UNIVERSITA' POLITECNICA DELLE MARCHE
PH.D. SCHOOL OF BIOMEDICAL SCIENCES
Department of Odontostomatologic and Specialized Clinical Sciences

Coordinator: *Prof. Carlo Catassi*

Bridging parenteral nutrition for the preterm infants

Nutrizione parenterale di transizione nel neonato pretermine

Ph.D. Dissertation of:
Giretti Ilaria

Advisor:
Prof. Virgilio Carnielli

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Abstract

Postnatal growth failure in preterm infants remains a reason of concern for the Neonatologist because it has been associated with poor outcomes, such as neurodevelopment. Nutrition is key for a proper infant growing and continuous improvements, especially for small preterm infants, are in place. In preterm infants with a low birth weight, bridging parenteral nutrition is a life-saving therapy and it is recommended from the first hours of life to reduce the risk of postnatal growth failure. However, it has often been associated with metabolic complications.

The aims of this thesis were to study the effect of the intravenous lipid emulsions on short- and long-term outcomes and to find factors associated to hypertriglyceridemia and elevated blood urea concentration in preterm infants on bridging parenteral nutrition.

Study patients were preterm infants with a birth weight of less than 1250g on bridging parenteral nutrition from birth, born between 2004 and 2018.

Preterm infants were able to tolerate markedly lower intravenous lipid intakes than the recommended target values of 2018 Parenteral Nutrition Guidelines and the low gestational age was associated both with hypertriglyceridemia and elevated blood urea. Infants with hypertriglyceridemia did not show significantly higher complications, worser growth and neurodevelopment than non-hypertriglyceridemic infants. Furthermore, intravenous lipid emulsions did not significantly affect neither lung growth nor neurodevelopment.

In conclusion, this thesis provides novel information on the management of bridging parenteral nutrition in the small preterm infant, pointing out the importance of the high standard of care for newborns admitted to the neonatal intensive unit care.

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Chapter 1. Introduction

a. Preterm infants: an overview

Preterm birth represents one of the major problems for neonatologists due to its increasing frequency, strong socio-economic impact and consequences for families. World Health Organization defined preterm birth as birth before 259 days of gestation, that correspond to 37 weeks post-menstrual age. Epidemiologic studies on premature birth in healthy woman with low-risk pregnancy reporting worldwide incidence varying from 3 to 18% (1, 2), while very and extremely preterm infants represent about 17% of the total premature birth (3).

Spontaneous preterm labor, caused by intra-amniotic infections, vascular disorders, uterine overdistension and cervical disease, is the main complication leading to preterm birth, followed by hypertensive during pregnancy, preterm premature rupture of the membranes, fetal intrauterine growth restriction and maternal infection as an indication for delivery (4-6).

Premature birth was often associated with early death and the development of several diseases, especially in infants born very preterm (7). During the last two decades, care of preterm infants was largely improved, and incidence of death was significantly reduced (**Figure 1.1**). However, in hospital growth, short- and long-term outcomes (8) and neurodevelopment remain major concerns for Neonatologists (9).

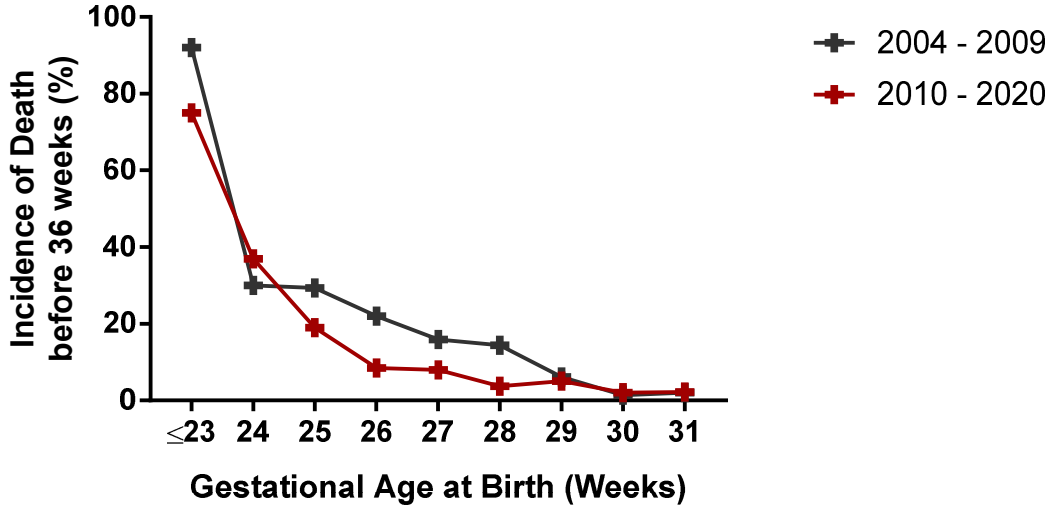

Figure 1.1 *Incidences of Death before 36 Weeks post-menstrual age in Very and Extremely Preterm Infants from 2004 to 2019 admitted to NICU of "G. Salesi" Children's Hospital of Ancona*

To date, survival in the neonatal period has increased up to 90% in very preterm infants (10) and nutrition and neonatal care are key elements for their short and long term health outcomes (11).

Nutrition is particularly important for preterm infants because the growth rate is faster than in term infants. Indeed, they must increase they weight of about 3 to 5 time from birth to 40 weeks post-menstrual age. Others factors that have contributed to the wellness of preterm infants are the use of prenatal steroids to enhance fetal maturation (12, 13) and the use of exogenous surfactant for the treatment of the respiratory distress syndrome (14).

Poor nutrition during the first few days of life and protein and energy deficit accumulated during the hospitalization, are often not recovered at discharge and are associated with subsequent postnatal growth failure (17). It has been shown that postnatal growth failure are related to adverse neurodevelopment outcomes (poor psychomotor and mental skills, higher rates of cerebral palsy, and autism (15)), alteration of organs and increased morbidity and mortality (16).

To reduce the risk of postnatal growth failure, caloric deficit and neonatal morbidities, early postnatal nutrition was recommended by many authors (18, 19). Due to gut immaturity it difficult to administered Enteral Nutrition (EN) immediately after birth and Parenteral Nutrition (PN) is required to cover nutritional needs (11). PN is indicated in infants who are unable to tolerate oral feeding, due to gastrointestinal immaturity, problems or surgery, and the time when PN should be started depend both on the age and size of the infant (20). In extremely and very preterm infants, with a Birth Weight (BW) of less than 1500 g, the use of PN from the first hours of life is highly encouraged (19). In this case, PN is defined as "bridging PN" and its role is to accompany newborns from birth to full EN.

Aggressive PN, early EN initiation and their rapid advancement, is well tolerated by preterm, could reduce caloric deficit and improve growth and clinical outcome (19, 21). From the first day of life, PN should be accompanied by Minimal Enteral Feeding (MEF) that consist in early provision of small amount of infant mother's milk (if possible) or infants' formula (up to 25 mL/kg/day). The role of MEF is not to provide optimal nutritive balance in premature infant, but to improve gastrointestinal maturation, digestion, absorption and activity. Infants receiving MEF results in a better feeding tolerance, lower incidence of necrotizing enterocolitis and sepsis and in a shorter hospitalization (22, 23). Generally, full EN is achieved in about 20 days in infants with a BW of less than 1250 g (24).

To date, PN is a standard of care for preterm infants but despite being widely used, their optimal composition is still unknown (25). PN should mimic the nutritional intake in utero and should guarantee a growth rate similar to growing fetus (26). It has been established that, after the initial postnatal weight loss, a weight gain of 17 – 20 g/kg/day is sufficient to guarantee an appropriate growth (27). Net protein gain, or protein accretion, however, is a much more appropriate indicator of nutritional status (most likely protein accretion reflects true growth) than weight gain alone.

Maintaining optimal body composition during PN is important, not only in maintaining the

adequate body composition in terms of lean body mass, fat, minerals and water, but perhaps even more importantly in assuring optimal tissue composition for organ function. This appears to be particularly important in the case of the central nervous system, the lung, the gut, the skeletal muscle and the immune system. Previous studies showed that about 4 g/kg/d of amino acid intake (28) and 90 – 120 kcal/kg/d of energy intake (27) should be provided to approximated intra-uterine lean body mass and an intravenous amino acid intake of 1.5 g/kg/day was found to be the minimum amino acid requirement to avoid negative nitrogen balance (29).

The European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) guidelines on pediatric PN suggests an intravenous energy intake of 45-55 kcal/kg in the first day of life to a maximum of 120 kcal/kg/day from the 2nd day of life (27), an amino acid intake of 1.5 g/kg in the first day of life up to 4.0 g/kg/day (29), a glucose intake of 5.8 – 11.5 g/kg during the first day of life up to 14.4 g/kg/day from day 2 onwards (30) and an intravenous lipid intake of 25-40% of the non-protein energy intake from the first day of life, to a maximum intake of 4.0 g/kg/day (31).

In the Neonatal Intensive Unit Care of "G. Salesi" of Ancona, intravenous lipids is infused at a dose of 1.0-1.5 g/kg/day on the day 1 up to 2.5-3.5 g/kg/day on the day of life 5, glucose was increased from 6.0-8.0 to 12.0-14.0 from day of life 1 to 5 and amino acids from 1.0-1.5 g/kg/day on the day 1 up to 2.5-3.5 g/kg/day on the day of life 5. Maximum intravenous lipid, amino acid and glucose intakes were kept constant from day of life 5 to day 7. Minimal enteral nutrition is started from the first day of life at a dose of 8 ml/kg/day during the first 4 days of life and 16 ml/kg/day from day 5 to day 7 and then it is gradually increased. Many infants in stable clinical conditions may tolerate the same infusate once the optimal intakes are reached for the total duration of PN. Others, particularly more immature and clinically unstable infants, require frequent adjustment of the intake of one or more nutrients. Indeed, although PN is considered a life-save therapy it is associated with metabolic complication (such as hyperglycemia, hypertriglyceridemia and high blood urea concentration), sepsis and cholestasis (31). For this reason, ability to change the composition of the infusate in response to clinical and chemical monitoring, or to increase or decrease the volume in response to greater or lesser fluid losses than expected is important.

Given the relevance of PN in very preterm infants, enhanced knowledge on the effect of PN components on growth, complication and short- and long-term outcome will be very helpful in order to improve neonatal care and the life expectancy of preterms.

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b. Practice of Parenteral Nutrition in Preterm Infants

Virgilio P. Carnielli[1,2], Alessio Correani[2], Ilaria Giretti[1], Rita D'Ascenzo[2], Maria P. Bellagamba[2], Ilaria Burattini[2], and Chiara Biagetti[2]

[1]: Department of Odontostomatologic and Specialized Clinical Sciences, Polytechnic University of Marche, Ancona (Marche), Italy

[2]: Department of Mother and Child Health, Division of Neonatology, "G. Salesi" Children's Hospital – Azienda Ospedaliero Universitaria Ospedali Riuniti di Ancona, Ancona, (Marche), Italy

Infants born preterm have very limited nutrient stores and their needs for tissue accretion are higher than in any other period of human life (1). Obtaining growth rates closer to the intrauterine accretion outside the uterus has been proven to be a very difficult objective to achieve as preterm infants also experience conditions that impair tissue growth, such as inflammation, use of medications and reduced physical activity (2). Furthermore, nutrient availability is often compromised by gastrointestinal (GI) immaturity that limits milk volume intakes and by the incomplete intestinal digestion and absorption (3).

Providing amino acids (AA) and energy immediately after birth with parenteral nutrition (PN) is nowadays a standard practice and a rather safe therapy if handled by an experienced team. Bridging PN is considered an integral part of the care of the smallest preterm infants during the first postnatal days, and full PN is a lifesaving therapy in case of impaired GI function secondary to congenital anomalies, prolonged GI intolerance or necrotizing enterocolitis (NEC) (4, 5). However, uncertainties persist about PN indications, compositional aspects, practical aspects of supply and delivery, need for central venous access, and monitoring. These uncertainties are often linked to variable local practices. Furthermore, it is difficult to disentangle the risk factors associated to the PN itself or to its individual components from the complication associated with the illness.

Indications and Benefits

The optimal population cut-off identifying patients that could benefit from a bridging has not been uniquely identified. Therefore, indications for PN are mainly based on clinical judgement, balancing pros and cons according to practice and expertise at the individual institutions.

Most often bridging PN is offered to infants born with a birth weight (BW) below 1500 g, and its average duration until full enteral nutrition (EN) is typically achieved in 1-2 weeks, duration is closely linked to the degree of prematurity (6).

Bridging PN in otherwise "healthy" and "moderately" preterm infants was found not to be associated with improved short-term outcomes (7).

In some neonatal intensive care units (NICU), PN is offered to patients with a BW of less than 1250 g and its duration is usually up to 3 weeks for the smallest infants (8). At our institution, we found that less than 25% of the infants with a BW between 1250 and 1499 g needed PN when "clinically indicated" and they fell into the following categories:

1. early high-risk patients: this group is comprised of infants in rather severe conditions at birth or soon after birth which make EN impossible or non-desirable (such as severe perinatal asphyxia, perinatal shock, GI malformations, severe intra-uterine growth retardation with reverse end-diastolic flow in the umbilical artery).
2. insufficient enteral intake: in this group fall the infants with critically low enteral intake usually for medical reasons (mean EN volume of 3 consecutive days of less than 30 ml/kg/day unless EN on the 3rd day is > 45 ml/kg/day.
3. late sickness: these are patients that developed major illnesses after a variable period of good GI tolerance (NEC, Severe late onset sepsis, Septic Shock and severe unexpected surgical conditions).

PN duration is usually longer in infants with GI surgery, with congenital or acquired short bowel, or with NEC. In these infants, PN is generally the main source of nutrients for several days or weeks and special attention should be dedicated to preventing nutrient deficits or nutrient overloads (e.g. zinc, copper, manganese, and iodine) and to optimize vitamin status (especially fat-soluble vitamins) (9-11).

PN allows a gradual increase in milk feeds during the recovery phase avoiding negative nitrogen balance and ensuring adequate growth (12).

Risks of Parenteral Nutrition and Monitoring

The use of PN has been associated with significant complications during its temporal course. It is sometimes difficult to sort out temporal relationship from causation and interactions. Common complications associated with the use of PN are:

- Central venous catheters (CVC)-related complications,
- Sepsis,
- Complications related to the composition of PN solution (admixture stability, drug toxins, etc),
- Metabolic complications (hepatobiliary, metabolic bone disease, etc).

CVC is the preferred route for PN in preterm neonates, as it represents a secure vascular access to deliver nutrient intakes in small volumes. However, CVC carries the inner risk of bloodstream

infections, thrombosis, or occlusion. Radiological or ultrasound guidance should be used during placement to ensure that the tip of the catheter is in a “safe place” (13).

Sometimes PN is administered via a peripheral vein, as a central venous access may be difficult to obtain or not desirable but in some centers the rate of sepsis is even higher with peripheral PN than with CVC (14). Moreover, peripheral PN carries the risk of extravasations, and nursing skills for prompt detection are very important. Low evidence data suggests that an upper limit of 1200 mOsm/L for CVC, and 800 mOsm/L for peripheral infusions are considered to be safe (15). In very low birth weight (VLBW) infants, only single lumen CVC or peripherally inserted central catheter lines are used, and withdrawals and transfusions are not feasible via these catheters (16).

Individualized PN bags in most centers are all-in-one mixtures containing lipids, AA, carbohydrates and electrolytes. Lipids and vitamins oxidation and photo-degradation should be avoided by using multi-layer bags (17). A review of clinical trials on the effect of light-exposed vs light-protected PN showed that mortality in the light-protected group was half of that in the light-exposed group and twice as high in males compared with females (18).

Hepatic dysfunction is a major issue for infants on long-term PN (19), however cholestasis is relatively rare in stable infants receiving bridging PN (8, 20). Aluminum may contaminate PN and it has been associated to short- and long-term metabolic bone disease and poor neurodevelopment (21-23). Metabolic bone disease in preterm infants on PN is mainly caused by insufficient intakes of calcium and more importantly of phosphorus. In recent years this condition has become less frequent with the use of the more soluble organic phosphorus (24).

Suggested monitoring for preterm infants on bridging PN is shown in **Table 1.1**.

Table 1.1 *Monitoring strategies for preterm infants on routine PN*

Parameter	Frequency of measurement*
Plasma triglycerides, urea and creatinine	Two or more times during the first week of PN and then weekly.
Glycaemia	Daily until PN steady state and during severe illness
Blood count	Twice during the first week of PN and then weekly
Hepatic function	Weekly
Calcium, phosphorus, magnesium and electrolytes	Weekly
Calcium and phosphorus in urine	Weekly
Weight	Daily
Length and head circumference	Weekly

* the frequency of measurement is referred to a stable patient. In critically ill patients, additional measurements are suggested.

Metabolic Complications

Hyperglycemia

Hyperglycemia is probably the most common metabolic complication during PN represents the most serious limitation to delivering adequate amounts of non-protein energy (NPE). Glucose infusions seem to have only a minimal impact on blood glucose concentrations; nonetheless, a daily prevalence of up to 30% of hyperglycemia >180 mg/dL (10 mmol/L) was observed during the first 2 postnatal weeks in 580 infants born before 27 weeks of gestation between 2004-2007 (EXPRESS cohort) (25). Hyperglycemia has been associated to an increased morbidity and mortality (26). The 2018 PN Guidelines recommend a lower glucose intake than in the previous 2005 guidelines. The recommended glucose supply in preterm newborns is from 5.8 to 11.5 on day 1 and up to 11.5-14.4 g/kg/day over the subsequent 2-3 days.

Blood glucose concentrations should be measured regularly and intravenous (IV) glucose titration is recommended at a glucose value of 175 mg/dL. Insulin therapy should be started if blood glucose exceeds 180 mg/dL and a reasonable adaptation of glucose infusion has been unsuccessful. Acutely ill preterm infants should temporarily receive the glucose intake of day 1 as glucose intake does not reduce protein catabolism in the acute phase of critical illness (27). A proposed IV glucose titration protocol is reported in **Figure 1.2**.

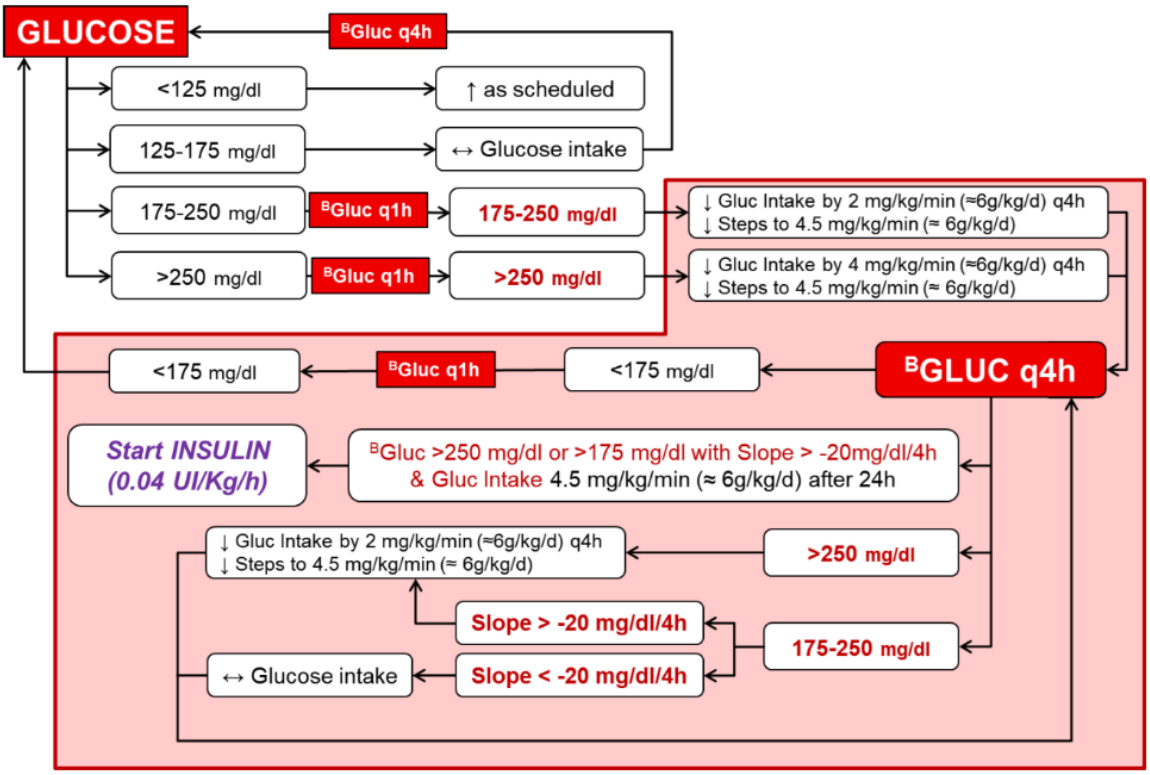

Figure 1.2 *Blood Glucose monitoring and IV glucose titration protocol*

Elevated Blood Urea

Elevated blood urea is often found during PN especially in VLBW infants during the first days of life. The usefulness of blood urea monitoring remains controversial. A study using a simple linear regression analysis, reported no association between AA intake and blood urea nitrogen and the authors concluded that limiting AA intake based on blood urea nitrogen concentration was not warranted (28). In more recent years, randomized clinical trials comparing 2 levels of AA intake reported significantly higher blood urea in the higher AA intake groups (29-31). Using multiple regression analysis, it has been recently found that blood urea concentrations were significantly and positively correlated with IV AA intake and negatively with IV NPE (32). A proposed IV AA titration protocol is reported in **Figure 1.3**.

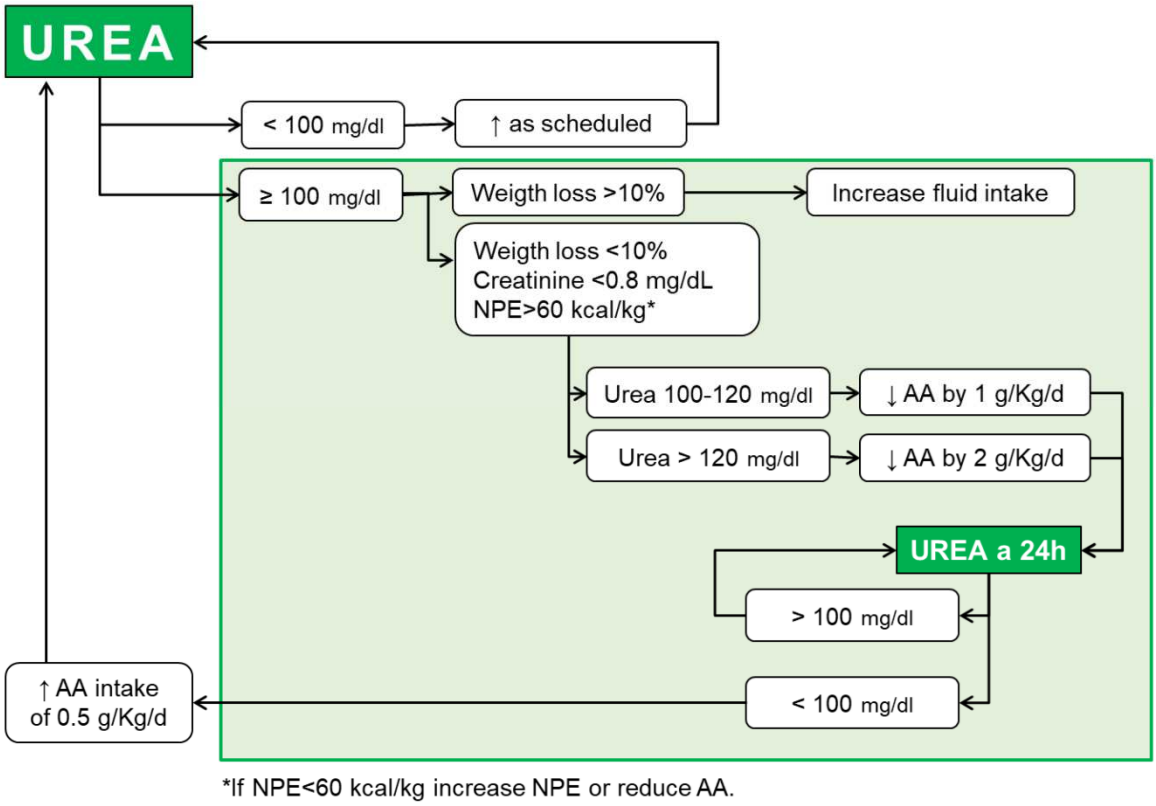

Figure 1.3 *Blood urea monitoring and IV AA titration protocol*

Hypertriglyceridemia

Hypertriglyceridemia typically occurs in infants receiving PN with lipids. The prevalence of hypertriglyceridemia is affected by the intravenous lipid emulsion (ILE) schemes, titration protocols and laboratory methods. The 2018 PN Guidelines recommended that in preterm neonates, "lipid intake should not exceed 4.0 g/kg/day" and "that serum triglyceride concentrations should be monitored regularly if receiving lipids and more frequently in cases with a marked risk for

hyperlipidemia" (33). Moreover, it was recommended that "reduction of the dosage can be considered if plasma triglyceride levels exceed 265 mg/dL" (33). Hypertriglyceridemia at a triglyceride level greater than 250 mg/dL was found to be rather common (20- 40) in the most preterm infants. Being small for gestational age and having a low gestational age are recognized risk factors for hypertriglyceridemia (34). In hospital complications and the 2-year follow up (anthropometry and neurodevelopment) of hypertriglyceridemic infants were not different from normolipemic controls (35). The algorithm in use at our institution for triglyceride monitoring is depicted in **Figure 1.4**.

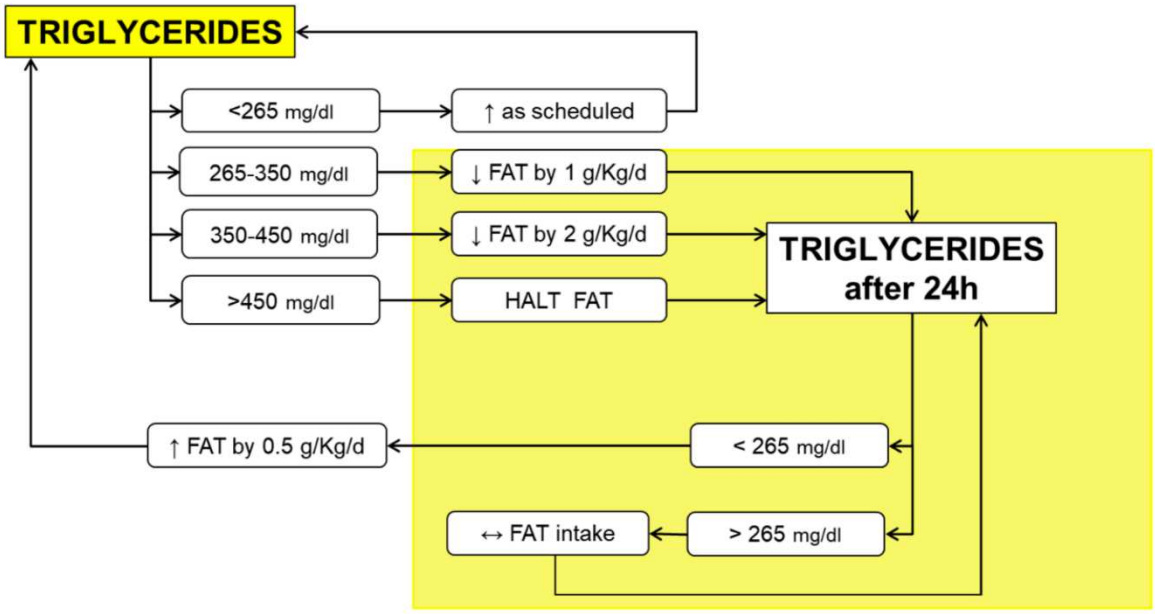

Figure 1.4 *Triglyceride monitoring and IV lipid titration protocol.*

Practical Aspects of Supply and Composition

Carbohydrates

Glucose intakes and glucose tolerance represent a very critical issue in the clinical care of VLBW infants, in general, and especially during PN. A significant percentage of infants has a poor glucose homeostasis (36) or develop significant hyperglycemia during critical illnesses. This may represent a significant problem when using high glucose intakes. Routine use of insulin should be avoided because of the risk of hypoglycemia and lactic acidosis (26, 37). Nonetheless, a recent report by Zamir et al. showed improved outcomes in hyperglycemic preterm infants treated with insulin (25).

Amino acids

Commencing PN with AA during the first hours of life as soon as the infant is stabilized has become part of the routine care as this improves nitrogen balance. The 2018 PN Guidelines recommend that AA supply should start on the first postnatal day with at least 1.5 g/kg/day to achieve an anabolic state while the maximum AA intake should be between 2.5 and 3.5 g/kg/day (38). These recommendations are based on 3 recent RCTs (29-31).

Twenty to twenty-five kcal of NPE per gram AA is the most often quoted figure for optimizing protein accretion (39), thus an AA intake of 3.0 g/kg/day should be given with a NPE >65 kcal/kg/day (38). Good quality studies on the effect of NPE on protein retention are lacking. The main difficulties in running these studies is that a significant proportion of VLBW infants develop hyperglycemia (about 30%) and/or hypertriglyceridemia (30-50% of cases) or both (about 20% of cases) (25, 35). These conditions represent a serious limitation to the administration of adequate amounts NPE and the impact on nitrogen balance is unknow. The effect of the NPE source on nitrogen retention has been studied only in term surgical neonates and it was found to be of limited importance (40), but such information is not available for VLBW infants. Insufficient NPE intakes may has been a limiting factor in trials examining the effect of AA intake on nitrogen balance (30). The impact of associated morbidities on NPE delivery also represents a challenge in sick patients and it requires to dedicated studies. The cost benefit ratio of “aggressive” PN during critical illness remains unexplored.

Lipids

ILE are integral part of PN for the preterm infants. Lipids are a good source of energy and should be started after birth and no later than day 2 of life (33). The dose of ILE to prevent essential fatty acid deficiency correspond to 0.25 g/kg/day of linoleic acid. The 2018 PN Guidelines recommend for preterm infants a maximum ILE intake of 4.0 g/kg/day, while ASPEN advises up to 3.0 g/kg/day (33). A lipid intake > 2.0 g/kg on the first day of life is not recommended. There is still limited information on the handling of the full dose ILE in critically ill preterm infants with circulatory failure and/or severe sepsis.

Composite ILE are preferred to pure soybean oil-based ones (33).

ILE containing fish oil have been used in recent years both in term and preterm infants, hoping to improve the fatty acid status (41), to reduce liver dysfunction and possibly to ameliorate neurodevelopment. To date, there is no evidence that IV fish oil containing ILE are superior to composite ILE but there is limited information on the effect of IV fish oil on neurodevelopment (42, 43).

To date, recent reports suggest caution on the use of large doses of fish oil during EN (44) and PN (45). The role of composite ILE with and without fish oil on liver function is still unclear (46, 47).

Electrolytes (Calcium, Phosphorus, Magnesium)

Calcium, and phosphorus are essential for bone mineralization. The use of the more stable organic calcium and phosphate salts has markedly reduced the incidence of PN associated bone diseases. Calcium and phosphate intakes in VLBW infants on PN can be increased until both are excreted simultaneously in low concentrations (>1.0 mmol/L) in the urine which is indicative of a slight surplus (48). During the first days of life, lower calcium, phosphate and magnesium intakes than in growing stable preterm infants are recommended. Once AA and energy intakes are optimized and the infants are in an anabolic status, a calcium to phosphate molar ratio below 1.0 (0.8-1.0) is recommended to reduce the incidence of early postnatal hypercalcemia and hypophosphatemia. Administering higher amounts of calcium and phosphate was associated with a significant reduction in the incidence of rickets and increased bone density (48).

Manufacture and Supply

PN solutions must be prepared under stringent conditions including a laminar flow hood and using appropriate ingredients. All processes should be validated and PN preparations checked for sterility and quality. All vitamins (water- and lipid-soluble) and trace elements should be added after appropriate tests for compatibility and solutions must be visually checked for clarity. This should be done before adding lipids in case of all-in-one preparations. Calcium and phosphate should be administered as organic salts as the risk of precipitation is greatly diminished.

Compositional/technical issues with PN can be found in the 20th edition of the Handbook of Injectable Drugs (49).

A dedicated software that generate warnings for osmolality, inadequate prescription and risk of precipitations is strongly recommended (50).

Some authors recommend the use of in-line filters to protect patients from precipitates, particles, or inadvertent microbial contamination (51). However, the incidence of sepsis does not appear to be reduced in neonatal patients by adding in-line filters. There are costs involved with filters and, in addition, they are not compatible with all-in-one PN preparations (52). Peroxides formation is reported in PN solutions by oxygenation reactions in the presence of light (53). Protecting the PN bags and infusion lines from light using amber tubing will reduce this problem. This issue is clinically relevant (18). Medications should not be added to PN solutions on the ward as this may

cause incompatibilities or contaminate the solution. Care should be taken in case of other solutions (usually vitamin and trace elements preparations) are infused though a Y connection with the PN line.

Standardized Parenteral Nutrition

Individualized prescriptions for PN are ordered and prepared every 24-48 h and are often not readily available for patients during the first day of life; moreover, after prescription changes, it takes extra time for new PN bags to be delivered to the ward. During the last years, standard PN bags have gained significant popularity and they are adequate for most of the patients in the NICU that are clinically stable to tolerate some variation in intakes (54). Standardized PN can be readily available in the NICU enabling initiation of PN within one hour after birth of very soon when needed. Standardized PN has other advantages over individualized PN including better provision of nutrients, less prescription and administration errors, decreased risk of infection, and possibly cost savings (55, 56). The 2018 PN Guidelines recommend that standard PN solutions should generally be used over individualized PN solutions in the majority of pediatric patients, in newborns and in VLBW infants. Individually tailored PN solution should generally be used when the nutritional requirements cannot be met by the available range of standard PN formulations (50). Different formulations of standard PN bags are available in different countries and at different institutions. Recently, a commercially designed multi-chamber ready-to-use solution became available for the use in preterm infants (57). To improve stability and reduce peroxidation, vitamins and trace elements should be added by the hospital pharmacy; Furthermore, standard PN allowed a high delivery of nutrients (if tolerated), but frequent electrolyte and fluid supplementation were necessary in nearly half of the patients (58).

Conclusion

PN is an integral part in the care of the VLBW infant. Administration of PN is safe when used judiciously with awareness of the local risk factors and complications. As more preterm infants than in the past do tolerate EN, the role bridging PN has changed during the last years, and its prescription has become less of a challenge. In case of exclusive PN, a careful formulation can meet all nutrient needs for months or years. Despite the clear short-term benefits of PN, data on long-term outcome are still lacking and further research is needed to better understand the role of nutrition vs sickness on long term health and neurodevelopment.

Key Messages

- Parenteral nutrition is a lifesaving therapy for newborn infants with congenital or acquired gut disorders or with prolonged feeding intolerance.
- Bridging parenteral nutrition for the very low birth weight preterm infant ensures adequate nutrient supply and reduces postnatal growth restriction while enteral nutrition is being established.
- The cost benefit ratio of parenteral nutrition for the very low birth weight infant vary from centre to centre because of different styles of care, different patient characteristics and disease severity.
- Strict monitoring is recommended, especially for the smallest infants who are more prone to complications (metabolic complications, growth failure, etc).
- Standardized parenteral nutrition is a valid alternative to individualized parenteral nutrition and computer guided prescriptions and monitoring algorithms are desirable.

Practice Tips

- PN should be preferably administered via CVC and it is recommended to guide the placement of the catheter by ultrasound (preferred) or by X-ray.
- PN should be started from the first hours of life after stabilization with a total IV energy of about 45-55 kcal/kg including at least 5.8 g/kg of glucose, 1.5 g/kg of AA and less than 2.0 g/kg of lipids.
- Target PN intakes of AA are between 2.5-3.5 g/kg/day, up to 14 g/kg/day of glucose and up to 4 g/kg/day lipids if tolerated.
- Metabolic complications commonly associated with PN (hypertriglyceridemia, hyperglycemia, elevated blood urea, cholestasis) should be regularly monitored and corrected according to predefined algorithms.
- EN should be started as soon as possible and PN should be tapered accordingly and stopped.
- Caveat
- Aggressive PN should not come to a price of increased risks of infections and other complications.
- PN should not divert the attention from optimizing EN.
- The optimal nutrition of the more critically ill VLBW infants is largely unknown.

Take-Home Messages

- PN is relatively safe and effective in reducing growth failure.
- Strict monitoring policy of patients on PN improves its safety.
- Long-term benefits of PN have not been studied.
- More research is mandatory in critically ill VLBW infants.

Research priorities

(1) Nutrition of the critically ill preterm infant: Sustaining anabolism vs putting the metabolism at rest soon after the onset critical illness. Which is better?
(2) Is non enzymatic clearance of ILE associated with increased inflammation?
(3) How much non-protein energy for optimal protein synthesis?
(4) Nutrition deficit vs sickness which affect neurodevelopment the most?

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c. The Aims of the thesis

The aims of thesis was to investigate on the effect of parenteral nutrition components on metabolic complications, short- and long-term outcome and to identify factors associated to hypertriglyceridemia and elevated blood urea concentration in a large cohort of preterm infants with a birth weight of less than 1250 g that received bridging parenteral nutrition from the first days of life.

Chapter 2. Hypertriglyceridemia and Intravenous Lipid Titration During Routine Parenteral Nutrition in Small Preterm Infants

Correani, Alessio∗; Giretti, Ilaria∗; Antognoli, Luca∗; Monachesi, Chiara∗; Cogo, Paola†; D'Ascenzo, Rita∗; Biagetti, Chiara∗; Carnielli, Virgilio P.∗Author Information
∗Division of Neonatology, Department of Clinical Sciences, Polytechnic University of Marche and Azienda Ospedaliero-Universitaria Ospedali Riuniti, Ancona
†Department of Medicine, University of Udine, Udine, Italy.
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Abstract

Objectives:

In case of hypertriglyceridemia (HiTG) during parenteral nutrition (PN), the 2018 European Society of Paediatric Gastroenterology, Hepatology and Nutrition guidelines recommend an intravenous (IV) lipid titration, but its consequences in small preterm infants are largely unknown. We compared macronutrient and energy intakes, growth, diseases associated with prematurity, and neurodevelopment in small preterm infants on PN who developed (cases) or did not develop HiTG (controls, CNTR).

Methods:

We retrospectively reviewed data of preterm infants with a birth weight (BW) <1250 g consecutively admitted to our neonatal intensive care unit (2004–2016) who received routine PN. HiTG infants were defined by at least 1 triglyceride (TG) measurement >250 mg/dL during the first 10 days of life. Patients with and without HiTG were match-paired for BW and gestational age.

Results:

A total of 658 infants were analyzed and 196 (30%) had HiTG. One hundred thirty-six HiTG patients were matched with 136 CNTR. In the first 10 days of life, IV lipid, non-protein energy and total energy intakes, but not IV amino acids and carbohydrates, were significantly lower in HiTG infants. We found no differences between groups in diseases associated with prematurity. Anthropometry at 36 weeks (W), anthropometry at 2-year (Y) corrected age (CA), and neurodevelopment at 2Y CA were not different.

Conclusions:

Growth, diseases associated with prematurity, and neurodevelopment at 2Y CA in HiTG infants were similar to CNTR. This occurred despite a statistically significant albeit small reduction in IV lipid and non-protein energy intakes due to a strict TG monitoring and IV lipid titration at TG levels >250 mg/dL.

What Is Known/What Is New

What Is Known

- Hypertriglyceridemia occurs in small preterm infants receiving parenteral nutrition.
- Plasma triglyceride monitoring and an intravenous lipid titration are recommended by the current pediatric parenteral nutrition guidelines.

What Is New

- Patients with hypertriglyceridemia had a statistically significant albeit small reduction in intravenous lipid and non-protein energy intakes due to triglyceride monitoring and titration.
- Growth, diseases associated with prematurity, and 2-year neurodevelopment were similar between patients with and without hypertriglyceridemia.
- A strict triglyceride monitoring and intravenous lipid titration at triglyceride levels higher than 250 mg/dL appear to be safe for small preterm infants.

Introduction

Hypertriglyceridemia (HiTG) is a metabolic complication often occurring in infants receiving parenteral nutrition (PN) (1). Preterm infants may be at higher risk of HiTG than term infants due to their relatively limited muscle and fat mass and, therefore, the decreased hydrolytic capacity of the enzyme lipoprotein lipase (2).

Current European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) guidelines recommend plasma triglycerides (TGs) monitoring to not exceed 265 mg/dL in infants on PN despite lower thresholds were considered in the past (eg, 150, 200, and 250 mg/dL) (3–6). Suggested management of HiTG involves titration or halting the intravenous (IV) lipid infusion until plasma TG levels normalize. As it is not advisable to administer excessive carbohydrates (CHOs), IV lipid titration may expose the small preterm infants to the risk of low non-protein energy (NPE) intakes whose consequences remain untested.

Adequate provision of amino acids (AAs) and NPE intakes is a primary goal of neonatal medicine, as inadequate nutrition has been associated with growth failure and poor neurodevelopment (7,8). So far, the relationship between sickness and inadequate nutrition remains unclear. It is still debated whether metabolic complications are mainly caused by sickness or they simply reflect the poor metabolic tolerance to nutrients of some preterm infants.

The purpose of this retrospective case-control study was to compare macronutrient and energy intakes (AA, lipids, CHO, NPE, and total energy), growth, diseases associated with prematurity, and neurodevelopment at 2-year (Y) corrected age (CA) in a large cohort of small preterm infants on routine PN who developed (cases) or did not develop HiTG (controls, CNTR).

Methods

Study Population

We retrospectively reviewed data of all preterm infants with a birth weight (BW) <1250 g, who routinely received an individualized all-in-one mixture PN from the first hours of life at the "G. Salesi" Children Hospital, Ancona, Italy from 2004 to 2016. Exclusion criteria were major congenital malformations, inborn errors of metabolism, admission to the neonatal intensive care unit (NICU) after 48 hours of life, death or transfer to another unit before 10 days of life (DOL), and no clinical data. The study was approved by the local ethics committee (Prot.2018201).

Study Design

The infants of the entire cohort were divided into 2 groups according to the plasma TG values: HiTG in case of at least 1 TG measurement >250 mg/dL during the first 10 DOL; CNTR in case of no TG measurements was >250 mg/dL during the first 10 DOL.

To overcome the association of both gestational age (GA) and BW with HiTG as confounding factors, we performed 2 separate case-control analyses (1:1 ratio, without replacement) between HiTG and CNTR infants: a) mconsidering all the study patients; b) excluding patients with hyperglycemia or elevated urea. Gestational age (GA) ± 2 days and BW ± 80 g were used as matching variables.

Plasma TG and urea were measured by Refloton dry chemistry system (Roche Diagnostics S.p.A, Monza, Italy), whereas blood glucose by Accu-Chek (Roche Diagnostics S.p.A, Monza, Italy).

According to the local NICU Nutrition Schemes, IV lipids were prescribed at a dose of 1.0 to 1.5 g/kg on DOL 1 up to 2.5 to 3.5 g/kg/d on DOL 5. CHO were increased from 6 to 8 to 12 to 14 g/kg/d from DOL 1 to DOL 5. AAs were prescribed at a dose of 1.0 to 1.5 g/kg/d in the first DOL and increased up to 2.5 to 3.5 g/kg/d on the DOL 5. Maximum IV lipids, AA, and CHO intakes achieved were then kept constant from DOL 5 to 7. Minimal enteral feeding was provided up to 8 mL/kg/d from DOL 1 to DOL 4, and 16 mL/kg/d from DOL 5 to DOL 7. PN was tapered after DOL 7 and stopped when the infant tolerated about 120 kcal · kg−1 · day−1 orally.

Individual patients received only one of IV lipid, CHO, and AA preparations assigned at birth by the neonatologist according to the pharmacy availability (more than 1 product is always available at our hospital pharmacy), or sometimes as part of clinical trials conducted in the past in our NICU (9–13). IV lipids were Lipidem 20%, BBraun SpA, Milan, Italy (40:50 medium chain TGs-MCT: soybean oil, 10% fish oil; MSF), SMOFlipid 20%, Fresenius-Kabi Italia Srl, Verona, Italy (30:30:25 MCT: soybean oil: olive oil, 15% fish oil; MSOF), Intralipid 20%, Fresenius-Kabi Italia Srl, Verona, Italy (100% soybean oil; S), Lipofundin MCT 20%, BBraun SpA, Milan, Italy (50% MCT and 50% soybean oil; MS) and Clinoleic 20%, Baxter SpA, Rome, Italy (80% olive oil and 20% soybean oil; OS). IV AA were TrophAmine 6% (TPH), BBraun SpA, Milan, Italy, and Primene 10% (PRI), Baxter, SpA, Rome, Italy, whereas IV CHO was Glucose 50%, Galenica Senese Srl, Siena, Italy.

HiTG was defined as plasma TG>250 mg/dL. Elevated urea was defined as plasma urea>100 mg/dL, and hyperglycemia as blood glucose>175 mg/dL. Both plasma TG and urea were monitored on DOL 3, 5, and 7 and then weekly during PN. Blood glucose was measured at least once daily. In case of metabolic complications, PN was handled according to the predefined algorithms: if TGs were >250 mg/dL, IV lipid intakes were reduced by 1.0 g/kg/d and TGs were checked again after 24 hours; if TGs reached 400 mg/dL, IV lipids were lowered to 0.5 g/kg/d. If plasma urea was >100 mg/dL (without excessive weight loss or urinary failure), IV AA intake was reduced by 1.0 g/kg/d. By contrast, if plasma urea was >120 mg/dL, the AA intake was reduced by 2.0 g/kg/d and urea was checked again after 24 hours. Blood glucose cut-off level was set at 175 mg/dL above which glucose intake was lowered by 2 mg · kg−1 · min−1 steps until blood glu□ose value fell <175 mg/dL and/or to a minimum glucose intake of 6.0 g/kg/d, whichever comes first. If hyperglycemia persisted at the minimum glucose intake, insulin infusion was started. Variations in blood sampling and intervention schemes might have occurred in selected cases at the discretion of the attending physician.

Administered IV AA, lipid and CHO intakes (g/kg/d) were collected from electronic medical records and calculated using hourly PN infusion (mL/h) provided by the syringe pump. PN intakes were normalized to 24 hours in the first and last PN day if incomplete. Day was defined as the 24-hour clock (from 00:00 to 24:00), and body weight (WT; g) was measured daily at about 24:00 using a digital infant scale (±5 g).

IV NPE and total energy were obtained from the actual IV lipid, AA, and CHO intakes (9, 4, and 4 kcal/g, respectively). Growth and other clinical information were prospectively recorded daily by a dedicated software (Neotools; Interactive, Milan, Italy). Length (cm) and head circumference (cm)

were measured at birth and weekly thereafter using a neonatal stadiometer and a flexible nonstretchable tape, respectively. Standard deviation scores were automatically computed by Neotools software using an Italian growth chart (14). The major complications of prematurity were defined according to the Vermont-Oxford definitions. Cholestasis was defined as plasma conjugated bilirubin >1.0 mg/dL. The 2Y follow-up included medical history, physical examination, anthropometry and neurodevelopmental assessment (Bayley III test).

Statistical Analysis

The primary objective of the study was to compare macronutrient and energy intakes (AA, lipids, CHO, NPE, and total energy) in small preterm infants who developed or not HiTG. As secondary objectives, we compared growth, diseases associated with prematurity, and neurodevelopment between groups. Because of the exploratory nature of the study, no formal sample size calculations were performed.

Depending on the distribution, data were expressed as mean ± SD, as the median |25P 75P| or as a number (percentage). The clinical characteristics of the study groups were compared using the paired t test, Mann-Whitney test, Wilcoxon test, or McNemar test as appropriate. Case-control pairs with missing data were discarded therefore some variables may have a lower number than that of matched pairs at birth.

Statistical significance was set at $P < 0.05$. SPSS software was used for the statistical analyses (v23.0; SPSS Inc, Chicago, IL). Case-control matching was performed using the FUZZY extension in SPSS.

Results

Study Cohort

A total of 848 consecutively admitted preterm infants with a BW<1250 g were screened for this study. One hundred forty-seven patients were excluded: 41 had malformations or inborn errors of metabolism, 24 were admitted to the NICU after 48 hours of life, 75 died or were transferred to another institution before DOL 10 and clinical information was not available for 7.

A total of 701 infants met the inclusion criteria (GA, weeks: $<27^{0/7}$, n= 208; $27^{0/7}$–$29^{6/7}$, n = 294; $\geq 30^{0/7}$, n = 199) and the TG measurements were available in 658 infants: 196 (30%) had at least one HiTG episode during the first 10 DOL and 462 (70%) had all the TG measurements<250 mg/dL (**Figure 2.1**).

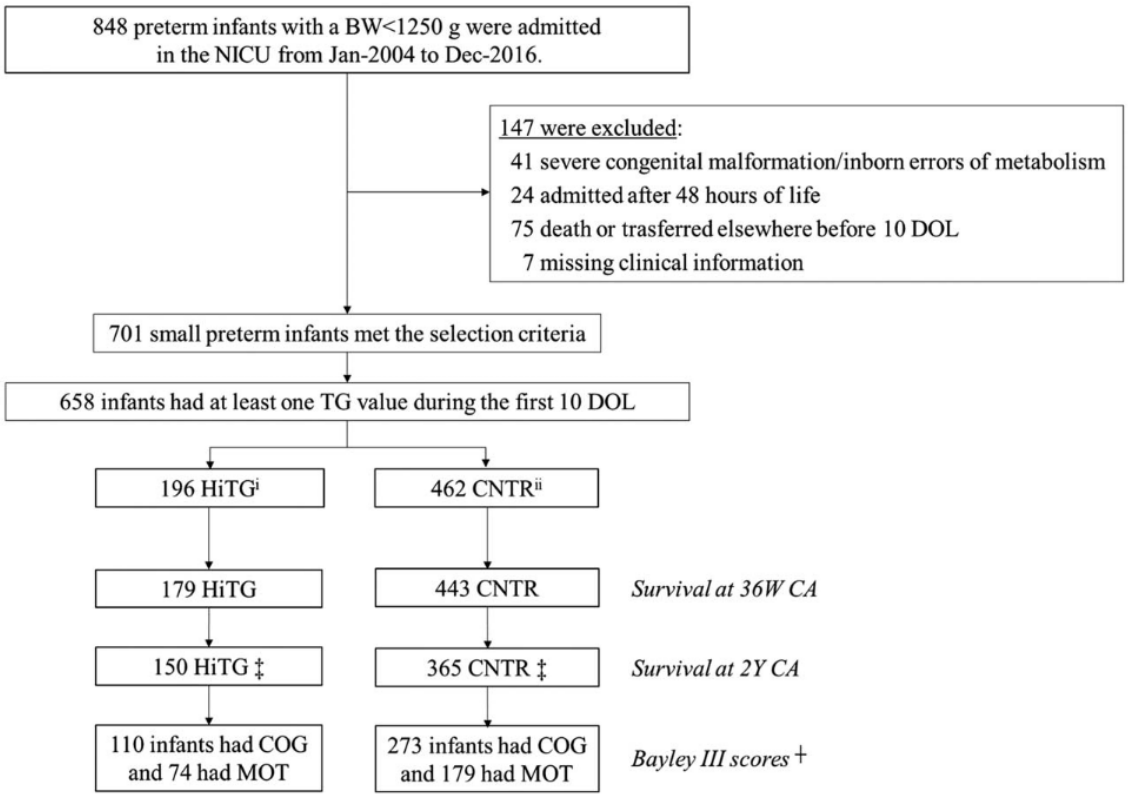

Figure 2.1 *Flow diagram of the patients included in the study.*
i At least 1 TG value >250 mg/dL during the first 10 DOL. ii Infants without HiTG. ‡ No information on survival at 2Y CA: 22/179 HiTG and 69/443 CNTR infants. † COG (cognitive composite score—Bayley III) started in 2007; MOT (motor composite score—Bayley III) in 2009. BW = birth weight; CA = corrected age; CNTR = controls; DOL = day of life; HiTG = hypertriglyceridemia; TG = trygliceride; W = weeks; Y = year.

In 94 HiTG infants (48%), the first TG value >250 mg/dL turned under the HiTG threshold in ≤24 hours, in 56 (29%) between 24 and 48 hours, in 30 (15%) in a longer time frame (>48 hours) and in 16 (8%) no other TG values <250 mg/dL were available until DOL 10.

Both GA and BW were significantly lower in infants with HiTG than those without HiTG (GA, weeks: 27.2 ± 2.2 vs 28.5 ± 2.2, P < 0.001; BW, g: 811 ± 192 vs 990 ± 178, P < 0.001, respectively).

Case-control Analyses

One hundred thirty-six case-control matched pairs (HiTG vs CNTR) were obtained using BW and GA as matching variables. Sixty unmatched cases were statistically different from the 136 matched cases for small for gestational age10thcentile and BW (47%, P = 0.03; 667 ± 150 g, P < 0.001). No differences in demographic and other baseline characteristics were found between the study-matched case-control infants (**Table 2.1**).

Table 2.1 *Demographic and other baseline characteristics of preterm infants*

	HiTG	CNTR^{BW-GA}	Differences	p
Birth weight (BW), g, n = 136	875 ± 173	877 ± 170	-2 ± 41	0.5
Gestational age (GA), wk, n = 136	27.4 ± 2.1	27.5 ± 2.1	-0.0 ± 0.2	0.7
Males, no. (%), n = 136	69 (51%)	64 (47%)	+5 (+4%)	0.5
BW-SDS, n = 136	-0.59 ± 1.02	-0.57 ± 1.03	-0.03 ± 0.35	0.4
Total length at birth (TL.B), cm, n = 136	34.7 ± 2.7	34.4 ± 2.8	+0.2 ± 2.4	0.3
TL.B-SDS, n = 136	-0.49 ± 1.00	-0.57 ± 1.02	+0.09 ± 0.86	0.3
Head circumference at birth (HC.B), cm, n = 136	24.5 ± 1.9	24.5 ± 1.8	-0.1 ± 1.5	0.6
HC.B-SDS, n = 136	-0.43 ± 1.08	-0.39 ± 1.03	-0.04 ± 1.07	0.6
SGA$^{10°centile}$, n (%), n = 136	41 (30%)	38 (28%)	+3 (+2%)	0.3
SGA^{2SDS}, n (%), n = 136	15 (11%)	13 (10%)	+2 (+1%)	0.5
Inborn—no. (%), n = 136	126 (93%)	120 (88%)	+6 (+4%)	0.2
Singleton birth—no. (%), n = 136	102 (75%)	103 (76%)	-1 (-1%)	0.9
Apgar5min—no., n = 136	8 |7 8|	8 |7 8|	0	0.4
Intubated 0-24 hours of life—no. (%), n = 136	101 (74%)	100 (74%)	+1 (+1%)	0.9
Surfactant therapy—no. (%), n = 136	97 (71%)	85 (63%)	+12 (+9%)	0.1
Perinatal steroid treatment—no. (%), n = 136	115 (88%)	117 (90%)	-2 (-2%)	0.7
Cesarean section—no. (%), n = 136	111 (85%)	113 (87%)	-2 (-2%)	0.7
IV lipid emulsions—no. (%), n = 136				
S	5 (4%)	4 (3%)	+1 (+1%)	0.8
MS	94 (69%)	80 (59%)	+14 (+10%)	0.049
OS	6 (4%)	2 (1%)	+4 (+3%)	0.2
MSF	23 (17%)	27 (20%)	-4 (-3%)	0.5
MSOF	8 (6%)	23 (17%)	-15 (-11%)	0.005
IV AA solutions—no. (%), n = 136				
TPH	121 (89%)	124 (91%)	-3 (-2%)	0.5
PRI	15 (11%)	12 (9%)	+3 (+2%)	0.5

Data are presented as mean SD, median j25P 75Pj or no. (%). Paired t test, Wilcoxon test, or McNemar test were used for the statistical analysis. AA = amino acid; HiTG = hypertriglyceridemia; IV = intravenous; MS = 50% MCT and 50% soybean oil (Lipofundin MCT 20%, B Braun SpA, Milan, Italy); MSF = 40:50 medium chain triglycerides-MCT: soybean oil (Lipidem 20%, B Braun SpA, Milan, Italy); MSOF = 30:30:25 MCT: soybean oil: olive oil, 15% fish

oil (SMOFlipid 20%, Fresenius Kabi Italia Srl, Verona, Italy); OS = 80% olive oil and 20% soybean oil (Clinoleic 20%, Baxter SpA, Rome, Italy); S = 100% soybean oil (Intralipid 20%, Fresenius Kabi Italia Srl, Verona, Italy); PRI = Primene 10%, Baxter, SpA, Rome, Italy; SDS = standard deviation score; SGA = small for gestational age; TPH = TrophAmine 6%, B Braun SpA, Milan, Italy.

PN duration was not different between HiTG and $CNTR^{BW\text{-}GA}$ (21 |18 26| vs 21 |18 25| days, respectively; P = 0.4). Daily IV lipid and NPE intakes were significantly lower in HiTG than $CNTR^{BW\text{-}GA}$ infants, whereas IV AA intakes were not different between study groups. CHO intake at DOL 4 was significantly lower in cases than in controls (**Figure 2.2 A**).

Cumulative IV lipid, AA, CHO, NPE, and total energy intakes in the first 10 DOL were 15.4 ± 3.8 vs 18.2 ± 3.1 g/kg (P < 0.001), 23.2 ± 4.8 vs 22.9 ± 4.2 g/kg (P = 0.4), 91.4 ± 11.1 vs 91.7 ± 10.9 g/kg (P = 0.8), 480 ± 64 vs 507 ± 60 kcal/kg (P < 0.001), and 573 ± 76 vs 599 ± 71 kcal/kg (P = 0.001) in HiTG and $CNTR^{BW\text{-}GA}$ infants, respectively. The mean reduction of IV lipids after an HiTG episode was −0.6 ± 1.1 g/kg, compared to the IV lipid progression of +0.1 ± 0.6 g/kg in controls (P < 0.001). Cumulative energy intake (EN and PN) in the first 10 DOL was also significantly lower in HiTG than controls (739 ± 101 vs 768 ± 87 kcal/kg, P = 0.002). There were no differences between HiTG and $CNTR^{BW\text{-}GA}$ in cumulative PN and EN AA and energy intakes from birth to 36 weeks (W) CA (AA: 203 ± 65 vs 193 ± 69 g/kg, P = 0.1; NPE: 5705 ± 1800 vs 5537 ± 1936 kcal/kg, P = 0.4; energy: 5908 ± 1859 vs 5730 ± 2000 kcal/kg, P = 0.3). A larger number of HiTG infants received fish oil–containing IV lipid emulsions than $CNTR^{BW\text{-}GA}$ infants (37% vs 23%, P = 0.012, respectively). The incidence of major complications of prematurity from birth to 36W CA were not different between HiTG and $CNTR^{BW\text{-}GA}$ infants (early-onset sepsis: 6% vs 5%, P = 0.8; late-onset sepsis: 22% vs 26%, P = 0.4; necrotizing enterocolitis ≥ grade II: 6% vs 4%, P = 0.6; surgical necrotizing enterocolitis: 4% vs 1%, P = 0.2; cholestasis: 10% vs 11%, P = 0.7; respiratory distress syndrome and Hyaline membrane disease: 91% vs 90%, P = 0.8; bronchopulmonary dysplasia: 25% vs 31%, P = 0.3; asphyxia: 6% vs 5%, P = 0.8; patent ductus arteriosus: 65% vs 70%, P = 0.4; periventricular leukomalacia ≥ grade II: 2% vs 4%, P = 0.3; intraventricular hemorrhage ≥ grade III: 9% vs 7%, P = 0.7; retinopathy of prematurity ≥ grade III: 1% vs 0%, P = 0.3, respectively). Anthropometry both at 36W and at 2Y CA and neurodevelopment at 2Y CA were also not different between groups (**Table 2.2**).

Table 2.2 *Growth and neurodevelopment of preterm infants*

	HiTG	CNTR^{BW-GA}	Differences	P
WT Nadir, g, n = 136	761 ± 162	759 ± 150	+2 ± 69	0.8
Age at Nadir, days, n = 136	4 |3 5|	4 |3 5|	0	0.5
Max WT loss, %, n = 136	12 |9 17|	13 |10 17|	-1	0.5
Time to regain BW, days, n = 136	12 |8 15|	11 |8 15|	+1	0.2
WT at 36W, g, n = 120	1861 ± 354	1895 ± 343	-34 ± 283	0.2
WT SDS at 36W—no, n = 120	-1.91 ± 0.88	-1.81 ± 0.86	-0.10 ± 0.71	0.1
TL at 36W, cm, n = 120	42.6 ± 2.5	42.7 ± 2.5	-0.1 ± 2.2	0.7
TL SDS at 36W—no, n = 120	-1.87 ± 0.99	-1.84 ± 0.96	-0.04 ± 0.85	0.7
HC at 36W, cm, n = 120	30.5 ± 1.5	30.6 ± 1.5	-0.1 ± 1.9	0.5
HC SDS at 36W—no, n = 120	-1.63 ± 1.02	-1.53 ± 1.08	-0.10 ± 1.36	0.4
WT gain (birth-36W), g kg 1 day 1, n = 120	15.4 ± 2.2	15.6 ± 2.5	-0.2 ± 3.3	0.5
WT gain (BW recovery-36W), g kg 1 day 1, n = 120	16.7 ± 2.8	16.6 ± 2.9	+0.1 4.0	0.8
WT at 2Y, g, n = 43	11627 ± 1759	11562 ± 2184	+65 2564	0.9
WT SDS at 2Y—no, n = 43	-0.29 ± 1.30	-0.24 ± 1.69	-0.05 ± 1.90	0.9
TL at 2Y, g, n = 43	87.5 ± 4.2	86.1 ± 4.6	+1.4 6.0	0.1
TL SDS at 2Y—no, n = 43	0.72 ± 1.36	0.44 ± 1.46	+0.28 1.82	0.3
HC at 2Y, g, n = 43	47.8 ± 1.9	48.0 ± 1.6	-0.2 ± 2.2	0.6
HC SDS at 2Y—no, n = 43	-1.05 ± 1.56	-0.72 ± 1.24	-0.33 ± 1.78	0.2
COG score (Bayley III)—no., n = 39	94 ± 13	95 ± 14	-2 ± 18	0.6
MOT score (Bayley III)—no., n = 22	100 ± 14	101 ± 11	-1 ± 17	0.8

Data are presented as mean± SD or median \|25P 75P\|. Paired t test or Wilcoxon test were used for the statistical analysis.BW= birth weight, COG= cognitive composite score; HC= head circumference; HiTG= hypertriglyceridemia, MOT= motor composite score; SDS= standard deviation score; TL= total length; W= weeks; WT= weight; Y= year.

To exclude the effect of hyperglycemia and/or elevated urea on macronutrient and energy intakes, we matched 68 isolated HiTG infants with 68 CNTR without these metabolic complications during the first 10 DOL. Daily IV intakes of infants included in this case-control analysis are reported in **Figure 2.2 B**.

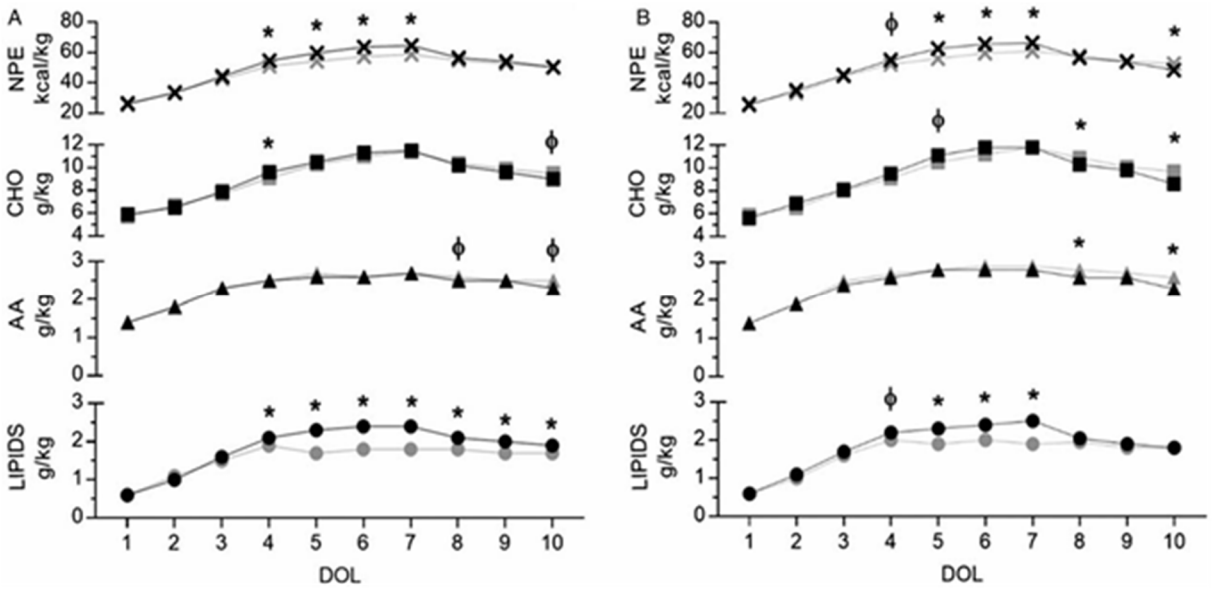

Figure 2.2 *Intravenous (IV) macronutrient and non-protein energy intakes in matched cases (grey) and controls (black): lipids, g/kg (●); AA, g/kg (▲); CHO, g/kg (□); NPE, kcal/kg (X).*

Data are presented as group means. Paired t test was used for the statistical analysis. P values <0.05 were indicated by '''' and <0.1 were indicated by ''Φ'' in the dotted graphs. A) Hypertriglyceridemia (HiTG) versus CNTR$^{BW\text{-}GA}$ considering all the study patients, n = 136. B) HiTG versus CNTR$^{BW\text{-}GA}$ excluding patients with hyperglycemia and/or elevated urea, n = 68. AA = amino acid; CHO = carbohydrates; DOL = day of life; NPE = non-protein energy.*

There were no differences in the perinatal characteristics, diseases associated with prematurity, cumulative PN and EN intakes (from birth to 36W), anthropometry at 36W and at 2Y CA, and neurodevelopment at 2Y CA between the 2 groups (data not shown).

Discussion

This study reports the consequences of HiTG and IV lipid titration on nutritional intakes and clinical outcomes in small preterm infants on routine PN. By using a case-control design, we overcame the well-known association of both GA and BW with HiTG. Of note, extreme small for gestational age and extremely low BW infants who carried at the highest risk of HiTG were excluded from the analysis. We reported that, in our unit, applying a strict TG monitoring and IV lipid titration at TG levels >250 mg/dL, HiTG infants did not have any growth, diseases, and neurodevelopment disadvantage compared to CNTR. This occurred despite the statistically significant albeit small reduction in IV lipid and NPE intakes.

Some physicians believe that intensive biochemical monitoring may lead to a marked reduction of PN intakes that in turn may result in poor growth and ultimately poor neurodevelopment. Our follow-up data did not support this concern. We found a 30% incidence of HiTG in our cohort that was in line with literature (1,5,15,16), and that HiTG resulted in a reduction of cumulative IV lipid intake of about 3.0 g/kg in the first 10 DOL, corresponding to −0.3 g/kg/d. This difference in the first DOL albeit statistically significant did not lead to any difference in short-term growth in small infants who received similar cumulative PN and EN intakes from birth to 36W CA. We speculate that halting IV lipid for 1 or 2 days which can happen with the use of the standard PN bags may have no or limited consequences on infant growth. Further studies are, however, required to confirm or refuse this hypothesis.

There are a few studies in preterm infants that reported significant associations between HiTG and disease/sickness (1,5,17). Some physicians consider HiTG to be associated with short-term illness, but our data did not support this notion. In our preterm infants, we did not find any association between HiTG and short-term complications of prematurity. Adequately powered studies are needed to confirm this finding.

The most important finding of the present study, in our opinion, is that the reduction in IV lipid and NPE intakes observed in HiTG infants did not significantly affect growth. Neurodevelopment at 2Y CA was also not different. Of note, our follow-up (cognitive composite score and motor composite score) started in 2009 and the low sample size did not allow us to come up with firm conclusions. The mean neurodevelopment score values of the groups were, however, very close, possibly suggesting that even a much larger number of infants would have not resulted in a statistically significant difference. We speculate that either compensatory mechanisms in place in small preterm infants or the modest increase in IV AA and CHO intakes found in our HiTG patients may have mitigated the potentially negative effects of reduced IV lipid intakes. It is also plausible that an IV lipid reduction of 0.3 g/kg/d represents a negligible fraction of the overall energy intake resulting in a negligible clinical relevance even if statistically significant.

Nowadays, IV lipid intakes of small preterm infants are set up to meet the target energy requirements during PN and to prevent essential fatty acid deficiency. The optimal IV lipid intake during the first DOLs, however, remains unknown. Some studies reported growth and neurodevelopment advantages in preterm infants receiving high IV lipid intakes from the first DOLs (18,19), whereas other authors did not find any difference (16,20). Cochrane meta-analyses suggested no differences in growth of very low birth weight infants between “early” and “not early” (>DOL 2) initiation of IV lipids (21,22). Based on our results, we speculate that the recommended

doses of IV lipids may still be in excess compared to those effectively required for adequate growth and metabolism of small preterm infants. The benefits of the extra IV lipids during HiTG remain to be clarified in this group of infants

We found that a smaller number of HiTG infants received fish oil–containing IV lipid emulsions than controls. Lipid-lowering effect of IV fish oil was previously reported in preterm infants (9). Our group in previous studies showed that IV fish oil may reduce lipogenesis in preterm infants (10), but it did not affect the overall growth (23). If IV fish oil, however, affects selected organ growth, such as brain, and in turn neurodevelopment requires further investigations. In this work, it is reassuring that although the greater number of infants in the control group received IV fish oil, this did not result in worse neurodevelopment compared to HiTG infants. Larger studies are needed to confirm it.

This study has limitations. Patients were not matched for year of birth. HiTG was defined as TG value >250 mg/dL rather than 265 mg/dL as currently suggested by ESPGHAN. About 85% of the study infants with HiTG, however, had plasma TG >265 mg/dL. Our case-control design did not consider a part of patients at high risk to develop HiTG (4,17). Plasma TGs were not measured at all time points: in several circumstances when the assay was not successful and there was no need to repeat other determinations than the TG, the caring physician often decided that it was not in the interest of the patients to repeat the test. The day after could have occurred that the test was repeated if blood was withdrawn for other biochemistry.

Neurodevelopment at 2Y CA was assessed from 2009 and about one-third of the case-control matched patients at birth were evaluated at 2Y CA. There were no differences in the perinatal data and hospital course of case-control matched patients who were/were not evaluated at 2Y CA follow-up (data not shown), and thus excluding a selection bias.

In conclusion, at our institution, TG monitoring resulted in a significant reduction of IV lipid and NPE intakes during the first 10 DOL in small preterm infants on routine PN who developed HiTG. Our patients with HiTG, however, did not have a reduced in-hospital growth, more complications of prematurity, or poorer neurodevelopment at 2Y CA than infants without HiTG. Information on the metabolic fate, utilization, and impact on growth of early and high IV lipid intakes in small preterm infants remain undefined.

This manuscript shows that a policy of IV lipid titration similar to the ESPGHAN 2018 recommendations is safe. The risk benefit ratio of IV lipid titration at plasma TG >250 mg/dL remains also unknown.

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Abbreviations

AA= amino acids; BW= birth weight; CA= corrected age; CHO= carbohydrates; CNTR= controls; COG= cognitive composite score; DOL: day of life; EN= enteral nutrition; GA= gestational age; HC= head circumference; HiTG= hypertriglyceridemia; IV= intravenous; M= months; MOT= motor composite score; NPE= non-protein energy; PN= parenteral nutrition; SGA = small for gestational age; TG= triglyceride; TL= total length; VLBW= very low birth weight; W= weeks; WT: weight; Y= year.

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Chapter 3. Hypertriglyceridemia and lipid tolerance in preterm infants with a birth weight of less than 1250 g on routine parenteral nutrition

Ilaria Giretti[a], Rita D'Ascenzo[b], Alessio Correani[b], Luca Antognoli[a], Chiara Monachesi[a], Chiara Biagetti[b], Adriana Pompilio[b], Luisita Marinelli[b], Ilaria Burattini[b], Paola Cogo[c], Virgilio P. Carnielli[ab].
a) Department of Odontostomatologic and Specialized Clinical Sciences, Polytechnic University of Marche and Azienda Ospedaliero-Universitaria Ospedali Riuniti Umberto I - G. M. Lancisi - G. Salesi, Ancona, Italy; b) Division of Neonatology, Department of Clinical Sciences, Polytechnic University of Marche and Salesi Children's Hospital, Ancona, Italy; c) Department of Medicine, University of Udine, Udine, Italy.

Abstract

Objectives:

To study the association of hypertriglyceridemia and of lipid tolerance with clinical and nutritional data in preterm infants receiving routine parenteral nutrition.

Design:

We retrospectively studied 672 preterm infants (gestational age <32 weeks) with birth weight <1250 g, consecutively admitted to our NICU, born between 2004 and 2018. Selected prenatal data and interventions, parenteral intakes and diseases were considered. Hypertriglyceridemia was defined as plasma triglycerides > 250 mg/dL. Lipid tolerance was defined as the ratio of plasma triglycerides to the intravenous lipid intake at the time of sampling. Variables associated to hypertriglyceridemia and to lipid tolerance were identified by multiple logistic and linear regression analyses.

Results:

Hypertriglyceridemia occurred in 200 preterm infants (30%), ranging from 67% at 23 weeks to 16% at 31 weeks' gestation. In 138 infants (69%) hypertriglyceridemia occurred at a lipid intake of 2.5 g/kg or less. Lipid tolerance was reduced especially in infants of less than 28 weeks' gestation (14.3±9.3 vs 18.8± 10.2, respectively, p<0.001). Lipid tolerance was negatively associated with respiratory distress syndrome (OR= -1.14, p=0.011), patent ductus arteriosus (OR= -1.73, p<0.001), small for gestational age (OR= -2.96, p<0.001), intraventricular haemorrhage (OR= -3.96, p<0.001), late onset sepsis (OR= -8.56, p=0.039).

Conclusion:

Preterm infants on routine parenteral nutrition were able to tolerate markedly lower intravenous lipid intakes than the recommended target values of current guidelines. Lipid tolerance was

associated with some of the major complication of prematurity, possibly at risk of developing hypertriglyceridemia.
Keywords: lipid metabolism, hypertriglyceridemia, parenteral nutrition, preterm infants, triglycerides.

Introduction

High intravenous (IV) lipid intakes in preterm infants have been associated with elevated plasma triglyceride (TG) concentrations (1, 2). Current ESPGHAN parenteral nutrition (PN) guidelines for preterm infants (3) recommend a maximum IV lipid intake of 4.0 g/kg/d, while ASPEN (4) advises up to 3.0 g/kg/d. However, there are studies in small preterm infants reporting hypertriglyceridemia (HiTG) already at 2.0 g/kg/d or less (5, 6).

Plasma TG monitoring is strongly recommended and 200 mg/dL (2.3 mmol/L) or 265 mg/dL (3.0 mmol/L) are the suggested thresholds for lipid titration according to ASPEN and ESPGHAN, respectively (3, 4, 7). Plasma TG monitoring may decrease IV lipid intake and this, in turn, may result in a lower energy intake, especially for the smallest infants. This remains of concern among neonatologists (8, 9).

Scanty information from small cohorts' studies is available on HiTG and lipid tolerance (LT) during PN in very preterm infants. Moreover, the association between HiTG and concomitant illnesses is often not studied and lipid intake at the time of TG determination is often not available (10-12). Furthermore, obtaining more information on LT in preterm infants on routine PN could help identifying the ability of infants to clear IV lipids before the development of HiTG.

We sought to study the association of HiTG and of LT with the perinatal characteristics, concomitant diagnoses and the simultaneous IV lipid intakes in a large cohort of small preterm infants on routine PN from birth.

Subjects and methods

Study population

We enrolled all infants consecutively admitted to the NICU of "G. Salesi" Children's Hospital, Ancona (Italy), between 2004 and 2018, with a birth weight (BW) lower than 1250 g and a gestational age (GA) lower than 32 weeks, who routinely received PN from the first hour of life. Exclusion criteria were major congenital malformations, inborn errors of metabolism, admission to the NICU after 24 hours of life, death or transfer to another unit before 10 days of life (DOL) and missing clinical information.

Nutrition protocol and biochemical monitoring

Nutrition protocols and biochemical monitoring policies were established in 2002, and they are still applied at our NICU. Accordingly, study infants with a BW lower than 1250 g received routine PN from the first hours of life. IV lipids were infused at a dose of 1.0-1.5 g/kg/d on the DOL 1 up to 2.5-3.5 g/kg/d on the DOL 5. From DOL 1 to 5, glucose was increased from 6.0-8.0 to 12.0-14.0 g/kg/d and amino acids (AA) from 1.0-1.5 g/kg/don the DOL 1 up to 2.5-3.5 g/kg/d. Maximum IV lipid, AA and glucose intakes were kept constant from DOL 5 to DOL 7. All infants received minimal enteral feeding from DOL 1 to DOL 7. The maximum amount of human milk or infant milk formula were supplied at 8 mL/kg/d from DOL 1 to DOL 4, 16 mL/kg/d from DOL 5 to DOL 7, and then it was gradually increased. PN was tapered after DOL 7 and stopped when the infant tolerated about 120 kcal/kg/d orally, generally during the 3rd week of life. Patients received only one of the 5 lipid emulsions routinely available at our hospital pharmacy, assigned by the attending neonatologist according to pharmacy availability, or sometimes as part of clinical trials conducted in the past in our NICU: Lipidem® 20%, B Braun (50:40 medium chain TG - MCT: Soybean oil, 10% fish oil), SMOFlipid® 20%, Fresenius Kabi (30:30:25 MCT: soybean oil: olive oil, 15% fish oil), Intralipid® 20%, Fresenius Kabi (100% soybean oil), Lipofundin MCT® 20%, B Braun (50%MCT and 50% soybean oil) and Clinoleic® 20%, Baxter spa (80% olive oil and 20% soybean oil).

Metabolic tolerance was assessed by measuring plasma TG, urea, creatinine and glycaemia using micro-methods (Reflotron® Plus, Sprint system, Roche Diagnostics International Ltd and Accu-chek Aviva Meter, Roche Diabetes Care, Inc.). During the study period HiTG episode was defined as TG concentration greater than 250 mg/dl; others metabolic complications were defined as previously described (13).

Biochemical TG monitoring was performed on the DOL 3, 5 and 7 and then weekly during PN. Additional determinations might have been performed in selected cases at discretion of the attending physician.

Data collection and definitions

During hospital stay, perinatal characteristics, nutritional and biochemical data were prospectively recorded by the nursing staff and attending neonatologists in the electronic medical record (Neotools; Interactive, Milan, Italy).

Administered IV lipid, AA and glucose intakes (g/kg/d) were calculated hourly using daily weight, PN bag composition and syringe pump rate (ml/h). GA was estimated by early ultrasound performed in the first trimester of pregnancy. The major complications of prematurity were defined according to the Vermont Oxford definitions. Asphyxia was defined as cord blood pH less than 7.0 and standard base excess less than -12.0 mmol/L. Small-for-gestational-age ($SGA^{10th\ centile}$) was defined as a BW less than the 10th percentile by using Italian reference growth charts (14).
LT was calculated as the ratio between administered IV lipid intake (g/kg) and plasma TG concentration (g/dL), where "administered IV lipid intake" is the mean IV lipid infusion during the 3 hours before TG determination. Patients received parenteral nutrition over 24 hours (continuous infusion) and lipid infusion was not halted before the blood withdrawal.

Ethics

The study was approved by the local ethics committee (ID 447790) and was in accordance with the principles of the Helsinki Declaration as revised in Fortaleza, October 2013.

Statistical analysis

Descriptive analysis was used to describe the study patients. Clinical characteristics, LT, IV lipid intake, plasma TG concentration and incidence of HiTG of the study groups were analysed by using the independent t-test, Mann-Whitney test, Kruskal-Wallis test or Chi square test as appropriate. A p-value of less than 0.05 was considered significant.
Binary logistic regression analysis with the enter method was performed to identify risk factors for HiTG episodes while multiple linear regression analysis with the enter method was used to assess the relationship between LT and clinical characteristics of our cohort of infants. All the TG determinations in the study period for each infant were used. Pearson's correlation test or Chi square test were performed to identify variables significantly associated with HiTG and with LT at univariate analysis. Variables were included in the logistic and linear regression analyses as independent variables if $p<0.1$ at univariate. All statistical analyses were performed by using SPSS software (v 23.0; SPSS Inc, Chicago, Illinois).
Independent variables for both logistic and linear regression analyses are listed below.
Perinatal and pregnancy characteristics: GA (weeks), $SGA^{10th\ centile}$, BW-SDS, gender, exposure to antenatal steroids (any steroids administered to the mother before birth), hypertension in pregnancy (HIP), type of delivery, singleton birth, Apgar score at 5 minutes, surfactant therapy.

Nutritional therapies: mean IV lipid intake (g/kg/d) administered during the 3 hours before plasma TG measurement, type of IV lipid emulsions and IV AA and glucose (g/kg/d) intakes administered during the 24 hours prior to the determination.
Complications of prematurity: Neonatal asphyxia, late onset sepsis (LOS), early onset sepsis (EOS), patent ductus arteriosus (PDA), intraventricular haemorrhage (IVH) grade III and IV, necrotizing enterocolitis grade II and III and respiratory distress syndrome (RDS).
Complication of prematurity and nutritional therapies were included in the analysis only when present at the time of TG measurement.
Both in the logistic and in the linear regression analysis the independent variables were considered significantly associated with the dependent variables if p value was lower than 0.05.

Results

Eight hundred and fifty-one infants with a BW of less than 1250 g and born before 32 weeks' gestation were studied. These infants represent nearly the 100% of the infants with such characteristics born in the Marche Region. One hundred and forty-five infants were excluded: 31 had major malformations, 29 were admitted in the NICU after 24 hours of life, 76 died or were transferred to another unit before DOL 10 and 9 had missing clinical information. Out of 706 patients who met the inclusion criteria, 672 were included, with at least one plasma TG determination before DOL 10 available. The BW and GA of the study infants were (mean±SD) 924±199 g and 28±2 weeks, respectively. Three hundred and twenty-seven infants were born at GA ranging from $23^{0/7}$ to $27^{6/7}$ weeks, whereas 345 infants were born between $28^{0/7}$ and $31^{6/7}$ weeks. Among prenatal factors infants with GA below 28 weeks differed from infants with 28 to 32 weeks' gestation for incidence of HIP, any antenatal steroids therapy and caesarean delivery (HIP: 19% vs 45%; p=0.001; antenatal steroid therapy: 85% vs 90%; p= 0.043; caesarean delivery: 75% vs 95%, p<0.001, respectively). The incidence of several prematurity complications was also significantly different between two GA groups (<28 weeks vs 28 to 32 weeks' gestation : $SGA^{10th\ centile}$: 14% vs 40%, p<0.001; asphyxia: 7% vs 3%, p=0.007; PDA: 73% vs 46%, p<0.001; IVH grade III and IV: 13% vs 1%, p<0.001; RDS: 97% vs 79%, p<0.001).

Incidence of HiTG, IV lipid intake and LT

Two hundred of the 672 infants (30%) exhibited at least one plasma TG concentration higher than 250 mg/dl. Lower GA was significantly associated with higher incidence of HiTG (p<0.001, **Figure 3.1 A**) as well as with greater plasma TG concentrations (p<0.001, **Figure 3.1 B**). Infants born

between $23^{0/7}$ and $27^{6/7}$weeks' gestation showed more than two-fold increase in the incidence of HiTG levels than infants born at $28^{0/7}$ and $31^{6/7}$ weeks (42% vs 19%; p<0.001).

Administered IV lipid intake was also significantly associated with GA (p<0.001, **Figure 3.1 C**). The mean IV lipid intake (g/kg/d) from day 1 to 3, from day 5 to 7 and from day 8 to 10 in infants born before 28 weeks' gestation and in those who were born between 28 and 32 weeks were: 1.1±0.3 vs 1.1±0.3 (p=0.5), 2.1±0.6 vs 2.3±0.6 (p<0.001), 1.9±0.5 vs 2.1±0.5 (p=0.001), respectively. The mean maximum IV lipid intake during the first 10 DOLs were 2.5±0.5 vs 2.6±0.5 (p=0.010), in infants with a GA lower than 28 weeks and in those with a GA between 28 and 32 weeks respectively.

Sixty-nine per cent (n=138) of the first HiTG episodes in the whole population occurred at IV lipid intake of 2.5 $g \cdot kg^{-1}$ or less.

Two hundred and forty-five patients (37%; 120 with a GA lower than 28 weeks' and 125 with GA between 28 and 32 weeks) received fish oil containing lipid emulsions (FO LEs). The incidence of HITG were significantly lower in infants receiving FOLEs compared to infants receiving conventional IV lipid emulsions (22% vs 34% respectively; p=0.002).

The incidence of HiTG did not change during the 14-year study period (p=0.154, data not shown).

LT was calculated for 1931 samples and it was lower in infants born before 28 weeks' compare to those with a GA between 28 and 32 weeks (14.3±9.3 vs 18.8± 10.2, respectively, p<0.001; **Figure 3.1 D**).

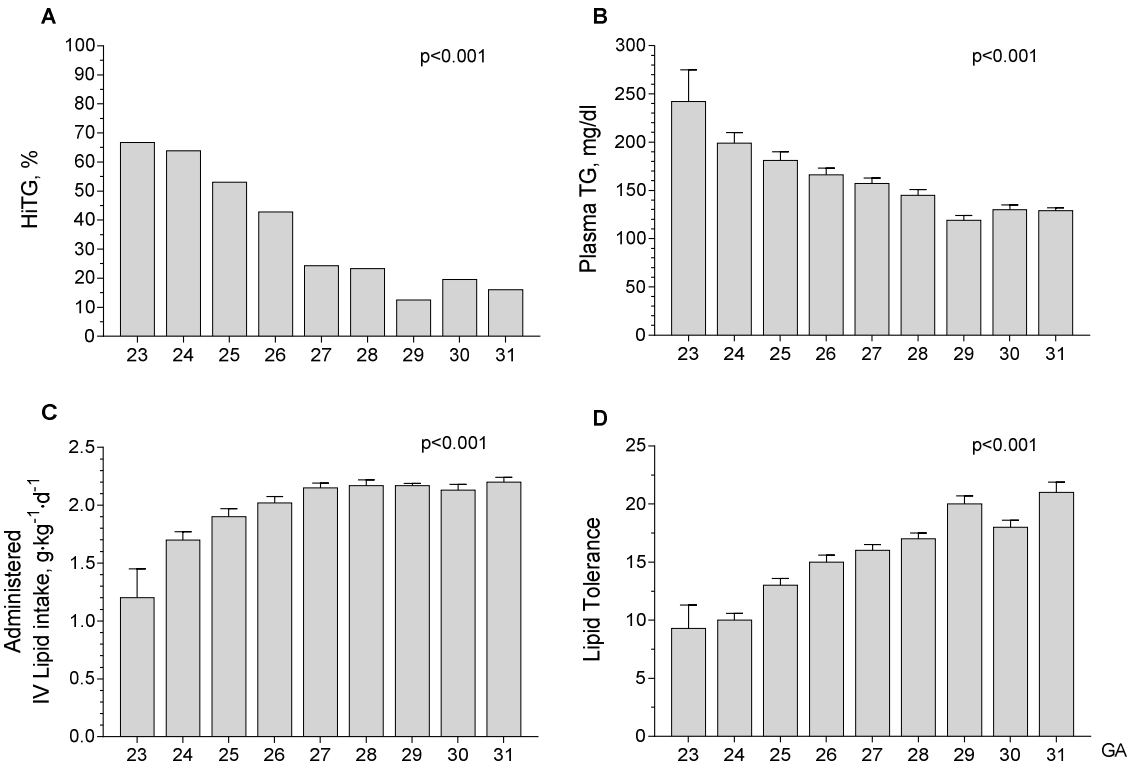

Figure 3.1 *Incidence of HiTG (A), plasma TG concentration (B), administered IV lipid intake (C) and LT (D) among GA weeks (230/7–236/7, n=12; 240/7–246/7, n=47; 250/7–256/7, n=49; 260/7–266/7, n=104; 270/7–276/7, n=115; 280/7–286/7, n=106; 290/7–296/7, n=80; 300/7–306/7, n=102; 310/7–316/7, n=57). Data were presented as mean±SE or percentage. One-way ANOVA or Chi-Square test were used for the analysis. P-value<0.05 was considered significant. LT was calculated as the ratio of intravenous lipid intake (g·kg-1) preceding sampling to plasma triglycerides (g·dL-1). Administered IV lipid intake was calculated as the mean administered intake from DOL 4 to 7. GA: gestational age; HiTG: hypertriglyceridemia; IV: intravenous; TG: triglyceride.*

Risk factors for HiTG

Perinatal variables associated with HiTG at univariate analysis were GA, SGA$^{10th\ centile}$, exposure to antenatal steroids, administered IV lipid intake, fish oil containing lipid emulsions (FO LEs), IV AA intake, DOLs, HIP, RDS, PDA, IVH III-IV, asphyxia and Apgar at 5 minutes (Pearson correlation, $p<0.1$). Logistic regression analysis showed that SGA$^{10th\ centile}$, IVH grade III and IV, PDA and IV lipid intake were risk factors for HiTG, whereas older GA, IV AA intake and the use of FO LEs were protective (**Table 3.1**). The risk of HiTG was about twice as high in infants diagnosed with IVH grade III and IV, PDA and SGA and it was reduced by 12% for each additional week of gestation and by 19% for each extra grams of IV AA. HiTG risk was increased by about 200% for each additional gram of IV lipid intake while the use of IV FO LEs reduced the risk of HiTG by about 40%.

Table 3.1 *Multiple logistic regression model to predict risk of HiTG*

Independent factors	Exp(B) (95% C.I.)	p-value
GA (weeks)	0.88 (0.85 - 0.92)	0.000
Apgar at 5 minutes	1.03 (0.92 - 1.15)	0.590
Antenatal steroids	0.98 (0.62 - 1.55)	0.916
HIP	0.50 (0.17 - 1.41)	0.189
IV Lipid Intake (g·kg-1d-1)	1.96 (1.59 - 2.4)	0.000
FO LEs	0.62 (0.46 - 0.84)	0.002
IV AA intake	0.81 (0.66 - 0.98)	0.034
DOLs	1.04 (0.97 - 1.12)	0.217
SGA10th centile	1.94 (1.43 - 2.65)	0.000
Asphyxia	1.83 (0.93 - 3.6)	0.078
IVH grade III and IV	1.80 (1.07 - 3.05)	0.028
PDA	1.36 (1.01 - 1.82)	0.042
RDS	1.19 (0.89 - 1.6)	0.238

P-value<0.05 was considered significant. AA: amino acid; GA: gestational age; DOLs: days of life; FO LEs: fish oil lipid emulsions; HIP: Hypertension in pregnancy; HiTG: hypertriglyceridemia; IV: intravenous; IVH: intraventricular haemorrhage; SGA: small-for-gestational-age; PDA: patent ductus arteriosus; RDS: respiratory distress syndrome. R^2*= 0.6, p<0.001 (no. of samples: 1812)*

The association between LT and clinical data

Pearson's correlation analysis indicated that LT was associated with GA, $SGA^{10th\ centile}$, exposure to antenatal steroids, FO LEs, DOLs, Apgar at 5 minutes, IV AA intakes, IV glucose intakes, RDS, asphyxia, PDA, LOS and IVH III-IV (Pearson correlation, p<0.1).

Multiple linear regression analysis showed that older GA, the use of FO LEs and the IV AA and glucose intakes were positively associated with LT. $SGA^{10th\ centile}$, PDA, RDS, IVH III-IV and LOS were negatively associated with LT (**Table 3.2**).

Table 3.2 *Multiple linear regression model to predict LT.*

Independent factors	B (95% C.I.)	p-value
GA (weeks)	0.24 (0.11 - 0.37)	0.000
Apgar at 5 minutes	0.16 (-0.19 - 0.52)	0.369
Antenatal steroids	1.05 (-0.44 - 2.54)	0.166
FO LEs	1.89 (1.00 - 2.78)	0.000
IV AA intake(g/kg/d)	1.18 (0.48 - 1.88)	0.001
IV glucose intake(g/kg/d)	0.61 (0.39 - 0.82)	0.000
DOLs	0.07 (-0.13 - 0.28)	0.486
RDS	-1.14 (-2.02 - -0.26)	0.011
PDA	-1.73 (-2.68 - -0.78)	0.000
Asphyxia	-2.28 (-4.87 - 0.31)	0.084
SGA10th centile	-2.96 (-3.94 - -1.90)	0.000
IVH grade III and IV	-3.96 (-5.90 - -2.02)	0.000
LOS	-8.56 (-16.70 - -0.42)	0.039

P-value<0.05 was considered significant. AA: amino acid; DOLs: days of life; FO LEs: fish oil lipid emulsions; GA: gestational age; IV: intravenous; IVH: intraventricular haemorrhage; LOS: late onset sepsis; SGA: small-for-gestational-age; PDA: patent ductus arteriosus; RDS: respiratory distress syndrome. R^2*=0.8, p<0.001 (no. of samples: 1767)*

Discussion

We studied HiTG in 672 preterm infants on routine PN from birth who were treated according to the same clinical guidelines and nutrition protocol over a 14 years' period.

Next to the incidence of HiTG we calculated, in all our patients, LT. In our unit where a tight lipid monitoring policy is effective in keeping plasma TG below the maximum threshold value of 250 mg/dl, we believe that LT could be used as a reliable index of the IV lipid metabolizing capacity of

a subject during PN. In addition, while HiTG is a dichotomous variable, LT offer the advantages of a continuous variable.

We would like to comment the following findings.

Our study patients received markedly lower lipid intakes than the recommended target values of current guidelines. Seventy per cent of HiTG episodes occurred at IV lipid intake of less than 2.5 g/kg/d and less than 25% of our patients were able to receive more than 3 g/kg/d without developing HiTG. Our data is in line with other reports (6, 10), that also show that lipid clearance is often impaired in very preterm infants.

We wonder if a more moderate IV lipid intake for these infants could avoid repeated blood sampling for lipid titration. On the other hand, additional studies are needed to clarify the optimal strategy for lipid titration both in terms of target lipid intakes and of plasma TG threshold values.

We recently published a case-control study comparing the clinical outcome of 136 pairs of preterm infants with and without HiTG (13), and surprisingly, we found no differences neither in the major complication of prematurity nor in anthropometry and neurodevelopment at 2 years of corrected age. These findings were quite reassuring to us and this information may prompt new study exploring the safety of higher threshold values.

We found that the incidence of HiTG was markedly higher in preterm infants below 28 weeks' gestation and in SGA infants. This information is in line with previous reports (1, 5, 10-12, 15) and it can be explained by the low lipoprotein lipase activity (6, 16) and/or worse endothelial function associated with prematurity and SGA status (17, 18).

In this paper we studied LT as the ratio of intravenous lipid intake (mg/kg/d) preceding sampling to plasma triglycerides (g/dL). Please note that, a low efficiency of TG metabolism can occur even in case of plasma TG concentration within the normal ranges (i.e. 200 mg/dL) with a low IV lipid intake (1.0 g/kg/d). Conversely, with high IV lipid intake (i.e. 2.5 g/kg/d) LT can be in the high range if plasma TG concentration is low (i.e.80 mg/dL).

Because of the continuous nature of this variable throughout the whole range of lipid intake and across all plasma TG values we are presently studying if LT could be a useful marker for lipid clearance and ultimately could prevent HiTG. Using a multiple approach, we found a significant association of both HiTG and LT with several of the major complications of prematurity.

We also found a significant association between LT and both AA and glucose intakes. We do not have a clear explanation for this finding, and we could not find any published data on the interaction

between IV AA and glucose intake with LT. We wonder if this association may occur in somewhat healthier babies or if higher glucose and AA intake could be capable of stimulating insulin production which in turn can stimulate lipoprotein lipase and improve LT (19, 20). In our view, the magnitude of this difference is, however, biologically small.

Of interest, the use of FO LEs was significantly associated with markedly and significantly lower incidence of HiTG and a higher LT. The effect of down-regulation of lipogenesis by FO LEs is well known in humans and it has also been reported by others and by our group in preterm infants on PN (21-24).

This study has some limitations. It is a retrospective study and it carries an inherent risk of biases. However, all data were prospectively recorded according to predefined criteria and nutrition protocols were unchanged during the study period. LT could not be calculated in case of nil IV lipid intake, but this occurred only in 3 patients.

In conclusion, in our large study cohort of 672 preterm infants on routine PN and with a strict TG monitoring, the IV lipid intakes were markedly lower than the target values recommended by ESPGHAN and ASPEN guidelines. LT was markedly reduced especially in preterm infants born between $23^{0/7}$ and $27^{6/7}$ weeks' gestation and in association with some of the major complication of prematurity. We believe that recommendations targeted to GA subgroups should be considered. The cost-benefit ratio of higher thresholds for plasma TG during PN remains to be studied.

Acknowledgments

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Abbreviations

AA: amino acid; BW: birth weight; DOL: day of life; EOS: early onset sepsis; FO LEs: fish oil lipid emulsions; GA: gestational age; HiTG: hypertriglyceridemia; IV: intravenous; IVH: intraventricular haemorrhage; LOS: late onset sepsis; NICU: neonatal intensive care unit; PDA: patent ductus arteriosus; PN: parenteral nutrition; RDS: respiratory distress syndrome; SGA: small-for-gestational-age; TG: triglycerides.

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Chapter 4. Oxygen Saturation (SpO2) to Fraction of Inspired Oxygen (FiO2) Ratio in Preterm Infants on Routine Parenteral Nutrition with Conventional or Fish Oil Containing Lipid Emulsions

Alessio Correani, MSc, PhD[1], Valentina Dell'Orto, MD[1], Stefano Nobile, MD, PhD[1], Luca Antognoli, MSc[2], Paolo Marchionni, MSc, PhD[1], Ilaria Giretti, MSc[2], Chiara Monachesi, MSc[2], Clementina Rondina, MD[1], Maria Laura Palazzi, MD[1], Chiara Biagetti, MD[1], Rita D'Ascenzo, MD[1], Adriana Pompilio, MSc[1], Manuela Simonato, MSc, PhD[3], Paola Cogo, MD, PhD[4], Ilaria Burattini, MD[1], Virgilio P Carnielli, MD, PhD[1,2]
1: Department of Mother and Child Health, Azienda Ospedaliero Universitaria Ospedali Riuniti di Ancona Umberto I, G M Lancisi, G Salesi, Ancona, Italy; 2:Polytechnic University of Marche, Ancona, Italy; 3: PCare Laboratory, Fondazione Istituto di Ricerca Pediatrica, "Città della Speranza", Padua, Italy; 4:Department of Medicine, University of Udine, Udine, Italy.

Abstract

Introduction

The benefits of intravenous (IV) fish oil (FO), as a source of n-3 long-chain polyunsaturated fatty acids, on lung growth in preterm infants remains controversial.

Aim

To evaluate if IV FO improves lung growth in small preterm infants on routine parenteral nutrition (PN).

Materials and Methods

We retrospectively reviewed prospectively collected data of preterm infants with a birth weight <1250g who received routine PN from birth. We compared patients who received FO containing IV lipid emulsions with infants who received conventional emulsions (CNTR). The oxygen saturation (SpO_2) to fraction of inspired oxygen (FiO_2) ratio (SFR) at 36 weeks (W) of gestation was chosen as primary outcome variable to assess lung growth.

Results

Four hundred and seventy-seven infants were studied: 240 received IV FO and 237 CNTR. While exposure to antenatal glucocorticoids was higher in IV FO group than in CNTR (95 vs 90%, p=0.04), there were no differences in birth data, enteral and parenteral nutrition intakes, ventilator supports and drug therapies. The incidence of the most common complications of prematurity at 36W was not different (bronchopulmonary dysplasia was 27 vs 21% in IV FO vs CNTR infants, p=0.1). Weight gain from birth to 36W was marginally, but significantly, higher (+0.5 g/kg/day, p=0.03) in IV FO group vs CNTR. SFR increased from 32W to 36W in all study patients (p<0.001). IV FO infants had significantly lower SpO_2 from 33W to 35W (p<0.001) and lower (worse) SFR at 36W (432±57 vs 444±51, p=0.026) compared to CNTR.

Conclusion

Contrary to our hypothesis, the use of FO containing IV lipid emulsions for the routine PN of the preterm infant did not improve lung growth compared to the infants who received conventional IV lipid emulsions.

Introduction

Fish oil (FO) is a rich source of n-3 long-chain polyunsaturated fatty acids (LCPUFA) which have been reported to suppress inflammatory responses and to modulate lipid metabolism(1, 2).

In adults, FO has been associated with an improvement in the arterial oxygen concentration (PaO2) to the fraction of inspired oxygen (FiO2) ratio, a reduction in the need and duration of mechanical ventilation (MV), a decreased risk of pulmonary complications, and shorter hospital stay (3, 4). In infants, the benefits of an n-3 LCPUFA rich diet on lung development and respiratory outcomes are still unclear. Some studies suggested a potential protective effect of n-3 LCPUFA on bronchopulmonary dysplasia (BPD) (5-8), whereas other studies did not show benefits (9-11).

Recently, Collins et al. studied 1273 preterm infants born before 29 weeks (W) of gestation in a randomized clinical trial. These authors investigated the effect of 60 mg/kg/day docosahexaenoic acid (DHA) given orally as FO supplement on the incidence of BPD. They observed a higher risk of BPD in the intervention group that received enteral FO (12, 13).

Based on these conflicting data, we investigated the effect of IV FO on lung growth in small preterm infants who routinely receive parenteral nutrition (PN) from birth. We decided to use SpO2 (oxygen saturation), FiO2 and the non-invasive SpO2 to FiO2 ratio (SFR) rather than FiO2 alone or the invasive PaO2 to FiO2 ratio to assess lung growth. SFR has been shown to be a reliable surrogate for PaO2 to FiO2 ratio and a proxy for oxygen diffusion in lungs (14-17).

The aim of this study was to evaluate if IV FO improves lung growth in small preterm infants on routine PN.

Materials and methods

Study infants

We retrospectively studied all infants with a birth weight (BW) of 400-1249 g, gestational age (GA) between 24 and 36W, inborn or outborn admitted before 24 hours of age, on routine PN from the first hours of life, who were consecutively admitted to the “G. Salesi” Children Hospital, Ancona, Italy, between October 2008 and June 2017. Individual patients received only one of the 5 IV lipid

emulsions routinely acquired by the hospital pharmacy and assigned at birth by the neonatologist according to pharmacy availability. Some patients participated to clinical trials conducted in our NICU (18-20). The IV lipid emulsions used were: FMS (10:40:50% - FO:soybean oil:medium chain triglycerides (MCT)), MOSF (15:30:30:25% - FO:MCT:soybean oil:olive oil), S (100% - soybean oil), MS (50:50% -MCT:soybean oil) and OS (80:20% - olive oil:soybean oil). Exclusion criteria were severe malformations, inborn errors of metabolism, neonatal transfer to another NICU before 24 hours of life, start of PN after 24 hours of life and death in the first day of life without receiving PN. The study was approved by the local ethics committee (n. 20/1359).

Nutrition Protocol Management

All preterm infants started full PN with glucose, amino acids, and lipids at about 1 hour after birth, according to the NICU nutrition scheme. The nutrition protocols of the different randomized clinical trials since 2008, and nutrition schemes used in the NICU as internal guidelines for the nutrition of extremely low BW infants had similar progression of nutrient intakes. IV lipid emulsions were infused at a dose of 1.0-1.5 g/kg/day on the first day of life (DOL) up to 2.5-3.5 g/kg/day on DOL 5. Glucose was increased from 6-8 g/kg/day to 12-14 g/kg/day from DOL 1 to DOL 5. Amino acids were started at a dose of 1.0-1.5 g/kg/day in the DOL 1 and increased up to 2.5-3.5 g/kg/day on DOL 5. Maximum lipid, amino acid and glucose intakes achieved were then kept constant from DOL 5 to 7. Minimal enteral feeding was provided up to 8 mL/kg/day from DOL 1 to DOL 4, and 16 ml/kg/day from DOL 5 to DOL 7. PN was tapered after DOL 7 and stopped when the infant tolerated about 120 kcal/kg/day orally.

Data collection

Interventions were prospectively recorded using a dedicated software (Neotools®; Interactive, Milan, Italy). SpO2 by pulse oximeter (Masimo®; Milan, Italy) and FiO2 as indicated by ventilators, continuous positive airway pressure (CPAP) devices or hood oximeters (depending on the ventilator support) were prospectively recorded hour-by-hour in the medical records. SFRs were calculated weekly, from 32W to 36W, as the mean of the SFR values recorded during 24 hours on the day that each infant reached a given GA (all GA were reported as post-menstrual age). At least 12 SFR individual values in 24 hours were used to calculate the weekly SFR (21). Data on nutrition, diseases of prematurity and growth were published elsewhere(22).

Outcomes

The primary outcome was the SpO2 to FiO2 ratio (SFR) in small preterm infants on routine PN with conventional or FO containing IV lipid emulsions when they reached 36W. The effect of IV FO on ventilator support, diagnoses and treatments were also studied.

Statistical Analysis

Due to the exploratory nature of the study, no formal sample size calculation was performed. Infants who received IV FO were compared to infants who received conventional IV lipid emulsions (CNTR). Data were reported as mean±SD or ±SE, median [25P-75P] or no. (%). Two-way repeated measures ANOVA, Independent t-test, Mann-Whitney test, and Chi-square test were used as appropriate. Significance was set at $p<0.05$. All statistical analyses were performed using SPSS (v 23.0; SPSS Inc, Chicago, Illinois) and Microsoft EXCEL (v 2016; Microsoft Corp, Redmond, Washington) software.

Results

Four hundred and seventy-seven of the 546 small preterm infants admitted to our NICU from October 2008 to June 2017 met the inclusion criteria and were studied. Eighteen infants admitted after 24 hours, 27 with congenital malformations, 10 who died in the first DOL, 1 transferred before 24 hours of life and 13 who started PN after 24 hours of life were excluded. Of the 477 study infants, 240 received one of the two FO containing IV lipid emulsions and 237 received one of the three conventional IV lipid emulsions.

We found no differences in GA (28±2 vs 28±2W, p=0.5; for details about GA subgroups see online supplement **Table 4.1**), gender (50 vs 46% male, p=0.4), BW (942±181 vs 960±197 g, p=0.3), BW-SDS (-0.7±1.0 vs -0.7±1.1, p=0.8), birth length (35.3±2.8 vs 35.5±2.8 cm, p=0.4), birth length-SDS (-0.7±1.0 vs -0.6±1.0, p=0.7), birth head circumference (25.2±1.9 vs 25.3±1.9 cm, p=0.6), birth head circumference-SDS (-0.5±1.0 vs -0.4±1.1, p=0.9), small for gestational age<-2 BW-SDS (12 vs 15%, p=0.4), and Apgar score at 5 min (8 |7-9| vs 8 |7-9|, p=0.5) between IV FO group and CNTR, respectively. More patients who received IV FO were exposed to antenatal glucocorticoids than CNTR (95 vs 90%, p=0.04). There were no differences in parenteral and enteral nutrition protein and energy intakes between the two study groups. At 36W, the incidence of the most common complications of prematurity were not different (BPD was 27 vs 21%, p=0.1 in the IV FO group vs CNTR). Weight gain from birth to 36W was marginally, but significantly higher (+0.5 g/kg/day, p=0.03) in IV FO group vs CNTR. These data were published elsewhere (22).

Table 4.1 *GA subgroups of the study preterm infants on routine PN.*

GA	All study patients, n=477	IV-FO, n=240	CNTR, n=237	p-value*
24-26W, no. (%)	143 (30.0)	71 (49.7)	72 (50.3)	0.8
27-29W, no. (%)	208 (43.6)	108 (51.9)	100 (48.1)	0.5
30-32W, no. (%)	112 (23.5)	55 (49.1)	57 (50.9)	0.8
33-35W, no. (%)	14 (2.9)	6 (42.9)	8 (57.1)	0.6

*CNTR: controls; FO: fish oil; GA: gestational age; IV: intravenous W: weeks; PN: parenteral nutrition. *Chi-square test was used for the statistical analysis.*

Selected interventions and respirator/ventilator parameters during the first week of life are reported in **Table 4.2**. No differences in the duration of mechanical ventilation and CPAP from birth to 36W between IV FO and CNTR infants were found (MV: 5 [1-13] vs 5 [1-12] days, p=0.9; CPAP: 24 [5-41] vs 21 [5-36] days, p=0.6, respectively).

Table 4.2 *Selected interventions and respirator/ventilator parameters from birth to DOL 7 in IV FO and CNTR infants.*

	IV FO n=240	CNTR n=237	Diff.	p
Antibiotics				
Ampicillin, no.(%)	204 (85%)	198 (84%)	+6(+1%)	0.7
duration (days)	6.0 [3.4-6.9]	6.0 [3.4-6.9]	+0.0	0.9
Gentamicin, no.(%)	204 (85%)	199 (84%)	+5(+1%)	0.8
duration (days)	6.0 [3.2-6.9]	5.7 [3.4-6.9]	+0.3	1.0
Vancomycin, no.(%)	5 (2%)	3 (1%)	+2(+1%)	0.7
duration (days)	1.4 [0.6-2.2]	0.3 [0.2-2.0]	+1.1	0.8
Meropenem, no.(%)	4 (2%)	5 (2%)	-1(-0%)	0.8
duration (days)	1.8 [0.6-3.6]	0.3 [0.3-1.0]	+1.5	0.4
Sodium bicarbonate, no.(%)	134 (56%)	139 (59%)	-5(-3%)	0.5
duration (days)	2.4 [1.6-3.9]	2.6 [1.8-3.8]	-0.2	0.6
RBC Transfusion, no. (%)	9 (4%)	15 (6%)	-6(-2%)	0.2
Patent ductus therapy				
Ibuprofen, no.(%)	105 (44%)	109 (46%)	-4(-2%)	0.6

duration (days)	2.0 [1.9-2.0]	2.0 [2.0-2.0]	+0.0	0.4
Indomethacin, no.(%)	11 (5%)	14 (6%)	-3(-1%)	0.5
duration (days)	1.0 [0.7-1.0]	0.8 [0.6-1.0]	+0.2	0.2
Systemic hypotension *				
Dopamine, no.(%)	34 (14%)	31 (13%)	+3(+1%)	0.7
duration (days)	1.7 [0.8-2.3]	1.2 [0.5-2.4]	+0.5	0.7
Dobutamine, no.(%)	23 (10%)	16 (7%)	+7(+3%)	0.3
duration (days)	1.2 [0.4-2.3]	1.4 [0.3-2.4]	-0.2	1.0
NO therapy, no.(%)	11 (5%)	4 (2%)	+7(+3%)	0.1
Intubated within 1 hour of life, no.(%)	91 (38%)	79 (33%)	+12(+5%)	0.3
Surfactant, no.(%)	134 (56%)	133 (56%)	+1(-0%)	1.0
FiO_2 **, %				
at 1-3 hours of life, %	31.9±16.8	30.3±13.6	+1.6	0.3
at 1-24 hours of life, %	25.6±9.2	25.5±8.6	+0.1	0.9
at 1-48 hours of life, %	25.2±8.2	25.1±7.5	+0.1	0.9
SpO_2 **, %				
at 1-3 hours of life, %	93.6±2.9	93.6±3.0	0	0.8
at 1-24 hours of life, %	94.1±3.0	94.0±2.9	+0.1	0.8
at 1-48 hours of life, %	94.1±2.9	94.0±3.0	+0.1	0.7
SFR **,				
at 1-3 hours of life,	353±109	360±98	-7	0.5
at 1-24 hours of life,	403±69	402±65	+1	0.9
at 1-48 hours of life,	405±64	404±61	+1	0.8
Ventilator support				
VAM, no.(%)	88 (37%)	95 (40%)	-7(-3%)	0.4
VNM, no.(%)	17 (7%)	13 (5%)	+4(+2%)	0.5
HFV, no.(%)	32 (13%)	31 (13%)	+1(+0%)	0.9
CPAP, no.(%)	85 (35%)	93 (39%)	-8(-4%)	0.4

** from birth to DOL 3; ** up to DOL 2. DOL: day of life; CPAP: continuous positive airway pressure; FiO_2: fraction of inspired oxygen; HFV: high frequency ventilation; NO: nitric oxide; RBC: red blood cells; SFR: SpO_2 to FiO_2 ratio; SpO_2: oxygen saturation; VNM: non-mandatory ventilation; VAM: artificial mechanical ventilation/assisted ventilation. Data are given as mean±SD, median [25P-75P] or no. (%). Mann Whitney test, Independent t-test and chi-square test were used for the statistical analysis.*

In all study patients, SFR increased from 32W to 36W (time: p<0.001). There were no differences in FiO2 from 32W to 36W, whereas SpO2 from 33W to 35W were significantly lower in IV FO group than CNTR (**Figure 4.1**).

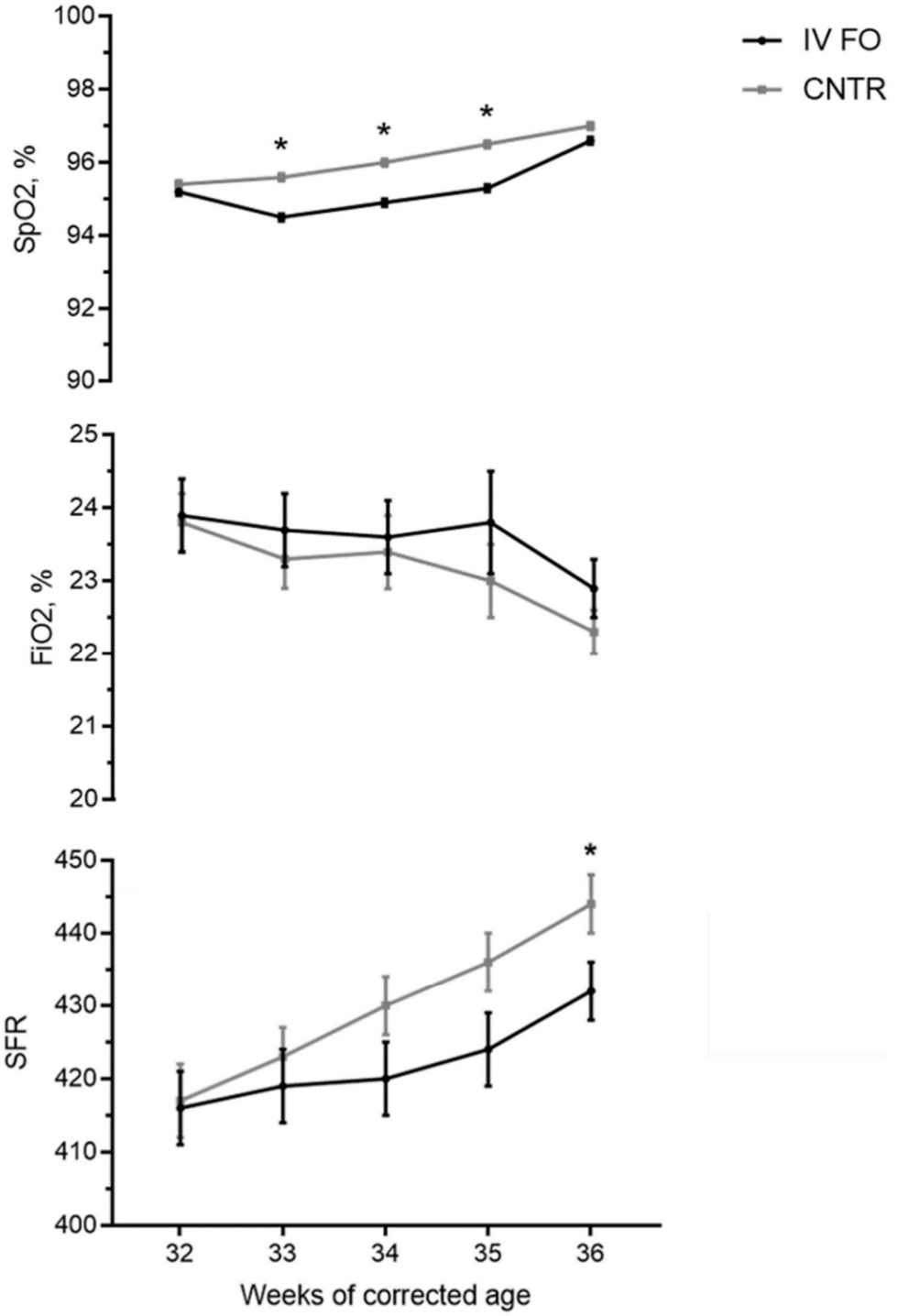

Figure 4.1 *SpO2, FiO2 and SFR from 32W to 36W in IV FO and CNTR infants.*

*Data are given as mean±SE. Independent t-test was used for the statistical analysis (*p<0.05). FiO2: fraction of inspired oxygen; SFR: SpO_2 to FiO_2 ratio; SpO_2: oxygen saturation.*

SFR up to 35W was not different between IV FO and CNTR (32W: 416±68 vs 417±66, p=0.8; 33W: 419±68 vs 423±64, p=0.5; 34W: 420±68 vs 430±62, p=0.1; 35W: 424±70 vs 436±60, p=0.057). Using two-way repeated measures ANOVA, both the overall effect of IV lipid emulsions and the interaction between IV lipid emulsions and time on SFR were not statistically significant (time: p<0.001; IV lipid emulsion: p=0.15; time*IV lipid emulsion: p=0.07). SFR at 36W (primary outcome) was significantly lower in IV FO group compared to CNTR (432±57 vs 444±51, p=0.026).

SFR at 36W was significantly lower in IV FO than in CNTR even when it was calculated as a mean of all weekly available SpO2 and FiO2 datapoints (see online supplement **Table 4.3**).

There were no differences neither in patient dropouts nor in missing SFR data (data not shown).

Table 4.3 *SFR by weeks of post menstrual age#*

Post menstrual age	IV-FO, n=240	CNTR, n=237	p-value*
32W	418±66	422±60	0.5
33W	419±68	427±58	0.2
34W	422±67	433±59	0.069
35W	426±66	437±59	0.053
36W	433±59	444±51	0.042

*#SFR was calculated as a mean of all weekly available FiO2 and SpO2 datapoints. CNTR: controls; FO: fish oil; IV: intravenous W: weeks. * Independent t-test was used for the statistical analysis.*

Discussion

In this retrospective study, including 477 preterm infants, we showed a significantly lower (worse) SFR value at 36W in IV FO infants than in CNTR. These data are in line with the findings of Collins et al. reporting a higher incidence of BPD in 1273 preterm infants who received high doses of enteral FO (12, 13). In our study, the incidence of BPD was not significantly higher in IV FO group compared to CNTR even if p-value was 0.1. Using the BPD data from this cohort, we performed an a posteriori sample size calculation and estimated that about 1000 infants are required to reach a statistical significance with a 90% power. In our view, our findings are important as FO containing IV lipid emulsions have gained popularity over the last few years because of the alleged benefits on infant neurodevelopment or on liver function. However, these preparations have not

been studied in large randomized clinical trials with adequate power to detect clinically significant outcomes in preterm infants such as BPD and neurodevelopmental outcomes.

Our group has previously shown that FO containing IV lipid emulsions in preterm infants on routine PN were associated with a statistically significant reduction in plasma lipids (free cholesterol, cholesterol esters and phospholipids) (23). We also reported a significant reduction of lipogenesis in preterm infants randomized to receive IV FO vs infants that received conventional IV lipid emulsions(18). Weight gain was slightly higher in patients who received IV FO even if lipogenesis could be significantly reduced (22). If reducing plasma lipids seems to be desirable in adults, the same may not apply to the fast-growing preterm infant in whom lipogenesis plays a key role in lipid biosynthesis which in turn is extremely important for lung function (surfactant synthesis), brain maturation (myelinization) and other growth-related phenomena.

FO has also been reported to reduce inflammation both in infants and in adults (24, 25). If the anti-inflammatory properties of FO in human adults with sepsis or polytrauma may be desirable, the role of FO in a stable uncomplicated preterm infant who receives PN for gastrointestinal intolerance and for sustaining growth is less clear. During the first stages of life low-grade inflammation may be required for an adequate lung development (26-28). Further studies are needed to clarify this issue as well as to investigate the risk benefit ratio of IV FO in preterm infants taking into account lung and brain development.
This retrospective study has limitations and it carries the risks of inherent biases. However, we did not find any significant difference at birth and in dropouts from birth to 36W in the two study groups. This is probably because of the well standardized population, as we enrolled all the consecutive admitted preterm infants with a birth weight of less than 1250 g and they all received routine PN starting within the first hours from birth. Our PN and enteral nutrition schemes remained unchanged over the last decade. Because of these considerations and because PN components were chosen based on product availability at the pharmacy we considered the present study as a quasi-randomized study.

We would like to briefly comment the statistically different prenatal steroid treatment which was administered more frequently in the IV FO infants than CNTR. Given the quasi-randomized nature of the study we believe that this difference is a chance finding as it often occurs in case of multiple comparisons. We speculate that even if this were to be a true difference this would have favoured

lung maturation in IV FO group that they had the worst respiratory outcome. If this were to be the case, we speculate that the difference in the primary outcome measure would have been even larger.

In conclusion, in a large cohort of small preterm infants on routine PN from birth, we found that early administration of FO containing IV lipid emulsions was not associated with a better lung growth. We strongly recommend that high-quality evidence from large randomized trials is necessary to assess the effect of IV FO on lung development and function before the widespread use of high doses of IV FO in small preterm infants.

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Abbreviations

BW= birth weight; BPD= bronchopulmonary dysplasia; CNTR= controls; DHA= docosahexaenoic acid; DOL= day of life; FiO_2: fraction of inspired oxygen; FO= fish oil; GA= gestational age; IV= intravenous; LCPUFA= long-chain polyunsaturated fatty acids; MV= mechanical ventilation; NO=nitric oxide; PN= parenteral nutrition; SDS: standard deviation score; SFR: SpO_2 to FiO_2 ratio; SpO_2: oxygen saturation; W= weeks.

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Chapter 5. Does intravenous fish oil improve neurodevelopment of extremely low birth weight preterm infants on parenteral nutrition?

Chiara Biagetti[a], Alessio Correani[a], Rita D'Ascenzo[a], Enrica Ferretti[a], Cecilia Proietti[a], Luca Antognoli[a], Ilaria Giretti[a], Adriana Pompilio[a], Paola Cogo[b], Virgilio P Carnielli[a]*
[a]Division of Neonatology, Polytechnic University of Marche, Salesi Children's Hospital, Via Corridoni 11, 60123 Ancona, Italy
[b]Department of Medicine, University Hospital S. Maria della Misericordia, University of Udine, Piazzale Kolbe 3, 33100 Udine, Italy

Abstract

Background and aims

Preterm infants are at increased risk of long-term neurodevelopmental disabilities (NDD). Long chain n-3 fatty acids (n-3 LCPUFA) play a key role during the development of the central nervous system and studies in preterm infants showed benefits of docosahexaenoic acid (DHA) and arachidonic acid (ARA) supplementation for visual and cognitive development. In recent years fish oil has been added to the fat blend of intravenous (IV) lipid emulsions (LE) but to date scanty data are available on neurodevelopmental outcome of preterm infants that received fish oil containing LE. We studied the effect of fish oil containing IV LE vs standard IV LE on neurodevelopment in a large cohort of preterm infants who received routine parenteral nutrition (PN) from birth.

Methods

We retrospectively reviewed the neurodevelopmental outcome of 440 preterm infants (birth weight (BW): 400-1249 g and gestational age (GA) at birth: 24^{+0} - 35^{+6} weeks (W) of postmenstrual age) admitted to our NICU between Oct-2008 and June-2017, who received routine PN with different LE, with and without fish oil (IV-FO vs CNTR). We compared neurodevelopment at 2 years corrected age by the Bayley III development scale and the incidence of NDD.

Results

Demographics, birth data and the incidence of the main clinical short-term outcomes of prematurity were similar in the two groups (IV-FO: n=178, GA 197±14 days, BW 931±182 g; CNTR: n=192, GA 198±15 days, BW 944±194 g). No differences were found in maternal demographics nor in both maternal and paternal educational levels between the two groups. Cognitive (COG) and language (LANG) scores were not significantly different between IV-FO and CNTR (92±15 vs 93±13, p=0.5; 93±17 vs 94±17, p=0.9, respectively), while the motor score (MOT) tended to be lower in the IV-FO group than CNTR (98±12 vs 100±13, p=0.055). No differences were found in the incidence of NDD in the two groups.

Conclusions

Contrary to our hypothesis, the use of fish oil containing LE in a large cohort of preterm infants on routine PN did not result in better neurodevelopment. Large randomized controlled trials are needed to clarify the impact of the widely used fish oil containing LE on neurodevelopment of preterm infants.

Introduction

Infants born preterm are at increased risk of long-term neurodevelopmental disabilities (NDD), such as cerebral palsy, cognitive disability and sensorineural defects (blindness and/or deafness) in the first 2 years of life, and memory or attention deficits at school-age. Among the different clinical risk factors that could be identified in these patients, an inadequate nutrient supply after birth has been hypothesized to contribute to poor neurodevelopment (1-5). Long-chain polyunsaturated fatty acids (LCPUFA) in general, and more specifically docosahexaenoic acid (DHA) and arachidonic acid (ARA), are important structural lipid components of neural cell membranes. Their accretion primarily occurs during the third trimester of gestation, thus infants born prematurely are deprived of the placental transfer of these essential lipids and may be at risk of postnatal LCPUFA nutritional deficit and subsequent impaired central nervous system development (6). Some studies in preterm infants showed benefits of DHA and ARA supplementation for retinal and cognitive development, especially in those born with an extremely low birth weight. Even if controversy still exists on whether they are to be considered essential nutrients, LCPUFA supplementation is now recommended for the preterm infant (7, 8). During the last decade, intravenous (IV) DHA has been provided to very low birth weight preterm infants, as fish oil was added to the fat blend of the lipid emulsions (LE) used for parenteral nutrition (PN). While data are available on red blood cells and plasma fatty acids concentrations, information on the impact of fish oil containing LE on the short term clinical outcomes of preterm infants is still scarce (9-11). To our knowledge, there are only two recent studies reporting on two years neurodevelopmental outcomes of preterm infants who received intravenous fish oil during early routine PN (12, 13). These studies show no effect on neurodevelopment of a fish oil containing multicomponent lipid emulsion in comparison with control infants who received a soy-bean oil LE.

We recently reviewed data from a large cohort of preterm infants admitted from 2008 to 2017 in our neonatal intensive care unit (NICU) who received routine PN with different LE, with and without fish oil. We were unable to find clinically significant differences in growth and short-term clinical outcomes (14).

The aim of this study was to report the two years neurodevelopmental data of this cohort, whose short-term outcomes have been published in this journal in 2018.

Materials and methods

Study design, participants

We retrospectively reviewed data of preterm infants (birth weight (BW): 400-1249 g and gestational age (GA) at birth: $24^{0/7}$ – $35^{6/7}$ weeks (W) of postmenstrual age (PMA)) who routinely received PN from the first day of life, admitted to the NICU of "G. Salesi" Children's Hospital, Ancona, Italy, between October 2008 and June 2017. Individual patients received only one of the 5 LE routinely acquired by the hospital pharmacy and assigned at birth by the neonatologist according to the pharmacy availability (more than one LE is always available at the hospital pharmacy), or sometimes as part of clinical trials (RCT) conducted in our NICU during the study period (15-17). LE were: 1=MSF (40:50 medium chain triglycerides-MCT: soybean oil, 10% fish oil-FO; Lipidem®, B Braun), 2=MOSF (30:30:25 MCT:soybean oil:olive oil, 15% FO; SMOFlipid, Fresenius Kabi), 3=S (100% soybean oil; Intralipid®, Fresenius Kabi), 4=MS (50% MCT and 50% soybean oil; Lipofundin MCT®, B Braun) and 5=OS (80% olive oil and 20% soybean oil; Clinoleic®, Baxter). Patients were grouped, according to the LE they received, into two groups: the IV-FO group, who received MSF and MOSF, and the control group (CNTR), who received S, MS and OS. Exclusion criteria were severe malformations, inborn errors of metabolism, admission after 24 hours of life, death in the first day of life without receiving PN, neonatal transfer in another NICU before 24 hours of life and start of PN after 24 hours of life. Information on the nutritional management was reported in details elsewhere (14).

To the purpose of this study we report the neurodevelopmental outcomes of patients who received fish oil containing LE (IV-FO) versus conventional LE (CNTR). The study was approved by the local ethics committee (Prot. N. 2019 100 OR).

Neurodevelopment assessment

According to our NICU policy, since 2006 all preterm infants with a BW below 1250 g and/or a GA at birth below $32^{0/7}$ W PMA are offered follow-up visits set at 3 month, one and two years of corrected age. To the purpose of this study, we analysed the data obtained at 2 years corrected age. The 2 years follow-up evaluation consisted in the collection of a detailed medical history and a clinical examination performed by the neonatologist, and a cognitive, motor and language

assessment performed by certified dedicated personnel. Information on growth, data about visual acuity or hearing controls, medical and rehabilitation records collected by the neonatologist, and the neurodevelopment evaluations performed during the follow up visits were prospectively recorded, using a dedicated software (Neotools; Interactive, Milan, Italy); information about maternal and paternal education, and perinatal/neonatal data were also prospectively recorded from birth until discharge for each study patient. Growth data were body weight (WT), measured using a digital infant scale, length (L) and head circumference (HC), which were measured using a neonatal stadiometer and a flexible non-stretchable tape respectively. Standard deviation scores (SDS) were computed using Italian growth charts. Caregivers involved with data collection and neurodevelopment evaluation were not aware of the LE assignment.

The Bayley Scales of Infant and Toddler Development (Bayley III) were used to assess cognitive, motor and language abilities of study patients; Bayley III scales are standardized to a mean score ± standard deviation (SD) of 100 ± 15. Composite cognitive (COG), motor (MOT) and language (LANG) scores were considered normal if the score was ≥ mean - 1SD, mildly impaired if the score was < mean-1SD and ≥ mean-2SD, moderately impaired if the score was < mean-2SD and ≥ mean-3SD, and severely impaired if the score was < mean-3SD. Cerebral Palsy (CP) was defined according to the diagnostic criteria of the SCPE Network (18), and the severity of the CP was graded through the Gross Motor Function Classification System (GMFCS) (19).

The overall composite outcome was characterized as no disability, and mild, moderate and severe NDD. No disability was defined as no CP, no hearing and visual impairment and a COG, MOT and LANG score more than mean-1SD (when available). In this category, all criteria must be fulfilled. Severe NDD was defined as any of the following criteria: non-ambulant CP (GMFCS levels 4-5), bilateral blindness, bilateral profound sensorineural hearing loss not improved by aids, or a COG, MOT or LANG score < mean-3SD. Moderate NDD was defined as any of the following criteria: ambulant CP (GMCFS level 3), unilateral blindness, hearing loss improved by aids, or a COG, MOT or LANG score < mean-2SD and ≥ mean-3SD. Mild NDD was defined as any of the following criteria: ambulant CP (GMCFS level 1-2), squints or refractive errors, hearing loss not sufficient to require aids, or a COG, MOT or LANG score < mean-1SD and ≥ mean-2SD. The worst outcome among the different domains classified for the NDD category assigned to each patient; Bayley III scores were considered when available.

The major diagnosis/complications of prematurity were defined according to the Vermont-Oxford definitions, and the physiological definition of bronchopulmonary dysplasia (BPD) was used according to Walsh et al, as previously described (14).

Primary and secondary outcomes

The primary outcome was the mean Bayley III COG score. Secondary outcomes were: growth at 2 years corrected age, the mean Bayley III MOT and LANG scores, the overall incidence of NDD and the incidence of its sub-categories (severe/moderate and mild disabilities); moreover, as neurodevelopment is affected by conditions such as asphyxia, intracranial hemorrhage (IVH), periventricular leukomalacia (PVL), retinopathy of prematurity (ROP) and BPD, we also calculated the mean COG, MOT and LANG scores in a subgroup of infants who did not receive during hospitalization the abovementioned diagnoses.

Data analysis

Sample size was calculated according to data available about NDD in our cohort: patients admitted to our NICU who received PN with a conventional LE and survived to 2 years corrected age had a Bayley III COG score of 94 ± 15. Assuming a patient drop of about one third after checking for death and losses at follow-up, and same variance in Bayley III COG scores in both study patients and controls, we estimated a sample of 155 patients per group to detect a difference of at least 5 points in the COG score with a 80% power at a significance level of 0.05.

Depending on the distribution, data were expressed as group means ± SD, as the median (interquartile range [IQR]) or as a number (percentage). The clinical characteristics of the two groups were compared using the Student's t-test, Mann-Whitney test, $\chi 2$ test or Fisher exact test as appropriate. Significant p-value was set at 0.05.

All statistical analyses were performed using SPSS (v 23.0; SPSS Inc, Chicago, Illinois) and Microsoft EXCEL (v 2016; Microsoft Corp, Redmond, Washington) software.

Results

Of the 477 preterm infants included in our previous paper (14), 44 died before 2 years corrected age (IV-FO=25, CNTR=19, p=0.4) and 35 could not be traced (IV-FO=22, CNTR=13, p=0.1). Of the 398 eligible children, 28 declined participation or moved to other regions and could not participate to the follow-up visits (IV-FO=15, CNTR=13). Three hundred seventy children (84%) could be evaluated for NDD: 178 belonged to the IV-FO group and 192 to the CNTR group. The cognitive assessment started in 2007 and was available in 344 children (IV-FO=167, CNTR=177). Motor assessment started in 2009 and was available in 312 children (IV-FO: 155, CNTR: 157), and the

language assessment started in 2016 and was available in 99 (IV-FO: 60, CNTR: 39). The median age at assessment was 24.8 months corrected age [24.4-25.3].

Demographics, clinical characteristics and the incidence of the main short-term outcome data of the 370 patients evaluated for NDD were not different between the IV-FO and CNTR groups (**Table 5.1**).

Table 5.1 *Demographics and clinical characteristics at birth*

	IV-FO (n=178)[a]	**CNTR (n=192)[b]**	**p**
M/F– no. (%)	92/86 (52/48)	90/102 (47/53)	0.4
GA (d)	197 ± 14	198 ± 15	0.5
BW (g)	931 ± 182	944 ± 194	0.5
Z- score	-0.6 ± 0.9	-0.6 ± 1.1	0.8
Birth length (cm)	35.1 ± 2.8	35.3 ± 2.8	0.6
Z-score	-0.6 ± 1.0	-0.6 ± 1.0	0.9
Birth Head Circumference (cm)	25.1 ± 1.9	25.1 ± 1.8	0.7
Z-score	-0.4 ± 1.0	-0.4 ± 1.1	0.9
SGA 10p – no. (%)	46 (26)	59 (31)	0.3
SGA 2SDS – no. (%)	14 (8)	20 (10)	0.4
Prenatal steroids – no. (%)	170 (96)	177 (92)	0.2
Multiple birth infant – no. (%)	48 (27)	69 (36)	0.06
BPD – no. (%)	50 (28)	43 (22)	0.2
LOS – no. (%)	35 (20)	36 (19)	0.8
IVH ≥ 3 – no. (%)	11 (6)	17 (9)	0.3
Cystic PVL – no. (%)	4 (2)	3 (2)	0.7
ROP ≥ 3 – no. (%)	0 (0)	1 (1)	1.0
NEC≥ 2 – no. (%)	8 (4)	10 (5)	0.9

PDA – no. (%)	105 (59)	113 (59)	1.0
OMM	111 (62)	110 (57)	0.3
Postnatal steroids – no. (%)	21 (12)	12 (6)	0.06
Median cumulative IV DHA intake (mg/kg)	637 [555-805]	133 [119-168]	0.000

[a]***MSF*** *(Lipidem®; B Braun): 50% medium chain triglycerides (MCT), 40% soybean oil (SO), and 10% fish oil (FO);* ***MOSF*** *(SMOFlipid®; Fresenius Kabi): 30:30:25 MCT:SO:olive oil, 15% (FO).*
[b]***MS*** *(Lipofundin MCT®; B Braun): 50% MCT and 50% SO;* ***S*** *(Intralipid®; Fresenius Kabi): 100% SO;* ***OS*** *(Clinoleic®; Baxter): 80% olive oil and 20% SO.*
Data are expressed as Mean ± SD, unless otherwise indicated, $p<0.05$ (Student's t-test, Mann-Whitney test, χ^2 test or Fisher's exact test, as appropriate).
BW: birth weight; DHA: docosahexaenoich acid; GA: gestational age; OMM: own mother's milk (66% of enteral intake as own mother's milk); PDA: patent ductus arteriosus; NEC: necrotizing enterocolitis; IV: intravenous; IVH: intraventricular hemorrhage; PVL: periventricular leukomalacia; ROP: retinopathy of prematurity; BPD: bronchopulmonary dysplasia; LOS: late onset sepsis; SDS: standard deviation score; SGA: small for gestational age.

During hospital stay, mean cumulative IV DHA intake was significantly greater in IV-FO than in CNTR. Baseline clinical data of the patients that underwent a neurodevelopment assessment were similar to those of the 440 patients analyzed in our previous paper (14). Drop out infants were more frequently out born, less likely to be exposed to antenatal glucocorticoids and affected by BPD, and their mother were in a significantly higher proportion of non-Caucasian origin and younger than mothers of patients who were evaluated at the 2 year follow-up visit. The remaining data that were tested were not significantly different between the two groups (data not shown). There were no differences between the drop out patients of IV-FO and CNTR groups (data not shown).
There were no differences in maternal demographics nor in both maternal and paternal educational levels between the two groups (**Table 5.2**).

Table 5.2 *Baseline characteristics of parents*

	IV-FO (n=178)[a]	**CNTR (n=192)[b]**	**p**
Mother			
Age, y – no. (%)			
<20	0 (0)	1 (1)	1.0
≥35	78 (44)	91 (47)	0.5

Ethnicity – no. (%)			
Hispanic	129 (72)	139 (72)	1.0
Black	4 (2)	7 (4)	0.4
Other	45 (25)	44 (23)	0.6
Mother education, y – no. (%)			0.7
≤ 5	0 (0)	1 (1)	1.0
6-8	42 (24)	42 (22)	0.7
9-13	76 (43)	92 (48)	0.3
14-16	14 (8)	10 (5)	0.3
≥ 17	43 (24)	42 (22)	0.7
Unknown	3 (2)	5 (3)	0.5
Father			
Education, y – no. (%)			0.7
≤ 5	1 (1)	2 (1)	0.6
6-8	68 (38)	63 (33)	0.3
9-13	73 (41)	90 (47)	0.3
14-16	8 (4)	6 (3)	0.5
≥ 17	25 (14)	22 (11)	0.5
Unknown	3 (2)	9 (5)	0.1

[a]***MSF*** *(Lipidem®; B Braun): 50% medium chain triglycerides (MCT), 40% soybean oil (SO), and 10% fish oil (FO);* ***MOSF*** *(SMOFlipid®; Fresenius Kabi): 30:30:25 MCT:SO:olive oil, 15% (FO).*
[b]***MS*** *(Lipofundin MCT®; B Braun): 50% MCT and 50% SO;* ***S*** *(Intralipid®; Fresenius Kabi): 100% SO;* ***OS*** *(Clinoleic®; Baxter): 80% olive oil and 20% SO.*
Data are expressed no. (%), $p<0.05$ (χ^2 test or Fisher's exact test, as appropriate).

The two groups were also similar in early respiratory severity: surfactant therapy (53% vs 55% in IV-FO and CNTR, p=0.6), days of respiratory support (37 d [6-64] vs 32 d [8-54] in IV-FO and CNTR, p=0.6) or duration of oxygen therapy (495 hours [6-1343] vs 495 hours [39-1183] in IV-FO and CNTR, p=0.9) from birth to 36^{+0}W PMA. No differences were found in the use of the main

medications nor in the mean saturation/FiO2 ratio (SFR) in the first period of life between the two groups (**Table 5.3**).

Table 5.3 *Selected interventions during the first week of life*

	IV-FO (n=178)[a]	CNTR (n=192)[b]	p
Ampicillin – no. (%)	152 (85)	162 (85)	0.9
Gentamycin – no. (%)	153 (86)	164 (85)	0.9
Sodium bicarbonate – no. (%)	101 (57)	116 (60)	0.5
Dopamine – no. (%) *	31 (17)	25 (13)	0.2
Dobutamine – no. (%) *	14 (8)	10 (5)	0.3
Ibuprofen – no. (%)	80 (45)	95 (49)	0.4
Indomethacin – no. (%)	11 (6)	14 (7)	0.7
SFR (0-3h)	370±93	372±88	0.8

[a]***MSF*** *(Lipidem®; B Braun): 50% medium chain triglycerides (MCT), 40% soybean oil (SO), and 10% fish oil (FO);* ***MOSF*** *(SMOFlipid®; Fresenius Kabi): 30:30:25 MCT:SO:olive oil, 15% (FO).*
[b]***MS*** *(Lipofundin MCT®; B Braun): 50% MCT and 50% SO;* ***S*** *(Intralipid®; Fresenius Kabi): 100% SO;* ***OS*** *(Clinoleic®; Baxter): 80% olive oil and 20% SO.*
*Data are expressed as no. (%) or Mean ± SD, $p<0.05$ (Student's t test, , χ^2 test, as appropriate). * from birth to DOL 3. DOL: day of life. SFR: saturation/FiO_2 ratio.*

No differences were found in anthropometry at 2 years corrected age between the two groups (**Table 5.4**).

Table 5.4 *Anthropometry at 2 years corrected age*

	IV-FO (n=178)[a]	CNTR (n=192)[b]	p
WT (g)	11504 ± 1718	11563 ± 1718	0.7
Z- score	-0.3 ± 1.2	-0.3 ± 1.3	0.8
L (cm)	86.6 ± 3.9	86.7 ± 3.8	0.9
Z-score	0.5 ± 1.3	0.6 ± 1.2	0.9
HC (cm)	48.1 ± 1.8	48.0 ± 1.8	0.6
Z-score	-0.7 ± 1.3	-0.7 ± 1.3	0.9

[a]***MSF*** *(Lipidem®; B Braun): 50% medium chain triglycerides (MCT), 40% soybean oil (SO), and 10% fish oil (FO);* ***MOSF*** *(SMOFlipid®; Fresenius Kabi): 30:30:25 MCT:SO:olive oil, 15% (FO).*

[b]***MS*** *(Lipofundin MCT®; B Braun): 50% MCT and 50% SO;* ***S*** *(Intralipid®; Fresenius Kabi): 100% SO;* ***OS*** *(Clinoleic®; Baxter): 80% olive oil and 20% SO.*
Data are expressed Mean ± SD, p<0.05 (Student's t test). WT: weight; L: length; HC: head circumference.

No differences were found in the incidence of NDD between the two study groups. The mean COG and LANG scores were not significantly different between IV-FO and CNTR, while the MOT score tended to be lower in the IV-FO group in comparison to the CNTR. Analyzing a subgroup of patients who did not receive a diagnosis of some conditions that affect neurodevelopment (asphyxia, IVH ≥ grade 3, PVL ≥ grade 2, ROP ≥ grade 3 and BPD), the mean COG and LANG scores were not different between IV-FO and CNTR, while the MOT score was significantly lower in the IV-FO group (**Table 5.5**).

Table 5.5 *Neurodevelopmental outcome*

	IV-FO[a]	**CNTR[b]**	**p**
NDD			
Overall disability			
Number of patients	178	192	0.1
Moderate/Severe impairment	8 (5)	3 (2)	0.09
Mild impairment	5 (3)	10 (5)	0.2
No disability	165 (95)	179 (96)	0.5
Both COG and MOT scores available			
Number of patients	155	157	0.7
Moderate/Severe impairment	10 (7)	7 (5)	0.4
Mild impairment	30 (19)	30 (19)	1.0
No disability	115 (74)	120 (76)	0.6
COG, MOT and LANG scores available			
Number of patients	60	39	0.6
Moderate/Severe impairment	10 (17)	4 (10)	0.4
Mild impairment	10 (17)	10 (26)	0.3
No disability	40 (67)	25 (64)	0.8

BAYLEY III SCORES			
All study patients			
COG	n=167 92 ± 15	n=177 93 ±13	0.5
MOT	n=155 98 ± 12	n=157 100 ±13	0.055
LANG	n=60 93 ±17	n=39 94 ±17	0.9
Healthy subgroup of patients*			
COG	n=110 94 ± 14	n=122 96 ± 11	0.3
MOT	n=107 100 ± 10	n=110 103 ± 10	0.02
LANG	n=39 95 ± 18	n=31 97 ± 16	0.6

[a]***MSF*** *(Lipidem®; B Braun): 50% medium chain triglycerides (MCT), 40% soybean oil (SO), and 10% fish oil (FO);* ***MOSF*** *(SMOFlipid®; Fresenius Kabi): 30:30:25 MCT:SO:olive oil, 15% (FO).*
[b]***MS*** *(Lipofundin MCT®; B Braun): 50% MCT and 50% SO;* ***S*** *(Intralipid®; Fresenius Kabi): 100% SO;* ***OS*** *(Clinoleic®; Baxter): 80% olive oil and 20% SO.*
[*]*Patients who did not receive a diagnosis of some conditions that affect neurodevelopment (asphyxia, intracranial hemorrhage ≥ grade 3, periventricular leukomalacia ≥ grade 2, retinopathy of prematurity ≥ grade 3 and bronchopulmonary dysplasia).*
Data are expressed as Mean ±SD or no. (%), $p<0.05$ (Student t-test, χ^2 test or Fisher's exact test, as appropriate).
COG: Bayley III composite cognitive score; LANG: Bayley III composite language score; MOT: Bayley III composite motor score; NDD: Neurodevelopmental Disabilities.

Discussion

Contrary to our hypothesis the use of fish oil containing LE in a large cohort of preterm infants on routine PN, in spite of the provision of the fetal accretion rate of DHA (≈40 mg/kg/day), did not result in a better neurodevelopment at 2 years corrected age.

We would like (1) to discuss the low risk of bias of our study groups, (2) to comment and possibly to interpret our findings and (3) finally to pledge for a RCT before IV FO becomes the standard of care for the PN of the preterm and term infants.

Studies on dietary interventions aiming at demonstrating improved neurodevelopment are difficult to perform, as they require a large sample size and because of the numerous confounding variables

affecting neurodevelopment. In the present study, we report data on neurodevelopment from a large homogenous population of preterm infants with a BW of less than 1250 g who received routine PN starting in the first day of life, as reported before in this journal (14). Because of the study design, the extensive collection of clinical and nutrition data according to predefined criteria and the very close match of the two study groups, we consider the present work as a "quasi randomized" controlled trial. Our study groups were rather similar for the most important variables known to affect neurodevelopment, including diseases/use of medications, demographics, pregnancy data, and perhaps most importantly maternal and paternal education (20, 21). In addition we can also reasonably exclude an effect of intra and extra-uterine growth restriction on the long-term neurologic outcome of these patients (22, 23), as body size (anthropometry) of the 2 study groups at 2 years corrected age was similar and the number of infants that were SGA at birth was not different (26% vs 31% in IV-FO and CNTR group respectively, p=0.3).

We found no differences in NDD between the two groups. We also did not find any difference in mental and language development, as measured by the mean COG and LANG score of the Bayley III scales, between the two groups, while the MOT score tended to be lower in the FO group. This finding is difficult to interpret. On one hand, it seems desirable to provide to preterm infants DHA amounts that meet the fetal DHA accretion rate (16). Moreover, omega-3 fatty acid are less likely to be "pro inflammatory" and may protect the brain from the pre and/or postnatal inflammatory insults, which are known to contribute to the central nervous system damage and affect the neurodevelopmental outcome of the preterm infant. We were therefore expecting a positive effect or at least a tendency to a better neurodevelopmental outcome in patients receiving FO containing LE. On the other hand, however, FO has a markedly higher proportion of DHA than ARA, but both fatty acids are important for brain accretion (24). We discussed in a previous paper by our group that the metabolic effects of FO in small infants may be different than in adults (15). We found that lipogenesis, in preterm infants on routine PN, who were randomized to receive a 10% fish oil containing LE or a standard LE, was significantly reduced in the IV-FO group (15). Moreover, we also recently found that early administration of FO containing IV LE during routine PN of the preterm infant was not associated with a better lung growth compared to the infants who received conventional IV lipid emulsions (25). In summary, our work on IV FO shows that FO does not exert a significant beneficial effect on complications of prematurity, even though we raised the hypothesis that it could impact on growth and function of specific organs in infants, such as brain. By analyzing the entire cohort of infants and the selected subgroup of "healthy" patients, we found a lower MOT score in IV-FO patients, although we cannot comment further these results, as this

study was not powered to detect such differences between the two groups. In addition, despite the reduction in the MOT score in the IV-FO group, given the small difference and the lack of a clear explanation for the result, we can conclude that overall data did not show a significant biological effect on neurodevelopment on preterm infants by using 10 to 15% FO containing LE during routine PN. Torgalkar et al., recently published a retrospective cohort study on neurodevelopment in preterm infants receiving a FO containing multicomponent LE vs an historical group that received soy-based LE, and they concluded there was not a harm in administering IV FO (12) but no clear benefit was demonstrated. Also a more recent RCT by Thanhaeuser et al., comparing two groups of preterm infants receiving a mixed LE containing fish oil vs a soy-based LE, concluded that the use of IV FO did not improve neurodevelopment of extremely low birth weight (ELBW) infants at 12 and 24 months corrected age.

Our study has strengths and limitations and some of these have been discussed above. As we mentioned in our previous paper on the growth of these infants, even if this was not a randomized controlled trial, the large cohort of preterm infants we analyzed and the homogeneity of the preterm population admitted, confer some degree of strength to our results and we believe selection bias if any was negligible. Moreover, we did not have an historical cohort of comparison, and all the patients received the same standard of care within each year. Finally, we have computed all the DHA intakes of the study patients, which allowed us to take into consideration also these more specific data in our analysis.

In conclusion, this large retrospective study showed that the use of 10 to 15% IV FO did not result in a better cognitive, motor and language development in small preterm infants on routine PN. However, to date there is still low-grade evidence for the use of FO LE in neonates and we urgently pledge for a large RCT. Such a study should clarify the impact of IV FO on long-term outcomes and neurodevelopment of ELBW preterm infants, which is one of the most relevant outcomes for this population of frail patients. A large RCT on the use of parenteral FO is likely to have very large social and economic impact on the families and the society, as the majority of these infants nowadays survive into adulthood with variable neurodevelopmental outcomes (26).

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Abbreviations

ARA: arachidonic acid; BW: birth weight; BPD: bronchopulmonary dysplasia; COG: Bayley III composite cognitive score; CP: cerebral palsy; DHA: docosahexaenoic acid; ELBW: extremely low birth weight; GA: gestational age; GMFCS: Gross Motor Function Classification System; HC: head circumference; IQR: interquartile range; IV: intravenous ; IVH: intraventricular hemorrhage; LANG: Bayley III composite language score; LE: lipid emulsion; L: length; LCPUFA**:** long chain polyunsaturated fatty acids; LOS: late onset sepsis.; MCT: medium chain triglycerides; MOT: Bayley III composite motor score; NEC: necrotizing enterocolitis; NDD: neurodevelopmental disabilities; NICU: neonatal intensive care unit; OMM: own mothers' milk; PDA: patent ductus arteriosus; PMA: post menstrual age; PN: parenteral nutrition; PVL: periventricular leukomalacia; RCT: randomized controlled trial; ROP: retinopathy of prematurity; SD: standard deviation; SDS: standard deviation score; SFR: saturation/FiO2 ratio; SGA: small for gestational age; W: week; WT: weight.

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Chapter 6. Blood Urea in Preterm Infants on Routine Parenteral Nutrition: A Multiple Linear Regression Analysis

Ilaria Giretti, MSc[1], Alessio Correani, MSc, PhD[2], Luca Antognoli, MSc[1], Chiara Monachesi, MSc[1], Paolo Marchionni, MSc, PhD[2], Chiara Biagetti, MD[2], Maria Paola Bellagamba, MD[2], Paola Cogo, MD, PhD[3], Rita D'Ascenzo, MD[2], Ilaria Burattini, MD[2], Virgilio P. Carnielli, MD, PhD[1,2].

[1] Department of Odontostomatologic and Specialized Clinical Sciences, Polytechnic University of Marche and Azienda Ospedaliero-Universitaria Ospedali Riuniti Umberto I - G. M. Lancisi - G. Salesi, Ancona, Italy; [2] Division of Neonatology, Department of Clinical Sciences, Polytechnic University of Marche and Salesi Children's Hospital, Ancona, Italy; [3] Department of Medicine, University of Udine, Udine, Italy.

Abstract

Background

Blood urea is considered a marker of amino acid utilization in preterm infants on routine parenteral nutrition. However, the association between blood urea and intravenous amino acid intake remains debated.

Aims

To evaluate the association between blood urea and both nutrition and clinical data, in a large cohort of preterm infants.

Subjects and methods

Consecutively admitted preterm infants with a gestational age of less than 32 weeks and a birth weight lower than 1250 g on routine parenteral nutrition from the first hour of life were studied. Clinical and nutrition data collected hourly during the hospitalization were used in multiple linear regression analysis.

Results

We studied 674 patients and 1863 blood urea determinations. Blood urea concentration was positively associated with blood creatinine concentration, intravenous amino acid intake, patent ductus arteriosus and respiratory distress syndrome, and negatively associated with intravenous non-protein energy intakes, daily weight change, gestational age, being small for gestational age, antenatal steroids therapy and reverse flow in the umbilical artery ($p<0.001$; $R=0.7$).

Conclusions

From a nutrition perspective, in our large cohort of small preterm infants blood urea was positively correlated with intravenous amino acid intake and negatively correlated with intravenous non-protein energy intake. This is in line with current knowledge in human physiology and suggest that a reduction of intravenous amino acid intake based on blood urea concentrations was justified.

Introduction:

In preterm infants on routine parenteral nutrition (PN), blood urea (^{B}Urea) is often considered a marker of protein intake adequacy and amino acid (AA) oxidation (1). However, ^{B}Urea concentration in unstable preterm infants on PN during the first days of life (DOLs) is affected by several clinical factors (2) such as renal function, hydration status, severity of illness and others (3, 4). For these reasons, studies on the association between ^{B}Urea and intravenous (IV) AA intakes showed conflicting results. Some cohort studies showed positive associations (5, 6), while others (1, 2, 7, 8) were unable to find significant correlations between AA intake and ^{B}Urea, leading to the interpretations that the preterm infants had higher requirement for AA and decreasing IV AA intake based on ^{B}Urea concentrations was not necessary. The majority of randomized clinical trials, however, demonstrated that ^{B}Urea was significantly higher in the groups of infants receiving the highest AA intake (9-16). To date, the relation between ^{B}Urea concentration and IV AA intake remains still unclear.

We studied the association between ^{B}Urea and both nutrition and clinical data, in a large cohort of preterm infants with a birth weight (BW) of less than1250 g on routine PN.

Subjects and methods:

Study design and patients

In this retrospective cohort study, all infants admitted to NICU of the "G. Salesi" Children's University Hospital between January 2004 and June 2018 were assessed for eligibility. Inclusion criteria were a BW lower than 1250 g and a gestational age (GA) of less than 32 weeks. Exclusion criteria were: major congenital abnormalities, admission to the NICU after 24 hours of life, death or transferred to another unit before 7 DOLs and missing clinical information.

Nutrition protocols and biochemical monitoring policies were established in 2002, and they are still applied. Accordingly, all study infants with a BW less than 1250 g received routine PN as all in one mixture from the first hours of life. IV AA were infused at dose of 1.5 g/kg/d on the DOL 1, and up to 3.5 g/kg/d on the DOL 5. From DOL 1 to 7, glucose was increased from 6.0 to 14.0 g/kg/d while IV lipids were administered at dose of 1.0-1.5 g/kg/d on the DOL 1 up to 3.5 g/kg/d on the DOL 5, and then kept constant until DOL 7. Infants from day 0 to day 7 were on minimal enteral feeding with human milk, when available, or infant milk formula at a maximum intake of 8 mL/kg/d, from day 1 to day 4 and 16 mL/kg/d from day 5 to day 7. For each infant after day 7, PN was tapered and stopped at a median age of 18 day of life while oral feeding was gradually increased to reach full feed and keep a maximum total fluid intake of 160 mL/kg/d. Elevated ^{B}Urea was arbitrary

defined as a ^{B}Urea concentration higher than 100 mg/dL. In case of elevated ^{B}Urea, after ruling out excessive postnatal weight loss, the IV AA intake was reduced by at least 1.0 g/kg/d and checked again after 24 hours.

Diagnoses and complication of prematurity were prospectively defined according to the Vermont-Oxford and prospectively recorded. BW standard deviation score (SDS) was electronically estimated by using Italian growth charts (17). Small for GA (SGA) was defined as SDS-BW less than 10th centile. Daily weight change was defined as the weight lost or gained expressed as a percentage of the BW.

Asphyxia was defined as cord blood pH less than 7.0 and standard base excess less than -12.0 mmol/L. Cholestasis was defined as plasma direct bilirubin concentration above 1.0 mg/dL.

Non-protein energy (NPE) intakes were obtained from the actual IV lipid and carbohydrates intakes (9 and 4 kcal/g, respectively). ^{B}Urea and blood creatinine were measured as part of routine monitoring on the 3rd, 5th and 7th DOL, by using micro-methods (Reflotron® Plus, Sprint system, Roche Diagnostics s.p.a, Monza – Italy).

Clinical data included in the analysis were prospectively recorded from birth to 7th DOL and were: GA at birth, BW, SDS-BW, Apgar at 5 minutes, hypertension during pregnancy, reverse flow in the umbilical artery, antenatal steroids therapy, daily weight change vs BW, ibuprofen and indomethacin therapy, hemodynamically significant patent ductus arteriosus (PDA), intraventricular hemorrhage (IVH), cholestasis, sepsis, Necrotizing Enterocolitis (NEC), perinatal asphyxia, respiratory distress syndrome (RDS), ^{B}Urea concentration, creatinine concentration, administered IV AA (g/kg/d) and IV NPE (kcal/kg/d). All PN intakes were collected on hourly basis throughout the study. IV AA and NPE intakes were calculated for the 24 hours before sampling by averaging the hourly values (g/kg/d).

The study was approved by the local ethics committee (Prot. 2018117).

Statistical analysis

Descriptive statistics was used to describe the study patients. Infants were divided arbitrarily into two groups depending whether ^{B}Urea value was > 100 at least in one occasion (elevated ^{B}Urea) or ≤ 100 mg/dL in all the determinations (normal ^{B}Urea).

Multiple linear regression analysis was performed in the all study patients to find predictors for ^{B}Urea concentrations in preterm infant on routine PN. Pearson's correlation test was performed to identify variables significantly associated to ^{B}Urea concentration at univariate analysis. Results of univariate analysis were considered statistically significant if p –value was less than 0.1. Multiple

linear regression analysis was developed by using enter method and p-value <0.05 was considered significant. Missing data were handled by using pairwise deletion. IBM SPSS software was used for the statistical analysis (version 23, SPSS Inc, Chicago, Illinois).

RESULTS:

Eight hundred and fifty-one infants with a BW lower than 1250 g and a GA of less than 32 weeks postmenstrual age were admitted to the NICU of "G. Salesi" Children's Hospital from January 2004 to June 2018. One hundred and thirty-five infants were excluded: 31 had congenital malformations; 29 were admitted to the NICU after 24 hours of life, 64 died or were transferred to another unit before 7 DOL and 11 had missing data.

A total of 1863 ^{B}Urea determinations were available for 674 infants. ^{B}Urea concentrations (mean±SD) were 54.5±26.8, 61.2±31.8 and 54.8±31.4 mg/dL on day 3, 5 and 7, respectively. ^{B}Urea concentration >100 mg/dL was found in 116 infants (17%) during the first 7 DOLs with a total of 183 episodes (85% of them occurred between day 5 and day 7). Infants with elevated ^{B}Urea were smaller than patients with normal ^{B}Urea (**Table 6.1**).

The incidence of major complications of prematurity from birth to discharge were: neonatal asphyxia 8% vs 4% (p=0.1), RDS 96% vs 85% (p=0.001), PDA 73% vs 55% (p<0.001), cholestasis 7% vs 11% (p=0.2), NEC grade II - III 6% vs 5% (p=0.5), IVH grade III – IV 16% vs 7% (p<0.001) and late onset sepsis 27% vs 19% (p=0.048) in elevated and normal ^{B}Urea group, respectively.

Table 6.1 *Demographic data and early diagnosis of the study preterm infants*

	Elevated ^{B}Urea (N=116)	**Normal ^{B}Urea (N=558)**	**p**
Gestation Age (days)	185±13	199±14	<0.001
Birth Weight (g)	864±202	934±200	0.001
SDS Birth Weight	-0.02±0.96	-0.76±0.95	<0.001
Total Length (cm)	34.7±3.0	35.3±3.0	0.031
SDS Total Length	0.08±0.82	-0.62±0.95	<0.001
Head Circumference (cm)	24.1±1.8	25.1±1.9	<0.001
SDS Head Circumference	0.08±0.92	-0.52±0.98	<0.001
Gender (male)	57 (48)	272 (49)	0.8
SGA^{10centile}	13 (11)	178 (32)	<0.001
Apgar at 5 minutes	8 |7 8|	8 |7 9|	<0.001
Antenatal Steroids	90 (81)	482 (91)	0.001
Hypertension during pregnancy	7 (6)	176 (32)	<0.001
Reverse Flow in umbilical artery	43 (8)	1 (1)	0.006

Data are presented as mean ± SD, median \|25th 75th perc. \| or no. (%). Independent t-test, Mann Whitney test or Chi-Square test were performed. *p<0.05. SDS: standard deviation score; SGA: small for gestational age.

The mean IV AA and NPE administered intakes during the first 7 DOLs were 2.1±0.9 g/kg/d and 46.7±18.8 kcal/kg/d, respectively. Daily intravenous amino acid and non-protein energy intakes in all patients are shown in **Figure 6.1**.

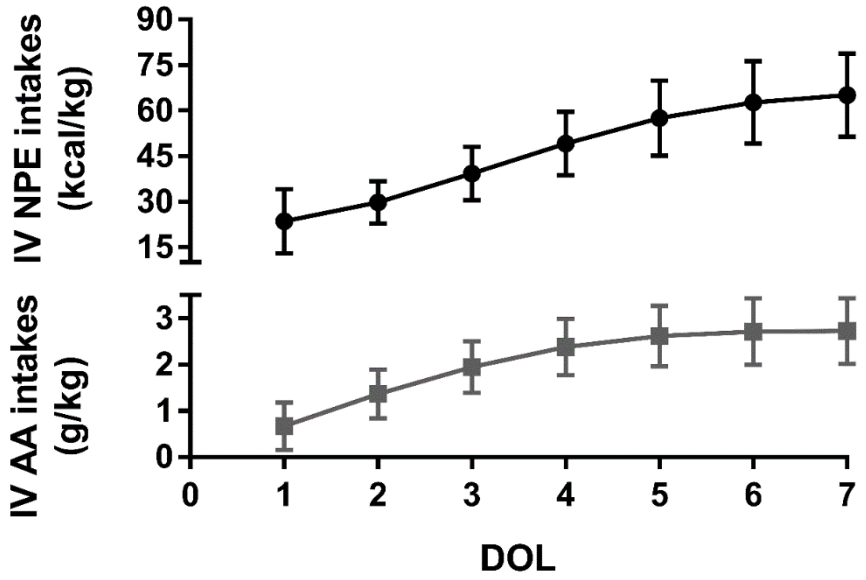

Figure 6.1 *Daily intravenous amino acid and non-protein energy intakes in all study patients.*

Data are shown as mean±SE. AA: amino acid; IV: intravenous; NPE: non protein energy.

Multiple linear regression analysis

Pearson correlation test showed that variables significantly associated with ^{B}Urea concentrations at univariate analysis were: GA (weeks), being SGA^{10centile}, Apgar at 5 minutes, DOL, daily weight change vs BW, hypertension during pregnancy, reverse flow in umbilical artery, antenatal steroids therapy, IV AA intakes, IV NPE intake, creatinine, RDS, sepsis, PDA, IVH grade 3 and 4 and ibuprofen therapy.

Multiple linear regression analysis for ^{B}Urea concentration is shown in **Table 6.2**.

Table 6.2 *Multiple linear regression model predicting* ^{B}*Urea concentration*

PARAMETER	B (95% C.I.)	p
Creatinine (mg/dL)	32.9 (28.8 - 37)	<0.001
IV AA Intake (g/kg/d)	11.5 (9.2 - 13.8)	<0.001
PDA	5.8 (2.6 - 9)	<0.001
IVH III-IV	4.5 (-1.8 - 10.8)	0.2
RDS	4.5 (0.7 - 8.2)	0.020
DOL	1.2 (0.0 - 2.5)	0.1
Apgar at 5 minutes	-0.8 (-1.8 - 0.1)	0.1
Daily weight change vs BW (%)	-1.1 (-1.3 - -0.9)	<0.001
Sepsis	-1.8 (-7.1 - 3.5)	0.5
GA (Weeks)	-3.5 (-4.2 - -2.8)	<0.001
Hypertension during pregnancy	-5.2 (-12.7 - 2.3)	0.2
IV NPE intake (10 kcal/kg/d)	-5.5 (-6.9 - -4.1)	<0.001
Antenatal Steroids therapy	-6.8 (-10.7 - -2.9)	0.001
Reverse Flow in umbilical artery	-7.0 (-12.3 - -1.8)	0.009
SGA10centile	-7.3 (-10.4 - -4.3)	<0.001
Constant	122.1 (101.2 - 142.9)	<0.001

Multiple linear regression analysis, R=0.7, $R^{2=}$ 0.5, p<0.001. AA: amino acids; DOL: day of life; GA: gestational age; IV: intravenous; IVH: intraventricular hemorrhage; NPE: non protein energy; PDA: patent ductus arteriosus; RDS: respiratory distress syndrome; SGA: small for gestational age.

^{B}Urea concentration was significantly positively associated with creatinine, IV AA intake, RDS and PDA disease, whereas it was negatively associated with the use of IV NPE intake, daily weight change vs BW, older GA, the use of antenatal steroids, reverse flow in umbilical artery and being $SGA^{10centile}$ (p<0.001; R=0.7; R^2=0.5). Briefly, ^{B}Urea increased by 32.9 mg/dL for each mg/dL of creatinine, by 11.5 mg/dL for each g/kg of IV AA intakes and by 5.8 and 4.5 if infants had PDA or RDS, respectively. ^{B}Urea decreased by 11 mg/dL for 10% daily weight change vs BW, 5.5 mg/dL

for each 10 kcal/kg of IV NPE, 3.5 mg/dL for week's gestation and about by 7 mg/dL if infants were SGA or had reverse flow in umbilical artery or in case of maternal antenatal steroids therapy.

Discussion:

In our large cohort study ^{B}Urea was positively correlated with IV AA intake, renal failure and weight loss, and negatively correlated with IV NPE. This is in line with current knowledge in human physiology. In addition, we found significant correlations with other clinical variables that we will discuss below.

According to data previously reported by other authors (5, 9-11, 13, 15, 16, 18, 19), high IV AA intake leads to increased ^{B}Urea concentration due to AA oxidation (20, 21). Our data suggest that this was also true in our cohort of small preterm on PN. In our model, administered IV AA intake was positively and significantly correlated with ^{B}Urea (odd ratio +11.5; 95% CI, +9.2 to +13.8; $p<0.001$).

Other cohort studies failed to demonstrate this association, leading to the concept that preterm infants could benefit from higher AA intake before increasing oxidation. We believe that some of the previous cohort studies could not demonstrate a significant correlation between ^{B}Urea and AA intake because of the small sample size or because of limitations in correcting for other important "covariates" as they used "simple univariate" rather than multivariate analyses (1, 2, 7, 8, 22). These studies in our view led to the unsupported interpretation that the preterm infants had higher requirement for IV AA and/or that a reduction of IV AA intake based on ^{B}Urea concentrations was not justified.

We also found that NPE intakes and ^{B}Urea concentrations were negatively correlated, suggesting that AA oxidation is reduced by energy intake favoring protein synthesis (16). In this respect we comment that there is limited information on the most efficient protein/energy ratio in small preterm infants on PN.

According to previous studies (6, 23), we found a strong association between ^{B}Urea and blood creatinine concentration, as a marker of reduced glomerular filtration. We did not find a significant correlation with acute kidney injury. This could be explained by the limited number of patients with acute kidney injury in our cohort or because, in fact, we believe that the major determinant of elevated ^{B}Urea in preterm infants is a reduced glomerular filtration without significant kidney injury. Elevated ^{B}Urea was strongly and significantly associated with postnatal weight loss possibly due to reduce glomerular filtration rate and haemoconcentration (24). In our unit, ^{B}Urea is considered a useful marker of excessive weight loss and it prompts us to check for dehydration and

kidney function. In our clinical practice we consider elevated ^{B}Urea a marker of excessive AA intake or insufficient NPE only when significant weight loss and glomerular filtration rate have been ruled out.

We found an interesting negative association between ^{B}Urea and antenatal steroids therapy which to the best of our knowledge has not been reported before. We speculate that a lower ^{B}Urea concentration in patients exposed to antenatal steroids may reflect a better overall metabolic maturation of the preterm infant including better kidney function (25). A positive effect of steroids on glomerular filtration rate and renal cells differentiation, in human and animal models, have been reported (26, 27).

We do not have a clear interpretation for the association between ^{B}Urea and RDS. Of note this diagnosis and their related therapies were reported to be associated with renal failure, increase in protein catabolism and uraemia (28). Regarding the association between hemodynamically significant PDA and elevated ^{B}Urea this could be explained by kidney hypoperfusion caused by blood-stealing through the open PDA and in addition by the effect of ibuprofen on glomerular filtration rate (29).

This study has limitations. As all retrospective studies, this study carries the risk of unknown confounders. Our model was able to predict about 50% of the ^{B}Urea variability. ^{B}Urea was not measured at all time points (8% of missing determinations). This often happens in clinical care and it could be applicable to several biochemical determinations that are performed in preterm infants during the first DOLs. We believe that missing data did not alter the main study findings as there were no differences in clinical characteristics between the patients with the complete ^{B}Urea determination and those with missing points. The present study was performed over a rather large period (about 14 years) however, all the data were prospectively recorded according to predefine criteria and nutrition protocols were unchanged during the study period. Furthermore, we did not find any differences in the incidence of elevated ^{B}Urea per year during the study period (data not shown).

In conclusion, we studied the association between ^{B}Urea and both nutrition and clinical data in a rather large cohort of preterm infants on routine PN. From a nutrition perspective, we found a strong positive association between ^{B}Urea and IV AA and a negative one between ^{B}Urea and IV NPE intakes. This is in line with current knowledge of human physiology and it may be useful to improve clinical management of infants with elevated ^{B}Urea concentrations. We believe that our work could help clinicians in identifying factors associated with ^{B}Urea in small preterm infants on

PN. Our regression is helping us in our daily clinical work to increase the awareness of factors associated with elevated ^{B}Urea. We speculate that the early recognition and correction, whenever possible, of the factors associated with elevated ^{B}Urea may improve the health status of preterm infants.

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No relevant conflict of interest to declare.

Abbreviations

AA: amino acid; ^{B}Urea: blood urea; BW: birth weight; DOL: day of life; GA: gestational age; IV: intravenous; IVH: intraventricular hemorrhage; NEC: necrotizing enterocolitis; NPE: non-protein energy; PDA: patent ductus arteriosus; PN: parenteral nutrition; RDS: respiratory distress syndrome; SDS: standard deviation score; SGA: small for gestational age.

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