outcomes will be 1) Cognitive impairment: below -1 SD on standard tests of development (toddlers) or cognition/intelligence quotient (later ages) and 2) Metabolic risk *(See Appendix 1 for definitions)*: any of overweight/obese; increased waist circumference; increased fat mass or fat mass percentage; elevated plasma triglyceride concentrations; low high-density lipoprotein (HDL) concentrations; elevated low-density lipoprotein (LDL) concentrations; elevated fasting plasma glucose concentrations; insulin resistance; impaired glucose tolerance; diagnosis of type 2 diabetes; high blood pressure and impaired flow-mediated vasodilatation.

The secondary outcomes will be *(See Appendix 2 for definitions):* 1) Composite of survival free of any disability (including death, cerebral palsy, motor development delay or impairment, cognition/intelligence delay or impairment, language delay, visual impairment, hearing impairment); 2) Cognition/intelligence delay or impairment; 3) Cognition/intelligence scores; 4) Motor delay or impairment; 5) Motor scores; 6) Cerebral palsy (any); 7) Severity of cerebral palsy; 8) Visual impairment; 9) Hearing impairment; 10) School performance; 11) Measures of psychological well-being; 12) Metabolic outcomes: waist circumference, overweight/obese, type-2 diabetes, blood lipid concentrations (triglycerides, HDL, LDL, HDL:LDL), fasting blood glucose concentration, insulin concentration, insulin resistance (HOMA), glucose tolerance, IGF-1 concentration; 13) Cardiovascular risk outcomes: blood pressure (systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP)), flow-mediated vasodilatation, measures of sympathetic and parasympathetic tone, cardiac size and structure; 14) 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; 15) Growth outcomes: 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), body composition (fat mass, lean mass, as

measured by bioimpedance or Dual-energy X-ray absorptiometry (DXA) or skin fold thickness or air displacement plethysmography or other method); 16) Bone development: bone mineral content; volumetric bone mineral density and fractures; 17) Health outcomes: allergies (eczema, asthma, hayfever), respiratory function, hospitalisation (duration), health care utilisation; 18) Nutrition: feeding tolerance; intake (milk, energy), appetite, breast feeding; 19) Death (neonatal or later death up to the time of follow-up and cause of death); 20) Quality of life; 21) General health and use of healthcare resources; 22) Adverse Events; 23) Cost.

Search strategy

We will search OvidMedline, Embase, Cochrane Library Central Registry of Controlled Trials (CENTRAL), and Cochrane Database of Systematic Review from inception to identify eligible trials. We will also search for registered trials in Current Controlled Trials (www.controlled-trials.com), Clinical Trials (www.clinicaltrials.gov) and Australian and New Zealand Clinical Trials Registry (www.anzctr.org.au) to identify eligible ongoing studies. The full search strategy and search terms are available as online supplementary material (Appendix 1). Experts in the field and trialists will be asked if they can identify other published or ongoing trials. Potentially eligible trials that are not yet completed will not be included in this IPD-meta analysis, but will be noted for inclusion in future updates.

Identify studies

The eligibility of trials will be assessed by two researchers. Discrepancies will be resolved by discussion. If IPD are unavailable from any eligible trial it will be included in the IPD-MA using aggregate data for sensitivity analysis where possible.

Contact Authors

Authors of eligible studies will be invited to join the ESSENCE IPD-MA Collaborative group. We will identify contact information from the published trials. An initial email will be sent to the main trial author (corresponding author) providing them with the summary IPD-MA protocol. Another investigator from the study will be contacted if initial emails fail to receive a response, followed by phone calls if needed.

Quality assessment

We will assess the quality of the eligible trials using the methods specified in the Cochrane Handbook for Systematic Reviews of Interventions:[15] random sequence generation (selection bias); allocation concealment (selection bias); blinding of participants, personnel, and outcome assessment (performance and detection bias); incomplete outcome data (attrition bias); selective reporting (reporting bias); (6) Other bias (checking for bias due to problems not covered by (1) to (5) above).

Development of the database

We will develop a set of pre-specified and defined variables for IPD-MA at both the outcome, participant and trial level (Appendix 3). We will seek data on all randomised infants which will be coded for anonymity (date of birth, study centre); baseline data for narrative purposes (birthweight, gestational age at birth, plurality, sex); details of the intervention and comparator (date of randomisation, allocated intervention, type and composition of intervention and comparator, enteral or parenteral administration, age at start of intervention, weight at start of intervention, duration of intervention) and the outcomes listed above.

Trialists will provide de-identified data in any format which will be recoded as required, verified and checked for consistency with published data and stored on a secure, password protected file on the University of Auckland servers. Data will only be accessible by authorised personnel in the ESSENCE Data Management Group.

Methodological details of the individual trials will be cross-checked against published reports, trial registration (where available) and trial data collection forms. Where inconsistencies are identified, discussions will be held with individual trial groups to attempt to resolve these. Each trial final dataset to be used in the IPD-MA will be returned to the trialists for verification. Trials will then be analysed individually using IPD-MA pre-specified variables and outcomes and the results returned to the trialists for verification. The individual trial datasets will then be combined to form the ESSENCE-IPD dataset and the IPD meta-analysis undertaken.

Data synthesis and statistical analysis

A detailed statistical analysis plan will be prepared and agreed on by the ESSENCE-IPD team.

We will use a one-stage approach to the analysis of each outcome so that the IPD from all eligible trials are included in a single model. We will make an assessment of heterogeneity to decide if combining data from trials is appropriate or if heterogeneity, if significant, can be explained.

Binary outcomes will be analysed using log binomial regression models and data will be reported as Risk Ratio (RR) with 95% Confidence Intervals (CI) and associated 2-sided p values. Continuous data will be analysed using linear regression models and data will be reported as mean differences (MD) with 95% Confidence Intervals (CI) and associated 2-sided p values.

A large number of outcomes are being investigated in this study. This increases the chance of observing false positive results. The overall probability of a type 1 error will be 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.

We will explore the effects of the sex of the infants by presenting data separately for each sex as pre-specified subgroups, and by testing a treatment by sex interaction term within the model.

Where data are missing, those infants will be removed from the analysis and, 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. Where there are large amounts of missing data or trials are unable to provide IPD we will conduct sensitivity analyses to explore the effect of removing such trials from the analysis.

Statistical analyses will be performed using SAS (v.9.4, SAS Institute, Cary, NC, USA).

Planned subgroup analysis

Where data are available, we will conduct subgroup analyses to explore whether the effects of supplements differ between subgroups and test for interaction terms.

1. Sex of infant (boys vs girls);
2. Size of infant at birth (≤ 1 kg vs >1 kg at birth);
3. Size for gestation of the infant (≤ 10th centile vs > 10th centile);
4. Gestational age of infant at birth (≤ 28 completed weeks vs 29 to 32 completed weeks vs 33 to 36 weeks).
5. Timing of supplement:

   In hospital nutrition: the intervention was commenced in hospital or on average ended at 42 weeks' postmenstrual age or earlier;

   Post-discharge nutrition: the intervention was commenced after discharge or on average started at or after 36 weeks' postmenstrual age;

   Both in hospital and post-discharge nutrition: the intervention was commenced in the hospital and continued post-discharge.
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 to 2 weeks vs 3 to 6 weeks vs more than 7 weeks).
9. Different epochs (commenced up to the year of 2000 vs commenced in or after the year of 2001).

Planned sensitivity analyses

We will perform sensitivity analyses to assess whether the results are robust to the trial design by excluding trials assessed as high risk of bias.

Where 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.

Patient and Public Involvement

It was not appropriate or possible to involve patients or the public in the design, or conduct, or reporting, or dissemination of our research.

DISCUSSION

This will be the first IPD-MA to investigate the effects of early macronutrient supplements on preterm and SGA infants. IPD meta-analysis has been described as the 'gold standard' of systematic review methodology as it allows for more powerful and flexible analysis of both subgroups and outcomes.[16] This IPD-MA, using existing data from the individual trials, may reveal the sex-specific effects of macronutrient supplement on preterm and SGA infants, and will allow assessment of important interactions that cannot be tested in standard, aggregate data meta-analysis.

ETHICS AND DISSEMINATION

The shared data will be de-identified, the data files will be transferred by secure means, and stored in in a secure password protected area on a Auckland University server.

Final results will be presented to the ESSENCE IPD-MA collaborators prior to publication and public dissemination. Results of the study will be published in peer-reviewed journals and presented at national and international conferences.

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DECLARATIONS

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Ethical approval and consent to participate

The ESSENCE-IPD project has been approved by University Of Auckland Human Participants Ethics Committee.

Participants in the individual trials have previously given informed consent to participate in their respective trials. The data for these projects are to be used for the purpose for which they were originally collected and are available through an agreement between all trialists of the collaborative group. These trialists remain custodians of their original trial data at all times. A date sharing agreement will be signed by a representative of the institution that owns the data and a representative of University of Auckland.

Consent for publication

Not applicable

Availability of data and material

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

The Chair of the ESSENCE-IPD Project Team (J.E. Harding) wrote the first draft of the ESSENCE-IPD protocol. L Lin revised the subsequent versions of the ESSENCE-IPD protocol and prepared the initial draft of the manuscript. The ESSENCE-IPD Project Team

and the ESSENCE-IPD Management Group participated in the protocol development and commented on all drafts of the manuscript. The ESSENCE-IPD Trialist Group participated in the development of the IPD protocol and have read and approved the final draft of the manuscript.

Contributors

The ESSENCE IPD-MA Group

ESSENCE-IPD Project Team: This is the project Steering Group which is responsible for the day-to-day management of the IPD. This group has drafted the IPD protocol, will liaise with trialists and prepare the draft publications. J.E. Harding (chair of ESSENCE-IPD project); L Lin; C.A. Crowther; F.H. Bloomfield; and G Gamble.

ESSENCE-IPD Management Group: This group is convened by the Chair of the Project Team and comprises the IPD-MA statistician and data manager who will be responsible for the collection, checking, storage and analyses of data. J.E. Harding (chair of ESSENCE-IPD project); L Lin; C.A. Crowther; and G Gamble.

ESSENCE-IPD Trialist Group: This group includes investigators from all eligible trials who have agreed to share their data for the IPD.

M Agosti[1]; S.A. Atkinson[2]; A Biasini[3]; R.D.S Da Cunha[4]; N.D. Embleton[5]; M Faraz[2]; M.S Fewtrell[6]; F Lamy Filho[7]; C. Fusch[2,8]; M.L. Gianni[9]; H.G. Kanmaz[10]; W.WK. Koo[11]; I Litmanovitz[12]; A Lucas[13]; C Morgan[14]; K Mukhopadhyay[15]; E Neri[16]; J Picaud[17]; E.V Rafael[18]; P Roggero[9]; A Singhal[19]; K Stroemmen[20]; M.J. Tan[21]; F.M. Tandoi[1]; C.L. Wood[22]; G Zachariassen[23]

[1] Division of Neonatology and Neonatal Intensive Care Unit, "F. Del Ponte" Hospital, Varese, Italy

[2] Department of Pediatrics, Faculty of Health Sciences, McMaster University, Hamilton, Ontario, Canada.

[3] Donor Human Milk Bank Italian Association (AIBLUD), Milan, Italy

[4] Hospital Universitário da Universidade Federal do Maranhão - Brasil

[5] Institute of Health and Society, Newcastle University, Newcastle upon Tyne, UK

[6] Childhood Nutrition Research Centre, UCL Great Ormond Street Institute of Child Health, London, UK

[7] Departamento de Medicina, Universidade Federal do Maranhão (UFMA), São Luís, MA, Brazil

[8] Department of Pediatrics, Nuremberg General Hospital, Paracelsus Medical University, 90471 Nuremberg, Germany

[9] Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, via Commenda 12, 20122 Milan, Italy

[10] Department of Neonatology, NICU, Zekai Tahir Burak Education andResearch Hospital, Ankara, Turkey

[11] Department of Nutrition and Food Science, Wayne State University, Detroit, MI, USA

[12] Department of Neonatology, Meir Medical Center, Kfar Saba, Israel

[13] MRC Childhood Nutrition Research Centre, Institute of Child Health, University College London, London, UK

[14] Department of Neonatology, Liverpool Women's Hospital, Liverpool, UK

[15] Department of Pediatrics, Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh, India.

[16] Department of Psychology, University of Bologna, Bologna, Italy

[17] Division of Neonatology, Hôpital de la Croix-Rousse, Lyon, France

[18] Departamento de Enfermagem da Universidade Federal do Maranhão, Brasil

[19] Department of Nutrition, Institute of Child Health, London, UK

[20] Department of Neonatal Intensive Care, Division of Pediatric and Adolescent Medicine, Rikshospitalet, Oslo University Hospital, Oslo, Norway

[21] Department of Developmental Paediatrics, Alder Hey Children's NHS Foundation Trust, Liverpool, UK

[22] Institute of Genetic Medicine, Newcastle University, Newcastle, UK

[23] H.C. Andersen Children's Hospital, Odense University Hospital and University of Southern Denmark, Odense, Denmark

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Supplement document

Appendix 1. Definitions for Primary Outcome of Metabolic Risk

Measurement	Guideline/ Equipment	Age	Abnormal	Notes
Size for gestation at birth	INTERGROWTH 21 Charts[1]	≤ 6 months	≤ 10th centile vs > 10th centile	INTERGROWTH 21 charts for babies younger than 6 months[1]
Overweight/obese	WHO Growth Charts[2 3]	<5 years[2]	Overweight: weight-for-height greater than 2 standard deviations above WHO Child Growth Standards median; Obesity: weight-for-height greater than 3 standard deviations above the WHO Child Growth Standards median.	Charts and tables: WHO child growth standards for children aged under 5 years[2]
		5-19 years[3]	Overweight: BMI-for-age greater than 1 standard deviation above the WHO Growth Reference median; Obesity: greater than 2 standard deviations above the WHO Growth Reference median.	Charts and tables: WHO growth reference for children aged between 5–19 years[2]
Waist Circumference	NHANES 2011-2014[4]	2- 60 years	≥90th percentile[5]	
Fat mass (FM)	DEXA-NHANES[6]	≥8 years	Fat Mass Index (kg/m^2) classification ranges for sex	
	BIA[7]	5-18 years	≥85th percentile (%FM)	
	ADP- BodPod[7]	5-18 years	≥85th percentile (%FM)	
	ADP- PedPod[8]	0.5-24 months	%FM greater than 1 standard deviation above the reference mean	
	skinfolds- NHES II, NHES III, NHANES I, NHANES II and NHANES III[9]	1.5-19 years	≥85th percentile[9]	
	Multicomponent model	0.5- 24 month[8]	%FM greater than 1 standard deviation above the reference mean	

Measurement	Guideline/ Equipment	Age	Abnormal	Notes
		5-20 years[10]	FM greater than 1 standard deviation above the reference mean	Fat mass reference data for males and females by Z-score or percentile[10]
Blood pressure	NHBPEP[11]	1 to 17 years	≥90th centile[5] (age, sex and height specific) Charts and tables: WHO Child growth standards for length/height	Compared with Jackson LV 2007[12], although the NHBPEP is older, it contains the appropriate age range and reported the actual numbers at each cut point.
Triglycerides	NHANES III, NHANES 1999–2004, Bogalusa, Muscatine, Fels, and Princeton[13]	4-18 years	≥90th centile[14]	Compared to NHANES III, NCEP, and NGHS, this includes a wider age range.
	NHANES	>18 years	≥150mg/dL (8.3 mmol/L)[13]	
HDL-C	NHANES III, NHANES 1999–2004, Bogalusa, Muscatine, Fels, and Princeton[13]	4-18 years	≤10th centile[14]	Compared to NHANES III, NCEP, and NGHS, this includes a wider age range.
	NHANES[13]	>18 years	<40 mg/dL (2.2 mmol/L)[13] for male	
			<50 mg/dL (2.8 mmol/L)[13] for female	
LDL-C	NHANES III, NHANES 1999–2004, Bogalusa, Muscatine, Fels, and Princeton[13]	4-18 years	≥90th centile[13]	Compared to NHANES III, this includes a wider age range.
	NCEP ATP III	>18 years	>130 mg/dL (7.2 mmol/L)[13]	
Fasting plasma glucose concentration	ADA criterion[15] (increased risk for diabetes or prediabetes)		FPG ≥100 mg/dL (5.6 mmol/L)	
Impaired glucose tolerance	ADA criterion[15] (increased risk for diabetes or prediabetes)		2-h PG ≥140mg/dL (7.8 mmol/L) during a 75g OGTT	

Abbreviation
WHO: World Health Organisation
NHANES: National Health and Nutrition Examination Survey
BIA: Bioelectrical impedance analysis
DEXA: Dual-energy X-ray absorptiometry
FM: Fat mass

Measurement	Guideline/ Equipment	Age	Abnormal	Notes
ADP: Air displacement plethysmography NHBPEP: National High Blood Pressure Education Program NCEP: National Cholesterol Education Program NGHS: National Lung, Heart and Blood Institute's Growth and Health Study HDL-C: High triglyceride concentration LDL-C: Low triglyceride concentration ATP III: Adult Treatment Panel III ADA: American Diabetes Association				

Appendix 2. Definitions for Secondary outcomes

Term	Classification	Definition	Note
Cerebral palsy		1. Cerebral palsy is a physical disability that affects movement and posture. Any definition that includes the following five key elements: (1) is an umbrella term for a group of disorders (2) is a condition that is permanent but not unchanging (3) involves a disorder of movement and/or posture and of motor function (4) is due to a non-progressive interference, lesion or abnormality, and (5) the interference, lesion or abnormality originates in the immature brain 2. As defined by investigators	Australian cerebral palsy register report - CP Register[16]
Severity of cerebral palsy	GMFCS Level I	Children walk at home, school, outdoors and in the community. They can climb stairs without the use of a railing. Children perform gross motor skills such as running and jumping, but speed, balance and coordination are limited.	Gross Motor Function Classification System (GMFCS)[17]
	GMFCS Level II	Children walk in most settings and climb stairs holding onto a railing. They may experience difficulty walking long distances and balancing on uneven terrain, inclines, in crowded areas or confined spaces. Children may walk with physical assistance, a handheld mobility device or used wheeled mobility over long distances. Children have only minimal ability to perform gross motor skills such as running and jumping.	
	GMFCS Level III	Children walk using a hand-held mobility device in most indoor settings. They may climb stairs holding onto a railing with supervision or assistance. Children use wheeled mobility when traveling long distances and may self-propel for shorter distances.	
	GMFCS Level IV	Children use methods of mobility that require physical assistance or powered mobility in most settings. They may walk for short distances at home with physical assistance or use powered mobility or a body support walker when positioned. At school, outdoors and in the community children are transported in a manual wheelchair or use powered mobility.	
	GMFCS Level V	Children are transported in a manual wheelchair in all settings. Children are limited in their ability to maintain antigravity head and trunk postures and control leg and arm movements.	
Developmental delay or intellectual	Mild	A score on scale from 2 SD to <1 SD below test mean	Scores were obtained relative to the mean and standard deviation (SD)
	Moderate	A score on scale from 3 SD to <2 SD below test mean	

impairment	Severe	A score on scale 3 SD below test mean	for the normal birth weight population.[18]
Visual impairment	None	Presenting visual acuity 6/18 or better in the better eye.	WHO Definition of visual impairment[19] Visual Standards- Aspects and Ranges of Vision Loss[20]
	Moderate/ low vision	Can see a toy and able to follow a toy. Presenting visual acuity worse than 6/18, equal to or better than 6/60 in the better eye in the better eye.	
	Severe/ no useful vision	Able to see light or gross movement up close (within 40cm). Presenting visual acuity worse than 6/60, equal to or better than 1/60 in the better eye.	
	Blindness/ no light perception	No useful vision. Presenting visual acuity worse than 1/60 in the better eye or no light perception.	
	Legal blindness	Medically diagnosed central visual acuity of 20/200 (6/60) or less in the better eye with the best possible correction, and/or a visual field of 20 degrees or less	American Foundation for the Blind[21]
Hearing impairment (Classification 1)	None	None diagnosed	WHO Grades of hearing impairment- Prevention of blindness and deafness[22]
	Mild	Hearing level in decibels: 26-40dB A child with this level of hearing loss will have trouble hearing and understanding soft speech, speech from a distance or speech against a background of noise	
	Moderate	Hearing level in decibels: 41-60db A child with this level of hearing loss will have difficulty hearing regular speech, even at close distance	
	Severe	Hearing level in decibels: 61-80dB A child with this level of hearing loss may only hear very loud speech or loud sounds in the environment, such as a fire truck siren or a door slamming. Most conversational speech is not heard.	
	Profound	Hearing level in decibels: over 81dB A child with this level of hearing loss may perceive loud sounds as vibrations.	
Motor dysfunction	mild impairment	Test score between 5th and 15th centile on the Movement ABC / A score from 2 SD to <1 SD below the population mean on the BOTMP	Movement Assessment Battery for Children （Movement ABC） Bruininks–Oseretsky Test of Motor Proficiency (BOTMP)[23]
	moderate to severe impairment	Test score less than 5th centile on the Movement ABC / more than 2 SD below the population mean on the BOTMP	
School performance	Defined by teachers based on their observation and academic scores; at or above vs below expected performance/level for age		Poor school performance[24]

Growth Z-scores	WHO Growth Charts	Charts and tables: WHO child growth standards for children[2]

Abbreviation:
CP: Cerebral palsy
GMFCS: Gross Motor Function Classification System
SD: Standard deviation
Movement ABC: Movement Assessment Battery for Children
BOTMP: Bruininks–Oseretsky Test of Motor Proficiency

Appendix 3. Data items to be collected

Trial level information

- Protocols of each individual trial
- Data forms or case report forms (CRFs) of each individual trial
- Country
- Setting (neonatal intensive care unit (NICU), hospital, or home)
- Dates of start and end of trial
- Number of infants randomised
- Informed consent procedures
- Methods of random allocation
- Methods of allocation concealment
- Blinding of outcome assessment
- Blinding of researchers/ caregivers
- Stratification factors used
- Purpose of intervention
- Planned intervention
- Specific details of the planned nutrition in the experimental and control arms of the trial (including composition, whether given as sole feed or in addition to breast milk and duration)
- Outcomes collected (primary and secondary)

Participant level information: Infant characteristics at trial entry

- Unique identification code
- Gestational age at birth
- Age at trial entry
- Birthweight
- Weight, length and head circumference at trial entry
- Sex
- Single/multiple (if multiple order of birth)

Participant level information: After trial entry

- Actual intervention or comparison received
- Age at commencement of nutritional supplement
- Age at ceasing the nutritional supplement
- Receipt of breast milk and whether mother's own or donor/banked
- Size at discharge or term-equivalent age (36-42 weeks' post-menstrual age)- including weight, length, head circumference, and measures of body composition e.g. skinfolds, boimpedence, plethysmography

Appendix 4. Search strategies

Embase from 1980	
#	Search strategies
1	exp prematurity/
2	exp low birth weight/
3	exp small for date infant/
4	exp very low birth weight/
5	(prematur* adj2 infant*).tw.
6	(prematur* adj2 newborn*).tw.
7	(prematur* adj2 neonate*).tw.
8	preterm.tw.
9	low birth weight.tw.
10	low birthweight.tw.
11	VLBW.tw.
12	LBW.tw.
13	ELBW.tw.
14	small for gestation*.tw.
15	SGA.tw.
16	(less than adj6 g).tw.
17	(less than adj3 32 weeks).tw.
18	birth weight below.tw.
19	(gestation* adj2 less than).tw.
20	or/1-19
21	exp breast feeding/
22	exp infant nutrition/
23	exp protein intake/
24	exp dietary supplement/
25	exp omega 3 fatty acid/ct, ad, dt, ig, pa [Clinical Trial, Drug Administration, Drug Therapy, Intragastric Drug Administration, Parenteral Drug Administration]
26	exp arachidonic acid/ae, ct, ad, dt, ig, pa, th [Adverse Drug Reaction, Clinical Trial, Drug Administration, Drug Therapy, Intragastric Drug Administration, Parenteral Drug Administration, Therapy]
27	exp unsaturated fatty acid/ct, dt, pa, th [Clinical Trial, Drug Therapy, Parenteral Drug Administration, Therapy]
28	exp fat intake/ae, ad, dt [Adverse Drug Reaction, Drug Administration, Drug Therapy]
29	exp enteric feeding/
30	exp parenteral nutrition/
31	exp artificial milk/
32	exp breast milk/
33	exp fortified food/
34	exp elemental diet/
35	exp baby food/
36	(breast milk or human milk).tw.
37	formula.tw.
38	PUFA supplement*.tw.
39	feed* regimen*.tw.
40	(protein* adj2 concentration*).tw.
41	probiotic$.tw.
42	parenteral*.tw.
43	enteral*.tw.
44	maternal milk.tw.
45	multinutrient supplement*.tw.

46	(breast fed or breastfed).tw.
47	prebiotic*.tw.
48	diet* supplement*.tw.
49	nutrient enriched.tw.
50	Docosahexaenoic Acid*.tw.
51	arachidonic acid*.tw.
52	(glutamine adj2 supplement*).tw.
53	(taurine adj2 supplement*).tw.
54	(calcium adj2 supplement*).tw.
55	palm olein.tw.
56	palmitic acid.tw.
57	(fortification or fortified).tw.
58	fatty acids.tw.
59	supplement* feed*.tw.
60	complementary feed*.tw.
61	nutrition*.tw.
62	Hydrolysed liquid.tw.
63	Hydrolyzed liquid.tw.
64	gamma-linoleic acid.tw.
65	(diet* adj3 protein*).tw.
66	or/21-65
67	20 and 66
68	Clinical Trial/
69	Randomized Controlled Trial/
70	exp randomization/
71	Single Blind Procedure/
72	Double Blind Procedure/
73	Crossover Procedure/
74	Placebo/
75	Randomi?ed controlled trial$.tw.
76	Rct.tw.
77	random allocation.tw.
78	randomly.tw.
79	randomly allocated.tw.
80	allocated randomly.tw.
81	(allocated adj2 random).tw.
82	Single blind$.tw.
83	Double blind$.tw.
84	((treble or triple) adj blind$).tw.
85	placebo$.tw.
86	prospective study/
87	or/68-86
88	case study/
89	case report.tw.
90	abstract report/ or letter/
91	or/88-90
92	87 not 91
93	67 and 92

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Reporting checklist for protocol of a systematic review.

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		Reporting Item	Page Number
Title			
Identification	#1a	Identify the report as a protocol of a systematic review	1
Update	#1b	If the protocol is for an update of a previous systematic review, identify as such	n/a

Registration			
	#2	If registered, provide the name of the registry (such as PROSPERO) and registration number	2
Authors			
Contact	#3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	1
Contribution	#3b	Describe contributions of protocol authors and identify the guarantor of the review	16
Amendments			
	#4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	n/a
Support			
Sources	#5a	Indicate sources of financial or other support for the review	15
Sponsor	#5b	Provide name for the review funder and / or sponsor	15
Role of sponsor or funder	#5c	Describe roles of funder(s), sponsor(s), and / or institution(s), if any, in developing the protocol	15
Introduction			
Rationale	#6	Describe the rationale for the review in the context of what is	4-5

		already known	
Objectives	#7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	6
Methods			
Eligibility criteria	#8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	6-8
Information sources	#9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	8
Search strategy	#10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	Appendix 4
Study records - data management	#11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	9
Study records - selection process	#11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	8
Study records - data collection	#11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate),	8-9 Appendix

process		any processes for obtaining and confirming data from investigators	3
Data items	#12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	6-8 Appendix 1-2
Outcomes and prioritization	#13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	7-8
Risk of bias in individual studies	#14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	9
Data synthesis	#15a	Describe criteria under which study data will be quantitatively synthesised	10
Data synthesis	#15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I2, Kendall's τ)	10
Data synthesis	#15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	11
Data synthesis	#15d	If quantitative synthesis is not appropriate, describe the type of summary planned	11
Meta-bias(es)	#16	Specify any planned assessment of meta-bias(es) (such as	n/a

		publication bias across studies, selective reporting within studies)	
Confidence in cumulative evidence	#17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	n/a

BMJ Open

Sex-specific effects of nutritional supplements in infants born early or small: Protocol for an individual participant data meta-analysis (ESSENCE IPD-MA)

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Sex-specific effects of nutritional supplements in infants born early or small: Protocol for an individual participant data meta-analysis (ESSENCE IPD-MA)

Luling Lin (0000-0002-8448-1504)[1], <luling.lin@auckland.ac.nz>

Caroline A. Crowther[1], <c.crowther@auckland.ac.nz>

Gregory D. Gamble (0000-0003-0412-3203)[1], <gd.gamble@auckland.ac.nz>

Frank H. Bloomfield[1], <f.bloomfield@auckland.ac.nz>

Jane E. Harding (0000-0003-2697-1422) [1], <j.harding@auckland.ac.nz>

for **the ESSENCE IPD-MA Group[2]**

[1] Liggins Institute, University of Auckland, Auckland, New Zealand

[2] Membership of the ESSENCE IPD-MA Group is listed in the Contribution section

Corresponding author

Jane E. Harding

Liggins Institute, University of Auckland

Building 503, Level 2, 85 Park Road, Auckland P Private Bag 92019, Auckland 1142, New Zealand

E-mail: j.harding@auckland.ac.nz

Telephone: +6499236439

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ABSTRACT

Introduction

Preterm and small for gestational age (SGA) infants are at increased risk of poor growth, disability and delayed development. While growing up they are also at increased risk of obesity, diabetes and later heart disease. The risk of such adverse outcomes may be altered by how preterm and SGA infants are fed after birth. Faltering postnatal growth is common due to failure to achieve recommended high energy and protein intakes, and thus preterm and SGA infants are often provided with supplemental nutrition soon after birth. Enhanced nutrition has been associated with improved early growth and better cognitive development. However, limited evidence suggests that faster growth may increase the risk for later adiposity, metabolic and cardiovascular disease, and that such risks may differ between girls and boys.

Methods and analysis

We will search OvidMedline, Embase, Cochrane CENTRAL, Cochrane Database of Systematic Reviews, controlled-trials.com, clinicaltrials.gov and anzctr.org.au for randomised trials that studied the effects of macronutrient supplements for preterm and SGA infants on i) developmental and metabolic and ii) growth outcomes after hospital discharge. The outcomes will be i) cognitive impairment and metabolic risk (co-primary) and ii) body mass index. Individual participant data (IPD) from all available trials will be included using an intention-to-treat approach. A one-stage procedure for IPD meta-analysis (MA) will be used, accounting for clustering of participants within studies. Exploratory subgroup analyses will further investigate sources of heterogeneity, including sex and size of infants, different timing, duration and type of supplements.

Ethics and dissemination: This IPD-MA is approved by the University of Auckland Human Participants Ethics Committee (reference number: 019874). Individual studies have approval from relevant local ethics committees. Results will be disseminated in a peer-reviewed journal and presented at international conferences.

PROSPERO registration number: CRD42017072683

Strengths and limitations of this study

- This IPD-MA will synthesise data from multiple randomised clinical trials using uniformly defined relevant outcomes for all available trials.
- Subgroup analyses may allow detection of potential sex differences that could not be achieved through aggregate analyses.
- This IPD-MA will explore the interactions between treatment and participant-level characteristics.
- A potential limitation is that the analyses planned will depend on obtaining the relevant individual participant data.
- There may be considerable heterogeneity as interventions are likely to be very varied and the trials performed over several decades.

INTRODUCTION

Infants born preterm or small for gestational age (SGA) are at increased risk of poor growth, disability and delayed development. [1-3] As adults, they are at increased risk of obesity, diabetes and later heart disease.[4] How infants born small are fed after birth may alter the risk of these adverse outcomes. Providing preterm and SGA infants with enhanced nutrition soon after birth is associated with improved early growth and better cognitive development,[5-7] but observational data suggest that early faster growth may increase the risk for later adiposity, metabolic and cardiovascular disease.[8]

It has been recognised for centuries that girls and boys grow differently, experience different metabolic and endocrine milieux, and have different cognitive and health outcomes. Little attention has been paid to improving outcomes following preterm birth by treating girls and boys differently, although it is well recognised that preterm boys compared to girls have higher mortality and morbidity,[9] and are more likely to have adverse developmental and educational outcomes.[10] There is substantial evidence that perinatal insults can result in different adult phenotypes in males and females.[11] For example, animal studies across many different species after a wide variety of prenatal insults show that males are more likely than females to exhibit adverse effects such as impaired renal function, hypertension, insulin resistance, altered hypothalamic-pituitary-adrenal (HPA) axis function and altered growth in later life.[12] The reasons for this sex difference in vulnerability to early environmental perturbations are not well understood, but may include faster growth and hence greater substrate demands in males, altered tempo of maturation, different exposure to sex steroids and sex-specific epigenetic mechanisms.[12]

There is limited evidence from human studies that these effects may also be important in babies. Preterm boys have been reported to have higher protein and calorie requirements to maintain growth than girls.[13] Preterm boys also were more vulnerable than girls to the adverse effects of delayed early nutrition[14], whereas enhanced nutrition improved cognitive outcomes for preterm boys, but had no effect on girls.[15] Unfortunately, most clinical studies have not reported findings separately by sex and are not adequately powered to do so. Further, because the majority of animal experiments are done in polytocous species, prenatal and postnatal sex effects cannot be separated in mixed-sex litters. There is little reliable

evidence about how best to feed preterm babies to optimise both short- and long-term health outcomes, and almost none about targeting nutrition by sex.

Hypotheses

The effects of early nutritional supplements on post-discharge development, markers of metabolic risk and growth are different in girls and boys.

Need for individual participant data meta-analysis

Systematic reviews using aggregate data meta-analyses are limited due to within-trial variation in gestational age of the infants at birth, co-morbidities of the infant, starting time and duration of the intervention, macronutrient content of the intervention and control groups. Outcomes within aggregate meta-analyses such as cerebral palsy, motor dysfunction or hearing loss also include a range of severity of disability. Aggregate meta-analyses tend to vary in completeness and in the definitions used for the outcomes. Not all trials combine the same outcomes in composite outcomes or use the same measures of neuro-developmental outcome. Few trials describe multiple subgroups, making meta-analysis of data almost impossible. Importantly, few trials to date have provided the sex of the infant as a subgroup variable.

One method to ameliorate some of the limitations of aggregate data meta-analysis is to combine the large volume of individual trial data available to perform an individual participant data (IPD) meta-analysis (MA). The estimates of treatment effects with an IPD-MA often differ from aggregate meta-analyses.[16] IPD-MA allows the inclusion of additional unpublished data provided by the trialists, and allows consistent re-categorisation of definitions of outcomes and populations in order to answer the clinical questions of interest. Further, IPD-MA may allow more detailed meta-analysis of key outcomes, taking into account both subject-level and study-level sources of heterogeneity in treatment effects. Thus, IPD-MA offers the potential to help clarify the sex-specific effects of early macronutrient supplements provided to preterm and SGA infants.

METHODS AND ANALYSIS

This study will use an IPD-MA approach and follow the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[17]

Aims

To assess, using IPD-MA, the effects of macronutrient supplements in nutrition of preterm and SGA infants on developmental, metabolic and growth outcomes after hospital discharge, and in particular, whether these effects differ in girls and boys.

Criteria for inclusion and exclusion

We will include published and unpublished randomised and quasi-randomised trials without restrictions on date of publication or language. Trials that studied infants born preterm (< 37 weeks' gestation) or born small (birthweight <2.5 kg or < 10th centile for gestational age) and in which 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 will be included.

Interventions can be enteral or parenteral or a combination, commence at any time during the hospitalisation or after discharge from hospital, and must be provided for a minimum duration of one week. Trials that report on comparisons between un-supplemented nutrition and supplemented nutrition with parenteral supplements, human breast milk supplements, formula milk or other macronutrients will be eligible for inclusion, for example: 1) Parenteral formulation A versus parenteral formulation B with different macronutrient composition; 2) Human milk (mother's own or donor) versus supplemented human milk (mother's own or donor); 3) Human milk (mother's own or donor) versus formula milk (term or preterm); 4) Supplemented human milk (mother's own or donor) versus formula (term or preterm); 5) Supplemented human milk A (mother's own or donor) versus supplemented human milk B (mother's own or donor); 6) Formula A versus formula B with different macronutrient composition (including preterm versus term formula, brand A versus brand B). We will exclude trials that examine the timing of the introduction of nutrition (early versus delayed feeding); that compare macronutrients of different composition (e.g. different types of lipids or proteins); studies whose outcomes focus on gastrointestinal development rather than growth and development; and studies reporting on variations in composition of

micronutrients (including sodium, potassium, calcium, phosphorus, vitamins, other minerals, amino acids, fatty acids).

Outcome data must be reported beyond term equivalent age (> 37 weeks' post-menstrual age) or following discharge from hospital after birth. Where the data are available, the outcomes will be categorised and evaluated in toddlers (less than 3 years), childhood (3 to 8 years), adolescence (9 to 18 years) and adulthood (more than 18 years).

We plan to report the findings as two reviews, one reporting developmental and metabolic outcomes, and the other reporting growth outcomes.

1. Developmental and metabolic outcomes:

The co-primary outcomes will be 1) Cognitive impairment: below -1 SD on standard tests of development (toddlers) or cognition/intelligence quotient (later ages) and 2) Metabolic risk *(See Appendix 1 for definitions)*: any of overweight/obese; increased waist circumference; increased fat mass or fat mass percentage; elevated plasma triglyceride concentrations; low high-density lipoprotein (HDL) concentrations; elevated low-density lipoprotein (LDL) concentrations; elevated fasting plasma glucose concentrations; insulin resistance; impaired glucose tolerance; diagnosis of type 2 diabetes; high blood pressure and impaired flow-mediated vasodilatation.

The secondary outcomes will be *(See Appendix 2 for definitions):* 1) Composite of survival free of any disability (including death, cerebral palsy, motor development delay or impairment, cognition/intelligence delay or impairment, language delay, visual impairment, hearing impairment); 2) Cognition/intelligence delay or impairment; 3) Cognition/intelligence scores; 4) Motor delay or impairment; 5) Motor scores; 6) Cerebral palsy (any); 7) Severity of cerebral palsy; 8) Visual impairment; 9) Hearing impairment; 10) School performance; 11) Measures of psychological well-being; 12) Metabolic outcomes: waist circumference, overweight/obese, type-2 diabetes, blood lipid concentrations (triglycerides, HDL, LDL, HDL:LDL), fasting blood glucose concentration, insulin concentration, insulin resistance, glucose tolerance, IGF-1 concentration; 13) Cardiovascular risk outcomes: blood pressure (systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP)), flow-mediated vasodilatation, measures of sympathetic and parasympathetic tone, cardiac size and structure; 14) 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; 15) Health outcomes: allergies (eczema, asthma, hayfever), respiratory function, hospitalisation (duration), health care utilisation; 16) Nutrition: feeding tolerance; intake (milk, energy), appetite, breast feeding; 17) Death (neonatal or later death up to the time of follow-up and cause of death); 18) Quality of life; 19) General health and use of healthcare resources; 20) Adverse Events; 21) Cost.

2. Growth outcomes:

The primary outcome will be body mass index (BMI) in childhood (3 to 8 years).

The secondary outcomes will be (1) 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), body composition (fat mass, fat free mass, measured by bioimpedance or DEXA or skinfold thickness or other method); (2) Bone development: bone mineral content, volumetric bone mineral density, bone fractures; (3) Nutrition: feeding tolerance; intake (protein, energy); appetite; breastfeeding and duration.

Search strategy

We will search OvidMedline, Embase, Cochrane Library Central Registry of Controlled Trials (CENTRAL), and Cochrane Database of Systematic Review from inception to identify eligible trials. We will also search for registered trials in Current Controlled Trials (www.controlled-trials.com), Clinical Trials (www.clinicaltrials.gov) and Australian and New Zealand Clinical Trials Registry (www.anzctr.org.au) to identify eligible ongoing studies. The full search strategy and search terms are available as online supplementary material (Appendix 1). Experts in the field and trialists will be asked if they can identify other published or ongoing trials. Potentially eligible trials that are not yet completed will not be included in this IPD-meta analysis, but will be noted for inclusion in future updates.

Identify studies

The eligibility of trials will be assessed by two researchers. Discrepancies will be resolved by discussion. If IPD are unavailable from any eligible trial it will be included in the IPD-MA using aggregate data for sensitivity analysis where possible.

Contact Authors

Authors of eligible studies will be invited to join the ESSENCE IPD-MA Collaborative group. We will identify contact information from the published trials. An initial email will be sent to the main trial author (corresponding author) providing them with the summary IPD-MA protocol. Another investigator from the study will be contacted if initial emails fail to receive a response, followed by phone calls if needed.

Quality assessment

We will assess the quality of the eligible trials using the methods specified in the Cochrane Handbook for Systematic Reviews of Interventions:[18] random sequence generation (selection bias); allocation concealment (selection bias); blinding of participants, personnel, and outcome assessment (performance and detection bias); incomplete outcome data (attrition bias); selective reporting (reporting bias); (6) Other bias (checking for bias due to problems not covered by (1) to (5) above).

Development of the database

We will develop a set of pre-specified and defined variables for IPD-MA at both the outcome, participant and trial level (Appendix 3). We will seek data on all randomised infants which will be coded for anonymity (date of birth, study centre); baseline data for narrative purposes (birthweight, gestational age at birth, plurality, sex); details of the intervention and comparator (date of randomisation, allocated intervention, type and composition of intervention and comparator, enteral or parenteral administration, age at start of intervention, weight at start of intervention, duration of intervention) and the outcomes listed above.

Trialists will provide de-identified data in any format which will be recoded as required, verified and checked for consistency with published data and stored on a secure, password protected file on the University of Auckland servers. Data will only be accessible by authorised personnel in the ESSENCE Data Management Group.

Methodological details of the individual trials will be cross-checked against published reports, trial registration (where available) and trial data collection forms. Where inconsistencies are identified, discussions will be held with individual trial groups to attempt

to resolve these. Each trial final dataset to be used in the IPD-MA will be returned to the trialists for verification. Trials will then be analysed individually using IPD-MA pre-specified variables and outcomes and the results returned to the trialists for verification. The individual trial datasets will then be combined to form the ESSENCE-IPD dataset and the IPD meta-analysis undertaken.

Data synthesis and statistical analysis

A detailed statistical analysis plan will be prepared and agreed on by the ESSENCE-IPD team.

We will use a one-stage approach to the analysis of each outcome so that the IPD from all eligible trials are included in a single model. We will make an assessment of heterogeneity to decide if combining data from trials is appropriate or if heterogeneity, if significant, can be explained.

Binary outcomes will be analysed using log binomial regression models and data will be reported as Risk Ratio (RR) with 95% Confidence Intervals (CI) and associated 2-sided p values. Continuous data will be analysed using linear regression models and data will be reported as mean differences (MD) with 95% Confidence Intervals (CI) and associated 2-sided p values.

A large number of outcomes are being investigated in this study. This increases the chance of observing false positive results. The overall probability of a type 1 error will be maintained at 5% for each review. For the review of developmental and metabolic outcomes, the p value will be split equally between the co-primary outcomes by testing each at $p = 0.025$. For the review of growth outcomes, $p < 0.05$ will denote statistical significance for the primary outcome. No further adjustment for multiplicity is planned for comparisons made in secondary and exploratory analyses.

We will explore the effects of the sex of the infants by presenting data separately for each sex as pre-specified subgroups, and by testing a treatment by sex interaction term within the model.

Where data are missing, those infants will be removed from the analysis and, 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. Where there are large amounts of missing data or trials are unable to provide IPD we will conduct sensitivity analyses to explore the effect of removing such trials from the analysis.

Statistical analyses will be performed using SAS (v.9.4, SAS Institute, Cary, NC, USA).

Planned subgroup analysis

Where data are available, we will conduct subgroup analyses to explore whether the effects of supplements differ between subgroups and test for interaction terms.

1. Sex of infant (boys vs girls);
2. Size of infant at birth (≤ 1 kg vs >1 kg at birth);
3. Size for gestation of the infant (≤ 10th centile vs > 10th centile);
4. Gestational age of infant at birth (≤ 28 completed weeks vs 29 to 32 completed weeks vs 33 to 36 weeks).
5. Timing of supplement:

   In hospital nutrition: the intervention was commenced in hospital or on average ended at 42 weeks' postmenstrual age or earlier;

   Post-discharge nutrition: the intervention was commenced after discharge or on average started at or after 36 weeks' postmenstrual age;

   Both in hospital and post-discharge nutrition: the intervention was commenced in the hospital and continued post-discharge.
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 to 2 weeks vs 3 to 6 weeks vs more than 7 weeks).
9. Different epochs (commenced up to the year of 2000 vs commenced in or after the year of 2001).

Planned sensitivity analyses

We will perform sensitivity analyses to assess whether the results are robust to the trial design by excluding trials assessed as high risk of bias.

Where 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.

Patient and Public Involvement

It was not appropriate or possible to involve patients or the public in the design, or conduct, or reporting, or dissemination of our research.

DISCUSSION

This will be the first IPD-MA to investigate the effects of early macronutrient supplements on preterm and SGA infants. IPD meta-analysis has been described as the 'gold standard' of systematic review methodology as it allows for more powerful and flexible analysis of both subgroups and outcomes.[19] This IPD-MA, using existing data from the individual trials, may reveal the sex-specific effects of macronutrient supplement on preterm and SGA infants, and will allow assessment of important interactions that cannot be tested in standard, aggregate data meta-analysis.

ETHICS AND DISSEMINATION

The shared data will be de-identified, the data files will be transferred by secure means, and stored in a secure password protected area on an Auckland University server.

Final results will be presented to the ESSENCE IPD-MA collaborators prior to publication and public dissemination. Results of the study will be published in peer-reviewed journals and presented at national and international conferences.

The ESSENCE-IPD project has been approved by University of Auckland Human Participants Ethics Committee (reference number: 019874). Individual studies have approval from relevant local ethics committees.

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DECLARATIONS

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Ethical approval and consent to participate

The ESSENCE-IPD project has been approved by University Of Auckland Human Participants Ethics Committee.

Participants in the individual trials have previously given informed consent to participate in their respective trials. The data for these projects are to be used for the purpose for which they were originally collected and are available through an agreement between all trialists of the collaborative group. These trialists remain custodians of their original trial data at all times. A date sharing agreement will be signed by a representative of the institution that owns the data and a representative of University of Auckland.

Consent for publication

Not applicable

Availability of data and material

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

The Chair of the ESSENCE-IPD Project Team (J.E. Harding) wrote the first draft of the ESSENCE-IPD protocol. L Lin revised the subsequent versions of the ESSENCE-IPD protocol and prepared the initial draft of the manuscript. The ESSENCE-IPD Project Team

and the ESSENCE-IPD Management Group participated in the protocol development and commented on all drafts of the manuscript. The ESSENCE-IPD Trialist Group participated in the development of the IPD protocol and have read and approved the final draft of the manuscript.

Contributors

The ESSENCE IPD-MA Group

ESSENCE-IPD Project Team: This is the project Steering Group which is responsible for the day-to-day management of the IPD. This group has drafted the IPD protocol, will liaise with trialists and prepare the draft publications. J.E. Harding (chair of ESSENCE-IPD project); L Lin; C.A. Crowther; F.H. Bloomfield; and G Gamble.

ESSENCE-IPD Management Group: This group is convened by the Chair of the Project Team and comprises the IPD-MA statistician and data manager who will be responsible for the collection, checking, storage and analyses of data. J.E. Harding (chair of ESSENCE-IPD project); L Lin; C.A. Crowther; and G Gamble.

ESSENCE-IPD Trialist Group: This group includes investigators from all eligible trials who have agreed to share their data for the IPD.

M Agosti[1]; S.A. Atkinson[2]; A Biasini[3]; R.D.S Da Cunha[4]; N.D. Embleton[5]; M Faraz[2]; M.S Fewtrell[6]; F Lamy Filho[7]; C. Fusch[2,8]; M.L. Gianni[9]; H.G. Kanmaz[10]; W.WK. Koo[11]; I Litmanovitz[12]; A Lucas[13]; C Morgan[14]; K Mukhopadhyay[15]; E Neri[16]; J Picaud[17]; E.V Rafael[18]; P Roggero[9]; A Singhal[19]; K Stroemmen[20]; M.J. Tan[21]; F.M. Tandoi[1]; C.L. Wood[22]; G Zachariassen[23]

[1] Division of Neonatology and Neonatal Intensive Care Unit, "F. Del Ponte" Hospital, Varese, Italy

[2] Department of Pediatrics, Faculty of Health Sciences, McMaster University, Hamilton, Ontario, Canada.

[3] Donor Human Milk Bank Italian Association (AIBLUD), Milan, Italy

[4] Hospital Universitário da Universidade Federal do Maranhão - Brasil

[5] Institute of Health and Society, Newcastle University, Newcastle upon Tyne, UK

[6] Childhood Nutrition Research Centre, UCL Great Ormond Street Institute of Child Health, London, UK

[7] Departamento de Medicina, Universidade Federal do Maranhão (UFMA), São Luís, MA, Brazil

[8] Department of Pediatrics, Nuremberg General Hospital, Paracelsus Medical University, 90471 Nuremberg, Germany

[9] Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, via Commenda 12, 20122 Milan, Italy

[10] Department of Neonatology, NICU, Zekai Tahir Burak Education andResearch Hospital, Ankara, Turkey

[11] Department of Nutrition and Food Science, Wayne State University, Detroit, MI, USA

[12] Department of Neonatology, Meir Medical Center, Kfar Saba, Israel

[13] MRC Childhood Nutrition Research Centre, Institute of Child Health, University College London, London, UK

[14] Department of Neonatology, Liverpool Women's Hospital, Liverpool, UK

[15] Department of Pediatrics, Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh, India.

[16] Department of Psychology, University of Bologna, Bologna, Italy

[17] Division of Neonatology, Hôpital de la Croix-Rousse, Lyon, France

[18] Departamento de Enfermagem da Universidade Federal do Maranhão, Brasil

[19] Department of Nutrition, Institute of Child Health, London, UK

[20] Department of Neonatal Intensive Care, Division of Pediatric and Adolescent Medicine, Rikshospitalet, Oslo University Hospital, Oslo, Norway

[21] Department of Developmental Paediatrics, Alder Hey Children's NHS Foundation Trust, Liverpool, UK

[22] Institute of Genetic Medicine, Newcastle University, Newcastle, UK

[23] H.C. Andersen Children's Hospital, Odense University Hospital and University of Southern Denmark, Odense, Denmark

Sex-specific effects of nutritional supplements in infants born early or small: protocol for an individual participant data meta-analysis (ESSENCE-IPD)

Supplement document

Appendix 1. Definitions for Primary Outcome of Metabolic Risk

Measurement	Guideline/ Equipment	Age	Abnormal	Notes
Size for gestation at birth	INTERGROWTH 21 Charts[1]	≤ 6 months	≤ 10th centile vs > 10th centile	INTERGROWTH 21 charts for babies younger than 6 months[1]
Overweight/obese	WHO Growth Charts[2 3]	<5 years[2]	Overweight: weight-for-height greater than 2 standard deviations above WHO Child Growth Standards median; Obesity: weight-for-height greater than 3 standard deviations above the WHO Child Growth Standards median.	Charts and tables: WHO child growth standards for children aged under 5 years[2]
		5-19 years[3]	Overweight: BMI-for-age greater than 1 standard deviation above the WHO Growth Reference median; Obesity: greater than 2 standard deviations above the WHO Growth Reference median.	Charts and tables: WHO growth reference for children aged between 5–19 years[2]
Waist Circumference	NHANES 2011-2014[4]	2- 60 years	≥90th percentile[5]	
Fat mass (FM)	DEXA-NHANES[6]	≥8 years	Fat Mass Index (kg/m^2) classification ranges for sex	
	BIA[7]	5-18 years	≥85th percentile (%FM)	
	ADP- BodPod[7]	5-18 years	≥85th percentile (%FM)	
	ADP- PedPod[8]	0.5-24 months	%FM greater than 1 standard deviation above the reference mean	
	skinfolds- NHES II, NHES III, NHANES I, NHANES II and NHANES III[9]	1.5-19 years	≥85th percentile[9]	
	Multicomponent model	0.5- 24 month[8]	%FM greater than 1 standard deviation above the reference mean	

Measurement	Guideline/ Equipment	Age	Abnormal	Notes
		5-20 years[10]	FM greater than 1 standard deviation above the reference mean	Fat mass reference data for males and females by Z-score or percentile[10]
Blood pressure	NHBPEP[11]	1 to 17 years	≥90th centile[5] (age, sex and height specific) Charts and tables: WHO Child growth standards for length/height	Compared with Jackson LV 2007[12], although the NHBPEP is older, it contains the appropriate age range and reported the actual numbers at each cut point.
Triglycerides	NHANES III, NHANES 1999–2004, Bogalusa, Muscatine, Fels, and Princeton[13]	4-18 years	≥90th centile[14]	Compared to NHANES III, NCEP, and NGHS, this includes a wider age range.
	NHANES	>18 years	≥150mg/dL (8.3 mmol/L)[13]	
HDL-C	NHANES III, NHANES 1999–2004, Bogalusa, Muscatine, Fels, and Princeton[13]	4-18 years	≤10th centile[14]	Compared to NHANES III, NCEP, and NGHS, this includes a wider age range.
	NHANES[13]	>18 years	<40 mg/dL (2.2 mmol/L)[13] for male	
			<50 mg/dL (2.8 mmol/L)[13] for female	
LDL-C	NHANES III, NHANES 1999–2004, Bogalusa, Muscatine, Fels, and Princeton[13]	4-18 years	≥90th centile[13]	Compared to NHANES III, this includes a wider age range.
	NCEP ATP III	>18 years	>130 mg/dL (7.2 mmol/L)[13]	
Fasting plasma glucose concentration	ADA criterion[15] (increased risk for diabetes or prediabetes)		FPG ≥100 mg/dL (5.6 mmol/L)	
Impaired glucose tolerance	ADA criterion[15] (increased risk for diabetes or prediabetes)		2-h PG ≥140mg/dL (7.8 mmol/L) during a 75g OGTT	

Abbreviation
WHO: World Health Organisation
NHANES: National Health and Nutrition Examination Survey
BIA: Bioelectrical impedance analysis
DEXA: Dual-energy X-ray absorptiometry
FM: Fat mass

Measurement	Guideline/ Equipment	Age	Abnormal	Notes
ADP: Air displacement plethysmography NHBPEP: National High Blood Pressure Education Program NCEP: National Cholesterol Education Program NGHS: National Lung, Heart and Blood Institute's Growth and Health Study HDL-C: High triglyceride concentration LDL-C: Low triglyceride concentration ATP III: Adult Treatment Panel III ADA: American Diabetes Association				

Appendix 2. Definitions for Secondary outcomes

Term	Classification	Definition	Note
Cerebral palsy		1. Cerebral palsy is a physical disability that affects movement and posture. Any definition that includes the following five key elements: (1) is an umbrella term for a group of disorders (2) is a condition that is permanent but not unchanging (3) involves a disorder of movement and/or posture and of motor function (4) is due to a non-progressive interference, lesion or abnormality, and (5) the interference, lesion or abnormality originates in the immature brain 2. As defined by investigators	Australian cerebral palsy register report - CP Register[16]
Severity of cerebral palsy	GMFCS Level I	Children walk at home, school, outdoors and in the community. They can climb stairs without the use of a railing. Children perform gross motor skills such as running and jumping, but speed, balance and coordination are limited.	Gross Motor Function Classification System (GMFCS)[17]
	GMFCS Level II	Children walk in most settings and climb stairs holding onto a railing. They may experience difficulty walking long distances and balancing on uneven terrain, inclines, in crowded areas or confined spaces. Children may walk with physical assistance, a handheld mobility device or used wheeled mobility over long distances. Children have only minimal ability to perform gross motor skills such as running and jumping.	
	GMFCS Level III	Children walk using a hand-held mobility device in most indoor settings. They may climb stairs holding onto a railing with supervision or assistance. Children use wheeled mobility when traveling long distances and may self-propel for shorter distances.	
	GMFCS Level IV	Children use methods of mobility that require physical assistance or powered mobility in most settings. They may walk for short distances at home with physical assistance or use powered mobility or a body support walker when positioned. At school, outdoors and in the community children are transported in a manual wheelchair or use powered mobility.	
	GMFCS Level V	Children are transported in a manual wheelchair in all settings. Children are limited in their ability to maintain antigravity head and trunk postures and control leg and arm movements.	
	Mild	A score on scale from 2 SD to <1 SD below test mean	Scores were obtained relative to the mean and standard deviation (SD)
	Moderate	A score on scale from 3 SD to <2 SD below test mean	

Developmental delay or intellectual impairment	Severe	A score on scale 3 SD below test mean	for the normal birth weight population.[18]
Visual impairment	None	Presenting visual acuity 6/18 or better in the better eye.	WHO Definition of visual impairment[19] Visual Standards- Aspects and Ranges of Vision Loss[20]
	Moderate/ low vision	Can see a toy and able to follow a toy. Presenting visual acuity worse than 6/18, equal to or better than 6/60 in the better eye in the better eye.	
	Severe/ no useful vision	Able to see light or gross movement up close (within 40cm). Presenting visual acuity worse than 6/60, equal to or better than 1/60 in the better eye.	
	Blindness/ no light perception	No useful vision. Presenting visual acuity worse than 1/60 in the better eye or no light perception.	
	Legal blindness	Medically diagnosed central visual acuity of 20/200 (6/60) or less in the better eye with the best possible correction, and/or a visual field of 20 degrees or less	American Foundation for the Blind[21]
Hearing impairment (Classification 1)	None	None diagnosed	WHO Grades of hearing impairment- Prevention of blindness and deafness[22]
	Mild	Hearing level in decibels: 26-40dB A child with this level of hearing loss will have trouble hearing and understanding soft speech, speech from a distance or speech against a background of noise	
	Moderate	Hearing level in decibels: 41-60db A child with this level of hearing loss will have difficulty hearing regular speech, even at close distance	
	Severe	Hearing level in decibels: 61-80dB A child with this level of hearing loss may only hear very loud speech or loud sounds in the environment, such as a fire truck siren or a door slamming. Most conversational speech is not heard.	
	Profound	Hearing level in decibels: over 81dB A child with this level of hearing loss may perceive loud sounds as vibrations.	
Motor dysfunction	mild impairment	Test score between 5th and 15th centile on the Movement ABC / A score from 2 SD to <1 SD below the population mean on the BOTMP	Movement Assessment Battery for Children（Movement ABC） Bruininks–Oseretsky Test of Motor Proficiency (BOTMP)[23]
	moderate to severe impairment	Test score less than 5th centile on the Movement ABC / more than 2 SD below the population mean on the BOTMP	
School performance	Defined by teachers based on their observation and academic scores;		Poor school performance[24]

	at or above vs below expected performance/level for age	
Growth Z-scores	WHO Growth Charts	Charts and tables: WHO child growth standards for children[2]

Abbreviation:
CP: Cerebral palsy
GMFCS: Gross Motor Function Classification System
SD: Standard deviation
Movement ABC: Movement Assessment Battery for Children
BOTMP: Bruininks–Oseretsky Test of Motor Proficiency

Appendix 3. Data items to be collected

Trial level information

- Protocols of each individual trial
- Data forms or case report forms (CRFs) of each individual trial
- Country
- Setting (neonatal intensive care unit (NICU), hospital, or home)
- Dates of start and end of trial
- Number of infants randomised
- Informed consent procedures
- Methods of random allocation
- Methods of allocation concealment
- Blinding of outcome assessment
- Blinding of researchers/ caregivers
- Stratification factors used
- Purpose of intervention
- Planned intervention
- Specific details of the planned nutrition in the experimental and control arms of the trial (including composition, whether given as sole feed or in addition to breast milk and duration)
- Outcomes collected (primary and secondary)

Participant level information: Infant characteristics at trial entry

- Unique identification code
- Gestational age at birth
- Age at trial entry
- Birthweight
- Weight, length and head circumference at trial entry
- Sex
- Single/multiple (if multiple order of birth)

Participant level information: After trial entry

- Actual intervention or comparison received
- Age at commencement of nutritional supplement
- Age at ceasing the nutritional supplement
- Receipt of breast milk and whether mother's own or donor/banked
- Size at discharge or term-equivalent age (36-42 weeks' post-menstrual age)- including weight, length, head circumference, and measures of body composition e.g. skinfolds, boimpedence, plethysmography

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Reporting checklist for protocol of a systematic review.

Based on the PRISMA-P guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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In your methods section, say that you used the PRISMA-Preporting guidelines, and cite them as:

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		Reporting Item	Page Number
Title			
Identification	#1a	Identify the report as a protocol of a systematic review	1
Update	#1b	If the protocol is for an update of a previous systematic review, identify as such	n/a

Registration			
	#2	If registered, provide the name of the registry (such as PROSPERO) and registration number	2
Authors			
Contact	#3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	1
Contribution	#3b	Describe contributions of protocol authors and identify the guarantor of the review	16
Amendments			
	#4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	n/a
Support			
Sources	#5a	Indicate sources of financial or other support for the review	15
Sponsor	#5b	Provide name for the review funder and / or sponsor	15
Role of sponsor or funder	#5c	Describe roles of funder(s), sponsor(s), and / or institution(s), if any, in developing the protocol	15
Introduction			

Rationale	#6	Describe the rationale for the review in the context of what is already known	4-5
Objectives	#7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	6
Methods			
Eligibility criteria	#8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	6-8
Information sources	#9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	8
Search strategy	#10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	Appendix 4
Study records - data management	#11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	9
Study records - selection process	#11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	8

Study records - data collection process	#11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	8-9 Appendix 3
Data items	#12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	6-8 Appendix 1-2
Outcomes and prioritization	#13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	7-8
Risk of bias in individual studies	#14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	9
Data synthesis	#15a	Describe criteria under which study data will be quantitatively synthesised	10
Data synthesis	#15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I2, Kendall's τ)	10
Data synthesis	#15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	11

Data synthesis	#15d	If quantitative synthesis is not appropriate, describe the type of summary planned	11
Meta-bias(es)	#16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	n/a
Confidence in cumulative evidence	#17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	n/a

Appendix. Search strategies

Embase from 1980	
#	Search strategies
1	exp prematurity/
2	exp low birth weight/
3	exp small for date infant/
4	exp very low birth weight/
5	(prematur* adj2 infant*).tw.
6	(prematur* adj2 newborn*).tw.
7	(prematur* adj2 neonate*).tw.
8	preterm.tw.
9	low birth weight.tw.
10	low birthweight.tw.
11	VLBW.tw.
12	LBW.tw.
13	ELBW.tw.
14	small for gestation*.tw.
15	SGA.tw.
16	(less than adj6 g).tw.
17	(less than adj3 32 weeks).tw.
18	birth weight below.tw.
19	(gestation* adj2 less than).tw.
20	or/1-19
21	exp breast feeding/
22	exp infant nutrition/
23	exp protein intake/
24	exp dietary supplement/
25	exp omega 3 fatty acid/ct, ad, dt, ig, pa [Clinical Trial, Drug Administration, Drug Therapy, Intragastric Drug Administration, Parenteral Drug Administration]
26	exp arachidonic acid/ae, ct, ad, dt, ig, pa, th [Adverse Drug Reaction, Clinical Trial, Drug Administration, Drug Therapy, Intragastric Drug Administration, Parenteral Drug Administration, Therapy]
27	exp unsaturated fatty acid/ct, dt, pa, th [Clinical Trial, Drug Therapy, Parenteral Drug Administration, Therapy]
28	exp fat intake/ae, ad, dt [Adverse Drug Reaction, Drug Administration, Drug Therapy]
29	exp enteric feeding/
30	exp parenteral nutrition/
31	exp artificial milk/
32	exp breast milk/
33	exp fortified food/
34	exp elemental diet/
35	exp baby food/
36	(breast milk or human milk).tw.
37	formula.tw.
38	PUFA supplement*.tw.
39	feed* regimen*.tw.
40	(protein* adj2 concentration*).tw.
41	probiotic$.tw.
42	parenteral*.tw.

43	enteral*.tw.
44	maternal milk.tw.
45	multinutrient supplement*.tw.
46	(breast fed or breastfed).tw.
47	prebiotic*.tw.
48	diet* supplement*.tw.
49	nutrient enriched.tw.
50	Docosahexaenoic Acid*.tw.
51	arachidonic acid*.tw.
52	(glutamine adj2 supplement*).tw.
53	(taurine adj2 supplement*).tw.
54	(calcium adj2 supplement*).tw.
55	palm olein.tw.
56	palmitic acid.tw.
57	(fortification or fortified).tw.
58	fatty acids.tw.
59	supplement* feed*.tw.
60	complementary feed*.tw.
61	nutrition*.tw.
62	Hydrolysed liquid.tw.
63	Hydrolyzed liquid.tw.
64	gamma-linoleic acid.tw.
65	(diet* adj3 protein*).tw.
66	or/21-65
67	20 and 66
68	Clinical Trial/
69	Randomized Controlled Trial/
70	exp randomization/
71	Single Blind Procedure/
72	Double Blind Procedure/
73	Crossover Procedure/
74	Placebo/
75	Randomi?ed controlled trial$.tw.
76	Rct.tw.
77	random allocation.tw.
78	randomly.tw.
79	randomly allocated.tw.
80	allocated randomly.tw.
81	(allocated adj2 random).tw.
82	Single blind$.tw.
83	Double blind$.tw.
84	((treble or triple) adj blind$).tw.
85	placebo$.tw.
86	prospective study/
87	or/68-86
88	case study/
89	case report.tw.
90	abstract report/ or letter/
91	or/88-90
92	87 not 91

93	67 and 92