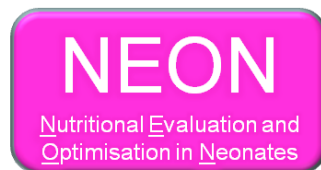


**Amino acid regimen and intravenous lipid composition in preterm  
parenteral nutrition: a randomised controlled trial of Nutritional Evaluation  
and Optimisation in Neonates**

**The NEON study**



Protocol version 3.0

Dated: 28<sup>th</sup> October 2010

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Sponsor: Imperial College

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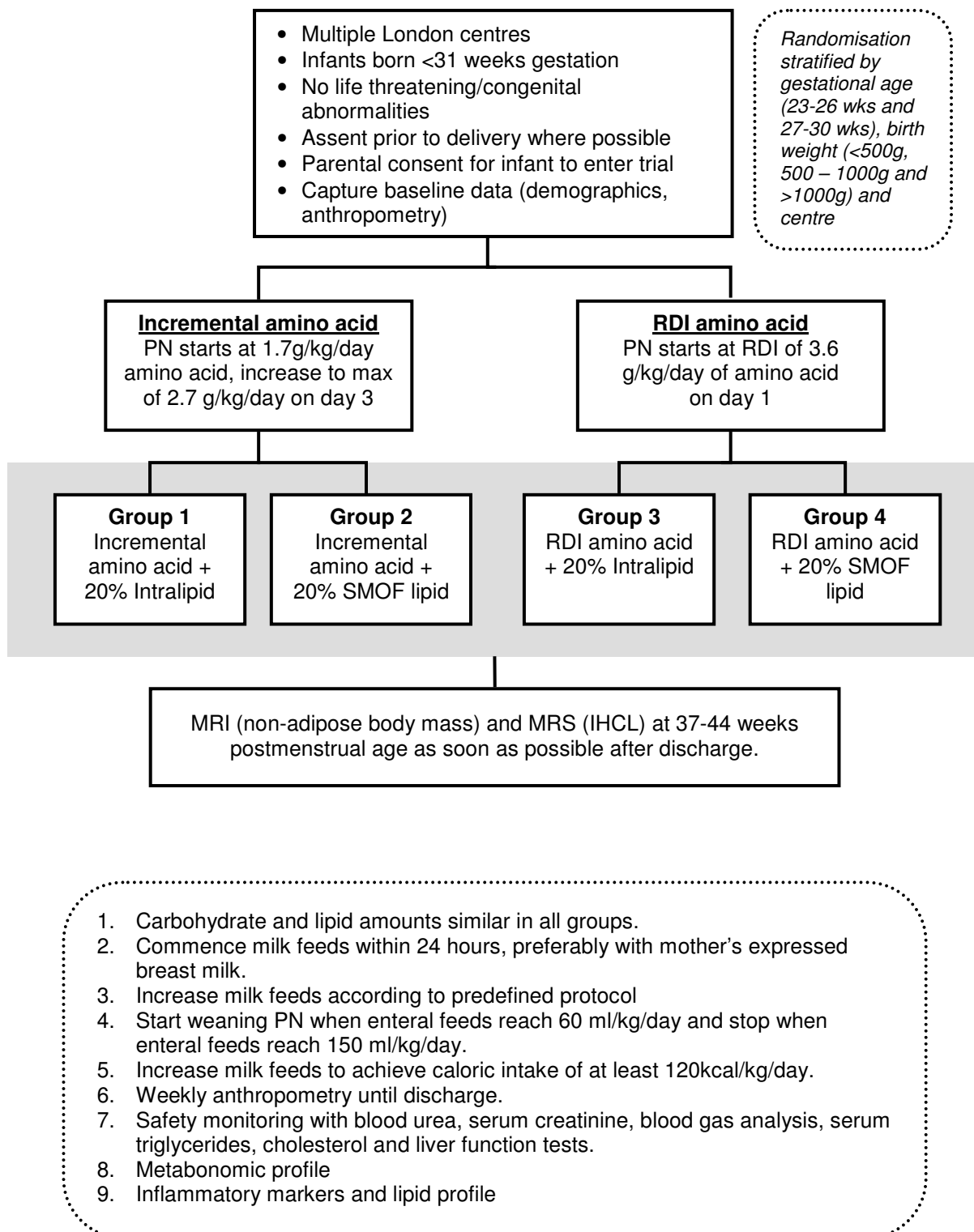
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## 1. PROTOCOL SUMMARY

<b>Title</b>	Amino acid regimen and intravenous lipid composition in preterm parenteral nutrition: a randomised controlled trial of Nutritional Evaluation and Optimisation in Neonates
<b>Acronym</b>	NEON
<b>Study centres</b>	Multiple UK centres
<b>Study Objective</b>	To confirm safety and demonstrate efficacy of immediate introduction of Recommended Daily Intake (RDI) of amino acids and SMOF lipid to decrease non-adipose (lean) body mass deficit and Intrahepatocellular Lipid (IHCL).
<b>Study Design</b>	Multicentre, double-blind 2x2 factorial randomised controlled trial
<b>Study Population</b>	<p><b>Inclusion criteria</b></p> <ul style="list-style-type: none"> <li>• Preterm infants born below 31 weeks gestation</li> <li>• Written informed consent from parents</li> </ul> <p><b>Exclusion criteria</b></p> <ul style="list-style-type: none"> <li>• Major congenital or life threatening abnormalities</li> <li>• Inability to randomise in time to allow administration of trial PN within 24 hours of birth</li> </ul>
<b>Interventions</b>	<ul style="list-style-type: none"> <li>• Recommended Daily Intake of intravenous amino acids</li> <li>• Incremental introduction of intravenous amino acids</li> <li>• 20% Intralipid</li> <li>• 20% SMOF lipid</li> </ul>
<b>Route of administration</b>	Intravenous
<b>Target number of patients</b>	160 patients (64 evaluable in each arm)
<b>Randomisation</b>	<ul style="list-style-type: none"> <li>• 2x2 factorial</li> <li>• Using minimisation</li> <li>• Stratifying factors are: gestational age (23-26 wks and 27-31 wks) birth weight ( &lt;500g 500-1000g, &gt;1000g) and centre</li> <li>• Telephone randomisation by Interactive Voice Recognition System</li> </ul>
<b>Primary outcomes</b>	<ul style="list-style-type: none"> <li>• Non-adipose body mass by whole body Magnetic Resonance Imaging</li> <li>• Intrahepatocellular lipid content (IHCL) by hepatic Magnetic Resonance Spectroscopy</li> </ul>
<b>Secondary outcomes</b>	<ul style="list-style-type: none"> <li>• Quantity and distribution of adipose tissue measured using whole body MRI</li> <li>• Anthropometry</li> <li>• Brain MRI (total and regional brain volumes)</li> <li>• Metabolic index of insulin sensitivity at term or near term age equivalent (QUICKI) using fasting serum glucose and insulin</li> <li>• Serum lipids</li> <li>• Metabonomic profile</li> <li>• Incidence of death</li> <li>• Number of infants with incomplete follow-up</li> <li>• Inflammatory markers and lipid profile</li> </ul>
<b>Duration of study</b>	<p>Recruitment period: 2.5 years</p> <p>Total trial duration: 3.5 years</p>
<b>Safety and efficacy assessments</b>	<ul style="list-style-type: none"> <li>• Routine assessments until discharge from Neonatal Intensive Care Unit</li> <li>• Safety tracking during hospitalisation</li> <li>• MRI and MRS scans after discharge at 37-44 weeks gestation</li> </ul>

## 2. STUDY FLOW CHART



### 3. ABBREVIATIONS

AE	Adverse Event
ALT	Alanine Aminotransferase
CTEU	Clinical Trials and Evaluation Unit
DMEC	Data Monitoring and Ethics Committee
eCRF	Electronic Case Record Form
HOMA	Homeostasis Model Assessment
IHCL	Intrahepatocellular lipid
IMP	Investigational Medicinal Product
IVRS	Interactive Voice Recognition System
LFT	Liver Function Tests
MR	Magnetic Resonance Scanning
MRS	Magnetic Resonance Spectroscopy
NICU	Neonatal Intensive Care Unit
PN	Parenteral Nutrition
PUFA	Polyunsaturated Fatty Acid
QP	Qualified Person
QUICKI	Quantitative insulin-sensitivity check index
RCT	Randomised Controlled Trial
REC	Research Ethics Committee
RDI	Recommended Daily Intake
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SD	Standard Deviation
SMOF	Soybean oil, Medium-chain triglycerides, Olive oil and Fish oil
SUSAR	Suspected Unexpected Serious Adverse Reaction
TSC	Trial Steering Committee
U+Es	Serum Urea and Electrolytes

## **4. BACKGROUND**

### **4.1 Preterm infants**

Extremely preterm infants, born below 31 weeks gestation, account for 1-1.5% of deliveries in the UK. Of around 60,000 premature 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).

### **4.2 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 is not 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.

There is good evidence that there are critical periods in development where nutrition has long term effects on later health. Embleton has shown that by end of the first week cumulative energy and protein deficits in infants < 30 weeks are 400 kCal / kg and 14 g/kg (9). Preterm formulas and fortified maternal milk are able to meet RDI of macronutrients but deficits accumulated in the period after birth combined with factors (such as sepsis) that increase requirements result in progressive deficit that are not made up or increase later catch-up growth.

Preterm infants have insulin resistance at pre-pubertal age compared to term born infants (10). As adults, compared to term born adults, they have higher blood pressure (11, 12), glucose intolerance (13), insulin resistance and dyslipidaemia (14). 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 all the preterm infants was characterised by 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 (15). Our group has 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 (16-18). 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 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 for the long term sequelae of prematurity. Mean health and societal costs for preterm children at 6 years of age exceed that of a child born at term by approximately three fold (19). The earliest extremely preterm survivors are now in their thirties and year on year, an ever increasing proportion is surviving in to adulthood. Thus, this problem is set to increase.

### **4.3 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 (20, 21) 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:

$$E \text{ intake} = E \text{ excreted} + E \text{ stored} + E \text{ expended} \text{ (E=energy)}$$

*\*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 (22-24). 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 (unpublished).

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) [946.7 (375.2) vs 1238 (407),  $P < 0.05$ ] but a significantly higher lean body mass accrual (approximately 4% higher LBM as % of body weight) than a low-protein-intake (<3g/kg/day) group (n = 22) (25).

Several recent reviews have concluded that current nutritional practices contribute to long term impairment and recommend early introduction of RDI of macronutrients (26, 27). However, the evidence for this is based on tolerability and growth outcomes, and not on body composition. To our best knowledge the impact on body composition, of providing RDI of macronutrients from immediately after birth has not been studied in extremely preterm infants.

#### **4.4 Parenteral Nutrition**

The intervention to be studied is 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 when the clinical condition precludes enteral feeding. Each day of PN per infant costs the NHS £80-100. A typical tertiary neonatal unit spends up to £150,000 per year on PN. There are currently various preparations of PN used routinely. They vary in composition and use. 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 recent scoping exercise that was commissioned by the Department of Health because of serious concern of clinical risk to patients. 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 and guidelines for use. One hundred and sixteen hospitals reported providing PN to neonates and completed the survey relating to neonates. Dr Uthaya was a member of the clinical group that developed and analysed the survey and prepared the report. This was submitted in November 2008 and calls 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.

Current widespread practice is to commence macronutrients in PN at a dose below that of the daily recommended 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 2 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 used in infants on long term PN (28).

There is a need for studies to investigate the efficacy of these newer lipid solutions in reducing liver impairment.

#### **4.5 Previous studies of PN**

Several recent reviews have concluded that current nutritional practices contribute to growth failure and recommend early introduction of RDI of macronutrients in parenteral nutrition (26, 27). However, the quality of the evidence on which this is based is grade B (RCTs 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 (17). Tan et al, studied the effect of 'hyperalimentation' on head growth (29, 30). 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 develop. The study was also underpowered to detect a significant effect on primary outcome.

#### **4.6 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 (32) and catheter related complications. However, these risks are difficult to avoid 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 (33). 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 (31). However, there was a small significant increase in serum bilirubin, without clinical implications. Other studies in preterm infants using this approach have not shown an increase incidence of this problem (31, 34, 35).

The currently used lipid solution, 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 (36). 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) (36). SMOF lipid 20% is well tolerated in infants without changing lipid peroxidation parameters (37) and beneficial effects on liver function and serum triglyceride concentrations have been described (28).

#### **4.7 Need for a 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.

### **5. HYPOTHESIS**

Introduction of RDI of amino acids will lead to an increase in non-adipose (lean) body mass; administration of SMOF lipid will reduce IHCL in preterm babies at term age equivalent.

## **6. TRIAL OBJECTIVES**

### **6.1 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)

### **6.2 Lipid intervention**

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

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

## **7. TRIAL DESIGN**

This will be a multi-centre, randomised 2x2 factorial double blind controlled trial in multiple UK centres. Eligible preterm infants will be randomised by 12 hours of age 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 will be four 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

## **8. TRIAL ENDPOINTS**

### **8.1 Primary endpoints**

- For the amino acid intervention the primary outcome measure will be non-adipose (lean) body mass measured by whole body magnetic resonance imaging (MRI) at term age equivalent.
- For the lipid intervention the primary outcome will be hepatic magnetic resonance spectroscopy (MRS) to measure intrahepatocellular lipid content (IHCL) at term age equivalent.

## **8.2 Secondary endpoints**

1. Anthropometry (weight, length and head circumference) measured at term age equivalent.
2. Brain MRI (brain volumes, white matter apparent diffusion co-efficient values, cerebral vessel tortuosity) measured at term age equivalent.
3. Metabolic index of insulin resistance at term age equivalent (QUICKI), calculated using fasting serum glucose and insulin.
4. Ratio of internal to subcutaneous adipose tissue at term age equivalent.
5. Serum triglyceride and serum bilirubin levels.
6. Metabonomic profile
7. Incidence of death
8. Number of patients with incomplete follow-up
9. Inflammatory markers and lipid profile

## **9. PATIENT POPULATION**

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

### **9.1 Inclusion criteria**

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

### **9.2 Exclusion criteria**

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

### **9.3 Screening and enrolment**

All preterm infants fulfilling the eligibility criteria will be considered for the trial. Where possible parents will be approached about the study before delivery and assent sought e.g. mothers at risk of premature delivery. In all cases, written informed consent will be sought following delivery.

### **9.4 Informed consent**

“Informed consent” requires individual discussion with the infant’s parents about the nature of the research to be conducted in a language that is easy to comprehend. The parents will have the study verbally explained to them and also be given a written information sheet about the study. The parents will understand that the infant can be allocated to one of four groups and that the trial is comparing standard nutritional regimens. The parents will also understand that refusal to participate in the study will not affect the quality of subsequent medical care and if they do consent to participate they may withdraw at any point without affecting their infant’s care.

Before any trial-related procedures may be performed, informed consent must be obtained from the infant's parents by the investigator by means of a signed declaration. The investigator must record in the eCRF to confirm that informed consent was obtained and store the original of the signed declaration of consent in the patient's notes. A copy should be given to the infant's parents and a copy filed in the investigator file.

## **9.5 Randomisation**

Randomisation will be performed using an interactive voice recognition telephone randomisation system (IVRS). The IVRS will be provided by Sealed Envelope Ltd. The method for randomisation will be minimisation with 25% chance of simple random allocation. Stratifying factors will be: gestational age (23-26 completed weeks and 27-31 completed weeks of gestation), birth weight (<500g, 500-1000g, >1000 g) and centre.

## **9.6 Patient withdrawal**

### **9.6.1 Temporary discontinuation of IMP**

There should be no reason to discontinue trial PN before the infant achieves milk feeds of at least 150 ml /kg /day. In exceptional circumstances however, a temporary discontinuation of the IMP could occur at any point during the intervention period for e.g. in acute renal failure.

It is unlikely that there will be problems with compliance with the intervention. Previous studies have shown that the intervention does not result in acidosis or increased blood urea. It is likely that in some situations babies may develop significant metabolic complications unrelated to the study.

### **9.6.2 Permanent discontinuation of IMP**

Parents may permanently withdraw their infant from treatment with the IMP if they decide to do so, at any time and for any reason, or this may be the Investigator's decision. If there is considered to be a concern about the batch of IMP, recall procedures will be in place and measures will be taken to ensure the safety of all trial participants.

### **9.6.3 Withdrawal of infant from trial procedures and incomplete follow-up**

It is possible that parents may choose to withdraw their infant from trial procedures resulting in incomplete patient follow-up and failure to capture outcome data. Likewise, if an infant dies before discharge or remains on the neonatal unit past 44 weeks of postmenstrual age they will not have their MRI scan. In these cases as much data as possible will be collected.

Parents may choose to withdraw their infant from trial procedures and request that further data are not collected.

## 10. TRIAL INTERVENTIONS

### 10.1 Description

There will be two interventions, namely the amount of amino acids in PN and the type of lipid formulation. The intervention will commence within 24 hours of birth. Nutritional intake, both parenteral and enteral, will be guided by pre-specified protocols that will be provided in the Investigator's Manual.

The interventions will cease once the infant is established on milk feeds of 150 ml/kg/day for at least 24 hours. Should the infant need to be nil by mouth after this point, PN will be prescribed in accordance with local practice as determined by the supervising consultant.

**Table 1. Summary of interventions**

<b>Group 1 (incremental amino acid and 20% Intralipid)</b>		<b>Day 1</b>	<b>Day 2</b>	<b>Day 3 onwards</b>
Volume ( <i>excluding lipid volume</i> )	ml/kg/day	90	90	120
Protein	g/kg/day	1.5	1.9	2.4
<i>Amino acid equivalent</i>	<i>g/kg/day</i>	<i>1.7</i>	<i>2.1</i>	<i>2.7</i>
Carbohydrate (glucose)	g/kg/day	8.6	8.6	8.6
20% Intralipid	g/kg/day	2	3	3
<b>Group 2 (incremental amino acid and 20% SMOF lipid)</b>				
<b>Group 2 (incremental amino acid and 20% SMOF lipid)</b>		<b>Day 1</b>	<b>Day 2</b>	<b>Day 3 onwards</b>
Volume ( <i>excluding lipid volume</i> )	ml/kg/day	90	90	120
Protein	g/kg/day	1.5	1.9	2.4
<i>Amino acid equivalent</i>	<i>g/kg/day</i>	<i>1.7</i>	<i>2.1</i>	<i>2.7</i>
Carbohydrate (glucose)	g/kg/day	8.6	8.6	8.6
20% SMOF lipid	g/kg/day	2	3	3
<b>Group 3 (RDI of amino acid and 20% Intralipid)</b>				
<b>Group 3 (RDI of amino acid and 20% Intralipid)</b>		<b>Day 1</b>	<b>Day 2</b>	<b>Day 3 onwards</b>
Volume ( <i>excluding lipid volume</i> )	ml/kg/day	90	90	120
Protein	g/kg/day	3.2	3.2	3.2
<i>Amino acid equivalent</i>	<i>g/kg/day</i>	<i>3.6</i>	<i>3.6</i>	<i>3.6</i>
Carbohydrate (glucose)	g/kg/day	8.6	8.6	8.6
20% Intralipid	g/kg/day	2	3	3
<b>Group 4 (RDI of amino acid and 20% SMOF lipid)</b>				
<b>Group 4 (RDI of amino acid and 20% SMOF lipid)</b>		<b>Day 1</b>	<b>Day 2</b>	<b>Day 3 onwards</b>
Volume ( <i>excluding lipid volume</i> )	ml/kg/day	90	90	120
Protein	g/kg/day	3.2	3.2	3.2
<i>Amino acid equivalent</i>	<i>g/kg/day</i>	<i>3.6</i>	<i>3.6</i>	<i>3.6</i>
Carbohydrate (glucose)	g/kg/day	8.6	8.6	8.6
20% SMOF lipid	g/kg/day	2	3	3

## **10.2 Unblinding**

In the case of an adverse event the code can only be broken in exceptional circumstances, when knowledge of the allocation is essential for treating the patient. If possible the CTEU should be contacted before breaking the code.

Pharmacies at the Trial Sites will have access to lists containing the randomisation allocation for each patient. These should be stored securely and access limited to authorised personnel who will only access these if unblinding is deemed necessary. Trial procedures for unblinding, including authorisation from an investigator, will be followed in all cases and the date and reason for breaking the code will be documented. Unblinding will occur at the individual patient level only.

## **10.3 Non-trial PN**

It is possible that infants may receive non-trial PN after completion of trial PN if the investigator should decide this is necessary. Centres will be asked to record any use of non-trial PN including the type and volume administered.

## **10.4 Investigational Medicinal Products**

### **10.4.1 Manufacture**

Vaminolact, SMOF lipid and Intralipid which are the IMPs for this trial are components used in the preparation of parenteral nutrition solutions. All three are produced by Fresenius-Kabi. These will be purchased by an external commercial supplier that holds a manufacturing license to produce IMPs. The supplier will compound the parenteral nutrition using the IMPs and other non IMPs to specified formulae. They will be responsible for preparing, packaging and labelling the parenteral nutrition. The PN will be despatched to the pharmacies at participating centres as required throughout the trial.

### **10.4.2 Composition**

- The amino acid intervention (RDI or incremental amino acid) will be produced by the supplier as aqueous bags containing the IMP, Vaminolact® in the required amounts. Vaminolact® is a solution of amino acids for intravenous infusion.
- The lipid intervention will be produced by the supplier as lipid bags or syringes containing either Intralipid® or SMOFlipid® in the required amounts. Intralipid® and SMOFlipid® are lipid emulsions for intravenous infusion.

### **10.4.3 Labelling**

The IMP supply will be labelled in accordance with regulatory requirements and specifications and will be approved by the MHRA as part of the application for Clinical Trial Authorisation.

### **10.4.4 Storage and dispensing**

- The IMPs will be provided to the local pharmacies as unblinded material.

- The IMP will be stored in a secure area of the pharmacy, under the conditions described in the respective Summary of Product Characteristics.
- The Pharmacist will be responsible for performing blinding procedures prior to dispensing the IMP to the ward using the approved trial labelling.
- The IMP supply will be dispensed by the local pharmacy which will be responsible for maintaining a record of accountability.

## **11. TRIAL OBSERVATIONS, TESTS AND INVESTIGATIONS**

### **11.1 Electronic CRF (eCRF)**

Trial data will be captured on a web-based electronic case record form (eCRF). The eCRF will be designed in accordance with the requirements of the trial protocol and will comply with regulatory requirements. Access to the eCRF will be password-protected.

### **11.2 Timescale for trial evaluations**

#### **11.2.1 Daily evaluations**

The first daily evaluation should occur when the first bag of trial PN is changed and on the first day of post-natal life. Subsequent evaluations should occur 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 are calculated from date PN was initiated.

#### **11.2.2 Weekly evaluations**

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

#### **11.2.3 Monthly evaluation**

The first monthly evaluation should occur 30 days +/- 5 days from randomisation and each 30 days +/- 5 days thereafter until 37 weeks corrected age or discharge from NICU.

#### **11.2.4 '37 week' evaluation**

This evaluation should take place when the infant reaches 37 weeks corrected gestational age (+/- 1 week) or when the infant is discharged from NICU, whichever occurs sooner.

#### **11.2.5 End of study evaluation**

This should take place as soon as possible after the infant is discharged from NICU at 37-44 weeks corrected gestational age.

## 11.3 Schedule of investigations

**Table 2. Summary of tests and investigations**

Evaluation	Baseline	Daily	Weekly	Monthly	37 weeks corrected age	End of study (37-44 wks and discharge from NICU)
Informed consent	✓					
Eligibility	✓					
Randomisation	✓					
Weight	#	#*	#			✓
Length	#		#			✓
Head circumference	#		#			✓
Blood pressure	#		✓			✓
Nutritional intake	✓	✓				
<b>Safety</b>						
Blood glucose (highest and lowest in previous 24 hrs)		#*				
Worst base deficit on blood gas (in previous 24 hrs)		#*				
Serum bilirubin, LFTs, serum urea, creatinine and electrolytes		#*			#	
Serum lipid and cholesterol			#*			
Trace elements (zinc, copper, manganese, aluminium, selenium)				#*		
Adverse event tracking		✓	✓		✓	✓
<b>Efficacy</b>						
QUICKI					✓	
Whole body and brain MRI, MRS						✓
<b>Metabonomic profile</b>						
Blood spot	✓				✓	
Urine sample and stool sample			✓			
<b>Inflammatory markers and lipid profile</b>						
Blood sample	✓	✓ <sup>5</sup>				

### **Key**

- ✓ For research purposes
- # Routine care
- \* While on parenteral nutrition
- <sup>5</sup> Day 5 only

## 11.4 Data to be collected

The data to be collected at each follow-up time-point are summarised below.

#### **11.4.1 Randomisation**

- Date and time of birth
- Gender
- Confirmation of eligibility (full eligibility check)
- Date of parental consent
- Name of person taking consent
- Gestational age (in weeks and days)
- Recruiting hospital
- Study Drug Number allocated

#### **11.4.2 Baseline evaluation**

- Birth weight (kg)
- Birth length (cm)
- Head circumference (cm)
- Ethnicity (NHS Ethnicity)
- Mother's date of birth
- Mother's ethnicity
- Mother's height and weight
- Father's ethnicity
- Father's height and weight
- Mode of delivery
- Use of antenatal steroids
- Diastolic blood pressure
- Systolic blood pressure
- Date and time first PN bag administered
- Metabonomic profile measurement (blood spot)
- Inflammatory markers and lipid profile (blood sample)

#### **11.4.3 Daily evaluations**

*Performed daily until discharge from NICU*

- Record nutritional intake (type of milk feed, PN or formula as applicable)
- Record level of care (BAPM 2001 criteria)
- Record if infant is nil by mouth and reason for this
- Check for SAEs
- Record of withdrawal information if relevant

*Performed daily throughout duration of PN in addition to the assessments listed above. These should be recorded irrespective of whether the infant is on trial or non-trial PN*

- Record bag code and volume of PN during previous 24 hrs
- Lowest blood glucose during previous 24 hrs
- Highest blood glucose during previous 24 hrs
- Worst base deficit during previous 24 hrs on blood gas
- Liver function tests (Alanine Aminotransferase, conjugated bilirubin, total serum bilirubin)
- Serum urea and creatinine
- Electrolytes (Sodium, Potassium, Phosphate, Calcium)
- Weight

- Use of insulin
- Use of additional electrolytes

Day 5 only

- Inflammatory markers and lipid profile (blood sample)

**11.4.4 Weekly evaluations**

*Performed weekly until discharge from NICU*

- Weight (when not on PN)
- Length
- Head circumference
- Diastolic blood pressure
- Systolic blood pressure
- Urine and stool collection for metabonomic profile

*Performed weekly throughout duration of PN (irrespective of whether the infant is on trial or non-trial PN) in addition to the above*

- Serum triglycerides
- Serum cholesterol

*When not on PN*

- Liver function tests (Alanine aminotransferase, conjugated bilirubin, total serum bilirubin)

**11.4.5 Monthly evaluation**

*Performed monthly throughout duration of PN (irrespective of whether the infant is on trial or non-trial PN)*

- Trace elements: zinc, copper, manganese, aluminium, selenium

**11.4.6 '37 weeks' evaluation**

This visit will take place at 37 weeks corrected gestational age or prior to discharge from NICU, whichever occurs sooner

- Liver function tests (Alanine aminotransferase, total serum bilirubin, conjugated bilirubin)
- Serum urea
- Paired pre-feed serum insulin and blood glucose (QUICKI)
- Metabonomic profile blood spot

**11.4.7 End of Study evaluation**

*Post discharge visit (>37 wks and 1-2 wks post-discharge)*

- Weight
- Length
- Head circumference
- Whole body MRI
- Brain MRI
- Hepatic MRS
- Diastolic blood pressure
- Systolic blood pressure
- Check for SAEs

- Urine for metabonomics if not done prior to discharge

## **11.5 Clinical investigations**

### **11.5.1 Anthropometry**

Weight, length and head circumference measurements are routinely used to monitor infant growth. Weight will be 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 will be recorded on a weekly basis until discharge and at the end of study visit.

### **11.5.2 Blood pressure measurements**

Record systolic and diastolic blood pressure measured in the right upper limb using a non invasive blood pressure monitor and a cuff that covers at least two-thirds of the right upper limb and encompasses the entire arm in a restful state.

### **11.5.3 MRI and MRS**

There are many ways to measure body composition. Several methods rely on assumptions and while they can measure absolute quantity of a certain tissue, they cannot measure the distribution. Other methods involve the use of radiation or are invasive and thus not appropriate in this patient group. MRI and MRS are non invasive and validated against the respective gold standards of cadaver dissection and liver biopsy. MRI has the advantage of directly quantifying quantity and distribution of lean and adipose tissue mass. We have pioneered the use of this technique in this age group. The details of the methods have been described in our previous publications (16, 17).

Efficacy of early introduction of RDI of amino acid will be assessed by whole body MRI to measure lean mass and the quantity and distribution of adipose tissue. This will be done at term age equivalent. The infants will be scanned as soon as possible after discharge from NICU between 37- 44 weeks post menstrual age.

Head circumference is used as a surrogate for brain growth but correlates poorly with brain volume as measured by MRI. Images will be acquired with T1 and T2 weighted volumetric acquisitions, diffusion tensor imaging, magnetic resonance angiography. All images will be visually analysed for the presence of congenital or acquired lesions. An automatic segmentation programme will be used to quantify central grey matter, white matter and cortical volumes. Diffusion Tensor Imaging datasets will be analysed using a TBSS approach to compare fractional anisotropy values in developing white matter tracts. Distance factor to measure vessel tortuosity will be calculated. (38-41)

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

#### **11.5.4 QUICKI**

QUICKI is a marker of insulin resistance calculated from pre-feed serum insulin and blood glucose. 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 (42) and been used in neonates before (18, 43). Measurement of QUICKI will be carried out at term age and samples taken at the time of routine (pre-feed) blood tests.

#### **11.5.5 Metabonomics**

Metabolic “profiles” can now be measured using small volumes of biological tissues and fluids, such as dried blood spots, urine and stool; this is termed metabonomics. Analysis is carried out using high throughput nuclear magnetic resonance (NMR) spectroscopy, mass spectrometry or other appropriate spectroscopic and chromatographic techniques. NMR spectroscopy provides the opportunity to analyse a large number of metabolites simultaneously and therefore offers extensive scope to study metabolic responses in relation to normal development, disease, physiological variation, nutritional intake and medications (44).

Gut microorganisms can drastically modify nutrient bioavailability and metabolism. Li et al have developed a technique to study the association between gut microbiota and host metabolism by combining spectroscopy, microbial community DNA fingerprinting and multivariate statistical tools (45).

Blood samples (3-4 drops) will be taken onto filter paper; urine will be obtained from a urine bag, and stool from the nappy. Samples will be stored at  $-80^{\circ}\text{C}$  and analysed in batches. Urine and blood samples will be analysed by nuclear magnetic resonance spectroscopy (or other appropriate spectroscopy or chromatography techniques). Faecal water will be analysed by NMR spectroscopy and stool samples will be analysed by denaturing gradient gel electrophoresis.

#### **11.5.6 Inflammatory markers and lipid profile**

The mechanism of SMOF lipid is believed to be via altering the inflammatory pathways, hence reducing damage to the liver. The lipid profile and markers of inflammation in the two lipid groups will be studied during the trial for patients recruited at the lead site, Chelsea and Westminster hospital. This will involve taking 2 additional blood samples of a few drops (0.5 – 1ml) on the first and fifth day after birth, at the time of routine blood samples.

### **12. PHARMACOVIGILANCE**

Use of PN is unavoidable for preterm infants and all the regimens in this trial are used in current standard practice. Infants that are not recruited to the trial will receive PN as part of their routine care. There is no alternative to PN for this infant population and any risks associated with this are therefore unavoidable. As a clinical trial of an IMP, any adverse events are subject to reporting procedures in accordance with regulatory requirements.

Any baby on PN would normally have at least daily measurement of blood gas, serum urea and electrolytes, serum bilirubin and liver function tests as part of routine care. In addition, monitoring of blood sugar would take place at least 4-6 hourly, more frequently if the infant was receiving insulin, and blood gas measurement at least once a day. As part of routine care any baby on lipid infusion would have weekly measurement of triglycerides and cholesterol. This data will be collected as part of the trial data capture. The worst base deficit measured on blood gas analysis and the highest and lowest blood sugar will be captured daily for safety related to the amino acid intervention and the serum triglycerides and liver function tests, including serum bilirubin for the lipid arm of the study. Incidence of metabolic acidosis and hypertriglyceridaemia and hyperbilirubinaemia defined as values falling outside a pre-specified range will be compared between the groups.

Definitions and reporting procedures are described in this section.

The Sponsor (Imperial College) has delegated the responsibility of pharmacovigilance to the trial management centre, the Clinical Trials and Evaluation Unit (CTEU) of the Royal Brompton & Harefield NHS Foundation Trust.

The CTEU will be responsible for recording all serious adverse events reported by the Trial Sites, reporting all serious adverse events to Chief Investigator (or designated deputy) for review, and expedited reporting of suspected unexpected serious adverse reactions (SUSARs) in accordance with statutory regulations. The Chief Investigator's designated deputies will be selected Investigators from the Trial Sites who can provide clinical expertise to adjudicate serious adverse events and determine if they are SUSARs, and advise the CTEU whether expedited reporting is required.

## 12.1 Adverse Event (AE)

An adverse event (AE) is defined as any untoward medical occurrence in a patient administered an investigational medicinal product (IMP).

## 12.2 Adverse Reactions (AR)

An adverse reaction (AR) is defined as any untoward or unintended response related to any dose of IMP administered. In the event that an AR is reported during the trial, investigators will assess the severity of the adverse event using the following criteria, detailed on the adverse event case report form (CRF).

- **Mild:** Awareness of signs or symptoms, but easily tolerated; of minor irritant type; causing no loss of time from normal activities; symptoms would not require medication.
- **Moderate:** Discomfort severe enough to cause interference with usual infant activities.
- **Severe:** Inability to carry out usual infant activities; signs and symptoms may be of systemic nature or require medical evaluation and/or treatment.

### 12.3 Expected drug-related Adverse Reactions

Expected Adverse Reactions are known side effects of the IMP reported in the Summary of Product Characteristics and those listed in appendix 1.

### 12.4 Specific AEs to be captured

Values of triglycerides, bilirubin and other safety parameters will be recorded throughout PN administration. Any values above pre-specified levels (Appendix 1) will be reported as adverse events through the data collection system. The management of these specific AEs is described in Appendix 2, 3 and 4. Additional guidance for reporting these AEs will be provided in the Investigator's Manuals.

### 12.5 Serious Adverse Events/Reactions

Serious adverse events (SAE) or reactions can be defined as any untoward medical occurrence or effect that at any dose:

- results in death
- is life-threatening
- prolongation of existing inpatient hospitalisation
- results in persistent or significant disability or incapacity

### 12.6 Expected SAEs/clinical outcomes due to preterm birth

There are multiple serious complications that could occur as a consequence of premature birth. It will be at the judgement of the investigator reporting the event and subsequently the Chief Investigator (or designated deputy) adjudicating the event, to reach a decision on the expectedness of an event. The reporting investigator and the Chief Investigator (or designated deputy) will be clinicians with substantial experience in treating preterm infants.

### 12.7 Reporting of Serious Adverse Events/Reactions (SAE/Rs)

The web-based eCRF will contain specific forms for reporting SAEs. The investigator will complete the SAE report in the eCRF, including date of event, admissions, details of diagnosis, date of discharge or death and an assessment of causal relationship to the IMP according to the criteria summarised in Table 3. The investigator will complete the required details and the SAE report within 24 hours. The web-based eCRF will send an email alert to the CTEU.

**Table 3. Definitions for assessment of causality**

Relationship	Description
<b>Unrelated</b>	There is no evidence of any causal relationship
<b>Unlikely</b>	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).
<b>Possible*</b>	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).
<b>Probable*</b>	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.
<b>Definitely*</b>	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.
<b>Not assessable</b>	There is insufficient or incomplete evidence to make a clinical judgement of the causal relationship.

\* If the AE is serious and unexpected, the possible, probable and definitely related events will be notified to the MHRA, the relevant REC and the Sponsor as SUSARS by the CTEU.

The CTEU will review all SAE reports before sending an electronic copy to the Chief Investigator (or designated deputy) for adjudication. All reported SAEs will be adjudicated by the Chief Investigator (or designated deputy) to assess the seriousness and causality between the IMP and/or concomitant therapy and serious adverse event/reaction. This assessment must take place within two working days upon receipt of the electronic copy of the form. If the Chief Investigator (or designated deputy) judges the SAE/R to constitute a SUSAR, the CTEU will ensure expedited reporting procedures are followed. A monthly list of SAEs will be notified to the sponsor.

## 12.8 Suspected Unexpected Serious Adverse Reactions (SUSARS)

A SUSAR is an Adverse Reaction that is classed as serious, is suspected to be caused by the investigational medicinal product and is *unexpected* i.e. not consistent with the information about the investigational medicinal product (Summary of Product Characteristics). SUSARs are subject to expedited reporting.

## 12.9 Reporting of SUSARs

The CTEU will unblind the SUSAR report before sending it electronically to the Chief Investigator (or designated deputy). The Chief Investigator (or designated deputy) will review the report within two working days and adjudicate whether the event constitutes a SUSAR. The Chief Investigator (or designated deputy) will inform the CTEU of their decision to ensure expedited reporting procedures are followed if necessary.

## 12.10 Expedited reporting of SUSARs

All SUSAR reports will be unblinded by the CTEU prior to submission. A SUSAR which is fatal or life threatening will be reported to the appropriate regulatory authority (MHRA) and the main Research Ethics Committee (REC) by the CTEU as soon as possible and within 7 days after the CTEU become aware of the event. A SUSAR which is not fatal or life threatening must be reported to the appropriate local regulatory authority and main REC as soon as possible and within 15 days after the CTEU become aware of the event. The CTEU will inform all trial investigators of all reported SUSARs within 15 working days.

## **12.11 Annual reporting**

The CTEU will submit annual safety reports of all serious adverse reactions (SARs) in accordance with regulatory requirements to the appropriate regulatory authority and REC. Annual safety reports will be submitted to the appropriate regulatory authority on the date of the original clinical trial authorisation. Annual progress reports will also be submitted to the REC and the sponsor on the same date. Annual safety reports will also be submitted to the Data Monitoring & Ethics Committee for review.

## **13. STATISTICAL CONSIDERATIONS**

### **13.1 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 would enable us to detect a 0.2 kg difference between the groups with 80% power and at 5% significance level. This is a clinically important increase in lean mass.

Since publication of our paper on IHCL (16), measurements are 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 enable us to detect a difference in means of 0.526 on the logarithmic scale as significant at the 5% significance level with 80% power. Transforming back to the original scale of measurement, this would enable us to detect a 40% decrease in IHCL in the intervention group.

Assuming 10% mortality prior to term and 10% drop out rate, 80 infants will be recruited in to each group or until 64 infants in each group has had their MRI and MRS, a total of 128 scans.

### **13.2 Statistical analysis**

This is a 2 x 2 factorial randomised trial. Analysis will be 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 calculated (46). A 'modified' intention to treat method will be used to analyse the results as it is accepted that a proportion of infants will not be able to attend the MR scan visit. With the exception of infants not completing the MR scan, all other infants will be 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 and centre), nutritional interventions (amino acid and lipid), sex and corrected gestational age at time of measurement will be used to estimate the effects of each intervention.

A planned secondary analysis will investigate the role of illness severity and post PN nutritional intake as potential modifiers of the effects of each intervention by adding these variables to the regression model.

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

Secondary analyses will investigate possible interactions between nutritional interventions and sex, and between nutritional interventions and gestational age at recruitment.

## **14. REGULATORY AND ETHICAL CONSIDERATIONS**

### **14.1 Regulatory framework and approval**

This study is a randomised trial of an investigational medicinal product (licensed product in new conditions of use; new dosing schemes / new target population) and as such will need to comply with the European Clinical Trials Directive and the Medicines for Human Use (Clinical Trials) Regulations 2004. The study has received clinical trials authorisation (CTA) from the Medicines and Healthcare Regulatory Agency (MHRA) prior to starting the study. The study is registered in the European Community with a EudraCT number.

### **14.2 Ethical Approval**

The trial will comply with the Declaration of Helsinki (<http://www.wma.net/>) on research involving human subjects. The study protocol, patient information sheet and consent form will be submitted to the Research Ethics Committee for approval and subsequently to the R&D departments of each participating centre for site-specific approval. A signed Clinical Trial Agreement with each of the centres will be required before the study commences.

### **14.3 Monitoring**

#### **14.3.1 Initiation Visit**

Before the study commences each trial site will receive a training visit from CTEU and the Chief Investigator where required. The purpose of these visits

will be to ensure that the local research team (local principal investigator, co-investigators, study co-ordinator and pharmacists) fully understand the protocol, eCRF and the practical procedures for the study.

#### **14.3.2 Interim monitoring visits**

At regular intervals during the study CTEU will perform monitoring visits to each trial site. The purpose of these visits is to ensure compliance with the protocol and that ethical and regulatory requirements are met. Source data verification (SDV) and checking of essential documents will be performed. Monitors will also visit the pharmacy departments to review study procedures, storage and accountability of IMP.

Monitoring visits also provide an opportunity for further training if required (e.g. new staff). Central review of study data will also be performed throughout the study by the data management team at CTEU.

#### **14.3.3 Closeout Visit**

At the end of the study each trial site will receive a closeout visit from CTEU to resolve any outstanding edit queries or adverse events and to verify the archiving procedures for study documentation.

### **15. TRIAL ORGANISATIONAL STRUCTURE AND RESPONSIBILITIES**

#### **15.1 Trial Sponsor**

Imperial College will act as the Sponsor. The Sponsor's role is clearly set out in the European Clinical Trials Directive and NHS Research Governance documents. Research agreements will be held with each of the participating centres.

#### **15.2 Trial Steering Committee**

A Trial Steering Committee (TSC) will be established to oversee the conduct of the study. It is anticipated that the TSC will comprise the lead investigators, an independent chair, two additional independent members and a user representative. The TSC will meet thrice during the course of the study, once before the start of the study and then annually or as required throughout the course of the study. Reports produced will be submitted to the EME programme.

#### **15.3 Data Monitoring and Ethics Committee**

An independent Data Monitoring and Ethics Committee (DMEC) will be established to review 'serious adverse event' reports and the result of the interim analysis. The data will be analysed for safety with the provision to drop one arm of the factorial design. Safety data will be studied after recruitment of 32 infants and the interim analysis carried out after 64 infants. The DMEC will be expected to develop, in agreement with the investigators and TSC, a charter outlining their responsibilities and operational details.

## **15.4 Trial Management**

The study will be managed by the Clinical Trials and Evaluation Unit (CTEU), a dedicated clinical trials unit within the Royal Brompton & Harefield NHS Foundation Trust. In addition to providing overall project co-ordination, the CTEU will assist in preparing the final protocol, the investigators' manuals, design the electronic Case Report Forms (eCRF), setting up the randomisation service and design the data management system in collaboration with colleagues at Imperial College.

The CTEU will ensure that the trial runs according to the pre-agreed timetable, recruitment targets are met, eCRFs are completed accurately, compliance with relevant ethical and regulatory standards, and that all aspects of the study are performed to the highest quality. The CTEU will also assist in the training of investigators and co-ordinators at the start-up of the study and for performing monitoring procedures throughout. A smaller trial management team consisting of lead investigators from the Steering Committee and members of CTEU will meet weekly either in person or by conference call.

## **15.5 Trial registration**

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

## **15.6 Trial sites**

The trial will be conducted in multiple London centres ('Trial sites'). Study coordinators will be responsible for screening and randomising patients, enrolling patients into the trial, providing a contact point for patients, liaising with CTEU, completing and submitting eCRFs, arranging all follow up visits and measurements, recording adverse events, ensuring forms are sent to CTEU and that all edit queries are resolved.

## **15.7 Investigator responsibilities**

Investigators will be responsible for ensuring that institutional (site specific) approval has been obtained as well as Agreements signed off by their Institution prior to the start of the study. Investigators are responsible for performing the study in accordance with the European Clinical Trials Directive and Medicines for Human Use (Clinical Trials) Regulations 2004. Investigators are required to ensure compliance to the Clinical Trial Protocol, eCRFs, Investigators File and any other study instructions as required by the Sponsor or its representatives. Investigators are required to ensure the accuracy of the trial data according to the instructions provided. Investigators are required to allow access to study documentation or source data on request for monitoring visits and audits performed by the CTEU, the Sponsor or any regulatory authorities. The Investigator may appoint co-investigators to assist with conduct of the study locally. All co-investigators must be listed as members of the research team and appropriately trained. The Investigator has overall responsibility for ensuring the conduct of the study locally.

## **16. END OF TRIAL**

The end of trial will be declared when the last infant recruited completes the last follow-up visit i.e. MRI scan at 37-44 weeks corrected age.

## **17. PUBLICATION POLICY AND DISSEMINATION OF RESULTS**

The results from the trial will be submitted for publication in a major journal irrespective of the outcome. The Trial Steering Committee will be responsible for approval of the main manuscript prior to submission for publication. At the end of the study, infants' parents will be able to request a copy of the results of the study from the investigator at that site.

Authorship of presentations and reports related to the study will be in the name of the collaborative group. The final follow-up study results paper will name local co-ordinators as well as those involved in central co-ordination and trial management.

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## 19. APPENDICES

### 19.1 Appendix 1. Safety assessments during trial

The following assessments will be done as part of safety monitoring during the trial. Any result above or below the levels indicated in the table should be recorded as Adverse Events on the appropriate form on the eCRF.

<b>Assessment (blood test)</b>	<b>Level at which Adverse Event recorded</b>
Glucose	< 2.6 mmol /l or > 15 mmol /l
Worst base deficit in previous 24 hours	> 15 mmol /l
Total serum bilirubin	> 150 µmol /l
Conjugated bilirubin	> 40 µmol /l
Cholesterol	> 6 mmol /l
Triglycerides	> 2.5 mmol /l
Sodium	< 131 mmol /l or > 150 mmol /l
Potassium	< 3.2 mmol /l or > 9 mmol /l
Phosphate	< 1.5 mmol /l or > 3 mmol /l
Calcium	< 1mmol /l or > 3 mmol /l
Urea	< 1.5 mmol /l or > 7 mmol /l
Creatinine	>170 µmol/l
ALT	> 60 IU / l
Zinc	<8 µmol/l
Copper	<2 µmol/l
Manganese	>30 nmol/l
Aluminium	>0.4 µmol/l
Selenium	<20 µg/l

## **19.2 Appendix 2. Suggested management of hyperglycaemia**

The aqueous PN in the control arm as well as the interventional arm contain 8.6 g /kg / day of glucose. This is the lower range of Recommended Daily Intake and the risk of hyperglycaemia is therefore low.

Within the trial, in order to achieve consistency in the management of hyperglycaemia, the following management is suggested.

- If an infant in the trial develops blood glucose level of > 12 mmol / l, repeat a level after 1 hour. If the level remains greater than 12 mmol / l commence an infusion of insulin.
- Titrate the dose of the infusion with hourly to two-hourly blood sugars to maintain blood sugars between 6 – 8 mmol / l.
- Stop the insulin infusion if blood glucose is < 6 mmol / l.
- Record the use of insulin in the eCRF.

## **19.3 Appendix 3. Suggested management of hypoglycaemia**

If an infant in the trial develops hypoglycaemia (defined as blood sugar level < 2.6 mmol / l) the following management is suggested.

- Check that the line being used to infuse the PN is patent and that the pumps are working.
- Repeat the level immediately on the ward-based blood gas analyser. If this is not available, send a sample of blood in a fluoride tube to the laboratory for analysis.
- Do not wait for the result to act. Give the infant a bolus of 2 ml /kg of 10% dextrose solution. Commence an additional infusion of 20 ml / kg / day of 10% dextrose infusion. This will provide an additional 2 g /kg /day of glucose (1.4 mg / kg / min). If sugar remains < 2.6 mmol /l after an hour of infusion change to 12.5 % dextrose at 30 ml /kg /day. This will provide an additional 3.75 g /kg /day (2.6 mg /kg /min) over that contained in the PN solution.

## **19.4 Appendix 4. Suggested management of hyponatraemia and hypokalaemia**

The PN solutions contain 2 mmol / kg / day of Sodium on day 1 and 2 of PN and 4 mmol /kg /day from day 3 onwards. The solutions contain 1 mmol /kg /day of Potassium on day 1 and 2 of PN and 2 mmol /kg /day from day 3 onwards.

Within the trial, in order to achieve consistency in the management of hyponatraemia and hypokalaemia, the following management is suggested.

- If an infant develops hyponatraemia (defined as serum sodium < 131 mmol / l) and is on at least 45 ml /kg day of milk feeds, oral sodium supplements may be commenced.
- If the infant is on less than 45 ml /kg /day of milk feeds then an additional infusion of 20 ml /kg / day of normal saline infusion is suggested. This will give an additional 3 mmol /kg /day of sodium. If higher amounts of sodium are required consider adding concentrated sodium chloride solution (for eg. 30% sodium chloride) to 10% dextrose infusion of 20 ml /kg /day to achieve desired intake.
- If the infant develops significant and persistent hypokalaemia (serum potassium < 3.2 mmol /l) and is tolerating at least 45 ml /kg / day of milk feeds, consider supplementing Potassium chloride enterally. If the infant is not tolerating at least 45 ml /kg / day of milk feeds, consider adding Potassium chloride to 20 ml /kg /day of 10% dextrose infusion.

### 19.5 Appendix 5. Central venous lines

The PN may be commenced via a peripheral venous line but it is recommended that a central line is inserted for ongoing parenteral feeding.

When a central line is inserted it is suggested that it is kept patent with a 1 ml /hr of 10% dextrose until the previous trial bag has run for 24 hours. When the next PN bag is prescribed the PN may be run through the central line.

This is to ensure that the new PN bag is commenced via the central line with aseptic precautions.

### 19.6 Appendix 6. Suggested milk feeding advancement template

Day of feeding	ml/kg/24h	suggested frequency
1	Up to 15	2 hourly
2	15 - 30	hourly
3	30 - 45	hourly
4	45 - 60	hourly
5	60 - 75	hourly
6	75 - 90	hourly
7	90 - 120	hourly
8	120 - 150	hourly or less frequent
9	Continue to increase to achieve caloric intake of at least 120 -130 kcal /kg/ day (see note below)	

Consider withholding feeds if:

- the pre-feed gastric aspirate exceeds 4ml/kg
- there are posits or vomits after 2 consecutive feeds
- there is marked or tender abdominal distension or the presence of visible bowel loops
- there is heavy bile staining of gastric aspirate or bilious vomiting

**Notes:**

130 kcal /kg /day is equivalent to:

- 180 mls /kg /day of Maternal expressed breast milk with Nutriprem breast milk fortifier (1 sachet per 100 mls) or
- 160 ml /kg /day of Nutriprem 1

Add breast milk fortifier when the infant has achieved 180 ml /kg/ day of maternal expressed milk, although commencing fortifier on 150 ml /kg /day is recommended if for any reason feeds are being restricted.

**Guide to nutritional composition of Nutriprem 1, Maternal expressed breast milk (MEBM) and Donor Expressed Breast Milk (DEBM):**

Per 100 ml	Nutriprem 1	MEBM	DEBM
Energy (kcal)	80	64 – 69	65 - 70
Protein (g)	2.4	1.2 – 1.9	1
Fat (g)	4.4	3.4 – 3.6	2.5 – 3.5
Carbohydrate (g)	7.9	6.3 – 6.7	7

Nutriprem Fortifier: 1 sachet per 100 ml of breast milk

Per sachet: protein 0.4 g; carbohydrate 1.5 g, fat 0.  
Calories: 8 Kcal