Title

Nutritional Evaluation and Optimisation in Neonates (NEON) trial of amino acid regimen and intravenous lipid composition in preterm parenteral nutrition: a randomised double blind controlled trial

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ABSTRACT

Objective: To confirm the safety and demonstrate efficacy of immediate introduction of Recommended Daily Intake (RDI) of amino acids and SMOF lipid in parenteral nutrition (PN) to increase non-adipose (lean) body mass and decrease Intrahepatocellular Lipid (IHCL)
Design: Multicentre, double-blind 2x2 factorial randomised controlled trial
Setting: Neonatal units in London and South East England
Participants: Extremely preterm infants born below 31 weeks gestation without major congenital or life threatening abnormalities who were able to be randomised to receive PN within 24 hours of birth.
Interventions: Infants were randomised within 24 hours of birth to receive PN containing either RDI of amino acids or incremental amino acids (control). In addition, infants were randomised to receive either 20% SMOFLipid or 20% Intralipid (control). In the control arm of amino acid intake infants received 1.7 g/kg/day of amino acid on day 1 of postnatal life. This increased to 2.1 g/kg/day on day 2 and a maximum of 2.7 g/kg/day from day 3. In the intervention group infants received 3.6 g/kg/day from day 1. On day 1 and 2 PN was provided in 90 ml/kg/day of aqueous increasing to 120 ml/kg/day from day 3 onwards. Carbohydrate intake was 8.6 g/kg/day from day 1. Lipid intake was 2 g/kg/day on day 1 increasing to 3 g/kg/day from day 2 onwards. Infants were also randomised to receive lipid as either 20% Intralipid or 20% SMOFLipid.
(Group 1: Incremental amino acids and 20% Intralipid, Group 2: Incremental amino acids and 20% Intralipid, Group 3: RDI of amino acids and 20% Intralipid, Group 4: RDI of amino acid and 20%). Intervention was continued until infants were receiving 150 ml/kg/day of enteral feeds for 24 hours. Nutritional intake, both parenteral and enteral, was guided by pre-specified protocols.
Primary outcome measure: For the amino acid intervention this was non-adipose body mass measured by Magnetic Resonance Imaging (MRI). For the lipid composition intervention this was IHCL measured by hepatic Magnetic Resonance Spectroscopy (MRS). Both primary outcomes were measured at term age equivalent between 37 and 44 weeks of post menstrual age.
Results: 168 infants were recruited to the study of which 133 infants completed the measurement of the primary outcome measures. There were no significant differences in the quantity of non-adipose tissue mass between the groups randomised to incremental vs RDI of

amino acids, (adjusted mean difference and 95% confidence interval: RDI – Incremental amino acid 1 (-108, 111) p=0.98). For the lipid composition intervention, there was no significant difference in IHCL between the groups randomised to receive 20% Intralipid vs 20% SMOFLipid (adjusted mean difference and 95% confidence interval 1.1 (0.8, 1.6) p=0.58). There were no significant differences in secondary outcome measures of the quantity and distribution of adipose tissue, measure of insulin sensitivity (QUICKI index), total cerebral volume, whole brain volume, weight and length at term age equivalent. There was however a significant difference in the mean head circumference at term age equivalent between the Groups randomised to receive incremental vs RDI of amino acids, (adjusted mean difference and 95% confidence interval: RDI – Incremental amino acid -0.8 (-1.5, -0.1) p= 0.02). The groups randomised to the RDI of amino acids had significantly greater proportion of infants with serum urea greater than 7 mmol / l and 10 mmol / l while on trial PN.

Conclusions: Provided PN is introduced within the first 24 hours after birth and accompanied by the early introduction and advancement of enteral nutrition, providing extremely preterm infants with RDI of amino acids within 24 hours of birth has no additional benefit on the accrual of lean body mass at term age equivalent. Administration of SMOFLipid from birth does not have a beneficial effect on the reduction of IHCL. Before either of the interventions studied in this trial can be recommended as routine practice long term follow up of brain and neuro-development as well as long term metabolic health is essential. The results do not support the calls for more aggressive nutrition in the extremely preterm infant nor the routine use of SMOFLipid.

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Table of Contents

Abstract 2
List of Tables 7
List of Figures 8
List of abbreviations/glossary 9
Scientific Summary 11
Plain English Summary 19
CHAPTER 1: Introduction 20
1. Background 20
1.1 Preterm infants 20
1.2 Rationale for trial 20
1.3 Nutritional requirements of preterm babies 21
1.4 Parenteral nutrition (PN) 22
1.5 Previous studies of parenteral nutrition 23
1.6 Risks and benefits of PN 25
1.7 Need for the NEON trial 26
CHAPTER 2: Research Objectives 27
1. Amino acid intervention 27
2. Lipid intervention 27
CHAPTER 3: Methods 28
1. Trial design 28
2. Participants 28
2.1 Inclusion criteria 28
2.2 Exclusion criteria 28
3. Interventions 28
4. Outcomes 30
4.1 Primary outcomes 30
4.1.1 Measurement of lean body mass 30
4.1.2 Measurement of intrahepatocellular lipid content 30
4.2 Secondary outcomes 30
5. Data collection 31
5.1 Electronic CRF (eCRF) 31
5.2 Timescale of trial evaluations 31
Daily evaluations: 31

5.3 Schedule of investigations 33
6. Clinical investigations 34
6.1 Anthropometry 34
6.2 Blood pressure measurements 34
6.3 Magnetic Resonance Imaging 34
6.3.1 MRI Body Composition 34
6.3.2 Hepatic MRS 36
6.3.3 Brain MRI 36
6.4 Quantitative Insulin- Sensitivity Check Index (QUICKI) 38
7. Pharmacovigilance definitions and procedures 38
7.1 Serious Adverse Events 38
7.2 Expectedness and Causality of SAEs 38
7.3 Reporting of Adverse Events 39
7.4 Specific Adverse Events 39
7.4.1 Annual Safety reports 41
8. Statistical considerations 41
8.1 Sample size 41
8.2 Randomisation 42
8.3 Blinding 42
8.4 Statistical methods 42
8.5 Missing data 44
8.6 Statistical Analysis Plan 45
9. Trial organisation 45
9.1 Trial Management 45
9.2 Trial Sponsor 45
9.3 Ethical considerations 46
9.3.1 Consent 46
9.4 Research governance 46
9.5 Regulatory requirements 46
9.6 Trial registration 46
9.7 NIHR CRN portfolio 46
9.8 Summary of protocol amendments 47
9.9 Trial Committees 47
9.9.1 Trial Steering Committee 47

9.9.2 Data Monitoring and Ethics Committee 48
9.10 Data management 48
9.11 Risk assessment and Monitoring Plan 48
9.12 Monitoring visits 49
9.13 Investigational Medicinal Product Manufacturer 49
9.14 Patient and Public Involvement 49
CHAPTER 4: Results 50
1. Participant flow 50
1.1 Screening 51
1.2 Recruitment and retention 51
1.3 Recruitment rate 52
1.4 Baseline data 54
CHAPTER 5: Discussion 98
CHAPTER 6: Conclusions and Recommendations 104
Acknowledgements 105
Contributions of authors 106
References 107
Appendix 1. Trial Protocol version 4.0 dated 16/10/2012 114
Appendix 2. Annotated Studybook (data collected on trial database) 115
Appendix 3. Parent information sheet, MR information Sheet and Consent Form 116
Appendix 4. Statistical analysis plan 117
Appendix 5. Formulae for derived variables (nutritional intake, MR outcomes, QUICKI index) 118

LIST OF TABLES

Table 1. Summary of interventions....29
Table 2. Summary of tests and investigations....33
Table 3. Definitions for assessment of causality....38
Table 4. Definitions of Specific Adverse Events including thresholds for reporting to DMEC....40
Table 5. Baseline characteristics for all infants randomised*....55
Table 6. Baseline characteristics for all infants completing MRI assessment....57
Table 7. Parenteral nutrition details and blood culture results for all infants randomised*....59
Table 8. Parenteral nutrition details and blood culture results for all infants completing MRI assessment*....60
Table 9. Trial Parenteral Nutrition intake during first 7 days for all infants randomised*....61
Table 10. Trial Parenteral Nutrition intake during first 7 days for all infants completing MRI assessment*....63
Table 11.Nutritional intake over first 2 weeks for all infants randomised*....65
Table 12. Nutritional intake over first 2 weeks for all infants completing MRI scan*....67
Table 13. Nutritional intake during study period for all infants randomised....69
Table 14. Nutritional intake during study period for all infants completing MRI scan*....72
Table 15. Safety data: Summary of laboratory adverse events by treatment for all infants randomised*....76
Table 16. Safety data: Summary of laboratory adverse events by treatment for all infants completing MRI scan*....78
Table 17. Safety data: Summary of serious adverse events by treatment*....80
Table 18. Reporting the factorial trial results all infants completing MRI assessment *....82
Table 19. Reporting the factorial original MRI results in volume for all infants completing MRI assessment *....86
Table 20. Summary of the covariates for secondary analysis*....89
Table 21. Reporting the factorial trial results for secondary analysis in all infants completing MRI assessment....90

LIST OF FIGURES

Figure 1. Classification of adipose tissue depots 36
Figure 2. CONSORT diagram 50
Figure 3. Summary of screening data for all trial sites 51
Figure 4.Cumulative recruitment vs target recruitment for the duration of the trial 52
Figure 5. Cumulative retention (number of MR scans) vs target retention for the duration of the trial 53
Figure 6. Total recruitment and retention per centre 53
Figure 7. Distribution of Primary and Secondary outcomes after transformation*.....87
Figure 8. Means (95% CIs) of incremental amino acid and recommended daily intake of amino acid in two lipid subgroups for: a) non-adipose body mass and b) IHCL on log scale 88
Figure 9. Time trend for babies' weight across four groups.....91
Figure 10. Daily protein intake from all sources (g/kg) in the first 2 weeks across four groups 92
Figure 11. Daily carbohydrate intake from all sources (g/kg) in the first 2 weeks across four groups.....93
Figure 12. Daily fat intake from all sources (g/kg) in the first 2 weeks across four groups....94
Figure 13. Daily protein intake from all sources (g/kg) after first 2 weeks across four groups 95
Figure 14. Daily carbohydrate intake from all sources (g/kg) after first 2 weeks across four groups.....96
Figure 15. Daily fat intake from all sources (g/kg) after first 2 weeks across four groups 97

LIST OF ABBREVIATIONS/GLOSSARY

ACA	Anterior Cerebral Artery
AT	Adipose tissue
CRN	Clinical Research Network
CTA	Clinical Trials Authorisation
CTEU	Clinical Trials and Evaluation Unit
DMEC	Data Monitoring and Ethics Committee
DSCAT	Deep Subcutaneous Adipose Tissue
DSCAAT	Deep Subcutaneous Abdominal Adipose Tissue
DSCNAAT	Deep Subcutaneous Non-Abdominal Adipose Tissue
DTI	Diffusion Tensor Imaging
eCRF	Electronic Case Record Form
EME	Efficacy and Mechanism Evaluation
FOV	Field of View
HOMA	Homeostatic Model Assessment
IAT	Internal Adipose Tissue
IAAT	Internal Abdominal Adipose Tissue
ICTU	Imperial Clinical Trials Unit
IHCL	Intrahepatocellular Lipid
IMP	Investigational Medicinal Product
INAAT	Internal Non-Abdominal Adipose Tissue
ISRCTN	International Standard Randomised Controlled Trial Number
IVRS	Interactive Voice Recognition System
LFT	Liver Function Test
MCA	Middle Cerebral Artery
MCRN	Medicines for Children Research Network
MHRA	Medicines and Healthcare products Regulatory Authority
MRA	Magnetic Resonance Angiography
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
NICU	Neonatal Intensive Care Unit
NIHR	National Institute for Health Research
PCA	Posterior Cerebral Artery

PN	Parenteral nutrition
QUICKI	Quantitative Insulin- Sensitivity Check Index
RDI	Recommended Daily Intake
REC	Research Ethics Committee
SAE	Serious Adverse Event
SMOF	Soyabean oil, Medium-chain triglycerides, Olive oil, Fish oil
SpAE	Specific Adverse Event
SSCAT	Superficial Subcutaneous Adipose Tissue
SSCAAT	Superficial Subcutaneous Abdominal Adipose Tissue
SSCNAAT	Superficial Subcutaneous Non-abdominal Adipose Tissue
SUSAR	Suspected Unexpected Serious Adverse Reaction
TAT	Total Adipose Tissue
TSC	Trial Steering Committee

SCIENTIFIC SUMMARY

Background

Extremely preterm infants, born below 31 weeks gestation, account for 1-1.5% of deliveries in the UK. By the time preterm infants reach term age, the overwhelming majority exhibit "growth failure" when compared with healthy term born infants. Growth failure is associated with neurodevelopmental impairment and cerebral palsy. Nutrition is a major factor influencing growth and possibly long-term metabolic health. Protein deficiency and a high fat, high carbohydrate diet characterises preterm nutrition during this period regardless of whether it is provided intravenously or enterally. A low protein diet and low protein:energy ratio in preterm infants results in a decrease in lean body mass and increased deposition of adipose tissue. Thus weight gain per se may not be as important as weight gain composition. In preterm infants, a low protein, high carbohydrate diet has also been shown to be associated with insulin resistance in adolescence. Preterm infants at present do not receive routine metabolic follow-up assessments and so the exact burden of subsequent metabolic ill health cannot be quantified. Current widespread practice is to commence parenteral nutrition (PN) several hours to days after birth and to commence macronutrients in PN at a dose below that of the Recommended Daily Intake (RDI) and increase slowly over 3-4 days, sometimes longer, often not achieving RDI. This practice is non-evidence based and results in cumulative deficits in protein and energy over the first two weeks of life. This practice is more prevalent with respect to amino acids than carbohydrates and fat. The evidence to support RDI of amino acids in preterm infants is based on historical data and observational studies. Increasingly there are calls for more aggressive early nutritional management in these babies but the evidence to support this is lacking.

There are now newer preparations of fat (SMOF lipid) that have been found to be liver protective and are currently only used in infants on long term PN. There is a need for studies to investigate the efficacy of these newer lipid solutions in reducing liver impairment.

Objectives

1. Amino Acid Intervention

To evaluate whether immediate rather than incremental introduction of Recommended Daily Intake (RDI) of amino acids in extremely preterm infants results in:

- Greater accrual of non-adipose (lean) body mass at term (Primary objective)
- Increased brain volume at term (Secondary objective)
- Reduced insulin resistance at term (Secondary objective)
- Reduced ratio of internal to subcutaneous adipose tissue at term (Secondary objective)
- Lower drop in weight standard deviation (SD) score between birth and term equivalent (Secondary objective)

2. Lipid Intervention

To evaluate whether 20% SMOF lipid (with lower ratio of n6 : n3 fatty acids) compared to 20% Intralipid in extremely preterm infants results in:

- Reduced IHCL at term age equivalent (Primary objective)
- Reduced incidence of hypertriglyceridaemia and hyperbilirubinaemia (Secondary objective)

Methods

1. Trial Design

This was a multi-centre, randomised 2x2 factorial double blind controlled trial in four London and South East of England centres. Eligible preterm infants were randomised within 24 hours of birth to receive 1) either incremental amino acids in parenteral nutrition or the RDI of amino acids from day one and 2) either 20% Intralipid or 20% SMOF lipid.

There were four randomised groups:

- Group 1: incremental amino acid and 20% Intralipid
- Group 2: incremental amino acid and 20% SMOF lipid
- Group 3: RDI of amino acids and 20% Intralipid
- Group 4: RDI of amino acids and 20% SMOF lipid

2. Participants

Preterm infants (<31 weeks gestation) requiring nutritional support in the form of parenteral nutrition.

Inclusion criteria

- Preterm infants born below 31 weeks of gestation (defined as ≤ 30 weeks and 6 days)
- Written informed consent from parents

Exclusion criteria

- Major congenital or life threatening abnormalities
- Inability to randomise in time to allow administration of trial PN within 24 hours of birth

3. Interventions

There were two interventions, namely the amount of amino acids in PN and the type of lipid formulation. All other components of PN were consistent across the four treatment groups. The intervention was commenced within 24 hours of birth. Nutritional intake, both parenteral and enteral, was guided by pre-specified protocols that were provided in an Investigator's Manual. In the control arm of amino acid intake infants received 1.7 g/kg/day of amino acid on day 1 of postnatal life. This increased to 2.1 g/kg/day on day 2 and a maximum of 2.7 g/kg/day from day 3. In the intervention group infants received 3.6 g/kg/day from day 1. On day 1 and 2 PN was provided in 90 ml/kg/day of aqueous increasing to 120 ml/kg/day from day 3 onwards. Carbohydrate intake was 8.6 g/kg/day from day 1. Lipid intake was 2 g/kg/day on day 1 increasing to 3 g/kg/day from day 2 onwards. Infants were also randomised to receive lipid as either 20% Intralipid or 20% SMOFLipid. Day 1 was defined as the duration between birth and when the first bag of PN was changed. Bag changes occurred at 1700 daily. PN was only dispensed between 0900 – 1700 hours. Depending on what time an infant was born and randomised infants received varying volumes from the first bag of PN. Subsequently all infants received the intended volumes as described above.

The interventions ceased once the infant was established on enteral feeds of 150 ml/kg/day for at least 24 hours. If the infant was subsequently placed nil by mouth after this point, PN was prescribed in accordance with local practice as determined by the supervising clinician.

4. Outcomes

a. Primary outcomes

Efficacy of early introduction of RDI of amino acid was assessed by whole body MRI to measure lean mass and the quantity and distribution of adipose tissue. This was done at term age equivalent. The infants were scanned between 37- 44 weeks post menstrual age.

i. Measurement of lean body mass

Lean body mass was calculated by subtracting adipose tissue mass from the weight of the baby on the day of the scan.

ii. Measurement of intrahepatocellular lipid content

Efficacy of SMOF lipid was assessed by liver magnetic resonance spectroscopy to measure intrahepatocellular lipid content. This was done at term age equivalent between 37 and 44 weeks post menstrual age.

b. Secondary outcomes

Quantity and distribution of adipose tissue

Total and regional brain volumes

Metabolic index of insulin sensitivity (QUICKI)

Serum lipids and bilirubin

Incidence of death

Anthropometry

5. Sample size and statistical analysis

The mean (standard deviation) of directly measured lean body mass of preterm infants when studied in 2003 was 2.1 (0.4) kg. The mean (SD) of healthy term infants is 2.6 (0.21) kg (mean difference 450g; 95% confidence interval for the difference 300, 610g). A sample size of 64 infants in each group was therefore chosen as this would allow detection of a 0.2 kg difference between the groups with 80% power and at 5% significance level (with a t-test). This was considered a clinically important increase in lean mass.

IHCL level used for sample size calculation was based on previous published work. Measurements were available for a total of 15 infants with gestational ages ranging from 24 to 32.6 weeks. IHCL had a mean = 1.75, sd = 1.85, range was 0.14 to 7.72. The distribution is clearly positively skewed. A $\log_e$ transformation was therefore used to achieve approximate normality. On the logarithmic scale the mean IHCL = 0.121, sd = 1.052, range was -1.97 to 2.04. A sample size of 64 infants in each group would therefore have 80% power to detect a difference in means of 0.526 on the logarithmic scale as significant at the 5% significance level (with a t-test). Transforming back to the original scale of measurement, this is equivalent to a 40% decrease in IHCL in the intervention group. Allowing for 10% mortality and 10% drop out rate the aim was to recruit 80 infants to each group or until 64 infants in each group had their MRI and MRS scans.

The analysis of this 2 x 2 factorial randomised trial was performed "at the margins" of the two by two table, assuming that the two factors are operating independently. In addition, summary measures were presented for each cell of the 2 x 2 table and an interaction ratio was calculated for binary outcomes. A 'modified' intention to treat method was used to analyse the results as it was accepted that a proportion of infants would not be able to attend the MR scan visit. With the exception of infants not completing the MR scan, all other infants were analysed according to their allocation.

The primary outcome measures for this trial are non-adipose (lean) body mass and intrahepatocellular lipid; the secondary outcomes are growth (weight, length and head circumference), brain growth and development (assessed by magnetic resonance imaging) and measure of insulin sensitivity (QUICKI). Growth parameters are the only outcomes that are measured sequentially; all other outcomes, including the two primary outcomes, are measured on a single occasion at term age equivalent.

For outcomes measured on a single occasion, a regression model containing the stratifying variables (gestational age, birth weight and centre), nutritional interventions (amino acid and lipid), sex and corrected gestational age at time of measurement were used to estimate the effects of each intervention.

For the amino acid intervention primary outcome we used multiple regression with non-adipose body mass (g) as the dependent variable and amino acids (incremental vs RDI), lipids (20% SMOF vs Intralipid), gestational age, birth weight, centre, gender and age at MRI scan as the independent variables to assess the effect of amino acids on non-adipose body mass. We also included an interaction term to assess whether the effect of amino acids regimen on non-adipose body mass is influenced by choice of lipids.

For the lipid intervention primary outcome we similarly used multiple regression with IHCL at natural logarithmic scale as the dependent variable and amino acid (incremental vs RDI), lipids (20% SMOF vs Intralipid), gestational age, birth weight, centre, gender and age at MRI scan as the independent variables to assess the effect of lipids on IHCL. Again, we included an interaction term so we could assess whether the effect of lipids on IHCL is affected by amino acid quantity.

A planned secondary analysis was used to investigate the role of illness severity and nutritional intake as potential modifiers of the effects of each intervention by adding these variables to the regression models.

A linear mixed model was used for the analysis of the secondary growth outcomes that were measured on several occasions during the trial. Here the interactions between interventions and time were interpreted as the difference in rate of change with time between the groups.

The analysis was performed "at the margins" of the two by two table, assuming that the two factors are operating independently. In addition, summary measures are presented for each cell of the 2 x 2 table and an interaction ratio/difference calculated. The secondary analysis investigates the role of illness severity, maternal breast milk and nutritional intake, including PN period and post PN period as potential modifiers of the effects of each intervention, by adding these variables to the regression model.

All analyses were performed using Stata 13, Stata Statistical Software: Release 13. College Station, TX: StataCorp LP).

All analyses were performed on an intention to treat basis but as the primary outcomes can only be ascertained in those infants attending the end of study evaluation up to 20% of primary outcomes are expected to be missing. We have assumed that these losses are missing at random.

Results

Of the 437 infants born less than 31 weeks of gestational age, 168 infants were randomised. A total of 133 infants were available for assessment of the primary outcome measures. Baseline characteristics of sex, gestational age at birth, anthropometry, maternal demographics, mode of delivery, antenatal steroid use, blood pressure on admission and time to commencing parenteral nutrition, were similar across the four groups.

The time to achieve a milk intake of 150 ml/kg/day for 24 hours for all infants randomised was similar across the four groups (median (IQR) in Groups A,B,C,D: 12(9-17.5), 11.5 (9-16), 11 (10 -14), 13 (9.5 -18) respectively. The length of hospital stay for all infants randomised was similar across the four groups (median (IQR) in Groups A,B,C,D 69.5 (52-95), 61 (45 – 88), 63 (45 – 95), 66.5 (44 – 98) respectively.

Nutritional intake from trial PN during the first week from PN was similar across the four groups except in the intake of protein. On day 4, when infants randomised to incremental amino acid intake achieved the maximum intake, the protein intake was 2.2 g/kg and 2.3 g/kg in Groups 1 and 2 compared to 3.7 g/kg and 3.5 g/kg in Group 3 and 4 respectively for all babies randomised. Median (IQR) cumulative protein intake from all sources in grams during the first 2 weeks after birth for all randomised babies in the incremental arm was 20.2 (14.6-25.6) and 18.6 (13.9-25.7) in Groups 1 and 2 compared to 28.6 (25.1-36.1) and 32.4 (25.4-41.4) in Groups 3 and 4 respectively. The median (IQR) cumulative protein intake in grams from all sources between birth and 34 weeks post- menstrual age for all babies randomised was 241.2 (189.3-468.2) and 215.5 (139.7-310.3) in Groups 1 and 2 compared to 275.0 (199.6-399.9) and 303.4 (221.9-419.8) in Groups 3 and 4 respectively.

There were no significant differences between the groups in the proportion of infants with abnormal biochemical indices namely, serum glucose, worst base deficit in the previous 24 hours, total serum bilirubin, conjugated bilirubin, serum cholesterol, serum triglycerides, serum sodium, serum potassium, serum phosphate, serum calcium, serum creatinine, and ALT). However, there were significantly more infants with blood urea nitrogen levels greater than 7 mmol/l (50% and 47.6 % in Groups 1 and 2 vs 70.7% and 79.1% in Groups 3 and 4 respectively, $p<0.01$) and 10 mmol/l (14.3% and 21.4% in Groups 1 and 2 vs 43.9% and 53.5% in Groups 3 and 4 respectively, $p<0.01$).

In relation to primary outcome measures, there were no significant differences in the quantity of non-adipose tissue mass between the groups randomised to incremental vs RDI of amino acids, (adjusted mean difference and 95% confidence interval: RDI – Incremental amino acid 1 (-108, 111) p=0.98). For the lipid composition intervention, there was no significant difference in IHCL between the groups randomised to receive 20% Intralipid vs 20% SMOFLipid (adjusted mean difference and 95% confidence interval 1.1 (0.8, 1.6) p=0.58). There were no significant differences in secondary outcome measures of the quantity and distribution of adipose tissue, measure of insulin sensitivity (QUICKI index), total cerebral volume, whole brain volume, weight and length at term age equivalent. There was however a significant difference in the mean head circumference at term age equivalent between the Groups randomised to receive incremental vs RDI of amino acids, (adjusted mean difference and 95% confidence interval: RDI – Incremental amino acid -0.8 (-1.5, -0.1) p= 0.02).

Conclusions

Provided PN is introduced within the first 24 hours after birth and accompanied by the early introduction and advancement of enteral nutrition, providing extremely preterm infants with RDI of amino acids within 24 hours of birth has no additional benefit on the accrual of lean body mass at term age equivalent. Administration of SMOFLipid from birth does not have a beneficial effect on the reduction of IHCL. Before either of the interventions studied in this trial can be recommended as routine practice long term follow up of brain and neuro-development as well as long term metabolic health is essential. The results do not support the calls for more aggressive nutrition in the extremely preterm infant nor the routine use of SMOFLipid.

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PLAIN ENGLISH SUMMARY

Infants born extremely preterm (defined as born less than 31 weeks of gestation) spend several weeks and months in intensive care and are subject to various complications relating to their prematurity. Outside the womb, meeting the nutritional demands of these infants presents challenges. Experts have called for changing how we feed babies but we do not know if giving more nutrition is better for babies. We studied two aspects of parenteral nutrition (PN), a fluid used to feed babies through their veins to overcome gut immaturity. One compared the incremental pattern of protein intake versus the Recommended Daily Intake (RDI). The second compared the type of fat in PN with a newer combination of fat that has shown to be less harmful to the liver. Babies were randomly allocated to one or other group by chance. This is so that both groups are similar at the start of the study so that any difference that is found at the end can be explained by the difference in the nutrition we gave them. Using special magnetic scans (to measure body muscle mass and fat in the liver) we studied the babies around the time they would have been born. We found that provided extremely preterm babies were fed milk from the start giving RDI of protein from birth instead of gradually increasing the intake did not result in any difference in muscle mass at term age. In addition the new type of lipid did not show any benefit over the old type of fat.

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CHAPTER 1: INTRODUCTION

1. BACKGROUND

1.1 Preterm infants

Extremely preterm infants, born below 31 weeks gestation, account for 1-1.5% of deliveries in the UK. Of around 70,000 preterm births in the UK each year, about 8,000 are born below 31 weeks gestation. The UK has one of the highest rates of preterm birth in Europe as well as one of the highest rates of neonatal mortality. These infants spend a prolonged period in hospital and are subject to long periods of poor nutrition. By the time preterm infants reach term age, the overwhelming majority exhibit "growth failure" when compared with healthy term born infants (1). Long term follow-up studies show that there appears to be catch-up growth in infancy and through adolescence (2). While this may be reassuring, catch-up growth is associated with adverse metabolic health and renal impairment (3, 4). However growth failure is associated with neurodevelopmental impairment and cerebral palsy (5, 6)

1.2 Rationale for trial

Nutrition is a major factor influencing growth and possibly long-term metabolic health. Protein deficiency and a high fat, high carbohydrate diet characterises preterm nutrition during this period regardless of whether it is provided intravenously or enterally. A low protein diet and low protein:energy ratio in preterm infants results in a decrease in lean body mass and increased deposition of adipose tissue (7). Thus weight gain per se may not be as important as weight gain composition. In preterm infants, a low protein, high carbohydrate diet has also been shown to be associated with insulin resistance in adolescence (8). Preterm infants at present do not receive routine metabolic follow-up assessments and so the exact burden of subsequent metabolic ill health cannot be quantified (9).

There is good evidence that there are critical periods in development where nutrition has long- term effects on later health. It has been shown that by end of the first week of life cumulative energy and protein deficits in infants born before 30 weeks are 400 kCal / kg and 14 g/kg (10, 11)... Preterm formulae and fortified maternal milk meet the Recommended Daily Intake (RDI) of macronutrients but deficits accumulated in the period after birth combined with factors that increase requirements result in a progressive deficit that is not made up or increases the magnitude of later catch-up growth.

Preterm infants have increased pre-pubertal insulin resistance compared to term born infants (12). Compared to term born infants, as adults they have higher blood pressure (13, 14), glucose intolerance (15), insulin resistance and dyslipidaemia (16). Insulin resistance in pre-pubertal children born extremely preterm has been associated with neonatal nutrition. Preterm infants were found to be insulin resistant compared to term infants. The diet of preterm infants was characterised as being low in protein in the first month and high in fat subsequently. Those that gained most weight in infancy were most insulin resistant and found to have a high carbohydrate intake in the first month of life (8).

Another group has demonstrated that a period of nutritional deprivation (though not specifically in any one macronutrient) in the early postnatal period may have beneficial effects on insulin resistance in preterm infants in adolescence (17). We have previously shown aberrant adipose tissue partitioning, increased intrahepatocellular lipid content and increased insulin resistance in preterm infants at term age equivalent compared to healthy term infants (18, 19). Our data suggest that even as early as term equivalent, preterm infants demonstrate the manifestations of cardiovascular risk factors.

Improving the quality and quantity of nutrition in this period has the potential to improve not just short- term outcomes but also the long- term neurodevelopmental and metabolic health of this vulnerable group of infants. Preterm infants comprise a group that continues to utilise NHS resources throughout life due to the long- term sequelae of prematurity. On average health and societal costs for preterm children at 6 years of age exceed that of a child born at term by approximately three fold (20).

1.3 Nutritional requirements of preterm babies

Traditionally, Recommended Daily Intakes (RDI) have been based on the composition of fetal and newborn weight gain. Source data are derived from the studies of Fomon and Ziegler (21, 22) on fetal cadavers of different gestational ages. Based on the weight gain composition at different periods of gestation and hence the accretion rate of lean mass and fat mass, the dietary intake of energy necessary for preterm newborns to achieve an intrauterine growth rate have been estimated as:

E *intake* = E *excreted* + E *stored* + E *expended* (E=energy)

**Where:*
Excreted energy: faeces and urine
Stored energy: as protein and fat (based on fetal accretion rate)
Expended energy = resting metabolic rate + energy of activity + thermoregulation (based on studies in growing preterm infants)

Using these data the American Academy of Pediatrics and the European Society for Paediatric Gastroenterology Hepatology and Nutrition have published RDI for preterm infants (23-25)... These have been used to inform this study. Putet has pointed out that knowledge of growth rate is insufficient to derive the optimum nutritional intake of preterm infants (7). He suggests that knowledge of weight gain composition (lean and fat mass) is essential to estimate the ideal ratio of protein to energy in order to avoid the deposition of excess energy as fat. Our previous work lends strength to this concept as we have shown that preterm infants receiving current conventional intakes have a carbohydrate-fat rich diet with a deficiency of protein and that they have a more adipose body composition when compared with term-born infants (11) .

Roggero et al, using whole body plethysmography at one month post term age, showed in a non-randomised study that a high protein intake (>3g/kg/day) preterm group (n = 26) had a significantly lower weight gain (g) [mean (SD): 946.7 (375.2) vs 1238 (407), $P < 0.05$] but a significantly higher lean body mass accrual (approximately 4 percent higher LBM as percent of body weight) than a low-protein-intake (<3g/kg/day) group (n = 22) (26).

Several recent reviews have concluded that current nutritional practices contribute to long-term impairment and recommend early introduction of RDI of macronutrients (27, 28). However, the evidence for this is based on tolerability and growth outcomes, and not on body composition.

1.4 Parenteral nutrition (PN)

Early nutritional intake in extremely preterm infants is wholly or in part delivered intravenously as PN because of immaturity of the gastrointestinal system. The median duration of PN after birth in infants born before 31 weeks of gestation is 12 days. Often PN is recommenced later in an infant's neonatal course if the clinical condition precludes enteral

feeding. Each day of PN costs the NHS £80-100 per infant. A typical tertiary neonatal unit spends up to £150,000 per year on PN. There are currently various PN preparations in routine use that vary in both composition and usage. None have previously been tested in this country in the setting of a large RCT. Some solutions are commercially prepared while others are made up in local hospital pharmacies.

This has been the focus of a scoping exercise that was commissioned by the Department of Health because of serious concern of clinical risk to patients. http://www.rpharms.com/support-pdfs/minimisingriskpnforchildren.pdf. The survey carried out as part of the exercise confirmed that current practice among neonatologists with respect to PN varies widely and is based on limited evidence. There was also considerable variation in the preparation of PN and guidelines for use. 116 hospitals reported providing PN to neonates and completed the survey relating to neonates. The principal investigator was a member of the clinical group that developed and analysed the survey and prepared the report. The report, which was published in November 2011, called for urgent measures to standardise practice both in the technical and clinical aspects of use of PN in neonates and children and for the development of evidence based guidelines for the use of PN. A further report from the NCEPOD that the principal investigator contributed to came to similar conclusions (http://www.ncepod.org.uk/2010pn.htm).

Current widespread practice is to commence PN several hours to days after birth and to commence macronutrients in PN at a dose below that of the RDI and increase slowly over 3-4 days, sometimes longer, often not achieving RDI. This practice is non-evidence based and results in cumulative deficits in protein and energy over the first two weeks of life. This practice is more prevalent with respect to amino acids than carbohydrates and fat. Long term use of PN results in liver impairment and even failure. This is a particular problem in neonatal units caring for infants with bowel problems that preclude or limit enteral feeding. There are now newer preparations of fat (SMOF lipid) that have been found to be liver protective and are currently only used in infants on long term PN (29). There is a need for studies to investigate the efficacy of these newer lipid solutions in reducing liver impairment.

1.5 Previous studies of parenteral nutrition

Several recent reviews have concluded that current nutritional practices contribute to growth failure and recommend early introduction of RDI of macronutrients in parenteral nutrition

(27, 28). However, the quality of the evidence on which this is based is grade B (RCT with minor limitations, overwhelming consistent evidence from observational studies) and only based on outcomes such as tolerability and growth despite recognition that the ideal postnatal growth rate of a preterm infant is unknown. No data exist on the effect on body composition.

We have shown that the body composition of preterm infants is different to that of healthy term infants. Preterm infants had significantly reduced lean body mass and a pattern of adipose tissue distribution associated with metabolic complications (19). Tan et al, studied the effect of 'hyperalimentation' on head growth (30, 31). No differences between the two groups was found but non-randomised analyses showed protein /energy deficits to be correlated with poor head growth. 80% of babies in the intervention group had significant protein /energy deficits at the end of the first four weeks. A major drawback of this study was that participants in this study were recruited up to 7 days after birth by which time significant deficits are known to have developed. The study was also underpowered to detect a significant effect on the primary outcome.

A systematic review of the effect of early administration of parenteral nutrition on growth outcomes in preterm infants included eight randomised controlled trials and thirteen observational studies. The review was limited by the disparate growth outcome measures. Early PN reduced the time to regain birth weight by 2.2 d (1.1, 3.2) in RCTs and 3.2d (2.0, 4.4) in observational studies. The maximum percentage weight loss with early PN was lower by 3.1% (1.7, 4.5) for RCTs and by 3.5% (2.6, 4.3) for observational studies. Early PN also improved weight at discharge or 36 weeks post menstrual age by 14.9 (5.3, 24.5 g) grams in observational studies but no benefit was shown for length or head circumference (32).

A trial comparing two different amounts of amino acids (2.4 g/kg/day vs 3.6 g/kg/day with lipid intake of 2-3 g/kg/day with an additional third arm of 2.4 g/kg/day of amino acids with delayed introduction of lipids) from birth demonstrated improved nitrogen balance on day 2 in the arms with early initiation of lipids. There was no improvement in nitrogen balance with greater amounts of amino acids (33).

A systematic review of the early introduction of lipids (defined as within the first two days after birth) and the use of new lipid emulsions included fourteen RCTs. Early initiation of lipids had no impact on any of the outcome measures including death, bronchopulmonary

dysplasia, necrotising enterocolitis, patent ductus arteriosis, sepsis, intraventricular haemorrhage, significant jaundice or hypertriglyceridaemia. The meta-analysis of the effects of lipid emulsions that are not purely soybean-based showed no difference in outcomes of death, duration of respiratory support or rate of weight gain. There was a lower rate of sepsis with the lipid emulsions that were not purely soybean-based but this was not statistically significant. However the authors concluded that large scale RCTs are needed to determine the efficacy of newer lipids (34).

We recently published a systematic review of preterm parenteral nutrition summarising the evidence to date (35) . The review concludes that the evidence base for current recommendations is based on historical evidence and there are no long- term studies of the impact of PN on health and neurodevelopment.

1.6 Risks and benefits of PN

PN is an independent risk factor for sepsis in neonates, associated with a 40 fold greater risk; which makes its judicious use a priority. The risks associated with any form of parenteral nutrition are metabolic disturbances (hyperglycaemia, hyperlipidaemia, electrolyte imbalances), infection (36) and catheter related complications. However, these risks are unavoidable as parenteral nutrition is the only option for feeding extremely preterm infants until they are established on enteral nutrition.

PN is also associated with cholestasis and liver impairment (37). The commencement of RDI of amino acids on the day of birth as proposed in the intervention arm may result in a higher incidence of metabolic acidosis and high blood urea nitrogen. Until now, only one study has investigated the efficacy of early introduction of amino acids (3.5 g/kg/d) combined with a lipid emulsion (3 g/kg/d) in high concentrations within the first 2 hours of life. Early lipid introduction resulted in an increased positive nitrogen balance without an increased incidence of metabolic or respiratory complications (38). However, there was a small statistically significant increase in serum bilirubin, without clinical implications. Other studies in preterm infants using this approach have not shown an increased incidence of this problem (38-40).

The lipid solution currently used, Intralipid 20%, is a first generation lipid emulsion based on soybean oil, which is very rich in n-6 polyunsaturated fatty acids (PUFA). However, an excess intake of n-6 PUFA in parenteral nutrition is associated with an unbalanced fatty acid

pattern in cell membranes, with possible modified function, and with increased lipid peroxidation(41). Second generation emulsions are represented by medium-chain/long-chain triglyceride (MCT/LCT) mixtures and olive oil containing emulsions. MCT/LCT mixtures have a faster clearance from the blood stream and a higher degree of immediate energy generation. Olive oil containing emulsions provide a more physiological fatty acid pattern with less lipid peroxidation. An example of a third generation emulsion is SMOF lipid, a mixture of soybean-LCT, MCT, olive oil and fish oil, supplemented with vitamin E. This emulsion is designed to increase the amount of n-3 fatty acids, thereby reducing the ratio n-6:n-3 fatty acids (in accordance with current recommended levels)(41). SMOF lipid 20% is well tolerated in infants without changing lipid peroxidation parameters (42)(29) and beneficial effects on liver function and serum triglyceride concentrations have been described(29).

1.7 Need for the NEON trial

In spite of evidence demonstrating that introducing RDI of macronutrients early appears to be safe and results in improved protein retention and better growth in the short term, clinical practice has remained variable because of the absence of evidence from randomised controlled trials with clinically meaningful outcomes. If early RDI introduction were shown in the setting of a randomised controlled trial to improve not just growth but a better measure of growth, i.e. increase in lean body mass and better brain growth, with the long term benefits that in turn result from these, it has the potential to impact the vast majority of neonatal unit graduates. There is an urgent need for therapy with PN to be evidence based.

CHAPTER 2: RESEARCH OBJECTIVES

1. AMINO ACID INTERVENTION

To evaluate whether immediate rather than incremental introduction of Recommended Daily Intake (RDI) of amino acids in extremely preterm infants results in:

- Greater accrual of non-adipose (lean) body mass at term (Primary objective)
- Increased brain volume at term (Secondary objective)
- Reduced insulin resistance at term (Secondary objective)
- Reduced ratio of internal to subcutaneous adipose tissue at term (Secondary objective)
- Lower drop in weight standard deviation (SD) score between birth and term equivalent (Secondary objective)

2. LIPID INTERVENTION

To evaluate whether 20% SMOF lipid (with lower ratio of n6 : n3 fatty acids) compared to 20% Intralipid in extremely preterm infants results in:

- Reduced IHCL at term age equivalent (Primary objective)
- Reduced incidence of hypertriglyceridaemia and hyperbilirubinaemia (Secondary objective)

CHAPTER 3: METHODS

1. TRIAL DESIGN

This was a multi-centre, randomised 2x2 factorial double blind controlled trial in four London and South East of England centres. Eligible preterm infants were randomised within 24 hours of birth to receive 1) either incremental amino acids in parenteral nutrition or the RDI of amino acids from day one and 2) either 20% Intralipid or 20% SMOF lipid.

There were four randomised groups:

- Group 1: incremental amino acid and 20% Intralipid
- Group 2: incremental amino acid and 20% SMOF lipid
- Group 3: RDI of amino acids and 20% Intralipid
- Group 4: RDI of amino acids and 20% SMOF lipid

2. PARTICIPANTS

Preterm infants (<31 weeks gestation) requiring nutritional support in the form of parenteral nutrition.

2.1 Inclusion criteria

- Preterm infants born below 31 weeks of gestation (defined as ≤ 30 weeks and 6 days)
- Written informed consent from parents

2.2 Exclusion criteria

- Major congenital or life threatening abnormalities
- Inability to randomise in time to allow administration of trial PN within 24 hours of birth

3. INTERVENTIONS

There were two interventions, namely the amount of amino acids in PN and the type of lipid formulation. All other components of PN were consistent across the four treatment groups. The intervention was commenced within 24 hours of birth. Nutritional intake, both parenteral and enteral, were guided by pre-specified protocols that were provided in an Investigator's Manual.

The interventions ceased once the infant was established on enteral feeds of 150 ml/kg/day for at least 24 hours. If the infant was subsequently nil by mouth after this point, PN was prescribed in accordance with local practice as determined by the supervising clinician.

A summary of the interventions is provided in Table 1.

Table 1. Summary of interventions

Group 1 (incremental amino acid and 20% Intralipid)		**Day 1**	**Day 2**	**Day 3 onwards**
Volume *(excluding lipid volume)*	ml/kg/day	90	90	120
Protein	g/kg/day	1.5	1.9	2.4
Amino acid equivalent	*g/kg/day*	*1.7*	*2.1*	*2.7*
Carbohydrate (glucose)	g/kg/day	8.6	8.6	8.6
20% Intralipid	g/kg/day	2	3	3

Group 2 (incremental amino acid and 20% SMOF lipid)		**Day 1**	**Day 2**	**Day 3 onwards**
Volume *(excluding lipid volume)*	ml/kg/day	90	90	120
Protein	g/kg/day	1.5	1.9	2.4
Amino acid equivalent	*g/kg/day*	*1.7*	*2.1*	*2.7*
Carbohydrate (glucose)	g/kg/day	8.6	8.6	8.6
20% SMOF lipid	g/kg/day	2	3	3

Group 3 (RDI of amino acid and 20% Intralipid)		**Day 1**	**Day 2**	**Day 3 onwards**
Volume *(excluding lipid volume)*	ml/kg/day	90	90	120
Protein	g/kg/day	3.2	3.2	3.2
Amino acid equivalent	*g/kg/day*	*3.6*	*3.6*	*3.6*
Carbohydrate (glucose)	g/kg/day	8.6	8.6	8.6
20% Intralipid	g/kg/day	2	3	3

Group 4 (RDI of amino acid and 20% SMOF lipid)	**Day 1**	**Day 2**	**Day 3 onwards**

Volume *(excluding lipid volume)*	ml/kg/day	90	90	120
Protein	g/kg/day	3.2	3.2	3.2
Amino acid equivalent	*g/kg/day*	*3.6*	*3.6*	*3.6*
Carbohydrate (glucose)	g/kg/day	8.6	8.6	8.6
20% SMOF lipid	g/kg/day	2	3	3

4. OUTCOMES

4.1 Primary outcomes

Efficacy of early introduction of RDI of amino acid was assessed by whole body MRI to measure lean mass and the quantity and distribution of adipose tissue. This was done at term age equivalent. The infants were scanned between 37- 44 weeks post menstrual age.

4.1.1 Measurement of lean body mass

Lean body mass was calculated by subtracting adipose tissue mass from the weight of the baby on the day of the scan.

4.1.2 Measurement of intrahepatocellular lipid content

Efficacy of SMOF lipid was assessed by liver magnetic resonance spectroscopy to measure intrahepatocellular lipid content. This was done at term age equivalent between 37 and 44 weeks post menstrual age.

4.2 Secondary outcomes

Quantity and distribution of adipose tissue
Total and regional brain volumes
Metabolic index of insulin sensitivity (QUICKI)
Serum lipids and bilirubin
Incidence of death
Anthropometry

5. DATA COLLECTION

5.1 Electronic CRF (eCRF)

Data management was through the InForm ITM (Integrated Trial Management) System, a web-based data entry system that builds an Oracle database for each individual clinical trial. Trial data was captured on a bespoke web-based electronic case record form (eCRF) with built-in validation rules to identify data entry errors in real time and a full audit trail of data entry and changes. All persons entering data were trained prior to start up and given personal login details with access to forms restricted according to site and role. The eCRF was designed in accordance with the requirements of the trial protocol and access to the eCRF was password-protected and included controlled level of access.

5.2 Timescale of trial evaluations

Daily evaluations:

The first daily evaluation started at the time of birth and was completed when the first bag of trial PN was changed and on the first day of post-natal life. Subsequent evaluations occurred 24 hours from this time point (+/- 2 hours) every day from birth and until 37 weeks corrected gestational age or discharge from NICU where days were calculated from date PN was initiated.

Weekly evaluations:

The first weekly evaluation occurred 7 days +/- 2 days from randomisation and each 7 days +/- 2 days thereafter until 37 weeks corrected age or discharge from NICU.

Monthly evaluation:

The first monthly evaluation occurred 30 days +/- 5 days from randomisation and each 30 days +/- 5 days thereafter until 37 weeks corrected age or discharge from NICU.
For infants that received long-term PN i.e. at least 28 continuous days, serum trace elements were measured.

'37 week' evaluation:

This evaluation took place when the infant reached 37 weeks corrected gestational age (+/- 1 week) or when the infant was discharged from NICU, whichever occured sooner.

End of study evaluation:

This took place as soon as possible after the infant was discharged from NICU at 37-44 weeks corrected gestational age. In the case of one hospital (Chelsea and Westminster NHS Foundation Trust) with on-site access to a MR scanner, infants aged between 37 – 44 weeks of gestation age who were otherwise well but not ready for discharge, were scanned prior to discharge.

5.3 Schedule of investigations

Table 2. Summary of tests and investigations

Evaluation	Baseline	Daily	Weekly	Monthly	37 weeks corrected age	End of study (37-44 weeks and discharge from NICU)
Informed consent	✓					
Eligibility	✓					
Randomisation	✓					
Weight	#	#*	#			✓
Length	#		#			✓
Head circumference	#		#			✓
Blood pressure	#		✓			✓
Nutritional intake	✓	✓				
Safety						
Blood glucose (highest and lowest in previous 24 hours)		#*				
Worst base deficit on blood gas (in previous 24 hours)		#*				
Serum bilirubin, LFTs, serum urea, creatinine and electrolytes		#*			#	
Serum lipid and cholesterol			#*			
Trace elements (zinc, copper, manganese, aluminium, selenium)				#*		
Adverse event tracking		✓	✓		✓	✓
Efficacy						
QUICKI					✓	
Whole body and brain MRI, MRS						✓
Blood spot	✓				✓	
Urine sample and stool sample			✓			

Key

✓ For research purposes
\# Routine care
* While on parenteral nutrition

6. CLINICAL INVESTIGATIONS

6.1 Anthropometry

Weight, length and head circumference measurements are routinely used to monitor infant growth. Weight was recorded on a daily basis while on PN and weekly when not on PN until discharge and at the end of study visit. Length and head circumference were recorded on a weekly basis until discharge and at the end of study visit.

6.2 Blood pressure measurements

Systolic and diastolic blood pressure was measured in the right upper limb using a non-invasive blood pressure monitor and a cuff that covered at least two-thirds of the right upper limb and encompassed the entire arm in a restful state.

6.3 Magnetic Resonance Imaging

The MRI measurements were carried out during normal sleep without the need for sedation. All the MRI measurements (body composition, hepatic MRS and brain MRI) took a total of 45 -60 min. The infants were monitored with pulse oximetry and a trained neonatal doctor was present throughout the scan. Parents were invited to be present in the console room.

6.3.1 MRI Body Composition

Acquisition of images

Scans were undertaken after discharge from hospital at the Robert Steiner MR unit at Imperial NHS Trust at a dedicated research scanning facility on a Phillips 1.5 Tesla system. Babies born at the lead site, Chelsea and Westminster NHS Foundation Hospital who were still in-patient between 37 and 44 weeks postmenstrual age and unlikely to be discharged home in time to be scanned in the research scanner, were scanned while in-patient at Chelsea and Westminster NHS Foundation Hospital scanner on a Siemens Avanto 1.5 Tesla system.

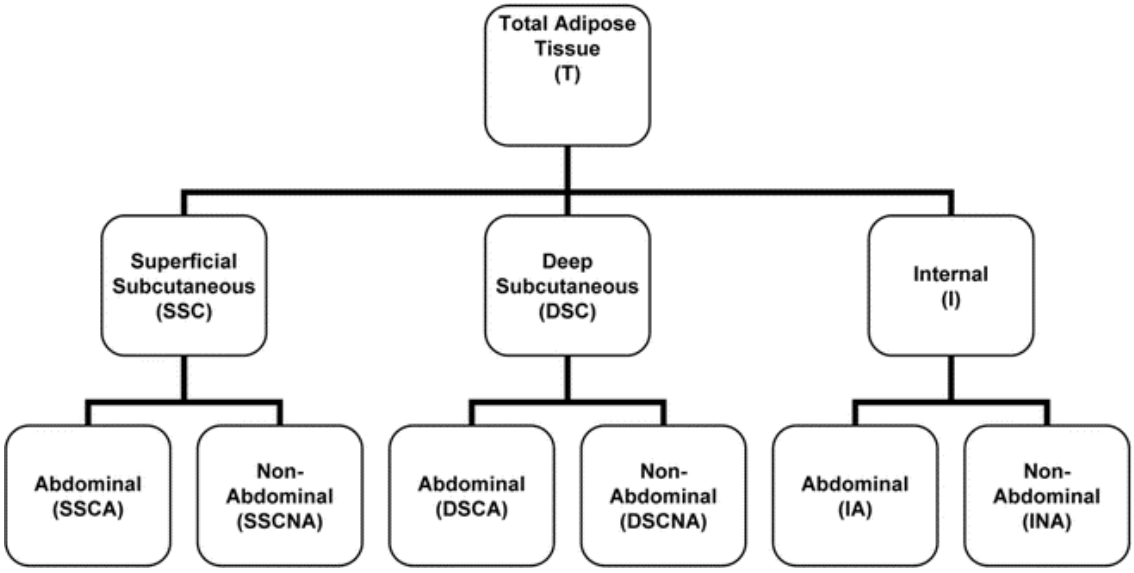
For images that were acquired on the Phillips 1.5 Tesla system a T1-weighted rapid-spin-echo sequence (repetition time of 500 ms, echo time of 17 ms, echo train length of 3) using a Q body coil was used. The slice thickness was 5 mm, and the interslice difference was 5 mm. Voxel size was 0.31 × 0.31 × 0.31 cm. Acquisition time was approximately 12 minutes. For

images acquired on the Siemens scanner a T1 turbo spin echo sequence was used (repetition time 514 ms, echo time of 11 ms).

Analysis of images

Analysis of all MR images was undertaken independently of the investigators, blind to subject identity and treatment, by VardisGroup, (London, UK, www.vardisgroup.com). Images were analysed by a single observer using a commercially available software program (SliceOMatic, Version 4.2, Tomovision, Montreal, Canada). A filter was used to distinguish between different gray level regions on each slice. This was then verified and, where necessary, edited using an interactive slice editor program. AT area (cm^2) for each slice was calculated as the sum of the voxels multiplied by the voxel area. AT volume (cm^3) for each slice was calculated by multiplying the tissue area by the sum of the slice thickness and the interslice distance. The coefficient of variation for these measurements was <3% (43)... AT volume in litres was converted to AT mass in kg, assuming a value for the density of AT of 0.90 kg/litre (44, 45). AT mass, kg = (AT volume, l) x 0.90. AT mass was used to determine percentage AT as: Percentage AT mass = (AT mass, kg) / (body mass, kg). Total adipose tissue volume was calculated as the sum of 6 individually quantified adipose tissue compartments: superficial subcutaneous abdominal (SSCA), superficial subcutaneous non-abdominal (SSCNA), deep subcutaneous abdominal (DSCA), deep subcutaneous non-abdominal (DSCNA), internal abdominal (IA), and internal non-abdominal (INA), as previously described (43) (Figure 1). Total subcutaneous adipose tissue was calculated as the sum of abdominal superficial subcutaneous, abdominal deep subcutaneous, non-abdominal superficial subcutaneous, and non-abdominal deep subcutaneous. Total internal adipose tissue was calculated as the sum of IA and internal non-abdominal.

Figure 1. Classification of adipose tissue depots



6.3.2 Hepatic MRS

Acquisition of spectra

^{1}H MR spectra were acquired at 1.5 T from the right lobe of the liver using a point-resolved spectroscopy sequence (PRESS) (TR 1500 ms/ TE 135 ms) without water saturation and with 128 signal averages. Transverse images of the liver were used to ensure accurate positioning of the (20×20×20 mm) voxel in the liver, avoiding blood vessels, the gall bladder, and fatty tissue. For spectra acquired on the Siemens scanner a voxel size of 15x15x15 mm was used.

Analysis of spectra

Spectra were analysed in the time domain using the AMARES algorithm included in the MRUI software package by a single investigator (ELT) who was blind to the treatment category (46, 47). Peak areas for all resonances were obtained and lipid resonances were quantified with reference to water resonance, after correcting for T_1 and T_2 hepatic water, known to be relatively constant, was used as an internal standard, and the results are presented as the percentage ratio of fat CH_2 to water.

6.3.3 Brain MRI

Acquisition of images

Brain imaging was performed on the infants using a dedicated 8 channel pediatric coil. Three dimensional T1 weighted fast gradient echo images were acquired in a sagittal plane: Field of view (FOV) 220 x 158 mm, slices 192, slice thickness 1mm, acquired voxel size 0.82mm x

0.97mm, matrix 256, echo time 4.6ms, repetition time 17ms, flip angle 13 degrees. Acquisition time 6 minutes.

Whenever possible and with time permitting the following brain scans were also undertaken:

T2W turbo spin echo sequence acquired in an axial plane. FOV 220 x 220 mm, slices 94, slice thickness 2mm, acquired voxel size 1.15 x 1.42, slice gap -1mm, matrix 256, echo time 160ms, repetition time 15077ms, flip angle 90 degrees. Acquisition time 2 minutes.

A three dimensional time of flight (TOF) Magnetic Resonance Angiography (MRA) sequence to assess the anterior cerebral artery (ACA), middle cerebral artery (MCA) and posterior cerebral artery (PCA). Imaging parameters: FOV 175 x 144, slices 75, stacks 1, slice thickness 0.8mm, slice gap 0mm, voxel size 0.61 x 0.61, echo time 12ms, repetition time 23ms, flip angle 16 degrees, matrix 512. Acquisition time 5 minutes.

15 direction Diffusion Tensor Imaging (DTI) imaging for assessment of white matter integrity also formed part of the protocol. FOV 224 x 224, slices 49, slices thickness 2.5mm, slice gap 0mm, acquired voxel size 2 x 2mm, matrix, 128, echo time 49ms, repetition time 49709ms, max b factor 750, nr of b factors 2. Acquisition time 6 minutes.

Analysis of images

All brain MRI images were reported by a specialist in neonatal neurology for clinical purposes. Note was made of any congenital or acquired lesions. The type and severity of these was recorded for all cases. Scans with parenchymal brain lesions were excluded from subsequent quantitative analysis.

A quantitative whole brain segmentation programme was used to segment the brain and its constituent structures using the T2 weighted image data (48). This volumetric data could only be provided from images that were of adequate quality, good signal to noise and absence of motion artefact.

The following outcomes were measured:

- Total cerebral volume: Sum of the volumes of basal ganglia, thalami (deep grey matter), cerebrospinal fluid, grey matter, white matter and lateral ventricles

- Whole brain volume: Sum of the volumes of basal ganglia, thalami (deep grey matter), grey matter and white matter
- Posterior fossa volume: Sum of the volumes of cerebellum and brainstem

6.4 Quantitative Insulin- Sensitivity Check Index (QUICKI)

QUICKI is a marker of insulin resistance calculated from pre-feed serum insulin and blood glucose. Homeostatic Model Assessment (HOMA) is the gold standard for measuring insulin resistance but is invasive and cannot be justified ethically in this patient group. QUICKI has been validated against HOMA (49) and been used in neonates before (50). Measurement of QUICKI was carried out at term age and samples taken at the time of routine (pre-feed) blood tests.

7. PHARMACOVIGILANCE DEFINITIONS AND PROCEDURES

7.1 Serious Adverse Events

An Adverse Event (AE) was defined as any untoward medical occurrence in a patient administered an investigational medicinal product (IMP), in accordance with clinical trial regulations. An AE was considered Serious and reportable via the eCRF if any of the following criteria occurred:

- resulted in death
- was life-threatening
- prolongation of existing inpatient hospitalisation
- resulted in persistent or significant disability or incapacity

7.2 Expectedness and Causality of SAEs

The trial protocol specified that a range of SAEs would be expected either as a consequence of preterm birth or if they were listed in any of the Summaries of Product Characteristics and this expectedness was recorded on the eCRF for each SAE report.

Causal relationship to the IMP was defined according to Table 3 below.

Table 3. Definitions for assessment of causality

Relationship	Description
Unrelated	There is no evidence of any causal relationship

Unlikely	There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the patient's clinical condition, other concomitant treatment).
Possible*	There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the patient's clinical condition, other concomitant treatments).
Probable*	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.
Definitely*	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.
Not assessable	There is insufficient or incomplete evidence to make a clinical judgement of the causal relationship.

***Suspected Unexpected Serious Adverse Reaction**

If an AE was considered serious, unexpected and related to the IMP (possible, probable or definitely related) this would have met the definition of Suspected Unexpected Serious Adverse Reaction requiring expedited reporting to the MHRA, REC and Sponsor. There were no SUSARs for the NEON trial.

7.3 Reporting of Adverse Events

The trial eCRF included dedicated forms for reporting SAEs. Investigators were advised to report SAEs via the eCRF within 24 hours of becoming aware of the event and to include an assessment of expectedness and causality in the SAE report. Each SAE report was reviewed by the Clinical Trials Unit and Chief Investigator within 2 working days.

7.4 Specific Adverse Events

The only non-Serious AEs that were reportable were values of triglycerides, bilirubin and other safety parameters above or below pre-specified levels and these are summarised in the table below. These were labelled as 'Specific AEs' (SpAEs) reportable via the eCRF. The eCRF incorporated in-built checks to flag any occurrence of a SpAE during the data entry

process to the local teams. Guidance for the management of these events was provided to the participating centres in a trial specific Investigator Manual. Specific Adverse Events related to safety parameters were collected daily during the period of trial PN administration.

As the levels selected for SpAEs were consistent with normal ranges used in standard neonatal clinical care and, in accordance with the new MHRA guidance on risk-adapted approach to managing clinical trials the NEON trial was equivalent to standard care, additional reporting and review of SpAEs was not required. The trial Data Monitoring and Ethics Committee (DMEC) reviewed a selection of SpAEs throughout the duration of the trial.

The thresholds for SpAEs as well as those requiring reporting to the DMEC are summarised in Table 4.

Table 4. Definitions of Specific Adverse Events including thresholds for reporting to DMEC

Assessment (blood test)	**Level requiring Specific Adverse Event (SpAE) report**	**Level requiring reporting to DMEC**
Glucose	< 2.6 mmol /l or > 15 mmol /l	Not reported to DMEC
Worst base deficit in previous 24 hours	> 15 mmol /l	> 15 mmol/l
Total serum bilirubin	> 150 μmol /l	> 150 μmol /l, only after 3 weeks on PN*
Conjugated bilirubin	> 40 μmol /l	> 40 μmol /l
Cholesterol	> 6 mmol /l	>10 mmol/l
Triglycerides	> 2.5 mmol /l	> 5 mmol/l
Sodium	< 131 mmol /l or > 150 mmol /l	Not reported to DMEC
Potassium	< 3. 2 mmol /l or > 9 mmol /l	Not reported to DMEC
Phosphate	< 1.5 mmol /l or > 3 mmol /l	Not reported to DMEC

Calcium	< 1mmol /l or > 3 mmol /l	Not reported to DMEC
Urea	< 1. 5 mmol /l or > 7 mmol /l	> 10 mmol/l
Creatinine	>170 μmol/l	Not reported to DMEC
Alanine aminotransferase	> 60 IU / l	Not reported to DMEC
Zinc	<8 μmol/l	Not reported to DMEC
Copper	<2 μmol/l	Not reported to DMEC
Manganese	>30 nmol/l	Not reported to DMEC
Aluminium	>0.4 μmol/l	Not reported to DMEC
Selenium	<20 μg/l	Not reported to DMEC

*Infant must have been on PN for at least 3 weeks for this to meet requirements for reporting to DMEC

7.4.1 Annual Safety reports

Annual Safety reports were provided to the REC and MHRA, in accordance with clinical trial regulations, on the anniversary of the Clinical Trial Authorisation each year. A total of three annual safety reports were submitted over the course of the trial.

8. STATISTICAL CONSIDERATIONS

8.1 Sample size

The mean (standard deviation) of directly measured lean body mass of preterm infants when studied in 2003 was 2.1 (0.4) kg (17). The mean (SD) of healthy term infants is 2.6 (0.21) kg (mean difference 450g; 95% confidence interval for the difference 300, 610g). A sample size of 64 infants in each group was therefore chosen as this would allow detection of a 0.2 kg difference between the groups with 80% power and at 5% significance level (with a t-test). This was considered a clinically important increase in lean mass.

Since publication of our paper on IHCL (16), measurements were available for a total of 15 infants with gestational ages ranging from 24 to 32.6 weeks. IHCL had a mean = 1.75, sd = 1.85, range was 0.14 to 7.72. The distribution is clearly positively skewed. A $\log_e$

transformation was therefore used to achieve approximate normality. On the natural logarithmic scale the mean IHCL = 0.121, sd = 1.052, range was -1.97 to 2.04. A sample size of 64 infants in each group would therefore have 80% power to detect a difference in means of 0.526 on the logarithmic scale as significant at the 5% significance level (with a t-test). Transforming back to the original scale of measurement, this is equivalent to a 40% decrease in IHCL in the intervention group.

Assuming 10% mortality prior to term and 10% drop out rate, the aim was to recruit 80 infants in to each group or until 64 infants in each group had had their MRI and MRS, a total of 128 scans.

8.2 Randomisation

Randomisation was performed using an interactive voice recognition telephone randomisation system (IVRS). The IVRS and randomisation list were provided by Sealed Envelope Ltd.

Randomisation was performed using minimisation with a 25% chance of simple random allocation (based on procedure outlined in Pocock SJ. Clinical Trials: A Practical Approach. Wiley, Chichester, 1983). Randomisation was stratified by gestational age at birth (23-26 completed weeks or 27-31 completed weeks of gestation), birth weight (<500g, 500-1000g, >1000 g) and centre.

8.3 Blinding

Unblinded trial PN was delivered to the pharmacy department at each participating centre. Trained pharmacy staff were responsible for blinding the trial PN prior to dispensing the supply for administration to each infant.

Secure copies of the randomisation list were held by each pharmacy team in case of the need for emergency unblinding. There was no requirement for unblinding at any point over the course of the study.

8.4 Statistical methods

The analysis of this 2 x 2 factorial randomised trial was performed "at the margins" of the two by two table, assuming that the two factors are operating independently. In addition,

summary measures were presented for each cell of the 2 x 2 table and an interaction ratio will be calculated for binary outcomes (53). A 'modified' intention to treat method was used to analyse the results as it was accepted that a proportion of infants would not be able to attend the MR scan visit. With the exception of infants not completing the MR scan, all other infants were analysed according to their allocation.

The primary outcome measures for this trial are non-adipose (lean) body mass and intrahepatocellular lipid; the secondary outcomes are growth (weight, length and head circumference), brain growth and development (assessed by magnetic resonance imaging) and measure of insulin sensitivity (QUICKI). Growth parameters are the only outcomes that are measured sequentially; all other outcomes, including the two primary outcomes, are measured on a single occasion at term age equivalent.

For outcomes measured on a single occasion, a regression model containing the stratifying variables (gestational age, birth weight and centre), nutritional interventions (amino acid and lipid), sex and corrected gestational age at time of measurement were used to estimate the effects of each intervention.

For the amino acid intervention primary outcome we used multiple regression with non-adipose body mass (g) as the dependent variable and amino acids (incremental vs RDI), lipids (20% SMOF vs Intralipid), gestational age, birth weight, centre, gender and age at MRI scan as the independent variables to assess the effect of amino acids on non-adipose body mass. We also included an interaction term to assess whether the effect of amino acids regimen on non-adipose body mass is influenced by choice of lipids.

For the lipid intervention primary outcome we similarly used multiple regression with IHCL at natural logarithmic scale as the dependent variable and amino acid (incremental vs RDI), lipids (20% SMOF vs Intralipid), gestational age, birth weight, centre, gender and age at MRI scan as the independent variables to assess the effect of lipids on IHCL. Again, we included an interaction term so we could assess whether the effect of lipids on IHCL is affected by amino acids quantity.

A planned secondary analysis was used to investigate the role of illness severity and nutritional intake as potential modifiers of the effects of each intervention by adding these variables to the regression models.

A linear mixed model was used for the analysis of the secondary growth outcomes that were measured on several occasions during the trial. Here the interactions between interventions and time were interpreted as the difference in rate of change with time between the groups.

Secondary analyses investigated possible interactions between nutritional interventions and sex, and between nutritional interventions and gestational age at recruitment. The analysis was performed "at the margins" of the two by two table, assuming that the two factors are operating independently. In addition, summary measures will be presented for each cell of the 2 x 2 table and an interaction ratio/difference calculated (46). The secondary analysis will investigate the role of illness severity, maternal breast milk and post PN nutritional intake, including PN period and post PN period as potential modifiers of the effects of each intervention, by adding these variables to the regression model. To explore the growth of the babies at every assessment, we will use time series plots to describe the change in weight for the groups of interest and explore whether the interventions interact. All analyses were performed using Stata 13 (ref to be added: StataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP).

All analyses were performed on an intention to treat basis but as the primary outcomes can only be ascertained in those infants attending the end of study evaluation up to 20% of primary outcomes are expected to be missing. We have assumed that these losses are missing at random.

8.5 Missing data

Owing to the nature of this study, it was expected that a number of infants would not attend the end-of study MR scan (primarily due to the death, ill-health or withdrawal of the subject). The reasons for non-attendance were recorded in the withdrawal form. We aimed to comment on the implications that the missing data patterns had on the results from the analysis.
No missing data imputation was carried out except for infant weight over study period. The weight of infant was measured every day during the trial PN study period and measured weekly after infants were off the trial PN. As the daily infant weight was used in the descriptive analysis only, we did not carry out multiple imputations. Instead, we used simple imputation by using the nearest measured weight, either before or after the day of missing weight, to impute the missing data.

8.6 Statistical Analysis Plan

A statistical analysis plan was prepared by the trial investigators and trial statistician and reviewed and agreed by the TSC and DMEC prior to the end of the recruitment period. A copy of the Statistical Analysis Plan is included in Appendix 4.

9. TRIAL ORGANISATION

9.1 Trial Management

The UKCRC registered Imperial Clinical Trials Unit (ICTU) was responsible for trial management, quality assurance, trial statistics and development and maintenance of the trial database. Trial and data management were carried out by the Clinical Trials and Evaluation Unit (CTEU) at the Royal Brompton and Harefield NHS Foundation Trust, which was one of the ICTU groups at the time of the trial.

ICTU core staff and the InForm team are supported by the National Institute for Health Research (NIHR) Biomedical Research Centre based at Imperial College Healthcare NHS Trust and Imperial College London. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health.

9.2 Trial Sponsor

The Sponsor of the trial was Imperial College London. The Sponsor's role is clearly set out in the European Clinical Trials Directive and NHS Research Governance documents. Imperial College London signed a clinical trial agreement with each of the participating centres prior to the start of the trial.

9.3 Ethical considerations

The trial was conducted in accordance with the Declaration of Helsinki (http://www.wma.net/) on research involving human subjects. The study protocol, parent information sheet and consent form were submitted to the Research Ethics Committee prior to the start of the study and a favourable opinion was obtained on the 8th December 2009.

9.3.1 Consent

Where possible, parents were approached prior to their infant's birth to give them the parent information sheet and discuss the trial. Full written informed consent was taken after birth using the ethically approved parent information sheet and consent form.

9.4 Research governance

The trial was carried out in accordance with the NHS Research Governance Framework and local NHS permission was granted by the Research and Development departments at each participating site prior to recruitment commencing.

9.5 Regulatory requirements

As a randomised trial of an IMP, NEON was conducted in accordance with the European Clinical Trials Directive and the Medicines for Human Use (Clinical Trials) Regulations 2004. The trial received clinical trials authorisation (CTA) from the Medicines and Healthcare Regulatory Agency (MHRA) on the 8th January 2010 and was registered in the European Community with a EudraCT number: 2009-016731-34.

9.6 Trial registration

The trial was registered on the ISRCTN clinical trial database with the following reference: ISRCTN29665319.

9.7 NIHR CRN portfolio

The NEON trial was adopted on the NIHR Clinical Research Network (CRN) and Medicines for Children Research Network (MCRN) portfolios. Accrual data were uploaded onto the NIHR CRN database on a monthly basis.

9.8 Summary of protocol amendments

The following amendments were made to the trial protocol following approval of the first version of the document by the Ethics committee and Medicines and Healthcare Products Regulatory Authority (MHRA):

- Version 2: Clarifications implemented following review by Trial Steering Committee, administrative corrections, addition of a metabonomic substudy
- Version 3: Addition of samples to assess inflammatory markers and lipid profile (not implemented), clarification of randomisation time-window, administrative corrections
- Version 4: Addition of follow-up visit for neurodevelopmental substudy at 2 years corrected age – not implemented

9.9 Trial Committees

9.9.1 Trial Steering Committee

A Trial Steering Committee (TSC) was established to oversee the conduct of the study. The TSC met three times over the course of the trial; on 26th February 2010, 14th December 2011 and 22nd November 2012. Copies of the minutes from each meeting were sent to the funder, the Efficacy and Mechanism Evaluation program (EME) of the National Institute of Health Research (NIHR). The TSC approved the trial protocol prior to the start of the study and received regular recruitment reports throughout the duration of the trial.

The TSC membership is listed below:
Independent members
Professor Richard Cooke – Chair
Mrs Lorraine Dob – Parent representative
Dr Paul Clarke
Professor Robert Hume

Investigators
Dr Sabita Uthaya – Chief Investigator
Professor Neena Modi
Caroline Doré
Professor Ian Wong
Professor Jimmy Bell

Professor Deborah Ashby

9.9.2 Data Monitoring and Ethics Committee

An independent Data Monitoring and Ethics Committee (DMEC) was established to review SAE reports and the results of interim analyses. The DMEC meetings took place on 2nd August 2010, 13th October 2011 and 27th September 2012.

The first DMEC meeting to agree the charter outlining operational details and responsibilities took place early in the trial, on 2nd August 2010. The second meeting to review interim data for the first 32 infants was on 13th October 2011 and the final interim analysis for 64 infants took place on 27th September 2012. The DMEC provided feedback reports for each meeting to the Chair of the TSC and this was reviewed at subsequent TSC meetings as applicable.

DMEC membership:
Professor Peter Brocklehurst – Chair
Professor Tim Cole – Independent Statistician
Professor Tony Nunn
Dr Helen Mactier

9.10 Data management

Pre-defined data ranges were included in the eCRF which raised automated queries if data outside of the expected range were entered. In addition to the automated queries, the trial data were reviewed on a regular basis by the Data Manager to look for discrepancies and errors. In addition to the regular checks performed by the Data Manager, the Trial Statistician also performed a series of checks on snapshots of data to look for inconsistencies. The checks performed by the Data Manager and Statistician were documented in a pre-specified data management plan which was updated over the course of the study as required.

9.11 Risk assessment and Monitoring Plan

A risk assessment was performed by the ICTU QA Manager prior to the start of the trial. The result of the risk assessment indicated that the study was low risk and that 20% of trial data, 100% consent forms and 100% SAEs should be source verified. A monitoring plan was prepared in accordance with the risk assessment to specify the frequency of monitoring visits and amount of source data verification required.

9.12 Monitoring visits

A site initiation visit was performed at all participating centres. Interim monitoring visits were carried out approximately annually, depending on the recruitment rate, and closeout visits were carried out at all centres following the final follow-up visit for the last patient recruited. The monitoring visits were conducted by the Trial Manager.

9.13 Investigational Medicinal Product Manufacturer

The investigational medicinal product (IMP) for the NEON trial was manufactured by Bath-ASU, an MHRA licensed manufacturing unit with expertise in producing aseptic products.

9.14 Patient and Public Involvement

The TSC membership included a parent representative who was invited to attend all TSC meetings and included in all relevant correspondence.

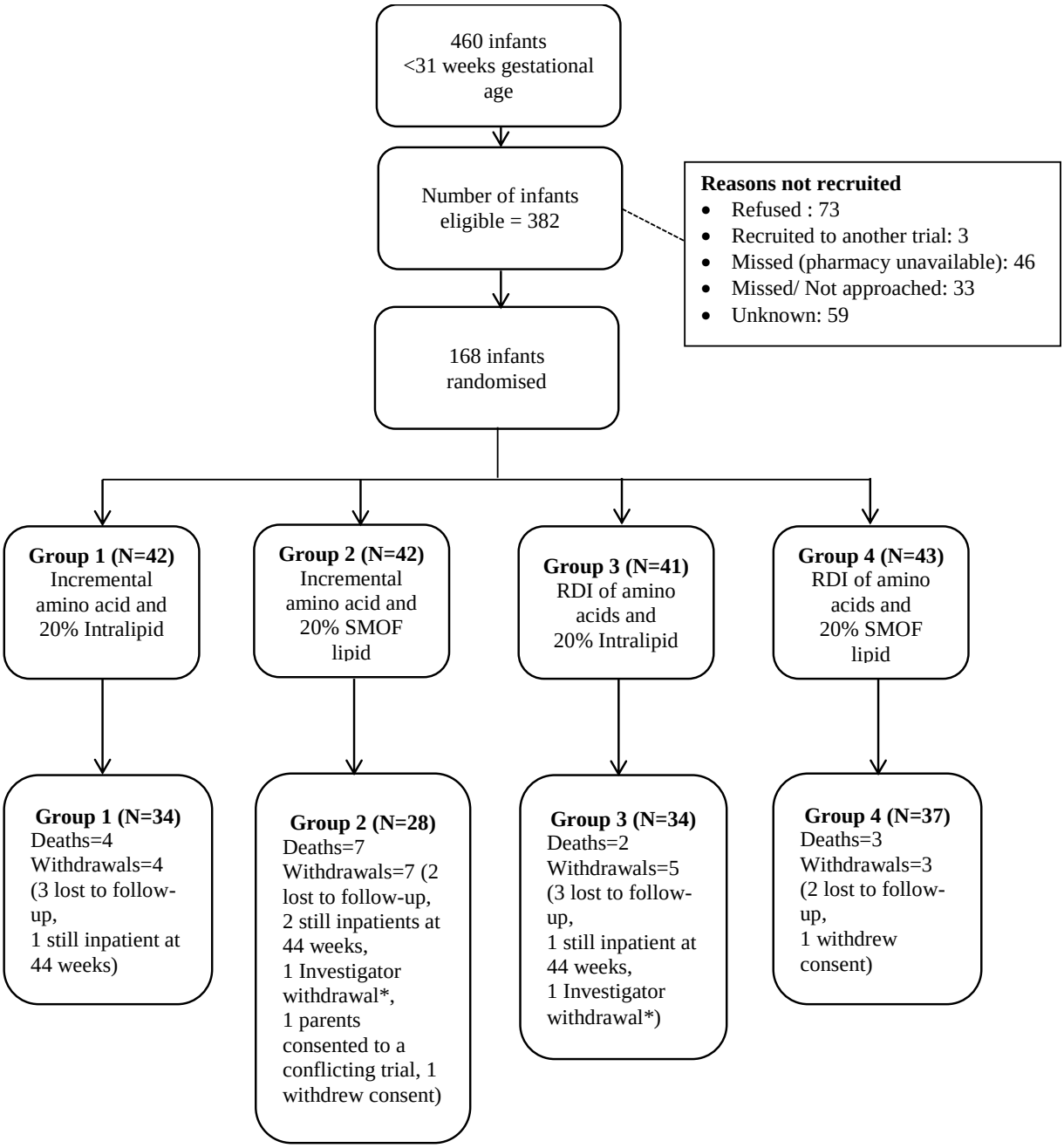
Parents were consulted during preparation of the parent information sheet and the charity, Bliss was also approached during the design phase of the study.

CHAPTER 4: RESULTS

1. PARTICIPANT FLOW

The flow of patients is summarised in Figure 2 including number of patients screened, randomised and completing the trial.

Figure 2. CONSORT diagram

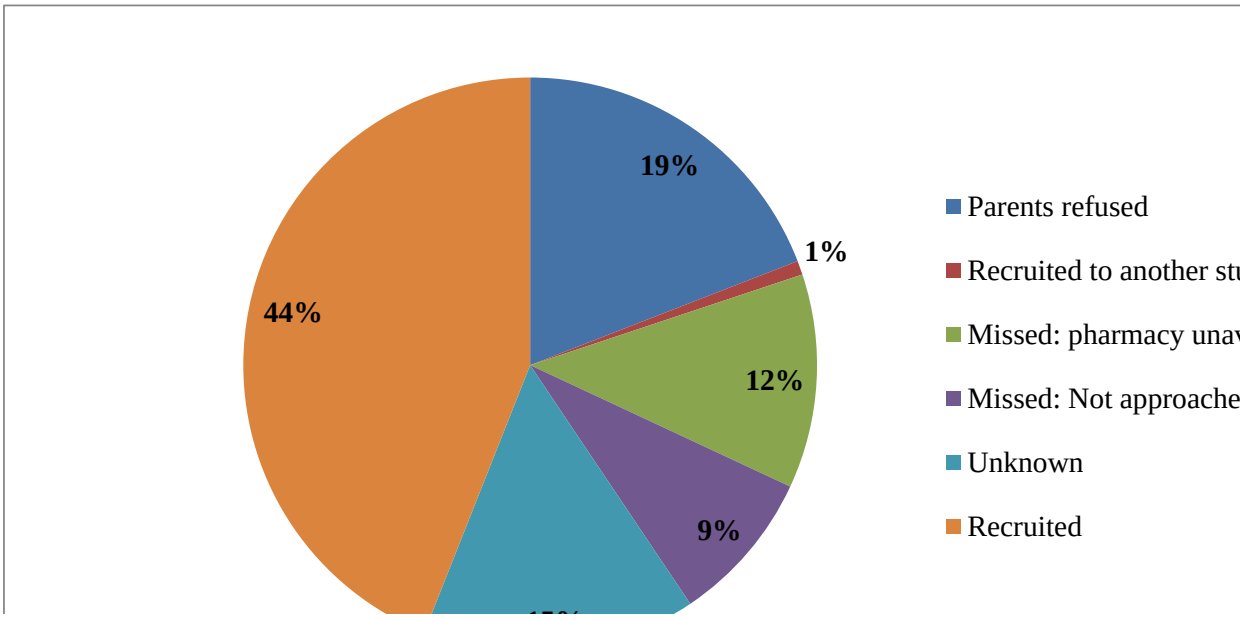


**Investigator withdrawal*: In both cases, this occurred when the infant was transferred to a non-trial site very soon after randomisation and was therefore unable to receive the trial intervention

1.1 Screening

460 infants below 3 weeks gestational age were admitted to the participating hospitals over the duration of the trial. Of the 382 infants meeting the eligibility criteria, 168 were randomised to the trial. Figure 3 summarises the percentage of eligible patients recruited to the trial and reasons for non recruitment.

Figure 3. Summary of screening data for all trial sites



1.2 Recruitment and retention

Recruitment lasted for 3 years, the first patient was recruited on 06/07/2010 and the last patient on 31/07/2013. The actual recruitment period was longer than the original target of two and a half years due to delays starting the trial at all sites. The delays in starting the trial were associated with the following:

a) Identifying a suitable manufacturer for the trial with an IMP license to produce parenteral nutrition and the capacity to support the trial
b) Agreement from each centre to support Excess Treatment costs due to cost difference between standard hospital PN and trial PN supply including signing a procurement contract for each participating pharmacy
c) Obtaining NHS permission at each site was lengthy, the procurement process was a factor for this

d) Inability to recruit during weekends and holidays. Pharmacy departments at three out of four sites could not support recruitment on weekends and during Christmas and Easter which reduced the recruitment rate

1.3 Recruitment rate

The target recruitment rate for the study was 6 patients per month, based on all four centres recruiting. The average monthly recruitment rate once all centres were activated (January 2012) was consistent with the target, i.e. 6 patients per month.

Figure 4 toFigure 6 below summarise cumulative recruitment and retention over the course of the study and recruitment and retention per centre.

Figure 4.Cumulative recruitment vs target recruitment for the duration of the trial

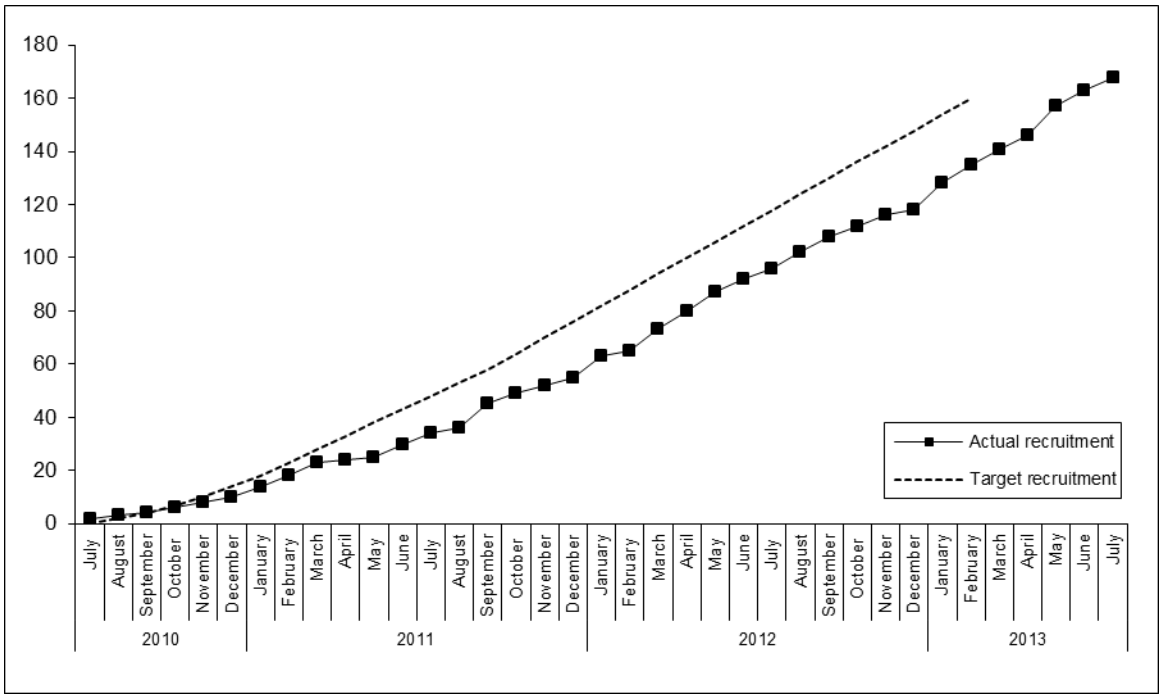


Figure 5. Cumulative retention (number of MR scans) vs target retention for the duration of the trial

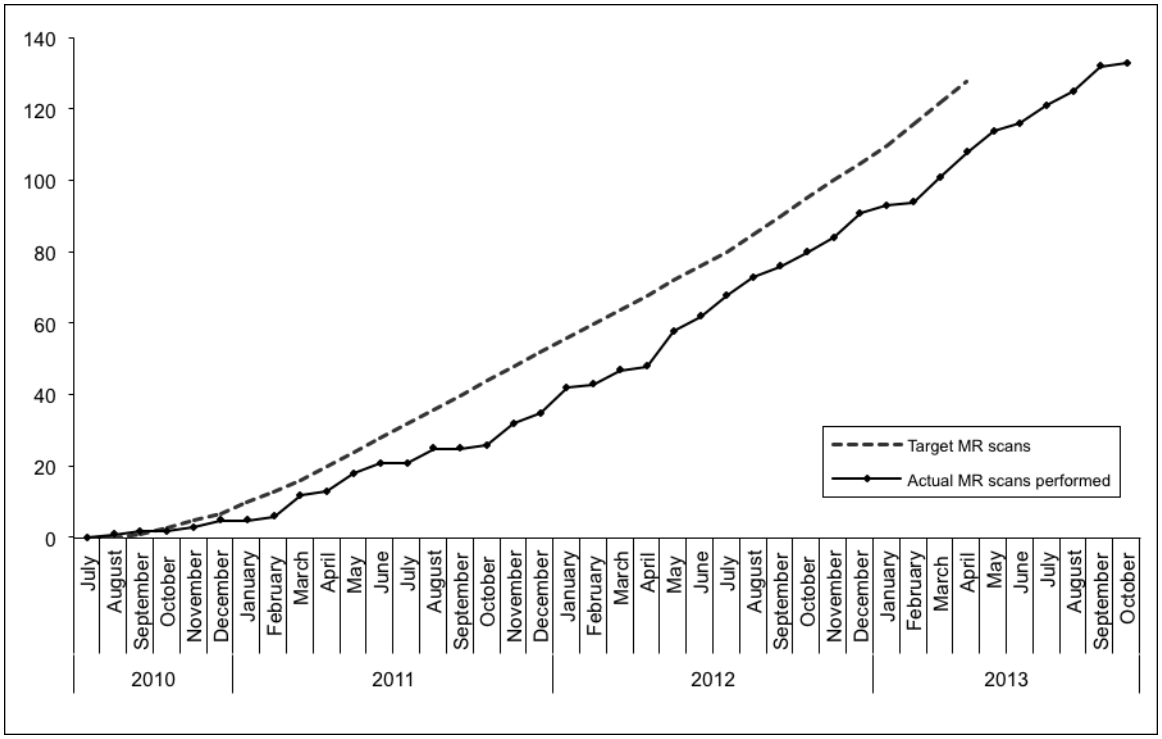
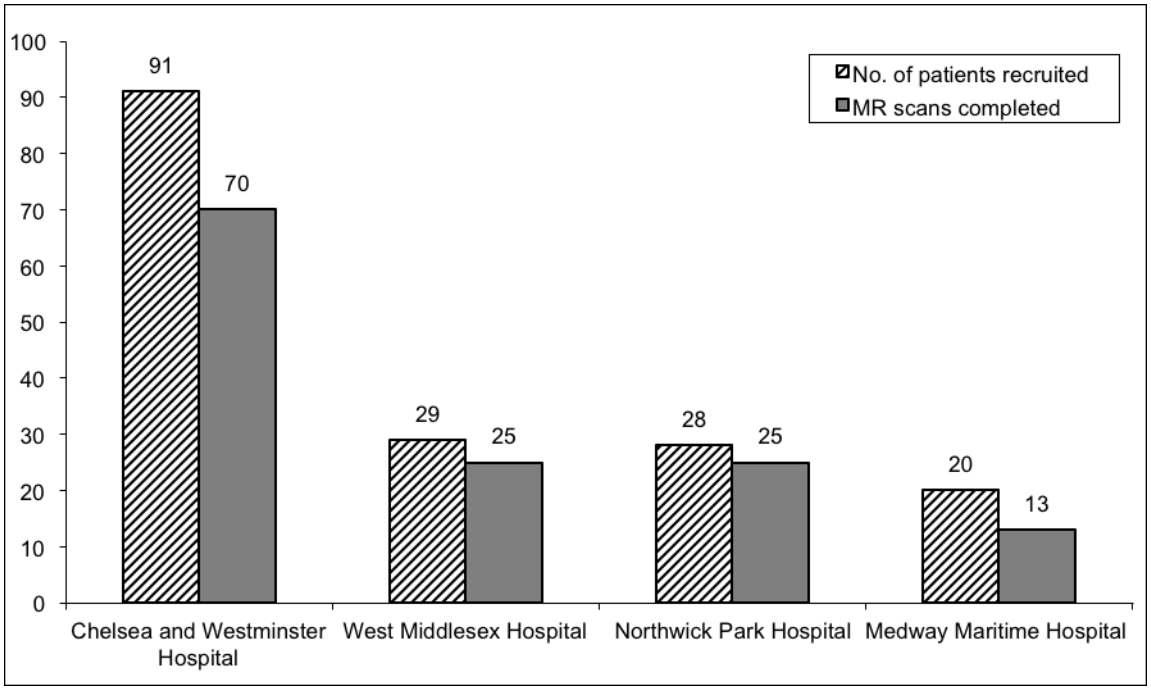


Figure 6. Total recruitment and retention per centre



1.4 Baseline data

The baseline characteristics of the infants recruited in to the study and those who completed the primary outcome measurement of MR scan are shown in Table 5 and Table 6 respectively. Of the 437 infants born less than 31 weeks of gestational age, 168 infants were randomised. A total of 133 infants were available for assessment of the primary outcome measures. Baseline characteristics of sex, gestational age at birth, anthropometry, maternal demographics, mode of delivery, antenatal steroid use, blood pressure on admission and time to commencing parenteral nutrition, were similar across the four groups.

Table 5. Baseline characteristics for all infants randomised*

	Incremental amino acid & 20% Intralipid	Incremental amino acid & 20% SMOF lipid	Recommended Daily Intake of amino acid & 20% Intralipid	Recommended Daily Intake of amino acid & 20% SMOF lipid
	N=42	N=42	N=41	N=43
Infant sex				
Boys	28 (66.7%)	26 (61.9%)	21 (51.2%)	22 (51.2%)
Gestational age (weeks)	27.8 (1.9)	27.5 (2.4)	28.1 (2.1)	27.8 (2.1)
Multiple birth				
Yes	6 (14.3%)	6 (14.3%)	9 (22.0%)	15 (34.9%)
Birth weight (kg)	1.03 (0.29)	1.05 (0.34)	1.04 (0.28)	1.06 (0.29)
Birth length (cm)	35.1 (3.5) [m=31]	34.6 (4.2) [n=32]	35.1 (3.9) [n=26]	35.2 (5.2) [n=32]
Head circumference (cm)	25.3 (2.0) [n=41]	25.0 (3.0) [n=40]	25.3 (1.9) [n=37]	25.6 (2.9) [n=39]
Birth weight (z-score)	-0.2 (1.0) [n=42]	0.1 (1.0) [n=41]	-0.2 (1.0) [n=41]	0 (0.9) [n=43]
Birth length (z-score)	-1.0 (1.0) [n=30]	-0.9 (1.2) [n=24]	-1.1 (1.0) [n=25]	-0.8 (1.5) [n=29]
Head circumference (z-score)	-0.5 (0.9) [n=41]	-0.3 (1.0) [n=39]	-0.7 (0.9) [n=37]	-0.2 (1.6) [n=41]
Mother's age (years)	32.9 (5.3) [n=42]	31.3 (7.7) [n=42]	32.9 (6.3) [n=40]	32.5 (6.6) [n=43]
Mother's weight (kg)†	66.4 (13.3) [n=34]	65.9 (11.4) [n=25]	64.9 (13.0) [n=30]	68.5 (15.2) [n=33]
Mother's height (cm)†	161.9 (7.8) [n=33]	164.9 (7.7) [n=27]	161.3 (9.2) [n=27]	164.5 (8.6) [n=32]
Father weight (kg)†	80.8 (10.7) [n=27]	82.3 (13.2) [n=22]	85.3 (16.1) [n=24]	86.3 (14.9) [n=31]
Father height (cm)†	178.4 (6.5) [n=28]	179.6 (6.8) [n=22]	175.7 (10.0) [n=22]	182.0 (9.7) [n=30]
Mother's ethnicity				
White	16 (38.1%)	19 (45.2%)	21 (51.2%)	21 (48.8%)
Asian	14 (33.3%)	7 (16.7%)	12 (29.3%)	12 (27.9%)
Black	6 (14.3%)	13 (31.0%)	6 (14.6%)	6 (14.0%)
Mixed	2 (4.8%)	2 (4.8%)	1 (2.4%)	2 (4.7%)
Other	3 (7.1%)	0 (0%)	1 (2.4%)	2 (4.7%)
Missing	1 (2.4%)	1 (2.4%)	0 (0%)	0(0%)
Mode of delivery				
Vaginal	8 (19.1%)	18 (42.9%)	16 (39.0%)	17 (39.5%)
Elective Caesarean	7 (16.7%)	3 (7.1%)	4 (9.8%)	2 (4.7%)
Emergency Caesarean	27 (64.3%)	21 (50.0%)	21 (51.2%)	24 (55.8%)
Antenatal steroids				
Yes	30 (71.4%)	34 (81.0%)	32 (78.1%)	35 (81.4%)

	Incremental amino acid & 20% Intralipid	Incremental amino acid & 20% SMOF lipid	Recommended Daily Intake of amino acid & 20% Intralipid	Recommended Daily Intake of amino acid & 20% SMOF lipid
	N=42	**N=42**	**N=41**	**N=43**
No	7 (16.7%)	6 (14.3%)	7 (17.1%)	4 (9.3%)
Unknown	5 (11.9%)	2 (4.8%)	2 (4.9%)	4 (9.3%)
Infant DBP (mmHg)	24.0 (9.0) [n=41]	24.5 (9.5) [n=42]	27.2 (9.8) [n=41]	25.8 (8.3) [n=42]
Infant SBP (mmHg)	49.1 (10.4) [n=41]	48.5 (9.7) [n=42]	50.7 (10.6) [n=41]	49.4 (11.8) [n=42]
Time from birth to starting Parenteral Nutrition, (hours)‡	18.4 (12.3-22.7) [n=42]	19.5 (13.6-22.8) [n=41]	20.4 (12.6-23.6) [n=40]	17.7 (13.0-22.4) [n=43]

* *Data presented are mean (SD) for continuous variables and frequency (percentage) for categorical variables*

† *Anthropometries measured at booking*

‡ *Data presented are median (IQR)*

Table 6. Baseline characteristics for all infants completing MRI assessment

	Incremental amino acid & 20% Intralipid	Incremental amino acid & 20% SMOF lipid	Recommended Daily Intake of amino acid & 20% Intralipid	Recommended Daily Intake of amino acid & 20% SMOF lipid
	N=34	N=28	N=34	N=37
Infant sex				
Boys	20 (58.8%)	18 (64.3%)	17 (50.0%)	19 (51.4%)
Gestational age (weeks)	28.0 (1.8)	28.0 (2.1)	28.4 (2.1)	27.7 (2.0)
Multiple birth				
Yes	4 (11.8%)	3 (10.7%)	8 (23.5%)	13 (35.1)
Birth weight (kg)	1.06 (0.29)	1.10 (0.32)	1.09 (0.28)	1.06 (0.29)
Birth length (cm)	35.5 (3.5) [n=28]	35.1 (4.0) [n=24]	35.6 (3.5) [n=24]	34.9 (4.9) [n=27]
Head circumference (cm)	25.3 (2.0) [n=34]	25.6 (2.6) [n=26]	25.5 (1.9) [n=32]	25.7 (2.9) [n=34]
Birth weight (z-score)	-0.1 (0.9)	0 (1.0)	-0.2 (1.0)	0.1 (0.9)
Birth length (z-score)	-0.9 (1.1) [n=28]	-1.0 (1.3) [n=21]	-1.0 (1.0) [n=23]	-1.1 (1.4) [n=25]
Head circumference (z-score)	-0.5 (0.9) [n=34]	-0.4 (1.0) [n=26]	-0.7 (0.9) [n=32]	-0.2 (1.7) [n=34]
Mother's age (years)	32.6 (5.4) [n=34]	30.3 (7.8) [n=26]	32.2 (6.4) [n=33]	32.7 (6.7) [n=34]
Mother's weight (kg)[†]	67.6 (14.5) [n=27]	63.8 (11.5) [n=17]	64.7 (13.3) [n=26]	68.5 (16.1) [n=29]
Mother's height (cm)[†]	162.4 (7.2) [n=26]	165.1 (7.3) [n=19]	162.1 (9.1) [n=23]	164.8 (9.1) [n=28]
Father weight (kg)[†]	82.3 (11.5) [n=21]	81.5 (14.0) [n=15]	84.1 (15.9) [n=22]	87.8 (14.7) [n=28]
Father height (cm)[†]	177.8 (6.1) [n=22]	179.3 (7.4) [n=15]	175.6 (10.2) [n=20]	182.8 (9.6) [n=27]
Mother's ethnicity				
White	13 (38.2%)	11 (39.3%)	17 (50.0%)	19 (51.4%)
Asian	10 (29.4%)	6 (21.4%)	11 (32.4%)	9 (24.3%)
Black	5 (14.7%)	10 (35.7%)	5 (14.7%)	6 (16.2%)
Mixed	2 (5.9%)	1 (3.6%)	0 (0%)	2 (5.4%)
Other	3 (8.8%)	0 (0%)	1 (2.9%)	1 (2.7%)
Missing	1 (2.94%)	0 (0%)	0 (0%)	0 (0%)
Mode of delivery				
Vaginal	6 (17.6%)	9 (32.1%)	13 (38.2%)	15 (40.5%)
Elective Caesarean	5 (14.7%)	2 (7.1%)	4 (11.8%)	1 (2.7%)
Emergency Caesarean	23 (67.7%)	17 (60.7%)	17 (50.0%)	21 (56.8%)
Antenatal steroids				
Yes	24 (70.6%)	21 (75.0%)	26 (76.5%)	30 (81.1%)
No	5 (14.7%)	5 (17.9%)	6 (17.7%)	4 (10.8)

	Incremental amino acid & 20% Intralipid	Incremental amino acid & 20% SMOF lipid	Recommended Daily Intake of amino acid & 20% Intralipid	Recommended Daily Intake of amino acid & 20% SMOF lipid
	N=34	**N=28**	**N=34**	**N=37**
Unknown	5 (14.7%)	2 (7.1%)	2 (5.9%)	3 (8.1%)
Infant DBP (mmHg)	23.6 (7.8) [n=33]	25.2 (9.1) [n=28]	27.1 (8.6) [n=34]	25.7 (8.3) [n=36]
Infant SBP (mmHg)	48.0 (10.0) [n=33]	49.8 (9.2) [n=28]	52.1 (10.0) [n=34]	49.0 (11.5) [n=36]
Time from birth to starting Parenteral Nutrition, (hours) ‡	16.9 (10.5-22.3) [n=34]	19.4 (12.1-22.3) [n=28]	20.0 (12.4-23.5) [n=34]	17.7 (13.2-22.4) [n=37]

* *Data presented are mean (SD) for continuous variables and frequency (percentage) for categorical variables*

† *Anthropometries measured at booking*

‡ *Data presented are median (IQR)*

Table 7. Parenteral nutrition details and blood culture results for all infants randomised*

	Incremental amino acid & 20% Intralipid	Incremental amino acid & 20% SMOF lipid	Recommended Daily Intake of amino acid & 20% Intralipid	Recommended Daily Intake of amino acid & 20% SMOF lipid
	N=42	N=42	N=41	N=43
Route of Parenteral Nutrition administration				
Peripheral (days)	0 (0-2) [n=42]	1 (0-5) [n=41]	0.5 (0-2.5) [n=40]	1 (0-3) [n=43]
Central (days)	11.5 (8-20) [=42]	13 (8-20) [n=41]	11 (9-15.5) [n=40]	12 (9-18) [n=43]
Days from delivery to achieve milk intake of 150ml/kg/day for 24hr	12 (9-17.5) [n=32]	11.5 (9-16) [n=28]	11 (10-14) [n=30]	13 (9.5-18) [n=36]
Reason for stopping Parenteral Nutrition†				
Investigator decision	5 (11.9%)	3 (7.1%)	3 (7.3%)	9 (20.9%)
Investigator decision & Investigator manual	0 (0%)	1 (2.4%)	0 (0%)	0 (0%)
Investigator manual	2 (4.8%)	1 (2.4%)	2 (4.9%)	2 (4.7%)
Operational	2 (4.8%)	1 (2.4%)	2 (4.9%)	3 (6.7%)
Withdrawal	1 (2.4%)	0 (0%)	0 (0%)	0 (0%)
SAE	0 (0%)	2 (4.8%)	0 (0%)	0 (0%)
Positive blood culture‡	15 (	13	9	14
Fungus	2 (13.3%)	1 (7.7%)	0 (0%)	1 (7.1%)
Gram negative bacilli	5 (33.3%)	4 (30.8%)	3 (33.3%)	1 (7.1%)
Gram positive bacilli	1 (6.7%)	0 (0%)	0 (0%)	0 (0%)
Gram positive cocci CoNS	2 (13.3%)	3 (23.1%)	4 (44.4%)	7 (50.0%)
Gram positive cocci excluding CoNS	3 (20.0%)	4 (30.8%)	1 (11.1%)	5 (35.7%)
Gram positive cocci not specified	2 (13.3%)	1 (7.7%)	1 (11.1%)	0 (0%)
Positive blood cultures (while on Parenteral Nutrition) ‡	8	9	8	8
Length of hospital stay in hospital (days)	69.5 (52-95) [n=38]	61 (45-88) [n=33]	63 (45-95) [n=38]	66.5 (44-98) [n=38]

The time to achieve a milk intake of 150 ml/kg/day for 24 hours for all infants randomised was similar across the four groups (median (IQR) in Groups 1,2,3,4: 12(9-17.5), 11.5 (9-16), 11 (10 -14), 13 (9.5 -18) respectively. The length of hospital stay for all infants randomised was similar across the four groups (median (IQR) in Groups 1,2,3,4 69.5 (52-95), 61 (45 – 88), 63 (45 – 95), 66.5 (44 – 98) respectively.

Table 8. Parenteral nutrition details and blood culture results for all infants completing MRI assessment[*]

	Incremental amino acid & 20% Intralipid	**Incremental amino acid & 20% SMOF lipid**	**Recommended Daily Intake of amino acid & 20% Intralipid**	**Recommended Daily Intake of amino acid & 20% SMOF lipid**
	N=34	**N=28**	**N=34**	**N=37**
Route of Parenteral Nutrition administration				
Peripheral (days)	0 (0-2)	1 (0-5.5)	1 (0-3)	1 (0-3)
Central (days)	11 (8-17)	13.5 (8.5-19.5)	10.5 (9-15)	12 (9-18)
Days from delivery to achieve milk intake of 150ml/kg/day for 24hr	11 (9-16) [n=28]	11.5 (9-16) [n=22]	11 (10-13.5) [n=28]	13 (10-18) [n=33]
Reason for stopping Parenteral Nutrition[†]				
Investigator decision	3 (8.8%)	2 (7.1%)	3 (8.8%)	6 (16.2%)
Investigator manual	2 (4.8%)	1 (2.4%)	2 (4.9%)	1 (2.7%)
Operational	2 (4.8%)	1 (2.4%)	2 (4.9%)	3 (8.1%)
Positive blood culture[‡]	9	4	5	12
Fungus	2 (22.2%)	0 (0%)	0 (0%)	1 (8.3%)
Gram negative bacilli	3 (33.3%)	3 (75.0%)	3 (60.0%)	0 (0%)
Gram positive bacilli	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Gram positive cocci CoNS	1 (11.1%)	0 (0%)	1 (20.0%)	6 (50.0%)
Gram positive cocci excluding CoNS	2 (22.2%)	0 (0%)	0 (0%)	5 (41.7%)
Gram positive cocci not specified	1 (11.1%)	1 (25.0%)	1 (20.0%)	0 (0%)
Positive blood cultures (while on Parenteral Nutrition)[‡]	5	3	4	7
Length of hospital stay in hospital (days)	69.5 (55-96) [n=34]	59 (44-85) [=28]	60.5 (44-88) [n=34]	67 (47-98.5) [n=36]

[*] *Data presented are median (IQR) for continuous variables and frequency (percentage) for categorical variables*

[†] *There can be more than one reason for each infant*

[‡] *Growth of known pathogen on culture; data presented are number of infants that had at least one positive result*

Nutritional intake from trial PN during the first week from PN was similar across the four groups except in the intake of protein. On day 4, when infants randomised to incremental amino acid intake achieved the maximum intake, the protein intake was 2.2 g/kg and 2.3 g/kg in Groups 1 and 2 compared to 3.7 g/kg and 3.5 g/kg in Group 3 and 4 respectively for all babies randomised. Median (IQR) cumulative protein intake from all sources in grams during the first 2 weeks after birth for all randomised babies in the incremental arm was 20.2 (14.6-25.6) and 18.6 (13.9-25.7) in Groups 1 and 2 compared to 28.6 (25.1-36.1) and 32.4 (25.4-41.4) in Groups 3 and 4 respectively. The median (IQR) cumulative protein intake in grams from all sources between birth and 34 weeks post- menstrual age for all babies randomised was 241.2 (189.3-468.2) and 215.5 (139.7-310.3) in Groups 1 and 2 compared to 275.0 (199.6-399.9) and 303.4 (221.9-419.8) in Groups 3 and 4 respectively.

Table 9. Trial Parenteral Nutrition intake during first 7 days for all infants randomised*

	Incremental amino acid & 20% Intralipid	**Incremental amino acid & 20% SMOF lipid**	**Recommended Daily Intake of amino acid & 20% Intralipid**	**Recommended Daily Intake of amino acid & 20% SMOF lipid**
	N=42	**N=42**	**N=41**	**N=43**
Day 1[†]	n=39	n=34	n=37	n=41
Aqueous Volume (ml/kg)	71.1 (36.2)	69.5 (34.3)	69.2 (36.7)	68.1 (35.6)
Lipid Volume (ml/kg)	8.8 (5.2)	8.6 (4.6)	8.5 (5.3)	7.9 (4.5)
Protein (g/kg)	1.2 (0.7)	1.2 (0.6)	2.5 (1.3)	2.4 (1.3)
Carbohydrate (g/kg)	6.8 (3.5)	6.7 (3.3)	6.6 (3.5)	6.4 (3.4)
Fat (g/kg)	1.8 (1.0)	1.7 (0.9)	1.7 (1.1)	1.6 (0.9)
Day 2	n=39	n=38	n=39	n=42
Aqueous Volume (ml/kg)	96.5 (20.8)	89.9 (31.1)	94.9 (16.8)	94.5 (20.9)
Lipid Volume (ml/kg)	14.8 (9.9)	12.8 (5.2)	13.7 (2.6)	14.1 (4.0)
Protein (g/kg)	2.0 (0.4)	1.8 (0.6)	3.2 (0.5)	3.2 (0.7)
Carbohydrate (g/kg)	8.6 (1.8)	8.1 (2.8)	8.4 (1.4)	8.2 (1.8)
Fat (g/kg)	3.0 (2.0)	2.6 (1.0)	2.7 (0.5)	2.8 (0.8)
Day 3	n=39	n=37	n=38	n=42
Aqueous Volume (ml/kg)	114.4 (22.4)	112.8 (29.1)	112.6 (25.1)	114.0 (23.2)
Lipid Volume (ml/kg)	14.3 (3.2)	14.7 (3.8)	13.4 (3.8)	13.4 (4.8)
Protein (g/kg)	2.3 (0.4)	2.3 (0.6)	3.4 (0.7)	3.4 (0.7)
Carbohydrate (g/kg)	8.5 (1.8)	8.3 (2.1)	8.4 (1.8)	8.3 (1.8)
Fat (g/kg)	2.8 (0.6)	2.9 (0.7)	2.6 (0.8)	2.6 (0.9)
Day 4	n=40	n=37	n=38	n=40
Aqueous Volume (ml/kg)	111.4 (26.0)	117.0 (21.5)	123.4 (14.7)	115.9 (20.2)
Lipid Volume (ml/kg)	14.2 (5.3)	14.6 (4.2)	15.3 (4.4)	14.0 (4.1)

	Incremental amino acid & 20% Intralipid	Incremental amino acid & 20% SMOF lipid	Recommended Daily Intake of amino acid & 20% Intralipid	Recommended Daily Intake of amino acid & 20% SMOF lipid
	N=42	**N=42**	**N=41**	**N=43**
Protein (g/kg)	2.2 (0.5)	2.3 (0.4)	3.7 (0.4)	3.5 (0.6)
Carbohydrate (g/kg)	8.1 (2.1)	8.4 (1.6)	8.9 (1.1)	8.4 (1.4)
Fat (g/kg)	2.6 (1.0)	2.7 (0.8)	2.8 (0.8)	2.5 (0.7)
Day 5	n=42	n=38	n=38	n=41
Aqueous Volume (ml/kg)	106.5 (29.9)	111.3 (23.7)	114.0 (25.1)	112.4 (22.1)
Lipid Volume (ml/kg)	14.4 (4.7)	14.9 (4.3)	14.8 (4.1)	13.7 (4.7)
Protein (g/kg)	2.1 (0.6)	2.2 (0.5)	3.4 (0.7)	3.4 (0.7)
Carbohydrate (g/kg)	7.7 (2.2)	8.1 (1.6)	8.2 (1.8)	8.1 (1.6)
Fat (g/kg)	2.5 (0.8)	2.6 (0.7)	2.6 (0.7)	2.4 (0.8)
Day 6	n=42	n=38	n=38	n=41
Aqueous Volume (ml/kg)	100.9 (34.0)	103.0 (31.6)	103.0 (31.3)	107.5 (27.8)
Lipid Volume (ml/kg)	13.1 (5.4)	13.8 (4.9)	13.2 (6.0)	12.8 (5.7)
Protein (g/kg)	2.0 (0.7)	2.1 (0.6)	3.1 (0.9)	3.2 (0.8)
Carbohydrate (g/kg)	7.3 (2.5)	7.4 (2.3)	7.4 (2.3)	7.7 (2.0)
Fat (g/kg)	2.3 (1.0)	2.4 (0.9)	2.3 (1.0)	2.3 (1.0)
Day 7	n=41	n=36	n=36	n=38
Aqueous Volume (ml/kg)	93.4 (35.7)	92.4 (31.3)	101.3 (28.2)	100.9 (25.6)
Lipid Volume (ml/kg)	11.1 (6.1)	11.1 (6.0)	13.1 (7.5)	11.6 (5.6)
Protein (g/kg)	1.9 (0.7)	1.8 (0.6)	3.0 (0.8)	3.0 (0.8)
Carbohydrate (g/kg)	6.8 (2.6)	6.7 (2.3)	7.3 (2.0)	7.3 (1.8)
Fat (g/kg)	2.0 (1.1)	1.9 (1.0)	2.3 (1.3)	2.1 (1.0)

[*] *Data presented are mean(SD)*

[†] *Day 1 is defined from birth to first 17:00*

Table 10. Trial Parenteral Nutrition intake during first 7 days for all infants completing MRI assessment*

	Incremental amino acid & 20% Intralipid	Incremental amino acid & 20% SMOF lipid	Recommended Daily Intake of amino acid & 20% Intralipid	Recommended Daily Intake of amino acid & 20% SMOF lipid
	N=34	N=28	N=34	N=37
Day 1[†]	n=32	n=25	n=31	n=36
Aqueous Volume (ml/kg)	69.6 (36.3)	70.1 (34.4)	70.9 (37.6)	67.8 (36.0)
Lipid Volume (ml/kg)	8.4 (5.0)	8.7 (4.5)	8.9 (5.5)	8.1 (4.7)
Protein (g/kg)	1.2 (0.7)	1.2 (0.6)	2.5 (1.3)	2.4 (1.3)
Carbohydrate (g/kg)	6.7 (3.5)	6.7 (3.3)	6.8 (3.6)	6.4 (3.4)
Fat (g/kg)	1.7 (1.0)	1.7 (0.9)	1.8 (1.1)	1.6 (0.9)
Day 2	n=33	n=27	n=34	n=36
Aqueous Volume (ml/kg)	92.9 (18.5)	89.5 (22.6)	97.3 (15.3)	97.1 (20.2)
Lipid Volume (ml/kg)	14.7 (10.7)	13.2 (4.4)	13.8 (2.6)	14.6 (3.8)
Protein (g/kg)	1.9 (0.4)	1.8 (0.5)	3.3 (0.5)	3.2 (0.6)
Carbohydrate (g/kg)	8.3 (1.7)	8.0 (1.9)	8.5 (1.3)	8.4 (1.7)
Fat (g/kg)	2.9 (2.1)	2.7 (0.9)	2.7 (0.5)	2.9 (0.7)
Day 3	n=32	n=28	n=34	n=36
Aqueous Volume (ml/kg)	116.8 (21.5)	112.2 (32.7)	111.8 (26.3)	117.5 (19.2)
Lipid Volume (ml/kg)	14.6 (3.4)	14.4 (4.3)	13.3 (4.0)	13.9 (4.6)
Protein (g/kg)	2.3 (0.4)	2.3 (0.7)	3.4 (0.8)	3.5 (0.6)
Carbohydrate (g/kg)	8.6 (1.8)	8.3 (2.4)	8.4 (1.9)	8.6 (1.6)
Fat (g/kg)	2.8 (0.6)	2.8 (0.8)	2.6 (0.8)	2.7 (0.9)
Day 4	n=33	n=28	n=34	n=35
Aqueous Volume (ml/kg)	112.4 (26.8)	117.4 (24.4)	123.2 (14.9)	118.5 (14.6)
Lipid Volume (ml/kg)	13.9 (5.6)	14.8 (4.7)	15.6 (3.7)	14.4 (3.5)
Protein (g/kg)	2.2 (0.5)	2.3 (0.5)	3.7 (0.4)	3.6 (0.4)
Carbohydrate (g/kg)	8.2 (2.1)	8.5 (1.8)	8.9 (1.1)	8.6 (0.9)
Fat (g/kg)	2.5 (1.0)	2.7 (0.8)	2.8 (0.7)	2.6 (0.6)
Day 5	n=34	n=28	n=34	n=36
Aqueous Volume (ml/kg)	107.6 (32.1)	111.0 (25.2)	113.3 (26.2)	112.3 (23.1)
Lipid Volume (ml/kg)	14.3 (5.1)	15.2 (4.1)	14.7 (4.3)	13.5 (5.0)
Protein (g/kg)	2.2 (0.6)	2.2 (0.5)	3.4 (0.8)	3.4 (0.7)
Carbohydrate (g/kg)	7.7 (2.3)	8.0 (1.8)	8.2 (1.9)	8.1 (1.7)
Fat (g/kg)	2.5 (0.9)	2.7 (0.7)	2.6 (0.7)	2.4 (0.9)
Day 6	n=34	n=28	n=34	n=35
Aqueous Volume (ml/kg)	101.4 (36.8)	104.6 (33.7)	101.5 (32.5)	111.0 (24.1)
Lipid Volume (ml/kg)	13.1 (5.8)	13.9 (5.4)	13.0 (6.1)	13.2 (5.6)
Protein (g/kg)	2.0 (0.7)	2.1 (0.7)	3.0 (1.0)	3.3 (0.7)
Carbohydrate (g/kg)	7.3 (2.7)	7.5 (2.4)	7.3 (2.3)	8.0 (1.7)
Fat (g/kg)	2.3 (1.0)	2.4 (0.9)	2.3 (1.1)	2.3 (1.0)
Day 7	n=33	n=26	n=32	n=33
Aqueous Volume (ml/kg)	93.8 (35.6)	93.2 (34.8)	101.9 (29.2)	103.1 (26.3)

	Incremental amino acid & 20% Intralipid	Incremental amino acid & 20% SMOF lipid	Recommended Daily Intake of amino acid & 20% Intralipid	Recommended Daily Intake of amino acid & 20% SMOF lipid
	N=34	N=28	N=34	N=37
Lipid Volume (ml/kg)	11.3 (6.0)	11.4 (6.3)	13.3 (7.8)	12.3 (5.4)
Protein (g/kg)	1.9 (0.7)	1.9 (0.7)	3.1 (0.9)	3.1 (0.8)
Carbohydrate (g/kg)	6.8 (2.6)	6.7 (2.5)	7.3 (2.1)	7.4 (1.9)
Fat (g/kg)	2.0 (1.1)	2.0 (1.1)	2.4 (1.4)	2.2 (1.0)

[*] *Data presented are mean(SD)*

[†] *Day 1 is defined from birth to first 17:00*

Table 11.Nutritional intake over first 2 weeks for all infants randomised*

	Incremental amino acid & 20% Intralipid	Incremental amino acid & 20% SMOF lipid	Recommended Daily Intake of amino acid & 20% Intralipid	Recommended Daily Intake of amino acid & 20% SMOF lipid
	N=42	N=42	N=41	N=43
Had MEBM *(number of babies)*	40 (95.2%)	38 (90.5%)	38 (92.7%)	42 (97.7%)
Cumulative maternal breast milk intake (l)	0.76 (0.42-1.56) [n=40]	0.94 (0.21-1.33) [n=38]	0.68 (0.34-1.44) [n=38]	0.59 (0.17-1.11) [n=42]
Had formula milk *(number of babies)*	12 (28.6%)	11 (26.2%)	16 (39.0%)	13 (30.2%)
Cumulative formula intake (l)	0.03 (0.01-0.29) [n=12]	0.16 (0.04-1.10) [n=11]	0.47 (0.03-1.00) [n=16]	0.37 (0.02-1.56) [n=13]
Had trial Parenteral Nutrition *(number of babies)*	42 (100.0%)	41 (97.6%)	40 (97.6%)	43 (100.0%)
Time on trial Parenteral Nutrition (days)	11.0 (8.0-14.0) [n=42]	12.0 (9.0-14.0) [n=41]	11.0 (9.0-13.0) [n=40]	12.0 (9.0-14.0) [n=43]
Cumulative trial Parenteral Nutrition Intake				
Aqueous Volume (l)	1.02 (0.74-1.29) [n=42]	0.93 (0.70-1.30) [n=41]	0.93 (0.81-1.18) [n=40]	1.04 (0.81-1.36) [n=43]
Lipid Volume (l)	0.13 (0.09-0.17) [n=42]	0.11 (0.08-0.17) [n=41]	0.12 (0.10-0.15) [n=40]	0.13 (0.09-0.18) [n=43]
Protein (g)	20.2 (14.6-25.6) [n=42]	18.6 (13.9-25.7) [n=41]	28.6 (25.1-36.1) [n=40]	32.4 (25.4-41.4) [n=43]
Carbohydrate (g)	76.6 (57.7- 96.1) [n=42]	69.3 (53.1- 96.6) [n=41]	69.8 (61.1- 87.8) [n=40]	79.5 (62.7-100.4) [n=43]
Fat (g)	23.6 (16.7-31.6) [n=42]	21.0 (15.1-31.4) [n=41]	21.4 (17.5-27.7) [n=40]	24.6 (16.7-31.8) [n=43]
Received non-trial Parenteral Nutrition *(number of babies)*	3 (7.1%)	1 (2.4%)	1 (2.4%)	3 (7.0%)
Time on non-trial Parenteral Nutrition(days)	4.0 (3.5-4.0) [n=3]	1.0 (1.0-1.0) [n=1]	1.0 (1.0-1.0) [n=1]	2.0 (1.5-5.0) [n=3]
Cumulative non-trial Parenteral Nutrition Intake[†]				
Aqueous Volume (l)	0.46 (0.40-0.47) [n=3]	0.02 (0.02-0.02) [n=1]	0.07 (0.07-0.07) [n=1]	0.37 (0.20-0.44) [n=3]
Lipid Volume (l)	0.02 (0.01-0.04) [n=3]	0.00 (0.00-0.00) [n=1]	0.00 (0.00-0.00) [n=1]	0.03 (0.01-0.03) [n=3]
Protein (g)	1.34 (1.17-1.54) [n=3]	0.07 (0.07-0.07) [n=1]	0.15 (0.15-0.15) [n=1]	1.04 (0.57-1.21) [n=3]
Carbohydrate (g)	42.57 (40.82-45.99) [n=3]	2.47 (2.47- 2.47) [n=1]	3.35 (3.35- 3.35) [n=1]	29.75 (17.04-35.48) [n=3]

	Incremental amino acid & 20% Intralipid	Incremental amino acid & 20% SMOF lipid	Recommended Daily Intake of amino acid & 20% Intralipid	Recommended Daily Intake of amino acid & 20% SMOF lipid
	N=42	**N=42**	**N=41**	**N=43**
Fat (g)	4.40 (2.20-7.90) [n=3]	0.30 (0.30-0.30) [n=1]	0.53 (0.53-0.53) [n=1]	4.44 (2.61-5.85) [n=3]
Cumulative non-trial and trial Parenteral Nutrition intake				
Aqueous Volume (l)	1.04 (0.81-1.29) [n=42]	0.93 (0.70-1.30) [n=41]	0.94 (0.81-1.18) [n=40]	1.04 (0.81-1.36) [n=43]
Lipid Volume (l)	0.13 (0.09-0.17) [n=42]	0.11 (0.08-0.17) [n=41]	0.12 (0.10-0.15) [n=40]	0.13 (0.09-0.18) [n=43]
Protein (g)	20.2 (14.6-25.6) [n=42]	18.6 (13.9-25.7) [n=41]	28.6 (25.1-36.1) [n=40]	32.4 (25.4-41.4) [n=43]
Carbohydrate (g)	78.0 (61.8- 96.1) [n=42]	69.3 (53.1- 96.6) [n=41]	70.3 (61.1- 87.8) [n=40]	79.5 (62.7-100.4) [n=43]
Fat (g)	23.6 (16.8-31.6) [n=42]	21.0 (15.1-31.4) [n=41]	21.4 (17.5-27.7) [n=40]	24.6 (16.7-31.8) [n=43]

[*] *Data presented are median (IQR)*

Table 12. Nutritional intake over first 2 weeks for all infants completing MRI scan*

	Incremental amino acid & 20% Intralipid	Incremental amino acid & 20% SMOF lipid	Recommended Daily Intake of amino acid & 20% Intralipid	Recommended Daily Intake of amino acid & 20% SMOF lipid
	N=34	N=28	N=34	N=37
Had MEBM *(number of babies)*	32 (94.1%)	27 (96.4%)	33 (97.1%)	36 (97.3%)
Cumulative maternal breast milk intake (l)	0.79 (0.60-1.63) [n=32]	0.97 (0.54-1.62) [n=27]	0.77 (0.42-1.54) [n=33]	0.76 (0.29-1.14) [n=36]
Had formula milk *(number of babies)*	10 (29.4%)	9 (32.1%)	14 (41.2%)	11 (29.7%)
Cumulative formula intake (l)	0.03 (0.01-0.19) [n=10]	0.26 (0.09-1.11) [n=9]	0.35 (0.03-1.12) [n=14]	0.37 (0.05-1.32) [n=11]
Had trial Parenteral Nutrition *(number of babies)*	34 (100.0%)	28 (100.0%)	34 (100.0%)	37 (100.0%)
Time on trial Parenteral Nutrition (days)	11.0 (8.00-14.0) [n=34]	12.0 (9.00-14.0) [n=28]	11.0 (9.25-13.0) [n=34]	12.0 (9.00-14.0) [n=37]
Cumulative trial Parenteral Nutrition Intake				
Aqueous Volume (l)	1.02 (0.74-1.30) [n=34]	1.03 (0.81-1.33) [n=28]	0.94 (0.86-1.21) [n=34]	1.07 (0.81-1.39) [n=37]
Lipid Volume (l)	0.13 (0.09-0.18) [n=34]	0.13 (0.10-0.18) [n=28]	0.12 (0.10-0.16) [n=34]	0.13 (0.09-0.18) [n=37]
Protein (g)	20.2 (14.6-25.9) [n=34]	20.3 (16.1-26.3) [n=28]	29.0 (26.5-36.9) [n=34]	33.6 (25.1-42.2) [n=37]
Carbohydrate (g)	76.6 (57.7- 97.2) [n=34]	79.1 (59.7-100.1) [n=28]	70.6 (64.4- 89.6) [n=34]	83.7 (62.0-102.1) [n=37]
Fat (g)	24.3 (17.0-32.2) [n=34]	24.5 (18.1-32.2) [n=28]	21.7 (18.1-28.4) [n=34]	25.0 (17.1-32.9) [n=37]
Received non-trial Parenteral Nutrition *(number of babies)*	2 (5.9%)	1 (3.6%)	1 (2.9%)	2 (5.4%)
Time on non-trial Parenteral Nutrition(days)	4.0 (4.0-4.0) [n=2]	1.0 (1.0-1.0) [n=1]	1.0 (1.0-1.0) [n=1]	5.0 (3.5-6.5) [n=2]
Cumulative non-trial Parenteral Nutrition Intake[†]				
Aqueous Volume (l)	0.47 (0.47-0.48) [n=2]	0.02 (0.02-0.02) [n=1]	0.07 (0.07-0.07) [n=1]	0.44 (0.41-0.47) [n=2]
Lipid Volume (l)	0.01 (0.01-0.02) [n=2]	0.00 (0.00-0.00) [n=1]	0.00 (0.00-0.00) [n=1]	0.03 (0.03-0.04) [n=2]
Protein (g)	1.37 (1.18-1.55) [n=2]	0.07 (0.07-0.07) [n=1]	0.15 (0.15-0.15) [n=1]	1.21 (1.12-1.29) [n=2]
Carbohydrate (g)	45.99 (44.28-47.69) [n=2]	2.47 (2.47- 2.47) [n=1]	3.35 (3.35- 3.35) [n=1]	35.48 (32.62-38.34) [n=2]

	Incremental amino acid & 20% Intralipid	Incremental amino acid & 20% SMOF lipid	Recommended Daily Intake of amino acid & 20% Intralipid	Recommended Daily Intake of amino acid & 20% SMOF lipid
	N=34	**N=28**	**N=34**	**N=37**
Fat (g)	2.20 (1.10-3.30) [n=2]	0.30 (0.30-0.30) [n=1]	0.53 (0.53-0.53) [n=1]	5.85 (5.14-6.56) [n=2]
Cumulative non-trial and trial Parenteral Nutrition intake				
Aqueous Volume (l)	1.04 (0.82-1.30) [n=34]	1.03 (0.81-1.33) [n=28]	0.95 (0.86-1.21) [n=34]	1.07 (0.81-1.39) [n=37]
Lipid Volume (l)	0.13 (0.09-0.18) [n=34]	0.13 (0.10-0.18) [n=28]	0.12 (0.10-0.16) [n=34]	0.13 (0.09-0.18) [n=37]
Protein (g)	20.2 (14.6-25.9) [n=34]	20.3 (16.1-26.3) [n=28]	29.0 (26.5-36.9) [n=34]	33.6 (25.1-42.2) [n=37]
Carbohydrate (g)	78.0 (63.0- 97.2) [n=34]	79.1 (60.5-100.1) [n=28]	71.7 (64.4- 89.6) [n=34]	83.7 (62.0-102.1) [n=37]
Fat (g)	24.3 (17.0-32.2) [n=34]	24.5 (18.4-32.2) [n=28]	21.7 (18.1-28.4) [n=34]	25.0 (17.1-32.9) [n=37]

[*] *Data presented is median (IQR)*

Table 13. Nutritional intake during study period for all infants randomised

	Incremental amino acid & 20% Intralipid	Incremental amino acid & 20% SMOF lipid	Recommended Daily Intake of amino acid & 20% Intralipid	Recommended Daily Intake of amino acid & 20% SMOF lipid
	N=42	N=42	N=41	N=43
Dextrose and Insulin				
Cumulative intravenous dextrose (g) †	15.2 (5.39-50.2) [n=42]	18.1 (7.20-54.0) [n=41]	11.8 (5.23-45.7) [n=40]	22.2 (7.99-55.5) [n=43]
Insulin (*number of babies that received insulin)*	11 (26.2%)	12 (28.6%)	5 (12.2%)	10 (23.3%)
Electrolytes				
Additional Sodium (mmol)	17.54 (8.96-24.4) [n=14]	7.46 (5.12-18.4) [n=14]	12.18 (9.01-42.7) [n=9]	10.01 (5.44-22.5) [n=14]
Additional Potassium (mmol)	9.97 (6.79-11.73) [n=8]	2.73 (1.47- 4.88) [n=8]	2.93 (1.73- 8.24) [n=8]	3.72 (1.64- 7.14) [n=10]
Received donor milk *(number of babies)*	15 (35.7%)	12 (28.6%)	13 (31.7%)	17 (39.5%)
Cumulative donor milk (l) *(total volume per baby)*	0.38 (0.08-1.07) [n=15]	0.30 (0.02-0.47) [n=12]	0.38 (0.05-1.10) [n=13]	0.19 (0.03-0.36) [n=17]
Received maternal breast milk *(number of babies)*	41 (97.6%)	38 (90.5%)	38 (92.7%)	43 (100.0%)
Cumulative maternal breast milk intake (l)				
During trial Parenteral Nutrition phase	0.72 (0.45-0.88) [n=41]	0.61 (0.43-0.81) [n=38]	0.57 (0.30-0.81) [n=38]	0.63 (0.26-0.89) [n=43]
During non-trial Parenteral Nutrition phase	1.92 (0.38-4.82) [n=8]	4.01 (1.83-5.08) [n=8]	1.79 (0.44-4.51) [n=8]	2.78 (0.70-4.50) [n=8]
Over study period	8.05 (4.50-13.2) [n=41]	7.16 (1.94-12.1) [n=38]	6.74 (1.27-12.1) [n=38]	7.61 (1.73-15.7) [n=43]
Number of days having Fortifier†	13.0 (10.0-24.0) [n=23]	23.0 (14.5-41.8) [n=12]	14.0 (1.0-29.0) [n=17]	27.0 (13.0-44.0) [n=17]
Received formula milk *(number of babies)*	30 (71.4%)	23 (54.8%)	29 (70.7%)	29 (67.4%)
Cumulative formula intake (l)				
During trial Parenteral Nutrition phase	0.02 (0.01-0.13) [n=10]	0.24 (0.02-0.52) [n=10]	0.28 (0.04-0.60) [n=15]	0.30 (0.04-0.39) [n=14]
During non-trial Parenteral Nutrition phase	2.05 (0.44-3.60) [n=6]	2.09 (0.00-4.35) [n=4]	0.78 (0.00-2.12) [n=7]	2.30 (0.95-5.06) [n=6]
Over study period	5.86 (1.22-10.87) [n=30]	6.39 (0.41- 9.36) [n=23]	8.44 (2.21-13.04) [n=29]	6.70 (3.86-12.63) [n=29]
Received trial Parenteral Nutrition (*number of babies)*	42 (100.0%)	41 (97.6%)	40 (97.6%)	43 (100.0%)

	Incremental amino acid & 20% Intralipid	Incremental amino acid & 20% SMOF lipid	Recommended Daily Intake of amino acid & 20% Intralipid	Recommended Daily Intake of amino acid & 20% SMOF lipid
	N=42	N=42	N=41	N=43
Time on trial Parenteral Nutrition (days)[†]	11.0 (8.00-16.8) [n=42]	12.0 (9.00-17.0) [n=41]	11.0 (9.75-13.2) [n=40]	12.0 (9.00-17.0) [n=43]
Cumulative trial Parenteral Nutrition Intake				
Aqueous Volume (l)	1.04 (0.77-1.56) [n=42]	0.97 (0.71-1.39) [n=41]	0.95 (0.81-1.33) [n=40]	1.19 (0.86-1.56) [n=43]
Lipid Volume (l)	0.13 (0.09-0.19) [n=42]	0.13 (0.08-0.18) [n=41]	0.12 (0.10-0.16) [n=40]	0.14 (0.10-0.19) [n=43]
During trial Parenteral Nutrition phase				
Protein (g)	20.5 (15.2-31.0) [n=42]	19.4 (14.0-27.6) [n=41]	29.2 (25.3-41.2) [n=40]	36.8 (26.4-47.3) [n=43]
Carbohydrate (g)	78.0 (57.7-116.0) [n=42]	72.7 (55.0-104.8) [n=41]	71.9 (62.6-101.3) [n=40]	90.4 (64.8-114.6) [n=43]
Fat (g)	23.9 (16.7-35.7) [n=42]	23.6 (15.1-33.0) [n=41]	21.6 (18.0-29.5) [n=40]	25.0 (18.6-35.0) [n=43]
Received non-trial Parenteral Nutrition *(number of babies)*	10 (23.8%)	8 (19.0%)	8 (19.5%)	10 (23.3%)
Time on non-trial Parenteral Nutrition(days)[†]	5.5 (3.25-19.8) [n=10]	22.0 (14.50-28.5) [n=8]	28.5 (9.50-58.5) [n=8]	12.0 (3.00-15.8) [n=10]
Cumulative non-trial Parenteral Nutrition Intake				
Aqueous Volume (l)	0.47 (0.39-2.44) [n=10]	2.12 (1.89-2.37) [n=8]	2.91 (0.87-9.50) [n=8]	1.23 (0.47-2.44) [n=10]
Lipid Volume (l)	0.06 (0.02-0.26) [n=10]	0.33 (0.32-0.39) [n=8]	0.38 (0.13-1.40) [n=8]	0.16 (0.05-0.26) [n=10]
During trial Parenteral Nutrition phase				
Protein (g)	0.0 (0.0-0.00) [n=10]	0.0 (0.0-0.00) [n=8]	0.0 (0.0-0.04) [n=8]	0.0 (0.0-0.07) [n=10]
Carbohydrate (g)	0.0 (0.0-0.00) [n=10]	0.0 (0.0-0.00) [n=8]	0.0 (0.0-0.84) [n=8]	0.0 (0.0-3.25) [n=10]
Fat (g)	0.0 (0.0-0.00) [n=10]	0.0 (0.0-0.00) [n=8]	0.0 (0.0-0.13) [n=8]	0.0 (0.0-0.59) [n=10]
Over study period				
Protein (g)	1.65 (1.34- 6.54) [n=10]	7.32 (5.60- 7.66) [n=8]	10.76 (4.60- 31.95) [n=8]	3.09 (0.39- 4.28) [n=10]
Carbohydrate (g)	49.4 (41.1- 220.6) [n=10]	222.7 (188.6- 265.4) [n=8]	338.7 (166.7- 1136.4) [n=8]	108.6 (11.3- 146.3) [n=10]
Fat (g)	10.5 (4.04- 46.5)	58.8 (56.76-	66.0 (23.68-	28.3 (8.78- 46.4)

	Incremental amino acid & 20% Intralipid	Incremental amino acid & 20% SMOF lipid	Recommended Daily Intake of amino acid & 20% Intralipid	Recommended Daily Intake of amino acid & 20% SMOF lipid
	N=42	N=42	N=41	N=43
	[n=10]	69.1) [n=8]	247.1) [n=8]	[n=10]
Cumulative non-trial and trial Parenteral Nutrition Intake				
Aqueous Volume (l)	1.12 (0.85-1.85) [n=42]	1.30 (0.83-1.64) [n=41]	1.06 (0.87-1.48) [n=40]	1.38 (0.95-2.04) [n=43]
Lipid Volume (l)	0.17 (0.10-0.24) [n=42]	0.16 (0.10-0.21) [n=41]	0.13 (0.11-0.20) [n=40]	0.18 (0.12-0.24) [n=43]
During trial Parenteral Nutrition phase				
Protein (g)	20.5 (15.2-31.0) [n=42]	19.4 (14.0-27.6) [n=41]	29.2 (25.3-41.2) [n=40]	36.8 (26.4-47.3) [n=43]
Carbohydrate (g)	78.0 (60.9-116.0) [n=42]	72.7 (55.0-104.8) [n=41]	71.9 (62.6-101.3) [n=40]	90.4 (66.3-114.6) [n=43]
Fat (g)	23.9 (16.8-35.7) [n=42]	23.6 (15.1-33.0) [n=41]	21.6 (18.0-29.5) [n=40]	25.0 (18.6-35.0) [n=43]
Over study period				
Protein (g)	21.5 (16.3-32.7) [n=42]	21.6 (16.4-28.3) [n=41]	31.8 (26.6-42.6) [n=40]	36.8 (28.1-47.3) [n=43]
Carbohydrate (g)	87.8 (64.5-139.6) [n=42]	94.8 (64.0-136.5) [n=41]	81.0 (65.1-113.4) [n=40]	101.4 (70.8-149.7) [n=43]
Fat (g)	31.3 (18.8-43.6) [n=42]	28.2 (19.0-37.4) [n=41]	23.2 (19.3-36.4) [n=40]	32.0 (22.6-44.1) [n=43]
Cumulative nutritional intake from birth until 34 weeks (includes Parenteral Nutrition and milk intake)				
Protein (g)	241.2 (189.3-468.2) [n=42]	215.5 (139.7-310.3) [n=41]	275.0 (199.6-399.9) [n=40]	303.4 (221.9-419.8) [n=43]
Carbohydrate (g)	1054.3 (768.0-1862.9) [n=42]	885.5 (581.7-1300.0) [n=41]	1226.8 (765.0-1574.6) [n=40]	1211.8 (818.0-1681.0) [n=43]
Fat (g)	491.6 (358.0-936.9) [n=42]	438.9 (279.6-628.6) [n=41]	552.7 (390.3-680.1) [n=40]	610.7 (389.2-825.5) [n=43]

[*] *Data presented are median (IQR) for continuous variables and frequency (percentage) for categorical variables*

Table 14. Nutritional intake during study period for all infants completing MRI scan*

	Incremental amino acid & 20% Intralipid	Incremental amino acid & 20% SMOF lipid	Recommended Daily Intake of amino acid & 20% Intralipid	Recommended Daily Intake of amino acid & 20% SMOF lipid
	N=34	N=28	N=34	N=37
Dextrose and Insulin				
Cumulative intravenous dextrose (g) †	9.27 (4.56-54.8) [n=34]	23.64 (7.04-75.1) [n=28]	11.81 (5.68-25.9) [n=34]	22.20 (8.89-53.4) [n=37]
Insulin (*number of babies that received insulin)*	7 (20.6%)	6 (21.4%)	4 (11.8%)	7 (18.9%)
Electrolytes				
Additional Sodium (mmol)	20.93 (11.81-34.2) [n=10]	11.13 (5.00-22.6) [n=11]	9.85 (8.87-13.5) [n=7]	10.01 (5.44-22.5) [n=14]
Additional Potassium (mmol)	10.38 (6.45-12.29) [n=7]	3.71 (1.62- 5.58) [n=7]	2.18 (1.46- 5.00) [n=7]	4.19 (1.60- 7.85) [n=9]
Received donor milk *(number of babies)*	11 (32.4%)	9 (32.1%)	12 (35.3%)	15 (40.5%)
Cumulative donor milk (l) *(total volume per baby)*	0.38 (0.08-1.07) [n=11]	0.27 (0.02-0.44) [n=9]	0.24 (0.04-1.15) [n=12]	0.19 (0.03-0.84) [n=15]
Received maternal breast milk *(number of babies)*	33 (97.1%)	27 (96.4%)	33 (97.1%)	37 (100.0%)
Cumulative maternal breast milk intake (l)				
During trial Parenteral Nutrition phase	0.73 (0.57-0.88) [n=33]	0.62 (0.45-0.83) [n=27]	0.62 (0.32-0.82) [n=33]	0.65 (0.36-0.90) [n=37]
During non-trial Parenteral Nutrition phase	1.75 (0.33-4.46) [n=5]	4.16 (2.86-5.13) [n=7]	1.79 (0.50-3.16) [n=6]	2.78 (0.70-4.50) [n=8]
Over study period	8.46 (5.90-14.6) [n=33]	7.86 (2.79-13.5) [n=27]	5.97 (1.53-12.3) [n=33]	8.86 (3.32-15.9) [n=37]
Number of days having Fortifier†	13.0 (10.0-21.0) [n=21]	20.0 (16.0-36.0) [n=9]	13.0 (1.0-28.2) [n=16]	25.5 (13.0-45.0) [n=16]
Received formula milk *(number of babies)*	25 (73.5%)	19 (67.9%)	25 (73.5%)	26 (70.3%)
Cumulative formula intake (l)				
During trial Parenteral Nutrition phase	0.02 (0.00-0.16) [n=9]	0.41 (0.13-0.53) [n=8]	0.13 (0.04-0.66) [n=13]	0.28 (0.05-0.38) [n=11]
During non-trial Parenteral Nutrition phase	2.34 (1.76-4.02) [n=5]	2.09 (0.00-4.35) [n=4]	0.78 (0.00-3.45) [n=5]	2.30 (0.95-5.06) [n=6]
Over study period	2.81 (0.99-14.37) [n=25]	8.04 (2.96- 9.93) [n=19]	8.44 (2.21-13.04) [n=25]	7.26 (3.90-12.61) [n=26]
Received trial Parenteral Nutrition (*number of babies)*	34 (100.0%)	28 (100.0%)	34 (100.0%)	37 (100.0%)

	Incremental amino acid & 20% Intralipid	Incremental amino acid & 20% SMOF lipid	Recommended Daily Intake of amino acid & 20% Intralipid	Recommended Daily Intake of amino acid & 20% SMOF lipid
	N=34	N=28	N=34	N=37
Time on trial Parenteral Nutrition (days)[†]	11.0 (8.0-16.0) [n=34]	12.5 (9.0-16.0) [n=28]	11.0 (10.0-13.0) [n=34]	13.0 (9.0-17.0) [n=37]
Cumulative trial Parenteral Nutrition Intake				
Aqueous Volume (l)	1.04 (0.77-1.56) [n=34]	1.06 (0.83-1.39) [n=28]	0.96 (0.86-1.32) [n=34]	1.19 (0.82-1.57) [n=37]
Lipid Volume (l)	0.14 (0.09-0.19) [n=34]	0.13 (0.10-0.19) [n=28]	0.12 (0.10-0.16) [n=34]	0.14 (0.10-0.20) [n=37]
During trial Parenteral Nutrition phase				
Protein (g)	20.5 (15.2-31.1) [n=34]	20.9 (16.4-27.6) [n=28]	29.6 (26.5-40.6) [n=34]	37.3 (25.6-47.4) [n=37]
Carbohydrate (g)	78.0 (57.7-118.5) [n=34]	81.6 (62.1-105.2) [n=28]	73.4 (64.4- 99.6) [n=34]	90.4 (63.4-114.6) [n=37]
Fat (g)	25.0 (17.0-35.7) [n=34]	24.9 (18.1-33.9) [n=28]	21.7 (19.1-28.4) [n=34]	25.0 (19.0-35.6) [n=37]
Received non-trial Parenteral Nutrition *(number of babies)*	6 (17.6%)	7 (25.0%)	6 (17.6%)	9 (24.3%)
Time on non-trial Parenteral Nutrition(days)[†]	13.5 (4.0-27.5) [n=6]	19.0 (13.0-26.0) [n=7]	43.5 (25.8-69.5) [n=6]	12.0 (3.0-16.0) [n=9]
Cumulative non-trial Parenteral Nutrition Intake				
Aqueous Volume (l)	1.77 (0.40- 3.76) [n=6]	2.13 (1.86- 2.48) [n=7]	6.26 (2.52-10.83) [n=6]	1.24 (0.74- 2.76) [n=9]
Lipid Volume (l)	0.18 (0.02-0.58) [n=6]	0.33 (0.32-0.35) [n=7]	0.90 (0.32-1.58) [n=6]	0.17 (0.07-0.29) [n=9]
During trial Parenteral Nutrition phase				
Protein (g)	0.0 (0.0-0.00) [n=6]	0.0 (0.0-0.00) [n=7]	0.0 (0.0-0.12) [n=6]	0.0 (0.0-0.00) [n=9]
Carbohydrate (g)	0.0 (0.0-0.00) [n=6]	0.0 (0.0-0.00) [n=7]	0.0 (0.0-2.51) [n=6]	0.0 (0.0-0.00) [n=9]
Fat (g)	0.0 (0.0-0.0) [n=6]	0.0 (0.0-0.0) [n=7]	0.0 (0.0-0.4) [n=6]	0.0 (0.0-0.0) [n=9]
Over study period				
Protein (g)	4.88 (1.44-12.63) [n=6]	7.44 (4.66- 7.69) [n=7]	19.86 (6.68-37.40) [n=6]	3.98 (1.07- 4.28) [n=9]
Carbohydrate (g)	159.4 (47.9-431.8) [n=6]	242.2 (186.9-267.0) [n=7]	701.5 (239.8-1339.7) [n=6]	139.6 (30.7-147.6) [n=9]
Fat (g)	31.2 (2.96-101.7)	58.2 (56.47-	159.0 (55.66-	30.4 (13.16-

	Incremental amino acid & 20% Intralipid	Incremental amino acid & 20% SMOF lipid	Recommended Daily Intake of amino acid & 20% Intralipid	Recommended Daily Intake of amino acid & 20% SMOF lipid
	N=34	N=28	N=34	N=37
	[n=6]	62.2) [n=7]	278.5) [n=6]	50.8) [n=9]
Cumulative non-trial and trial Parenteral Nutrition Intake				
Aqueous Volume (l)	1.11 (0.85-1.85) [n=34]	1.34 (0.96-1.80) [n=28]	1.12 (0.89-1.43) [n=34]	1.53 (0.97-2.05) [n=37]
Lipid Volume (l)	0.17 (0.10-0.25) [n=34]	0.18 (0.13-0.25) [n=28]	0.13 (0.11-0.20) [n=34]	0.18 (0.13-0.25) [n=37]
During trial Parenteral Nutrition phase				
Protein (g)	20.5 (15.2-31.1) [n=34]	20.9 (16.4-27.6) [n=28]	29.6 (26.5-40.6) [n=34]	37.3 (25.6-47.4) [n=37]
Carbohydrate (g)	78.0 (61.2-118.5) [n=34]	81.6 (62.1-105.2) [n=28]	73.4 (64.4- 99.6) [n=34]	92.7 (66.4-114.6) [n=37]
Fat (g)	25.0 (17.0-35.7) [n=34]	24.9 (18.4-33.9) [n=28]	21.7 (19.1-28.4) [n=34]	25.0 (19.0-35.6) [n=37]
Over study period				
Protein (g)	21.5 (16.3-33.0) [n=34]	23.8 (18.7-28.4) [n=28]	32.9 (27.5-42.1) [n=34]	37.3 (28.6-47.4) [n=37]
Carbohydrate (g)	85.5 (64.5-139.6) [n=34]	101.9 (72.2-145.1) [n=28]	83.5 (67.2-110.6) [n=34]	112.0 (71.9-152.7) [n=37]
Fat (g)	30.3 (18.8-45.5) [n=34]	31.8 (23.5-46.0) [n=28]	23.6 (20.2-35.7) [n=34]	32.9 (22.8-45.6) [n=37]
Cumulative nutritional intake from birth until 34 weeks (includes Parenteral Nutrition and milk intake)				
Protein (g)	254.3 (193.9-518.3) [n=34]	257.1 (187.7-336.0) [n=28]	275.0 (203.5-393.6) [n=34]	342.8 (243.5-425.0) [n=37]
Carbohydrate (g)	1096.3 (778.6-2041.2) [n=34]	1056.7 (812.0-1323.2) [n=28]	1226.8 (785.5-1513.0) [n=34]	1286.4 (874.5-1714.2) [n=37]
Fat (g)	517.8 (405.8-1002.3) [n=34]	494.6 (379.9-641.8) [n=28]	569.7 (395.9-678.0) [n=34]	618.3 (446.5-849.7) [n=37]

[*] *Data presented are median (IQR) for continuous variables and frequency (percentage) for categorical variables*

There were no significant differences between the groups in the proportion of infants with abnormal biochemical indices namely, serum glucose, worst base deficit in the previous 24 hours, total serum bilirubin, conjugated bilirubin, serum cholesterol, serum triglycerides, serum sodium, serum potassium, serum phosphate, serum calcium, serum creatinine, and ALT). However, there were significantly more infants with blood urea nitrogen levels greater than 7 mmol/l (50% and 47.6 % in Groups 1 and 2 vs 70.7% and 79.1% in Groups 3 and 4 respectively, $p<0.01$) and 10 mmol/l (14.3% and 21.4% in Groups 1 and 2 vs 43.9% and 53.5% in Groups 3 and 4 respectively, $p<0.01$).

Table 15. Safety data: Summary of laboratory adverse events by treatment for all infants randomised*

		Incremental amino acid & 20% Intralipid (N=42)	Incremental amino acid & 20% SMOF lipid (N=42)	Recommended Daily Intake of amino acid & 20% Intralipid (N=41)	Recommended Daily Intake of amino acid & 20% SMOF lipid (N=43)	P for Amino acid	P for lipid
Glucose	Low (< 2.6mmol/l)	12 (28.6%)	19 (45.2%)	15 (36.6%)	16 (37.2%)	1.0	0.32
	High (>15mmol/l)	8 (19.0%)	11 (26.2%)	3 (7.3%)	7 (16.3%)	0.10	0.25
Worst base deficit in previous 24 hours	> 15 mmol /l	5 (11.9%)	3 (7.1%)	5 (12.2%)	8 (18.6%)	0.35	1.0
Total serum bilirubin	> 150 μmol /l	30 (71.4%)	27 (64.3%)	26 (63.4%)	31 (72.1%)	1.0	1.0
Conjugated bilirubin	> 40 μmol /l	6 (14.3%)	4 (9.5%)	3 (7.3%)	4 (9.3%)	0.61	0.96
Cholesterol	> 6 mmol /l	0 (0%)	2 (4.8%)	1 (2.4%)	0 (0%)	0.56	0.57
	>10 mmol /l	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	NA	NA
Triglycerides	> 2.5 mmol /l	15 (35.7%)	13 (31.0%)	14 (34.1%)	12 (27.9%)	0.87	0.55
	> 5 mmol /l	2 (4.8%)	2 (4.8%)	1 (2.4%)	2 (4.7%)	0.70	0.72
Sodium	Low (<131 mmol/l)	9 (21.4%)	7 (16.7%)	10 (24.4%)	9 (20.9%)	0.70	0.65
	High (>150 mmol/l)	5 (11.9%)	10 (23.8%)	4 (9.8%)	5 (11.6%)	0.27	0.3
Potassium	Low (<3.2 mmol/l)	5 (11.9%)	6 (14.3%)	11 (26.8%)	7 (16.3%)	0.22	0.63
	High (>9 mmol/l)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	NA	NA
Phosphate	Low (<1.5 mmol/l)	17 (40.5%)	12 (28.6%)	14 (34.1%)	19 (44.2%)	0.63	1.0
	High (>3 mmol/l)	4 (9.5%)	5 (11.9%)	5 (12.2%)	4 (9.3%)	1.0	1.0
Calcium	Low (<1 mmol/l)	0 (0%)	1 (2.4%)	0 (0%)	2 (4.7%)	0.56	0.08
	High (>3 mmol/l)	0 (0%)	0 (0%)	3 (7.3%)	2 (4.7%)	0.02	0.63
Urea	Low (<1.5 mmol/l)	13 (31.0%)	11 (26.2%)	5 (12.2%)	8 (18.6%)	0.15	0.55

		Incremental amino acid & 20% Intralipid (N=42)	Incremental amino acid & 20% SMOF lipid (N=42)	Recommended Daily Intake of amino acid & 20% Intralipid (N=41)	Recommended Daily Intake of amino acid & 20% SMOF lipid (N=43)	P for Amino acid	P for lipid
	High (>7 mmol/l)	21 (50.0%)	20 (47.6%)	29 (70.7%)	34 (79.1%)	<0.01	0.78
	High(>10 mmol/l)	6 (14.3%)	9 (21.4%)	18 (43.9%)	23 (53.5%)	<0.01	0.30
Creatinine	>170 µmol/l	0 (0%)	0 (0%)	0 (0%)	3 (7.0%)	0.08	0.08
ALT	> 60 IU / l	5 (11.9%)	2 (4.8%)	4 (9.8%)	4 (9.3%)	1.0	0.56
Zinc	<8 µmol/l	0 (0%)	0 (0%)	0 (0%)	0 (0%)	NA	NA
Copper	<2 µmol/l	0 (0%)	0 (0%)	0 (0%)	0 (0%)	NA	NA
Manganese	>30 nmol/l	0 (0%)	0 (0%)	0 (0%)	0 (0%)	NA	NA
Aluminium	>0.4 µmol/l	0 (0%)	0 (0%)	0 (0%)	0 (0%)	NA	NA
Selenium	<20 µg/l	0 (0%)	1 (2.4%)	0 (0%)	0 (0%)	0.32	0.32

[*] *Data presented are number of infants (percentage); NA: Not applicable*

Table 16. Safety data: Summary of laboratory adverse events by treatment for all infants completing MRI scan*

		Incremental amino acid & 20% Intralipid (N=34)	Incremental amino acid & 20% SMOF lipid (N=28)	Recommended Daily Intake of amino acid & 20% Intralipid (N=34)	Recommended Daily Intake of amino acid & 20% SMOF lipid (N=37)	P for Amino acid	P for lipid
Glucose	Low (< 2.6mmol/l)	8 (23.5%)	12 (42.9%)	13 (38.2%)	14 (37.8%)	1.0	0.32
	High (>15mmol/l)	6 (17.6%)	5 (17.9%)	3 (8.8%)	5 (13.5%)	0.1	0.25
Worst base deficit in previous 24 hours	> 15 mmol /l	2 (5.9%)	0 (0.0%)	2 (5.9%)	6 (16.2%)	0.35	1.0
Total serum bilirubin	> 150 μmol /l	24 (70.6%)	21 (75.0%)	23 (67.6%)	28 (75.7%)	1.0	1.0
Conjugated bilirubin	> 40 μmol /l	4 (11.8%)	3 (10.7%)	2 (5.9%)	3 (8.1%)	0.61	0.96
Cholesterol	> 6 mmol /l	0 (0.0%)	1 (3.6%)	1 (2.9%)	0 (0.0%)	0.56	0.57
	>10 mmol /l	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	NA	NA
Triglycerides	> 2.5 mmol /l	10 (29.4%)	7 (25.0%)	11 (32.4%)	10 (27.0%)	0.87	0.55
	> 5 mmol /l	2 (5.9%)	0 (0.0%)	1 (2.9%)	2 (5.4%)	0.7	0.72
Sodium	Low (<131 mmol/l)	7 (20.6%)	3 (10.7%)	9 (26.5%)	7 (18.9%)	0.7	0.65
	High (>150 mmol/l)	3 (8.8%)	3 (10.7%)	4 (11.8%)	4 (10.8%)	0.27	0.3
Potassium	Low (<3.2 mmol/l)	2 (5.9%)	4 (14.3%)	9 (26.5%)	6 (16.2%)	0.22	0.63
	High (>9 mmol/l)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	NA	NA
Phosphate	Low (<1.5 mmol/l)	12 (35.3%)	8 (28.6%)	13 (38.2%)	17 (45.9%)	0.63	1.0
	High (>3 mmol/l)	4 (11.8%)	3 (10.7%)	4 (11.8%)	3 (8.1%)	1.0	1.0
Calcium	Low (<1 mmol/l)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (5.4%)	0.56	0.08
	High (>3 mmol/l)	0 (0.0%)	0 (0.0%)	3 (8.8%)	1 (2.7%)	0.02	0.63
Urea	Low (<1.5 mmol/l)	10 (29.4%)	10 (35.7%)	5 (14.7%)	7 (18.9%)	0.15	0.55

		Incremental amino acid & 20% Intralipid (N=34)	Incremental amino acid & 20% SMOF lipid (N=28)	Recommended Daily Intake of amino acid & 20% Intralipid (N=34)	Recommended Daily Intake of amino acid & 20% SMOF lipid (N=37)	P for Amino acid	P for lipid
	High (>7 mmol/l)	14 (41.2%)	14 (50.0%)	25 (73.5%)	29 (78.4%)	<0.01	0.78
	High(>10 mmol/l)	1 (2.9%)	4 (14.3%)	16 (47.1%)	18 (48.6%)	<0.01	0.3
Creatinine	>170 μmol/l	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (5.4%)	0.08	0.08
ALT	> 60 IU / l	3 (8.8%)	2 (7.1%)	3 (8.8%)	2 (5.4%)	1.0	0.56
Zinc	<8 μmol/l	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	NA	NA
Copper	<2 μmol/l	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	NA	NA
Manganese	>30 nmol/l	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	NA	NA
Aluminium	>0.4 μmol/l	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	NA	NA
Selenium	<20 μg/l	0 (0.0%)	1 (3.6%)	0 (0.0%)	0 (0.0%)	0.32	0.32

[*] *Data presented are number of infants (percentage); NA: Not applicable*

Table 17. Safety data: Summary of serious adverse events by treatment*

Variable	Incremental amino acid & 20% Intralipid (N=42)	Incremental amino acid & 20% SMOF lipid (N=42)	Recommended Daily Intake of amino acid & 20% Intralipid (N=41)	Recommended Daily Intake of amino acid & 20% SMOF lipid (N=43)
Number of infants that experience SAE	7 (16.7%)	12 (28.6%)	7 (17.1%)	9 (20.9%)
SAE classification[†]				
Death	4 (9.5%)	7 (16.7%)	2 (4.9%)	3 (7.0%)
Life threatening	3 (7.1%)	3 (7.1%)	4 (9.8%)	4 (9.3%)
Prolongation of existing inpatient hospitalization	2 (4.8%)	2 (4.8%)	1 (2.4%)	3 (7.0%)
Persistent or significant disability or incapacity	0 (0.0%)	1 (2.4%)	0 (0.0%)	1 (2.3%)
Sepsis *(diagnosis on SAE form)*[‡]	2 (4.8%)	6 (14.3%)	1 (2.4%)	1 (2.3%)
Necrotising Enterocolitis (NEC)[‡]	3 (7.1%)	4 (9.5%)	1 (2.4%)	4 (9.3%)

[*] *Data presented are number of infants (percentage;* all SAEs were classified by local Investigators on the SAE reporting form

[†] *One infant may have more than one SAE*

In relation to primary outcome measures, there were no significant differences in the quantity of non-adipose tissue mass between the groups randomised to incremental vs RDI of amino acids, (adjusted mean difference and 95% confidence interval: RDI – Incremental amino acid 1 (-108, 111) p=0.98). For the lipid composition intervention, there was no significant difference in IHCL between the groups randomised to receive 20% Intralipid vs 20% SMOFLipid (adjusted mean difference and 95% confidence interval 1.1 (0.8, 1.6) p=0.58). There were no significant differences in secondary outcome measures of the quantity and distribution of adipose tissue, measure of insulin sensitivity (QUICKI index), total cerebral volume, whole brain volume, weight and length at term age equivalent. There was however a significant difference in the mean head circumference at term age equivalent between the Groups randomised to receive incremental vs RDI of amino acids, (adjusted mean difference and 95% confidence interval: RDI – Incremental amino acid -0.8 (-1.5, -0.1) p= 0.02).

Table 18. Reporting the factorial trial results all infants completing MRI assessment *

	Incremental amino acid & 20% Intralipid (N=34)	Incremental amino acid & 20% SMOF lipid (N=28)	Recommended Daily Intake of amino acid & 20% Intralipid (N=34)	Recommended Daily Intake of amino acid & 20% SMOF lipid (N=37)	Adjusted mean difference (Immediate Recommended Daily Intake amino acid – Incremental amino acid) *	Adjusted mean difference(20% SMOF lipid – 20% Intralipid)*	Interaction (P value)
Gestational age (weeks)	28.0 (27.3, 28.6)	28.0 (27.2, 28.9)	28.4 (27.7, 29.2)	27.7 (27.1, 28.4)			
Birth weight (g)	1064 (962, 1166)	1103 (979, 1226)	1090 (993, 1186)	1059 (962, 1155)			
Sex: Boys	58.8% (40.7%, 75.4%)	64.3% (44.1%, 81.4%)	50.0% (33.4%, 67.6%)	51.4% (34.4%, 67.5%)			
Age at scan (weeks)	12.5 (11.3, 13.7)	12.4 (10.9, 14.0)	12.1 (10.8, 13.4)	13.3 (12.0, 14.5)			
Non-adipose body mass (g)	2450(2246, 2655)	2337 (2164, 2510)	2344 (2244, 2444)	2485 (2327, 2643)	1 (-108, 111) p=0.98	-41 (-150, 68) p=0.46	216 (0, 432) p=0.05
IHCL[†]	0.6 (0.4, 0.9) [n=34]	0.7 (0.5, 1.0) [n=28]	0.5 (0.4, 0.6) [n=34]	0.5 (0.3, 0.7) [n=36]	0.7 (0.5, 1.1) [n=132] p=0.11	1.1 (0.8, 1.6) [n=132] p=0.58	0.8 (0.4, 1.7) [n=132] p=0.53
Total cerebral volume (cm^3)	468 (419, 518) [n=13]	480 (425, 534) [n=10]	468 (414, 523) [n=11]	511 (440, 583) [n=15]	15 (-42, 71) [n=49] p=0.61	24 (-32, 80) [n=49] p=0.40	-26 (-142, 90) [n=49] p=0.66
Whole brain volume (cm^3)	339 (304, 373) [n=13]	352 (319, 385) [n=10]	344 (296, 393) [n=11]	365 (321, 410) [n=15]	9 (-29, 47) [n=49] p=0.64	14 (-24, 52) [n=49] p=0.47	-29 (-107, 49) [n=49] p=0.46
Posterior fossa volume (cm^3)	30 (26, 33) [n=13]	31 (28, 34) [n=10]	30 (27, 34) [n=11]	35 (29, 38) [n=15]	1.44 (-1.99, 4.87) [n=49] p=0.41	2 (-2, 5) [n=49] p=0.35	-2 (-9, 5) [n=49] p=0.64
QUICKI	0.18 (0.17, 0.19)	0.19 (0.18, 0.20)	0.19 (0.18, 0.20)	0.18 (0.17, 0.20)	0.01 (0, 0.02)	0.01 (-0.01, 0.02)	-0.01 (-0.04, 0.02)

	Incremental amino acid & 20% Intralipid (N=34)	Incremental amino acid & 20% SMOF lipid (N=28)	Recommended Daily Intake of amino acid & 20% Intralipid (N=34)	Recommended Daily Intake of amino acid & 20% SMOF lipid (N=37)	Adjusted mean difference (Immediate Recommended Daily Intake amino acid – Incremental amino acid) *	Adjusted mean difference(20% SMOF lipid – 20% Intralipid)*	Interaction (P value)
	[n=11]	[n=6]	[n=11]	[n=11]	[n=39] p=0.20	[n=39] p=0.28	[n=39] p=0.46
End of Study Weight (g)	3060 (2780, 3340)	2924 (2686, 3162)	2932 (2780, 3085)	3151 (2934, 3368)	17 (-136, 170) p=0.83	-35 (-187, 117) p=0.65	293 (-8, 593) p=0.06
End of Study Length (cm)	47.7 (46.4, 49.0)	48.0 (46.6, 49.4)	48.2 (47.4, 49.0)	49.1 (47.8, 50.3)	0.5 (-0.3, 1.3) p=0.20	0.2 (-0.6, 1.0) p=0.56	0.5 (-1.1, 2.1) p=0.56
End of Study Head circumference (cm)	36.0 (34.9, 37.1)	35.3 (34.6, 36.0)	34.8 (34.3, 35.3)	35.2 (34.5, 35.9)	-0.8 (-1.5, -0.1) p=0.02	-0.2 (-0.9, 0.5) p=0.56	1.1 (-0.2, 2.5) p=0.09
Superficial subcutaneous adipose tissue mass (g)	515 (437, 593)	495 (431, 559)	493 (431, 554)	564 (499, 629)	12 (-44, 68) p=0.67	9 (-46, 64) p=0.75	73 (-38, 183) p=0.20
Internal adipose tissue mass (g)	67.2 (55.5, 79.0)	65.0 (52.4, 77.5)	69.1 (57.2, 81.0)	71.2 (59.8, 82.5)	2.5 (-7.5, 12.6) p=0.62	-3.4 (-13.4, 6.6) p=0.50	0.1 (-19.9, 20.1) p=0.99
Deep subcutaneous abdominal adipose tissue mass (g)	14.2 (11.0, 17.3)	13.0 (10.7, 15.2)	14.9 (12.3, 17.5)	17.8 (14.8, 20.7)	2.0 (-0.5, 4.4) p=0.11	0.4 (-2.0, 2.8) p=0.74	3.5 (-1.3, 8.3) p=0.15
Internal abdominal adipose tissue mass (g)	14.8 (12.2, 17.3)	14.1 (11.0, 17.2)	15.9 (12.8, 18.9)	16.5 (13.6, 19.3)	1.4 (-1.2, 4.1) p=0.28	-0.8 (-3.4, 1.8) p=0.56	0.5 (-4.8, 5.7) p=0.86
Superficial	87.0 (72.4, 101.6)	84.5 (72.8, 96.2)	85.2 (73.8, 96.5)	102.6 (87.2,	5.2 (-6.5, 17.0)	5.0 (-6.7, 16.6)	16.3 (-7.0, 39.6)

	Incremental amino acid & 20% Intralipid (N=34)	Incremental amino acid & 20% SMOF lipid (N=28)	Recommended Daily Intake of amino acid & 20% Intralipid (N=34)	Recommended Daily Intake of amino acid & 20% SMOF lipid (N=37)	Adjusted mean difference (Immediate Recommended Daily Intake amino acid – Incremental amino acid) *	Adjusted mean difference(20% SMOF lipid – 20% Intralipid)*	Interaction (P value)
subcutaneous abdominal adipose tissue mass (g)				118.1)	p=0.38	p=0.40	p=0.17
Total adipose tissue mass (g)	610 (518, 702)	587 (509, 664)	589 (514, 663)	666 (589, 743)	16 (-51, 82) p=0.64	6 (-60, 72) p=0.85	77 (-55, 208) p=0.25
Total adipose tissue mass as percentage of weight (%)	19.4 (17.9, 20.9)	19.7 (18.4, 21.0)	19.6 (17.8, 21.4)	20.8 (19.4, 22.3)	0 (-0.01, 0.02) p=0.56	0.01 (-0.01, 0.02) p=0.45	0.01 (-0.02, 0.03) p=0.72
Ratio of internal abdominal and deep subcutaneous abdominal (metabolically active) to non metabolically active adipose tissue	0.05 (0.04, 0.05)	0.05 (0.04, 0.05)	0.05 (0.05, 0.06)	0.05 (0.05, 0.6)	0 (0, 0.01) p=0.06	0 (0, 0) p=0.54	0 (-0.01, 0.01) p=0.87
Ratio of internal to subcutaneous adipose tissue	0.13 (0.12, 0.15)	0.13 (0.12, 0.15)	0.14 (0.13, 0.15)	0.13 (0.11, 0.14)	0 (-0.01, 0.01) p=0.85	-0.01 (-0.02, 0) p=0.12	-0.01 (-0.04, 0.01) p=0.29

	Incremental amino acid & 20% Intralipid (N=34)	Incremental amino acid & 20% SMOF lipid (N=28)	Recommended Daily Intake of amino acid & 20% Intralipid (N=34)	Recommended Daily Intake of amino acid & 20% SMOF lipid (N=37)	Adjusted mean difference (Immediate Recommended Daily Intake amino acid – Incremental amino acid) *	Adjusted mean difference(20% SMOF lipid – 20% Intralipid)*	Interaction (P value)
Incidence of hypertriglyceridaemia Triglycerides > 2.5 mmol /l§	29.4% (15.1%, 47.5%)	25.0% (10.7%, 44.9%)	32.4% (17.4%, 50.5%)	27.0% (13.8%, 44.1%)	1.15 (0.48, 2.74) p=0.76	0.68 (0.28, 1.62) p=0.38	0.71 (0.12, 4.06) p=0.70
Incidence of hyperbilirubinaemia based on Total serum bilirubin > 150 µmol /l§	70.6% (52.5%, 84.9%)	75.0% (55.1%, 89.3%)	67.6% (49.5%, 82.6%)	75.7% (58.8%, 88.2%)	0.92 (0.41, 2.04) p=0.83	1.32 (0.59, 2.94) p=0.50	1.29 (0.26, 6.47) p=0.75
Incidence of hyperbilirubinaemia based on Conjugated bilirubin > 40 µmol /l§	11.8% (3.3%, 27.5%)	10.7% (2.3%, 28.2%)	5.9% (0.7%, 19.7%)	8.1% (1.7%, 21.9%)	0.47 (0.12, 1.85) p=0.28	0.93 (0.24, 3.54) p=0.92	1.65 (0.11, 25.34) p=0.72
Incidence of abnormal ALT based on ALT>60IU/l§	8.8% (1.9%, 23.7%)	7.1% (0.9%, 23.5%)	8.8% (1.9%, 23.7%)	5.4% (0.7%, 18.2%)	0.99 (0.22, 4.41) p=0.99	0.45 (0.09, 2.12) p=0.31	0.59 (0.03, 12.69) p=0.74

** Adjusted for: age at scan, gender, gestational age, birth weight and centre; body mass components are derived from body mass volumes*

† Log transformation was used in the regression model, the results transformed back from the log scale

§Logistic regression was used for modelling and odds ratio was reported

Table 19. Reporting the factorial original MRI results in volume for all infants completing MRI assessment *

	Incremental amino acid & 20% Intralipid (N=34)	Incremental amino acid & 20% SMOF lipid (N=28)	Recommended Daily Intake of amino acid & 20% Intralipid (N=34)	Recommended Daily Intake of amino acid & 20% SMOF lipid (N=37)	Adjusted mean difference (Immediate Recommended Daily Intake amino acid – Incremental amino acid) *	Adjusted mean difference(20% SMOF lipid – 20% Intralipid)*	Interaction (P value)
Total Internal adipose tissue (L)	0.07 (0.06, 0.09)	0.07 (0.06, 0.09)	0.08 (0.06, 0.09)	0.08 (0.07, 0.09)	0 (-0.01, 0.01) p=0.62	0 (-0.01, 0.01) p=0.50	0 (-0.02, 0.02) p=0.99
Superficial adipose tissue (L)	0.57 (0.49, 0.66)	0.55 (0.48, 0.62)	0.55 (0.48, 0.62)	0.63 (0.55, 0.7)	0.01 (-0.05, 0.08) p=0.67	0.01 (-0.05, 0.07) p=0.75	0.08 (-0.04, 0.2) p=0.20
Deep subcutaneous adipose tissue (L)	0.03 (0.03, 0.04)	0.03 (0.02, 0.03)	0.03 (0.03, 0.03)	0.03 (0.03, 0.04)	0 (0, 0) p=0.55	0 (0, 0) p=0.71	0 (0, 0.01) p=0.24
Internal abdominal adipose tissue (L)	0.02 (0.01, 0.02)	0.02 (0.01, 0.02)	0.02 (0.01, 0.02)	0.02 (0.02, 0.02)	0 (0, 0) p=0.28	0 (0, 0) p=0.55	0 (-0.01, 0.01) p=0.86
Superficial subcutaneous abdominal adipose tissue (L)	0.1 (0.08, 0.11)	0.09 (0.08, 0.11)	0.09 (0.08, 0.11)	0.11 (0.1, 0.13)	0.01 (-0.01, 0.02) p=0.38	0.01 (-0.01, 0.02) p=0.40	0.02 (-0.01, 0.04) p=0.17
Deep subcutaneous abdominal adipose tissue (L)	0.02 (0.01, 0.02)	0.01 (0.01, 0.02)	0.02 (0.01, 0.02)	0.02 (0.02, 0.02)	0 (0, 0) p=0.11	0 (0, 0) p=0.74	0 (0, 0.01) p=0.15
Total adipose tissue (L)	0.68 (0.58, 0.78)	0.65 (0.57, 0.74)	0.65 (0.57, 0.74)	0.74 (0.65, 0.83)	0.02 (-0.06, 0.09) p=0.64	0.01 (-0.07, 0.08) p=0.85	0.09 (-0.06, 0.23) p=0.25

** Adjusted for: age at scan, gender, gestational age, birth weight and centre.*

Figure 7. Distribution of Primary and Secondary outcomes after transformation*

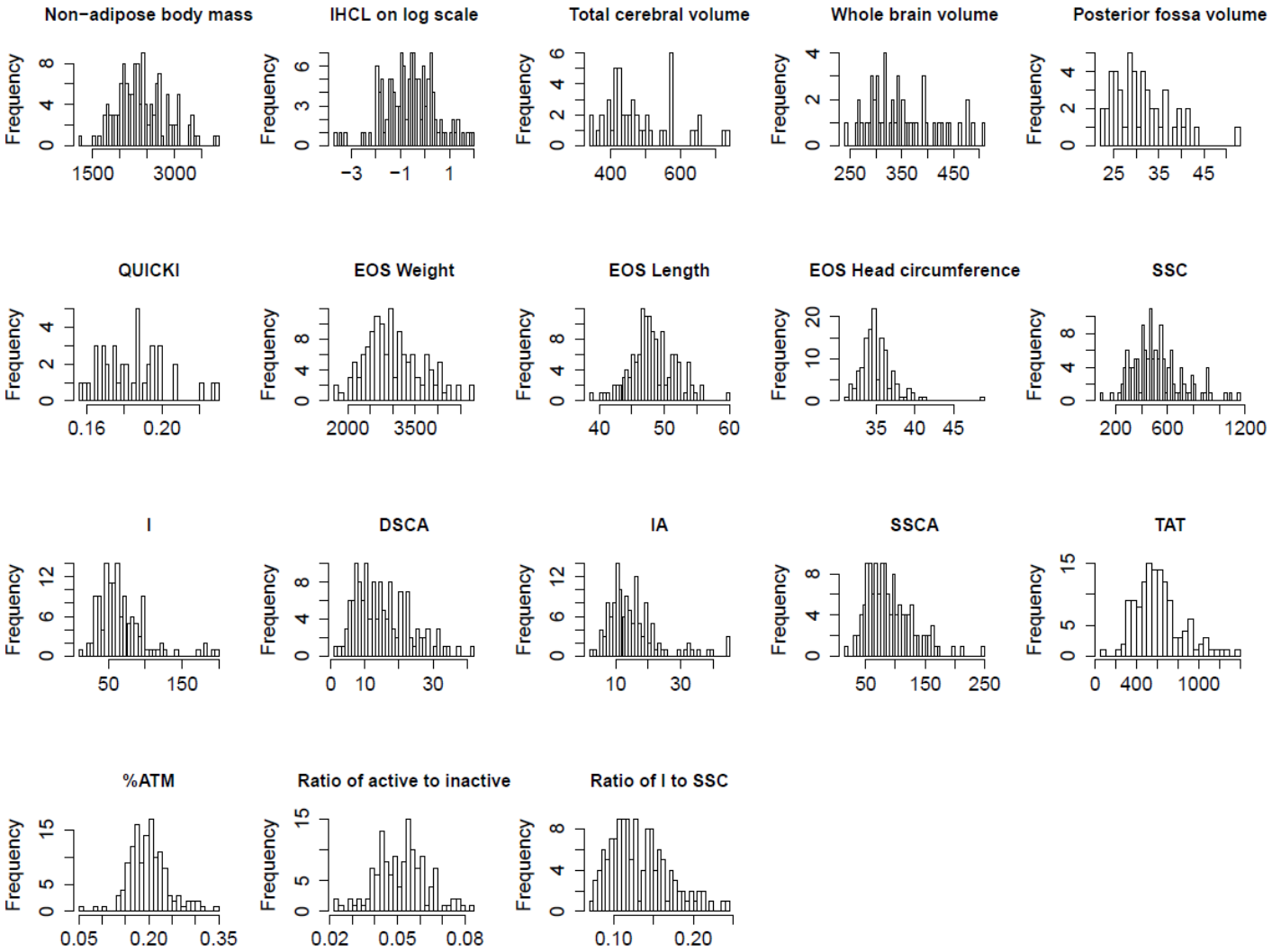
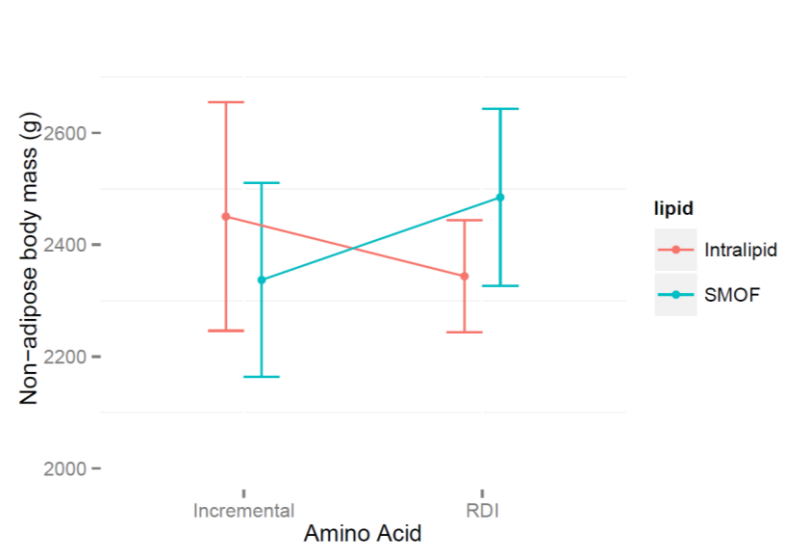


Figure 8. Means (95% CIs) of incremental amino acid and recommended daily intake of amino acid in two lipid subgroups for: a) non-adipose body mass and b) IHCL on log scale

a)



b)

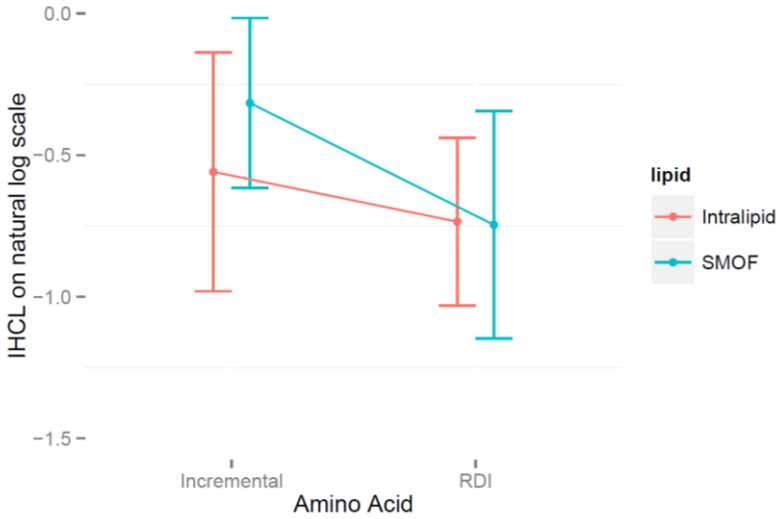


Table 20. Summary of the covariates for secondary analysis*

	Incremental amino acid & 20% Intralipid	Incremental amino acid & 20% SMOF lipid	Recommended Daily Intake of amino acid & 20% Intralipid	Recommended Daily Intake of amino acid & 20% SMOF lipid
	N=34	N=28	N=34	N=37
Proportion of level 1 care	0.11(0.07-0.21)	0.11 (0.06-0.35)	0.12 (0.08-0.31)	0.16 (0.07-0.32)
Proportion of level 2 care	0.42 (0.21-0.59)	0.30 (0.20-0.46)	0.29 (0.20-0.44)	0.31 (0.21-0.42)
Proportion of MEBM in all milk intake	0.80 (0.44-1.00) [n=33]	0.95 (0.28-1.00) [n=27]	0.65 (0.10-0.99) [n=33]	0.70 (0.26-1.00) [n=37]
Trial-PN phase protein intake (g)	31.5 (24.2-46.2)	34.4 (27.8-44.5)	42.8 (36.1-54.1)	48.5 (36.2-63.9)
Trial-PN phase carbohydrate intake (g)	124.6 (98.5-178.7)	139.6 (110.8-173.2)	129.9 (109.6-158.9)	148.2 (111.8-182.6)
Trial-PN phase fat intake (g)	52.8 (35.5-68.6)	51.6 (42.7-68.6)	51.8 (38.1-59.5)	53.1 (38.2-70.2)
Post-trial-PN phase protein intake (g)	213.0 (168.7-449.4)	213.4 (154.0-302.2)	234.5 (155.3-335.4)	257.4 (187.3-365.2)
Post-trial-PN phase carbohydrate intake (g)	969.0 (699.6-1786.1)	867.7 (643.5-1196.1)	1042.9 (644.8-1390.7)	1080.4 (696.3-1531.5)
Post-trial-PN phase fat intake (g)	479.8 (375.0-942.3)	434.8 (329.5-584.4)	515.9 (346.9-613.8)	550.5 (374.9-779.6)

* *Data presented are median (IQR) for continuous variables*

Table 21. Reporting the factorial trial results for secondary analysis in all infants completing MRI assessment

	Adjusted e mean difference (Immediate Recommended Daily Intake amino acid – Incremental amino acid)*	Adjusted mean difference(20% SMOF lipid – 20% Intralipid*	Interaction (P value)	Adjusted mean difference (Immediate Recommended Daily Intake amino acid – Incremental amino acid)†	Adjusted mean difference(20% SMOF lipid – 20% Intralipid) †	Interaction (P value)
Non-adipose body mass (g)	1 (-108, 111) p=0.98	-41 (-150, 68) p=0.46	216 (0, 432) p=0.05	-44 (-226, 139) [n=130] p=0.64	-14 (-114, 86) [n=130] p=0.78	184 (-22, 390) p=0.08
IHCL‡	0.7 (0.5, 1.1) [n=132] p=0.11	1.1 (0.8, 1.6) [n=132] p=0.58	0.8 (0.4, 1.7) [n=132] p=0.53	0.81 (0.37, 1.80) [n=129] p=0.61	0.89 (0.61, 1.31) [n=129] p=0.57	0.86 (0.39, 1.92) [n=129] p=0.71

* *Adjusted for: age at scan, gender, gestational age, birth weight and centre*

† *Adjusted for: age at scan, gender, gestational age, birth weight z-score, centre, level of care, nutritional intake*

‡ *Log transformation was used in the regression model, the results transformed back from the log*

Figure 9. Time trend for babies' weight across four groups

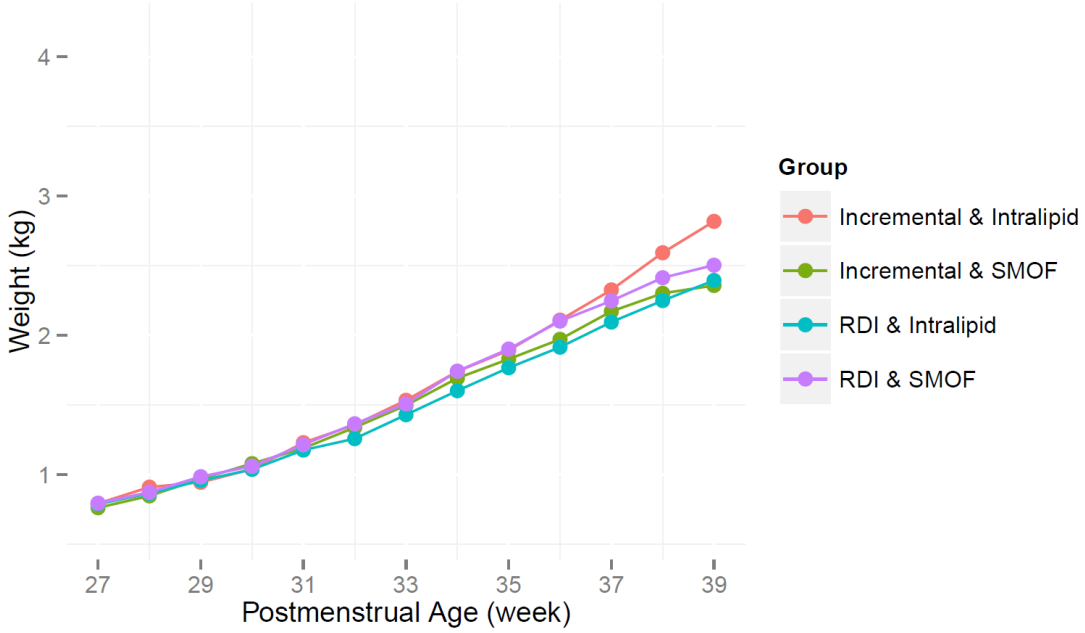


Figure 10. Daily protein intake from all sources (g/kg) in the first 2 weeks across four groups

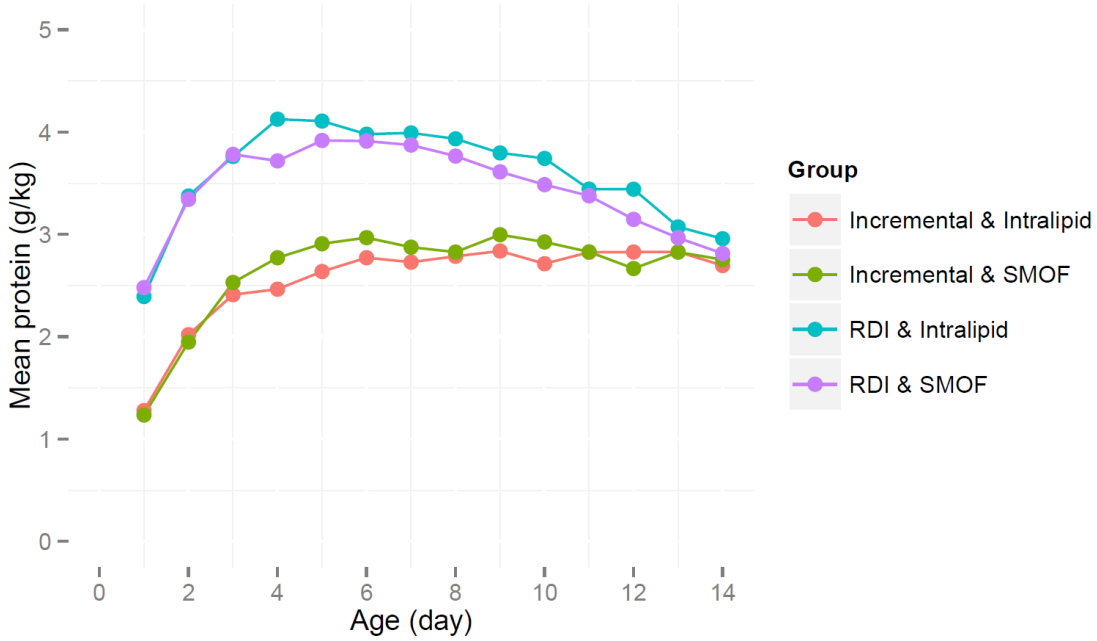


Figure 11. Daily carbohydrate intake from all sources (g/kg) in the first 2 weeks across four groups

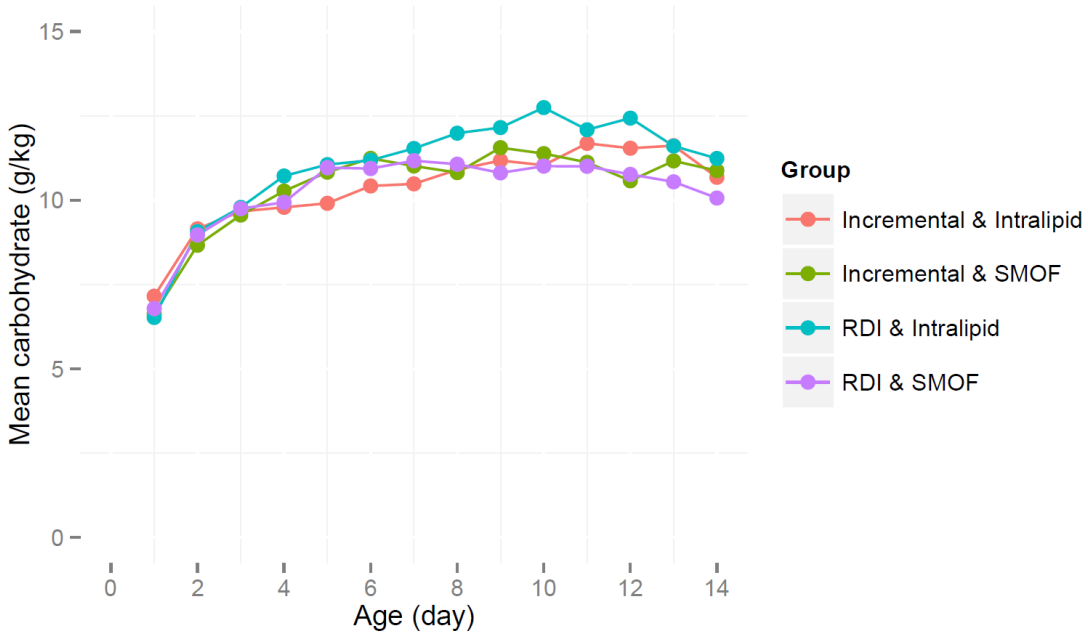


Figure 12. Daily fat intake from all sources (g/kg) in the first 2 weeks across four groups

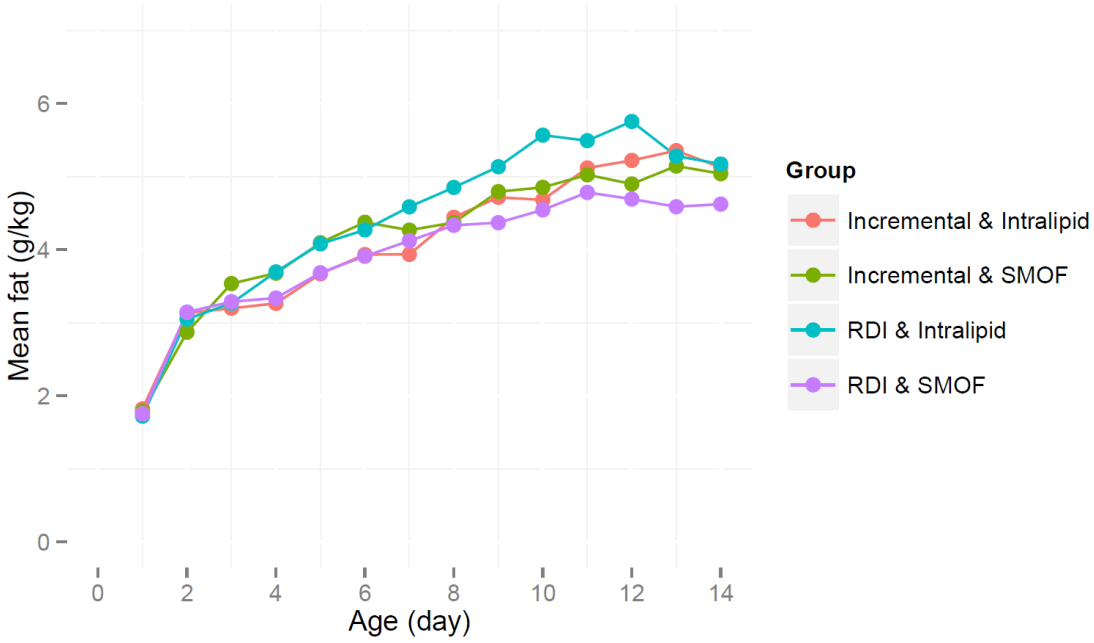


Figure 13. Daily protein intake from all sources (g/kg) after first 2 weeks across four groups

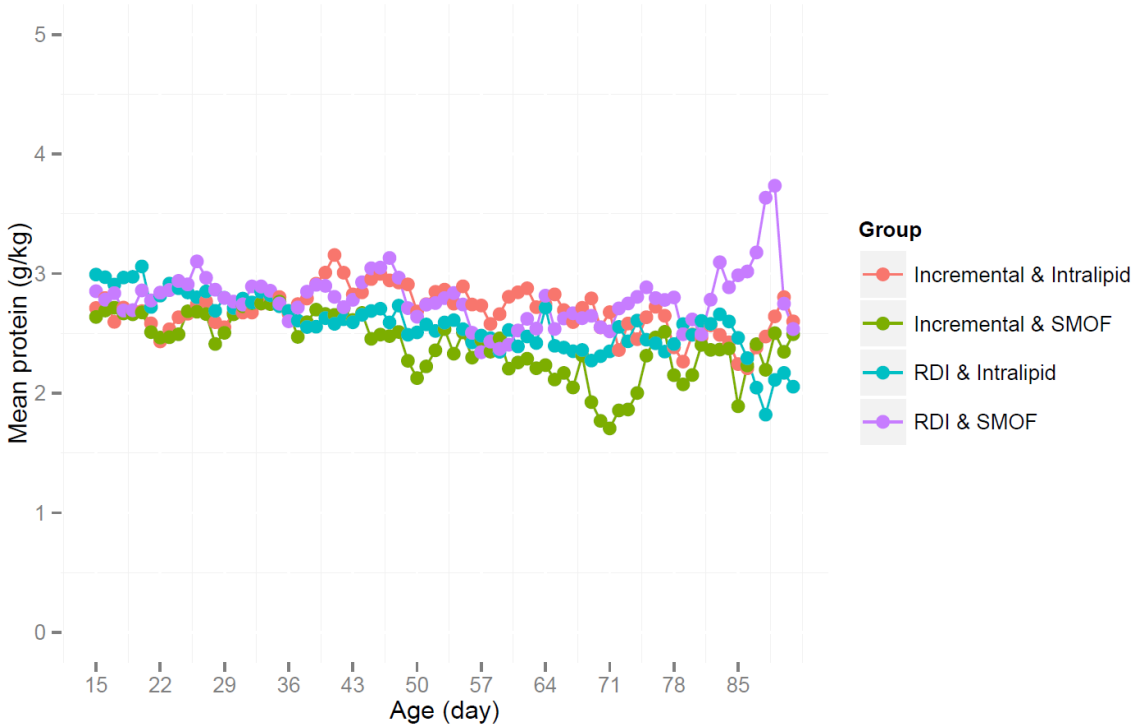


Figure 14. Daily carbohydrate intake from all sources (g/kg) after first 2 weeks across four groups

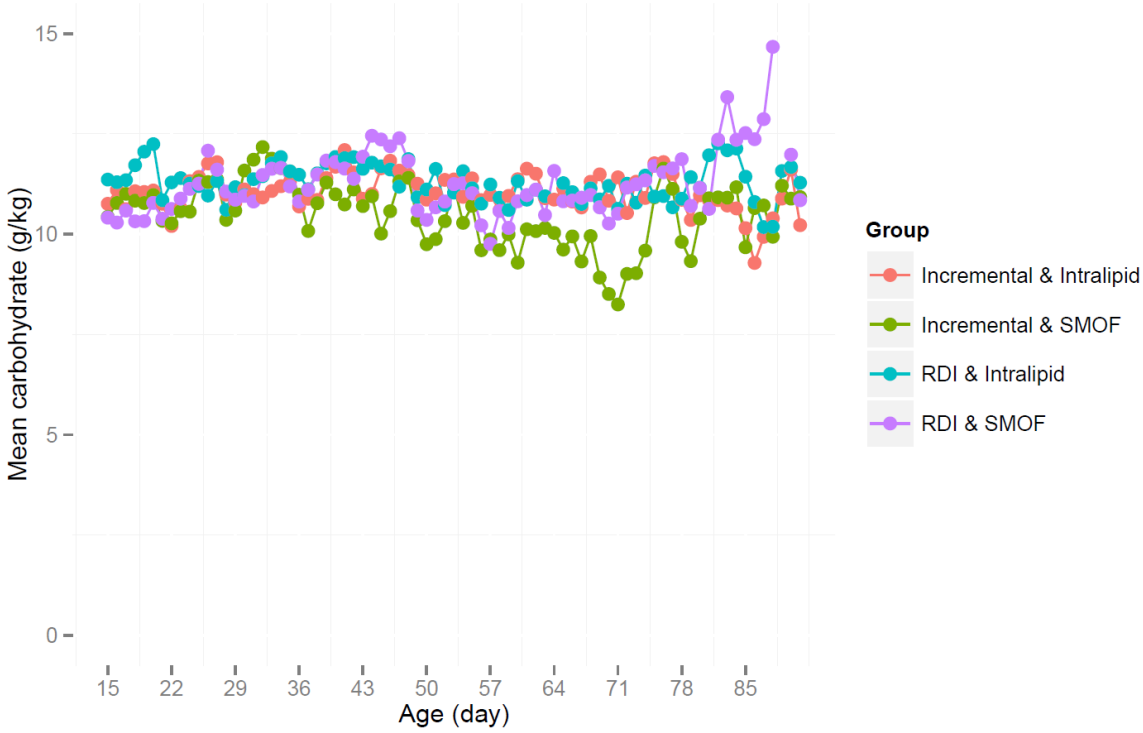
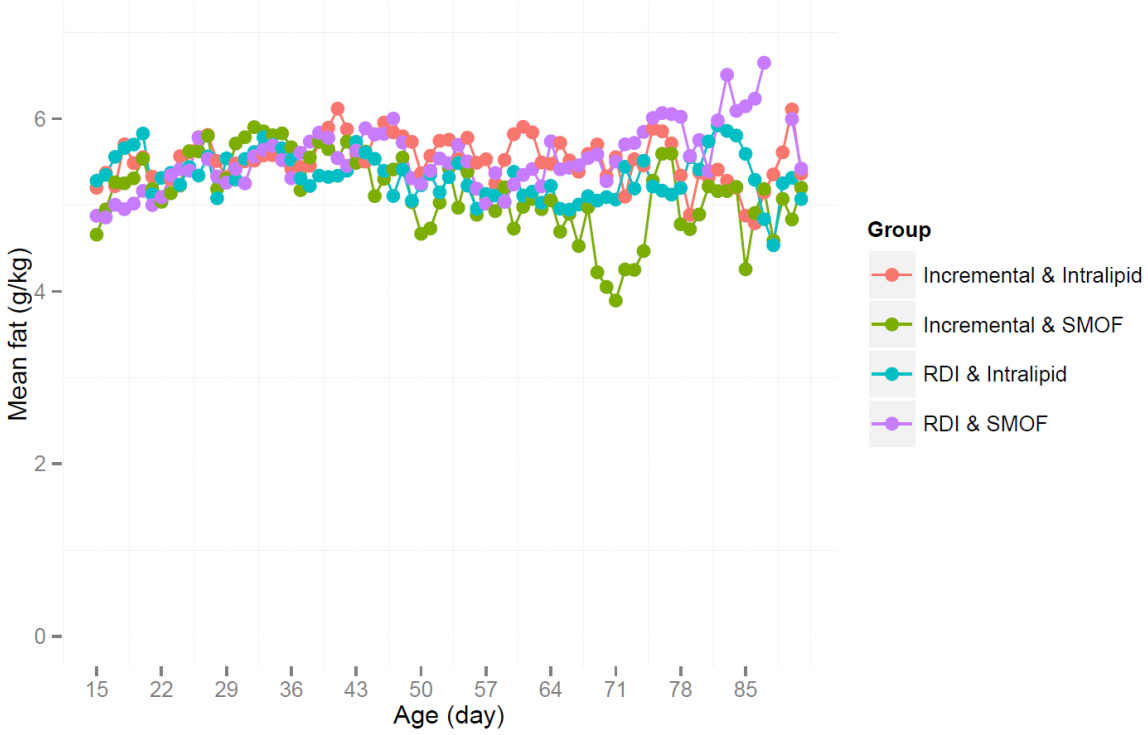


Figure 15. Daily fat intake from all sources (g/kg) after first 2 weeks across four groups



CHAPTER 5: DISCUSSION

Amino acid intervention

This is the first randomised controlled trial of the impact of amino acids intake in PN on body composition in extremely preterm infants. Despite several guidelines and reviews recommending that extremely preterm infants be given RDI of protein and calling for more aggressive nutritional management especially in the early postnatal period we have shown that this does not have an impact on lean body mass at term age equivalent. Similarly, the use of 20% SMOFLipid as the primary lipid composition resulted in similar IHCL levels as those infants who received 20% Intralipid.

The strengths of this study are that there was prospective collection of detailed data of nutritional intake from birth to discharge and the proportion of missing nutritional data was less than 0.1% for data on trial PN and < 5% for non-trial PN. The cohort, for this reason, lends itself to the long- term study of early nutritional intervention on outcomes such as neurodevelopment and later metabolic health. As evident from Table 9 and Table 10, the trial interventions were delivered according to protocol both in the commencement of PN within 24 hours (barring a few protocol violations) and in the subsequent immediate postnatal period. By a reduction in the concentration of glucose, it was possible to commence PN without the need for central access, which can be a rate- limiting factor in the early commencement of PN. Additionally, we were able demonstrate adherence to the use of a standardised regimen with a standardised pre-specified approach to the management of electrolyte disturbances. Previous non- randomised studies comparing standardised versus individualised PN have been inconclusive on the effect of these regimens on delivery of required amounts of nutrition. We have demonstrated that using a standardised PN regimen in the context of a randomised controlled trial is feasible. This is an important outcome as increasingly there is recognition that current practices in relation to the prescription, preparation and use of PN pose a potential clinical risk to patients. There are several standard bags and regimens commercially available on the market. However none of these regimens has been subjected to the rigour of a large randomised controlled trial with clinically meaningful outcomes and the concurrent

collection of a host of safety data. Data collection for this study included daily electrolyte and biochemical data while infants were on trial PN and weekly thereafter.

The study was carried out in four neonatal units in London and the South East of England. Two of the units were tertiary in nature while the remaining two were level two units that cared for infants of greater than 27 weeks of completed gestation. All units serve a varied population both in terms of ethnic and socio-economic backgrounds. Due to the lack of MR facilities for research use on- site, the original trial protocol dictated that infants had to have been discharged from hospital before the measurement of the primary outcome measure as the MR facility was located at a site separate from the location of the four hospitals and it would have been unethical to transfer a baby for a MR scan purely for the purpose of research if the baby was not fit to be discharged. This potentially could have resulted in a bias with the sickest babies being excluded from the measurement of primary outcome. However, early on in the trial the hospital where the majority of infants were recruited was able to scan infants who were still in-patient resulting in 10 infants who were otherwise not fit for discharge to be safely scanned on-site. This allowed a total of 10 infants who would have otherwise been excluded from primary outcome measure analysis to be included. There were a total of 4 babies who were excluded from analysis of primary outcome measure because they were still in-patient in hospital during the window of measurement of the primary outcome measure. There was also concern during the trial design stage that the most immature infants with the highest mortality may be under-represented in the final results. The Trial Steering Committee therefore considered ceasing recruitment to the strata of infants in the higher gestational age category if this was found to be an issue in the interim analysis. However this was not found to be the case and hence the results are generalisable to not just the sicker infants but the most immature as well. In the group of infants between 31 – 33 weeks, or the very growth restricted more mature infant where the use and justification of PN remains uncertain definite recommendations cannot be made from the results of this study.

There has been previous concern about the use of aggressive nutrition in a study comparing standard intake to ‘aggressive’ nutrition where the intervention included higher protein and energy intake .(54). The authors terminated the study early as there

was an increased rate of sepsis in the intervention group, and an association between low serum phosphate levels in the intervention group (despite increased phosphate delivery) and sepsis. In this study both groups received similar intake of electrolytes and there was no significant difference in the incidence of abnormalities in electrolytes.

Previous studies have suggested that the provision of increased early amino acids in PN is safe and not associated with an increased incidence of metabolic acidosis or elevation in blood urea nitrogen.(55, 56). We showed a significantly higher incidence of elevated blood urea concentrations in the groups receiving RDI of amino acids. This is also in keeping with the studies of Vlaardingerbroek et al (33)(57) although there was no associated increased incidence of metabolic acidosis. The significance of elevated urea in the early postnatal period in preterm infants is unclear. It may reflect increased amino acid oxidation but is also dependent on renal function and hydration status. The long term outcomes of providing increased intake of amino acids from birth require evaluation before this approach can be recommended in practice.

Several studies have shown growth failure in preterm infants in the postnatal period and leading in to adulthood (2, 58). There are calls for a more aggressive approach to early postnatal nutrition to prevent this growth failure (40, 59). Various published guidelines recommend the early introduction of amino acids with recommended intakes of up to 4 g /kg /day (60). However, these recommendations are based on limited evidence and there is no long-term data to support the safety of such an approach. A recent paper with a similar intervention of amino acids demonstrated that the early introduction of parenteral amino acids given in conjunction with lipids improved nitrogen balance. However higher intake of amino acids from day one did not further improve the nitrogen balance but led to increased amino acid oxidation.(33). While there is concern that under-nutrition is associated with adverse neuro-developmental outcomes there is also some suggestion that ‘over-nutrition’ may be detrimental to neuro-development (61). There is animal data reporting of an association between parenteral nutrition and adverse neurodevelopment when compared to enteral nutrition (62). Interestingly in that study the pigs fed on the enteral diet showed a slowing of growth before recovery of growth rate to match the PN fed pigs. The PN fed pigs showed a positive growth trajectory in the immediate

postnatal period, which excludes poor postnatal growth as being the cause of adverse neurodevelopment. We have shown that commencing amino acids within the first 24 hours and increasing the quantity to a maximum of 2.7 g /kg/ day when accompanied by the early introduction of enteral feeds results in an increase in lean body mass compared to historical controls and no significant difference between the intervention and controls. Our sample size calculation was based on our previous work (63). Practice in neonatal parenteral nutrition has changed with the emphasis on commencing PN earlier. Data from the UK National Data Analysis Unit (NDAU) show that year on year more infants born at less than 30 weeks commence PN within the first 48 hours after birth but at the present time up to a fifth to a quarter of babies have not commenced PN by day 3 (35). In the incremental group the mean protein intake from parenteral and enteral intake by day 3 was 3.4 g /kg /day. The difference in mean protein intake between the incremental and RDI groups was only significant in the first two weeks when the infants were establishing enteral nutrition. It is of note that the confidence interval for the mean difference in lean mass between the groups excludes the deviation of pre-specified difference in lean mass upon which the sample size calculation was based. The trial was therefore adequately powered to detect any clinically important differences between the groups.

Our own work and that of others have shown that preterm infants have a significantly reduced lean body mass at term age equivalent compared to healthy term infants. A systematic review, including our work, has shown that the magnitude of mean difference between preterm and term infants is about 460g (64). The median lean body mass across the four groups ranged from 2337 to 2485g. These values of lean mass are higher than the mean (SD) of our previous cohort of preterm infants of 2.1(0.4) kg on which the sample size calculation was based and closer to the mean (SD) of our term cohort of 2.6 (0.21) kg and comparable to the mean lean mass seen in a more recent cohort of preterm infants of 2490g (95% CI 2450-2540) published after the commencement of this study (11). As is evident from the NDAU data, increasingly babies are receiving PN earlier. Babies randomised to the standard arm in this trial commenced PN earlier than is routine practice and hence were not exposed to deficits that may arise from delaying PN. Despite the difference in early protein intake in the first week this did not result in differences in lean body mass at term age equivalent. This suggests that provided PN is initiated early and established according

to a standardised regimen, irrespective of whether this is incremental or RDI of amino acids, it is possible to achieve the lean body mass nearer the healthy term infant at term age equivalent when accompanied by early introduction and advancement of milk feeds.

The finding of a statistically significant lower head circumference in the group that received higher amino acid intake at term age (but not at baseline) is at odds with the SCAMP study (65). In that study preterm infants who received higher amounts of protein and energy had greater increase in head circumference from birth to 28 days with the difference in means (95% confidence interval) of 5 mm (2 – 8). The differences persisted to 36 weeks. This contradictory finding is likely to be a chance finding given the inter- and intra- observer variability that the measurement of head circumference is prone to.

One of the weaknesses of this study is that a significant proportion of babies did not have the appropriate MR images of the brain required to measure volumes. The brain volumes were derived from T2- weighted images. This sequence followed the longer T1- weighted sequence in the scanning protocol and babies often woke up at the end of the T1-weighted scan. This was a missed opportunity to test the hypothesis that amino acid intake and SMOFLipid influences brain growth and volume by using a direct measure of brain growth instead of previously used surrogate measure of head circumference .(65). However, in the third of infants for whom there were images of sufficient quality to analyse, there were no differences seen between groups in relation to either intervention. If early nutritional intervention at a period of rapid brain development has long- term impact on neuro-development then it is plausible that a difference in total and regional brain volumes persists beyond the neonatal period. Follow up of this cohort in which detailed nutritional intake has been captured offers the unique opportunity of studying the long- term impact of early nutrition on brain development as well as neuro-development. Establishing the long- term safety of the introduction of higher amino acid intake is of particular importance given the calls for early aggressive nutrition without the accompanying evidence of the lack of harm, both in the short and long term. There are several examples of interventions in preterm infants that while in the short term showed benefit, in the long term or when studied in trials with adequate power have shown adverse long- term impact.

SMOFLipid intervention

This is the first randomised controlled trial of SMOFLipid versus Intralipid in preterm infants to study the impact of lipid composition on intrahepatocellular lipid content. Preterm infants are known to have elevated IHCL compared to term infants and this is correlated with early lipid intake (66). IHCL measured with MRS in adults has been shown to have good diagnostic accuracy and compares favourably with the gold standard of liver biopsy for the quantitative measurement of hepatic steatosis .(67). SMOFLipid has been shown to be liver protective in the context of intestinal failure and parenteral nutrition in children and adults. Increasingly the use of SMOFLipid has been adopted for use in neonates with liver impairment. However to date there have been no studies showing benefit for its use. Previous studies of SMOFLipid in preterm infants published to date have focused mainly on lipid profiles, one small study on the incidence of Retinopathy of Prematurity and a further one the impact on growth outcomes (68-72). Unlike the paper by Vlaardingerbroek, we did not show any difference in growth outcomes in either weight or head circumference in this study between the group receiving SMOFLipid and that receiving Intralipid. Although this study was not powered to detect a significant difference in rates of sepsis there was a higher rate of sepsis associated with SAE reports in the SMOF group (15.5% vs 3.6%) although this finding could be due to chance. A systematic review, comparing soybean versus non-soybean lipid preparations showed a trend towards lower incidence of sepsis, which did not reach statistical significance, in the non-soybean lipid preparation. 20% SMOFLipid in this study did not result in a reduction in IHCL in preterm infants at term when used as a primary lipid composition. While there were no differences between the groups, the quantity of IHCL in the cohort of babies was similar to that in our previously published work comparing preterm infants with term infants.(18, 73).

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

Provided PN is introduced within the first 24 hours after birth with the early introduction and advancement of enteral nutrition, there is no difference between groups receiving incremental amino acids and those receiving RDI of amino acids in the amount of lean body mass between extremely preterm infants at term age equivalent as measured with MR scan after correcting for age at scan, gender, gestational age, birth weight and centre of birth. Extremely preterm infants at term age equivalent can achieve the body composition nearer that of healthy term infants with the early provision of PN according to a standardised regimen.

There was no impact of SMOFLipid on the amount of IHCL at term age equivalent as measured by MRS.

Before either of the interventions studied in this trial can be recommended as routine practice, long term follow up of brain and neuro-development as well as long term metabolic health is essential. The results do not support the calls for more aggressive nutrition in the extremely preterm infant nor the routine use of SMOFLipid.

This trial has demonstrated that the use of standard PN regimens is feasible, acceptable to clinicians, can deliver desired nutritional intake without manipulation and is safe. We recommend that standard regimens that have been tested in the context of a RCT be adopted in routine clinical practice.

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Participating sites:

- Chelsea and Westminster Hospital: Dr Sabita Uthaya, Izabela Andrzejewska, Sara Abdula, Laura Read, Elaine Smith, Kathryn McCormick
- Medway Maritime Hospital: Dr Aung Soe, Dr Helen McElroy, Abimbola Ojo, Helen Harizaj, Parool Darbar
- Northwick Park Hospital: Dr Richard Nicholl, Rosemond Owoo, Cecilia Lam, Matilda Lang, Theo Emmanuel
- West Middlesex Hospital: Dr Nour Elhadi, Dr Hashir Ariff, Siew Koay, Mylene Erese, Isabel Munoz

Parents and babies participating in the trial

Prof Mary Rutherford: Professor of Perinatal Imaging, Centre for the Developing Brain, King's College London

Chris Gale (scanning)

Imperial Clinical Trials Unit (ICTU): Juan Gonzalez-Maffe, Alexina Mason
Clinical Trials and Evaluation Unit at Royal Brompton and Harefield NHS Foundation Trust

Sealed Envelope; provision of randomisation list and Interactive Voice Response system (IVRS)

Trial Steering Committee members: Richard Cooke, Lorraine Dob, Paul Clarke, Robert Hume
Data Monitoring and Ethics Committee members: Peter Brocklehurst, Tim Cole, Tony Nunn, Helen Mactier

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Caroline Dore: design of study, analysis and interpretation of data, drafting/revising report, approval of report
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Ash Ederies: acquisition, analysis of data, drafting report, approval of report
Monica Yanez-Lopez: analysis of data, revising report, approval of report
Neena Modi: conception and design of study, analysis and interpretation of data, drafting/revising report, approval of report

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APPENDIX 1. TRIAL PROTOCOL VERSION 4.0 DATED 16/10/2012

APPENDIX 2. ANNOTATED STUDYBOOK (DATA COLLECTED ON TRIAL DATABASE)

APPENDIX 3. PARENT INFORMATION SHEET, MR INFORMATION SHEET AND CONSENT FORM

APPENDIX 4. STATISTICAL ANALYSIS PLAN

APPENDIX 5. FORMULAE FOR DERIVED VARIABLES (NUTRITIONAL INTAKE, MR OUTCOMES, QUICKI INDEX)