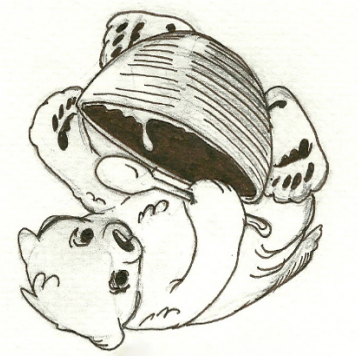
Supplemental Parenteral Nutrition in Pediatric Respiratory Failure
SUPPER Study
Manual of Operations
03.06.2017
NCT: NCT01937884



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Project Summary

Acute hypoxemic respiratory failure (AHRF) accounts for 10% of all pediatric intensive care unit (PICU) admissions. Nutritional support is central to appropriate PICU management of AHRF, and yet fundamental gaps in knowledge exist regarding best practice for timing, route, dose, and type of nutrition. Optimized nutritional support during *pediatric* critical illness is important because even brief periods of malnutrition in infancy result in permanent negative effects on neurocognitive development, an outcome with lifelong impact. Parenteral nutrition (PN) supplementation could improve long-term neurocognitive outcome for pediatric AHRF by preventing acute malnutrition, but has unknown effects on intestinal barrier function; a proposed mechanism for late sepsis and infectious complications during critical illness.

**In this research protocol we will randomize mechanically ventilated infants and children with AHRF who can be enterally fed and do not have a baseline intestinal disorder to early vs standard care PN. PN will be in combination with early EN to provide nearly immediate and constant goal calories and protein over the first week of mechanical ventilation in the early PN arm. We want to examine the impact of a combined early EN + PN approach on intestinal barrier functions and nutritional biomarkers in children with AHRF.** *The central hypothesis of this study is that combined early EN and PN support will improve both nutritional outcome and intestinal barrier function for critically ill infants and children with AHRF.* The overall goals of this project are to evaluate the efficacy and safety of an early PN strategy to improve nutritional delivery, nutritional outcomes, and intestinal barrier function

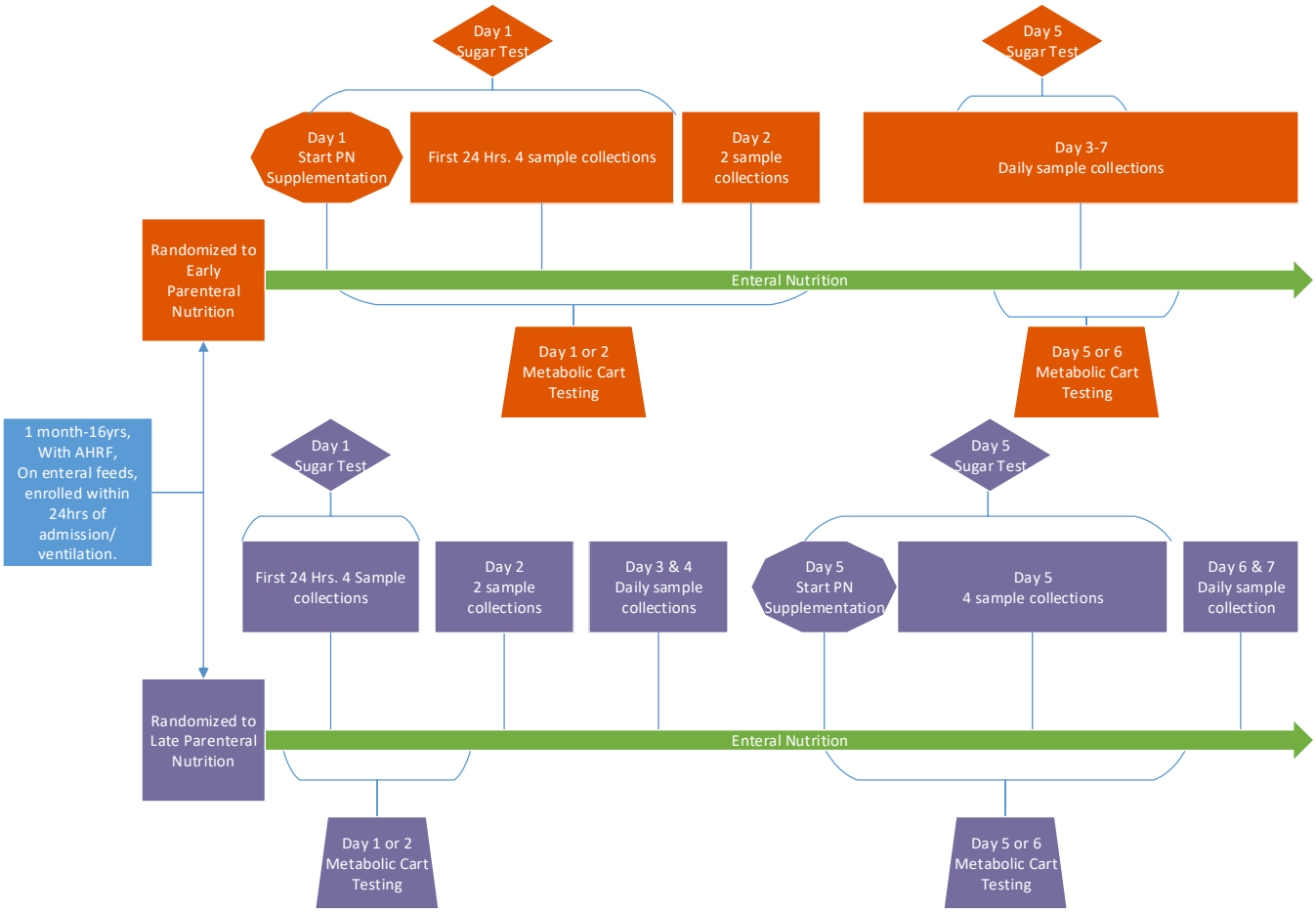
for children with AHRF in the PICU when used in combination with early EN. These goals will be addressed in 2 specific aims:

**Aim 1: Perform a prospective, single-blind, randomized pilot trial to determine the effect of early PN supplementation of EN versus late PN supplementation of EN in children with a functional gastrointestinal tract to meet measured resting energy expenditure on nutritional indices for infants and children with AHRF.**

**Aim 2a: Determine the effect of early combined EN + PN on intestinal barrier function in children with AHRF.**
**Aim 2b: Identify patient, disease, and treatment factors, which act as effect modifiers for non-invasive markers of intestinal barrier function for children with AHRF.**
**Aim 2c: (Exploratory) Determine the influence of EN +/- PN on the intestinal microbiome in children with AHRF.**

*This proposal is innovative*, because will evaluate the extent to which early combined enteral and parenteral nutrition improves delivery of daily and cumulative goal calories, protein, and indices of nutrition (Aim 1), while monitoring for potential negative consequences of early PN on intestinal barrier function (Aim 2). *This proposal is significant*, because it is expected to improve our understanding of optimal timing for parenteral nutritional support and define the effect of route and dose of nutrition, patient, disease, and treatment characteristics on intestinal barrier function. By understanding how patient, disease, and treatment factors impact intestinal barrier function during pediatric AHRF, we may be able individualize dose of enteral nutrition to protect against gut barrier dysfunction and promote new strategies to reduce septic morbidity; meeting the goals of NIH Healthy People 2020.

Timeline of Interventions




Screening and Eligibility

Screening is to take place twice daily (~8am and ~5pm) for newly intubated patients that meet inclusion criteria. All screened patients will receive a study ID. Enter the patient on the SUPPER Enrollment log for study ID. Complete the SUPPER Screening form for all screened patients. If the patient does not meet eligibility, complete the SUPPER Enrollment log and the Supper Screening form and place the Screening form in the study binder under Completed Screenings. If the patient meets all inclusion criteria, with no exclusion criteria, the patient is eligible and the family may be approached for consent. If unsure whether an individual patient can be enrolled in the SUPPER study, please call the University of Arizona PI.

**Inclusion Criteria-**
**Intent: To identify PICU patients who have Acute Hypoxemic Respiratory Failure and who will receive early enteral feeding.**
**Inclusion Criteria**

1. Admitted to study hospital pediatric intensive care unit (PICU),
2. One month to 16 years of age,
3. Exhibits Acute Hypoxemic Respiratory Failure as defined as:

a. PaO2/FiO2 ≤ 300 or SpO2/FiO2 ≤ 260
b. No evidence of left ventricular systolic dysfunction
c. Mechanically ventilated,

4. Require enteral tube feeding for nutrition,
5. Anticipate placement of central venous line within 48 hours of intubation.

**Exclusion Criteria**

**Intent: To exclude patients with a baseline intestinal disorder (which would alter biomarkers of intestinal barrier function), or who have contraindications to premixed PN solutions or sugar permeability testing.**

Patients will be excluded if at screening they have one or more of the following:

1. Premature infants and neonates < 37 weeks corrected gestational age,
2. Transfer patient from an outside facility and mechanically ventilated for >24 hours,
3. Receiving parenteral nutrition for > 24 hours,
4. Known allergy to lactulose or mannitol,
5. Pregnant,
6. Thoracic trauma, abdominal trauma, and/or active intracranial bleeding,
7. Anuric renal failure
8. Previous bowel resection and/or short gut syndrome, inflammatory bowel disease, or any patient without a functional gastrointestinal tract.
9. Cannot be enterally fed within 24 hours of admission according to the admitting physician,
10. On extracorporeal membrane oxygenation (ECMO),
11. Expected survival <24 hours or limitations to aggressive ICU care (DNR),
12. Receiving active CPR when admitted to the PICU,
13. A pre-existing bronchopleural fistula,
14. At goal enteral feeds at time of screening,
15. Previously enrolled and randomized into this protocol,
16. Actively enrolled in another clinical trial, which at the discretion of the PI would conflict with this study.

Consent and Assent

This project involves both infants and children up through the age of 16 and is categorized as 45 CFR §46.405, greater than minimal risk with potential benefit, and requires parental consent. Approach for consent must be within 24 hours of PICU admission or initiation of mechanical ventilation. Document consent approach on the Supper Screening form and whether parental consent was obtained on the SUPPER Enrollment log. Original parental consent forms are to be filed in the patient study file. Make two copies of the parental consent form, one for the family and one for bedside. Note in the patient medical record that patient was enrolled in the SUPPER Study.

Patients ≥8 years of age are to be approached for assent if the patient is extubated, no longer under the influence of sedatives and analgesics, and the patient is actively receiving early or late parenteral nutrition per study. If no assent obtained because the patient is sedated and unable to participate in the assent process, document this on the assent form and keep in patient study file. If the child does not provide assent once able to cooperate with assent, the intervention is discontinued at that point. All data obtained prior to assent will be utilized. File original assent forms with original parental

consent form in the patient study file and document assent on the SUPPER Enrollment form. Make two copies of the assent form, one for the family and one for bedside.

Randomization

Randomization takes place within 48 hours of PICU admission or initiation of mechanical ventilation. Patients are blocked randomized by age and pre-existing acute or chronic malnutrition (based on admission weight for age BMI z score) to either early or late parenteral nutrition (PN) supplementation. There are 9 randomization stratifications (see table below). Randomization cards are pre-generated; random sequence was created using a random generator with atmospheric noise at random.org. BMI z-score is derived from one of two websites. If <2 years of age, use www.emmes.cpm/study/ped/resources/htwtcalc.htm; if ≥2 years of age, use www.stokes.chop.edu/web/zscore/index/php.

| | 1month to <3 years | 3-10 years of age | 11-16 years of age |
|---|---|---|---|
| BMI z score < -1 and >-2 (Low) | A1 | B1 | C1 |
| BMI z score -1 to +1 (Normal) | A2 | B2 | C2 |
| BMI z score > +1 and < +2 (High) | A3 | B3 | C3 |

To randomize a successfully consented patient, use age and BMI z score to determine which randomization block the patient should be in. Choose the first randomization envelope in the pack. The envelope will have a card stating the intervention arm. Record the patient's study ID, patient age, BMI z-score, name of study personnel randomizing, and date/time of randomization on the card, and staple to the SUPPER Screening Form. Document date, time and study intervention randomized on the patient's SUPPER Screening form and SUPPER Enrollment Log.

Once randomized, email the study PI the patient's study ID, age BMI z score, date and time of randomization, and assigned intervention.

Patient Kits and Supplies

Each successfully enrolled and randomized patient will have a patient study file. This file will contain all forms for that patient. SUPPER Study Sample Kits have been provided for the collection of blood, and stool for this study. One kit is to be used per patient.

Early Parenteral Nutrition Supplementation (EPN)
Supplies, Medications, and Forms to have on hand when starting:
SUPPER Tablet with SUPPER Titration
Supplemental PN (Baxter Clinimix for patients 1 year of age and older; in-house custom PN for infants 1 month and <1 year)
SUPPER Patient Study Binder
SUPPER Sample Kit
Tape measure to perform mid upper arm circumference

Once randomized to Early Parenteral Nutrition (EPN), patients are to be started on the PN intervention within 2 hours of randomization. Patients randomized to EPN will receive enteral nutrition with supplemental PN for 168 hours. Calorie goal and titration of PN/IL are according to the SUPPER Titration Tool. **If at any point the clinical team feels that it is unsafe to continue PN, discontinue PN immediately and record this as a protocol violation**. **Ensure appropriate monitoring of blood glucose after abrupt discontinuation of PN.** Reducing the hourly rate of PN to achieve clinically indicated fluid restriction is also acceptable for the study protocol. All attempts should be made, and the study protocol is designed to avoid fluid overload as a result of PN. The SUPPER Titration Tool does not allow PN to exceed MIVF rates. Obtain mid upper arm circumference.

Prior to EPN Hour 0, set up SUPPER Tablet at bedside. Open the SUPPER Titration and enter all of the patient demographics, formula, TPN, and fluid information at the top. Calculate Schofield 100% BMR (height/weight). Rates for EN, PN, Lipids, and remaining available hourly fluids will populate. Print SUPPER Titration for bedside and study binder and review with the bedside nurse. A new SUPPER Titration Tool will need to be created for any change in formula kcal content, calorie goal, or new total fluid order (such as in the case of fluid restriction). As EN feeds are advanced, PN and Lipids decrease. If EN is held or reduced, titrate PN and Lipids according to SUPPER Titration sheet. Choice of PN is based on patient age. If the patient is 1 year of age or older, patients will receive Baxter Clinimix. Patients between 1 month of age and <1 year of age will receive in-house custom PN. The early EN guideline in place for clinical use at CHOP will be used to increase enteral feeds. The goal should be to reach full enteral feeds within 48 hours of initiating EN.

The SUPPER Algorithm uses Schofield's equation to calculate the initial kcal/day goal. Patients are to initially receive a max of 75% of calorie goal and 1.5 gm/kg/day protein for the first 24 hours. Patients will receive 100-120% of MREE (or 100-120% of Schofield predicted BMR in the setting of no MREE) and 2-3gm/kg/day protein with combined PN and EN by study day 2, not to exceed 1600mL/$M^2$/day in fluids (See Table at end of MOO). If there is >20% discrepancy between the unit dietician's usual practice for determination of kcals/day for a given patient and the Schofield predicted BMR, please call the U of A PI to discuss altering the individual patient's kcal goal during the 7-day study intervention. The bedside nurse will titrate PN to maintain calorie goal and protein every 4 hours with the aid of the SUPPER Titration Sheet in place of usual total IVF orders. As patient tolerates increased EN, PN and IL are decreased. In this manner, in the Early PN arm, the patient's delivered calories and protein remain constant, although the route of delivery changes as enteral feeds advance.

| | 0 Hour | 12 Hour | 24 Hour | 48 Hour | 72 Hour | 96 Hour | 120 Hour | 144 Hour | 168 Hour |
|---|---|---|---|---|---|---|---|---|---|
| **Blood Sample 2mLs** | X | X | X | X | X | X | X | X | X |
| **Fecal Samples (if patient has stool)** | X | | X | X | X | X | X | X | X |
| **Metabolic Cart (REE)** | Occurs Between 0 hour and 48 hours (DCMC Only) | | | | | Occurs Between 96 hours and 144 hours (DCMC Only) | | | |

Before initiation of EPN, collect 0 hour blood sample. This may be collected within 4 hours prior to beginning EPN to coordinate with clinical laboratory sample collection. Continue with timed sample collections as shown in the table above, using the time of EPN initiation as the starting point. All samples may be collected ± 2 hours from target time. If patient has continuous vasoactive infusions and the infusion has been titrated within the last hour of the time of sample collection, wait at least 1 hour from time of last vasoactive infusion change. (For example, sample collection is to be drawn at 8:00am, and vasoactive infusion was titrated last at 7:30am, wait until 8:30am for sample collection). Where ever possible, match sample collection with clinical labs to reduce line access and risk of infection. For clinical variables recorded on the sample collection log use clinical data obtained within the hour prior to sample collection. (i.e. if sample collection is at 0805, record CVP from the clinical record between 0705-0805.)

While writing initial orders for EN/PN, also include orders for PINI. This includes albumin, c-reactive protein, fibrinogen, transferrin, and prealbumin. If these values are not already available pre-randomization, then obtain within 4 hours of randomization.

If the care team decides the patient does not require artificial nutrition and/or central access lines are no longer needed, PN regimen is to be discontinued.

After Initiation of EPN:

SUPPER Pre-Admit Questionnaire and SUPPER Baseline Form

Once the patient has been initiated on EPN, retrieve the SUPPER Pre-Admit Questionnaire from the patient study file. SUPPER Pre-Admit Questionnaire should be completed within 24 hours of randomization. Ideally, study personnel should sit with family and complete the questionnaire to assist the family with any questions and ensure that questionnaire is successfully completed.

Using the patient's medical record, complete the SUPPER Baseline Form. Include only information prior to time of randomization. For variables with multiple values, choose that which is closest to randomization.

SUPPER Daily Data Collection and SUPPER EN/PN Tracking Log

SUPPER Daily Data Collection forms are provided for each of the intervention days and can be found in the patient's study file. For variables with multiple values use value closest to 8:00am, unless otherwise noted. For Day 0 Daily Data Collection, only use post randomization information. These forms do not need to be completed in real time if normal bedside charting clearly provides required information, but we recommend completing them the day after they occur (ex: Day 0 completed on Day 1).

Due to frequent collection of study labs, it is recommended to speak with bedside nurse at these times to gather information about possible adverse events or signs of feeding intolerance. SUPPER study labs may be delayed by 1-2 hours, if necessary, to ensure that blood collection occurs at a time when vasoactive infusions have not been titrated for 1 hour.

Using nutrition charting and tracking of in/out fluids, complete a SUPPER EN/PN Tracking Log for each study day.

Metabolic Cart Testing (University of Arizona only)

Within the first 48 hours of the intervention, the patient's Resting Energy Expenditure (REE) is to be tested using the site's available metabolic cart. Print at least two copies of the REE results; one for the patient study file and one for the patient's medical record. Attending physician caring for the patient should be updated on REE results. Record REE results on the SUPPER Sample log and file in the patient's study file. Repeat REE between Hour 96 and 144 of the intervention.

Prescribed kcal/day from the REE maybe different than the calculated kcal/day goal from the SUPPER Titration. If it is different, enter the new Measured REE kcal/day in the space provided. The SUPPER Titration sheet will utilize this new goal and adjust EN, PN, and Lipid rates accordingly.

**At Hour 96 document PINI.** This includes albumin, C - reactive protein, fibrinogen, transferrin, and prealbumin.

Discharge and Follow Up

Prior to patient PICU discharge, complete the SUPPER Contact Form with the parent/guardian. This form will be used for the 28 Day Vitals check if patient is not in the hospital or deceased.

There are 5 key sections to the SUPPER Discharge Form, Extubation, PICU Discharge, Hospital Discharge, Early Cessation, and 28 Day Vitals and Patient Death. Complete each of these sections as they occur.

To assess 28 Day Vitals first check to see if the patient is still hospitalized or if the patient died while hospitalized. If patient is not hospitalized, use the SUPPER Contact form to contact the patient's parents/guardians. Three attempts should be made to contact the parents/guardians. If the third attempt is unsuccessful, attempt to contact the patient's primary care physician. If the patient has not

been seen by the primary care physician since hospitalization, assess vital status from county death records. If you can successful contact either the patient's parents/guardians or primary care physician obtain information on whether the patient's condition is better, the same, or worse from condition at discharge. Also obtain information on whether the patient was readmitted to the hospital on or before 28 Days.

Late Parenteral Nutrition Supplementation (LPN)

Supplies and forms for have on hand after randomization.
SUPPER Tablet with SUPPER Titration
SUPPER Patient Study Binder
SUPPER Sample Kit
Tape measure to measure mid upper arm circumference

Once randomized to Late Parenteral Nutrition (LPN), patients are to be started on EN within 2 hours of randomization if not already started. Patients randomized to LPN will receive enteral nutrition for 168 hours and supplemental PN if not reaching 80% goal feeds starting at Hour 96 for a total of 72 hours. Calorie goal and titration of feeds are according to the SUPPER Titration. Obtain mid upper arm circumference.

Prior to LPN Hour 0, set up SUPPER Tablet at bedside. Open the SUPPER Titration and enter all of the patient demographics, formula, and fluid information at the top. Rates for EN and remaining fluids will populate. Print SUPPER Titration for bedside and study binder and review with the bedside nurse.

The SUPPER Titration uses Schofield's equation to calculate the initial kcal/day goal. Patients are to initially receive a max of 75% of calorie goal and 1.5gm/kg/day protein for the first 24 hours. Patients will receive 100-120% of MREE and 2-3gm/kg/day protein with combined EN by study day 2, not to exceed 1600mL/$M^2$/day in fluids. As the patient can tolerate, EN is to be escalated every 4 hours to full kcal/day goal.

The LPN intervention starts with the initiation of enteral feeds. Initiation of EN should take place within 2 hours of randomization if the patient was not already on EN. Within 2 hours of randomization, collect 0 hour blood sample and continue with timed sample collections as shown in the table below using the time of LPN initiation as the starting point. All samples may be collected ± 2 hours from target time. Where ever possible, match sample collection with clinical labs to reduce line access and risk of infection.

While writing initial orders for EN also document PINI. This includes albumin, C - reactive protein, fibrinogen, transferrin, and prealbumin. If these values are not already available pre-randomization, then obtain within 4 hours of randomization.

| | 0 Hour | 12 Hour | 24 Hour | 48 Hour | 72 Hour | 96 Hour | 120 Hour | 144 Hour | 168 Hour |
|---|---|---|---|---|---|---|---|---|---|
| **Blood Sample 2mLs** | X | X | X | X | X | X | X | X | X |
| **Fecal Samples (if patient has stool)** | X | | X | X | X | X | X | X | X |
| **Metabolic Cart (REE)** | Occurs Between 0 hour and 48 hours (DCMC Only) | | | | | Occurs Between 96 hours and 144 hours (DCMC Only) | | | |

SUPPER Pre-Admit Questionnaire and SUPPER Baseline Form

Once LPN is initiated and 0 hour sample is collected, retrieve the SUPPER Pre-Admit Questionnaire from the patient study file. SUPPER Pre-Admit Questionnaire should be completed within 24 hours of randomization. Ideally, study personnel should sit with family and complete the questionnaire to assist the family with any questions and ensure that questionnaire is successfully completed.

Using the patient's medical record, complete the SUPPER Baseline Form. Include only information prior to time of randomization. For variables with multiple values, choose that which is closest to randomization.

SUPPER Daily Data Collection and SUPPER EN/PN Tracking Log

SUPPER Daily Data Collection forms are provided for each of the intervention days and can be found in the patient's study file. For variables with multiple values use value closest to 8:00am, unless otherwise noted. For Day 0 Daily Data Collection, only use post randomization information. These forms do not need to be completed in real time if normal bedside charting clearly provides required information, but we recommend completing them the day after they occur (ex: Day 0 completed on Day 1).

Due to frequent collection of study labs, it is recommended to speak with bedside nurse at these times to gather information about possible adverse events or signs of feeding intolerance. Supper study labs may be delayed by 1-2 hours, if necessary, to ensure that blood collection occurs at a time when vasoactive infusions have not been titrated for 1 hour. Data on the daily sample collection forms (vital signs and clinical data) are to be collected as the most recent vital sign in the hour PRIOR to blood sample collection.

Using nutrition charting and tracking of in/out fluids, complete a SUPPER EN/PN Tracking Log for each study day.

Metabolic Cart Testing

Within the first 48 hours of the intervention, the patient's Resting Energy Expenditure (REE) is to be tested using the site's available metabolic cart. Print at least two copies of the REE results; one for the patient study file and one for the patient's medical record. Attending physician should be updated on REE results. Record REE results on the SUPPER Sample log and file in the patient's study file. Repeat REE between Hour 96 and 144 of the intervention.

Prescribed kcal/day from the REE maybe different than the calculated kcal/day goal from the SUPPER Titration. If it is different, enter the new Measured REE kcal/day in the space provided. The SUPPER Titration sheet will utilize this new goal and adjust EN rates accordingly.

**At Hour 96 record PINI.** This includes albumin, C - reactive protein, fibrinogen, transferrin, and prealbumin.

Initiation of PN at Hour 96

At hour 96, late supplemental PN is initiated if the patient is not meeting 80% of calorie goal on EN feeds alone. Choice of PN is based on patient age. If patient is 1 year of age or older, patients will receive Baxter Clinimix E 5/15. Patients between 1 month of age and 1 year of age will receive in-house custom PN. Enter PN/EN doses into SUPPER Titration, and start tracking PN on the PN/EN Hourly Log with first PN entry under the hour PN is initiated. The bedside nurse will titrate PN to maintain calorie goal and protein every 4 hours with the aid of the SUPPER Titration Sheet. As patient tolerates increased EN, PN is decreased.

If the care team decides the patient does not require artificial nutrition and/or central access lines are no longer needed, PN regiment is to be discontinued, or foregone if before 96 hours.

Discharge and Follow Up

Prior to patient PICU discharge, complete the SUPPER Contact Form with the parent/guardian that provided consent for this study. This form will be used for the 28 Day Vitals check if patient is not in the hospital or deceased.

There are 5 key sections to the SUPPER Discharge Form, Extubation, PICU Discharge, Hospital Discharge, Early Cessation, and 28 Day Vitals and Patient Death. Complete each of these sections as they occur.

To assess 28 Day Vitals, first check to see if the patient is still hospitalized or if the patient died while hospitalized. If patient is not hospitalized, use the SUPPER Contact Form to contact the patient's parents/guardians. Three attempts should be made to contact the parents/guardians. If the third attempt is unsuccessful, attempt to contact the patient's primary care physician. If the patient has not been seen by the primary care physician since hospitalization, assess vital status from county death records. If you can successful contact either the patient's parents/guardians or primary care physician obtain information on whether the patient's condition is better, the same, or worse from condition at

discharge. Also obtain information on whether the patient was readmitted to the hospital on or before 28 Days.

Subject Withdrawal

Subjects may withdraw from the study at any time. Should the patient or the patient's parents/guardians wish to withdraw, note the date, time, and reason for withdrawal on the SUPPER Discharge Form. If the patient is receiving supplemental PN when they wish to withdraw from the study, review the risks associated with abrupt discontinuation of PN, and advise that the patient will require blood sugar monitored 2-3 times over a 24 hour period to ensure they do not develop hypoglycemia. Ask parents/guardians if we may continue to collect information from the medical record without continuing any study procedures for outcome analysis. If they gave permission to bank samples for future research, ensure this has not changed.

Data Collection and Entry into REDCap

Prior to starting the study, sites will provide a written plan on how and from what system data is obtained. This is to assist with consistency of source of information, and to provide a guide for data monitoring. For instance, if vitals and medications are found in different applications of the medical record system, it should be noted where source documentation is obtained.

Data is to be collected onto the provided SUPPER study forms from source documentation. All data forms are to be kept in the patient's study file and the file kept in a secure location. Study personnel at each site will have a username and password to REDCap. REDCap is serviced and maintained by the University of Arizona's Clinical and Translational Sciences Research Center (CATS) Informatics Core. All SUPPER data should be entered into REDCap no later than 2 week from Study Day 7. SUPPER Enrollment Log information from the previous week should be entered every Monday when ever possible.

Sample Collection

Each patient will have a max of 11 blood samples and 8 stool samples. Blood samples are to be taken from indwelling catheters placed for clinical care. Stool samples will be collected from diapers, or in older patients, bed pans. Each blood sample collected will amount to 2mLs for a total of 22mLs.

Collect samples per the sample collection table provided and coordinate these samples collections so that they are obtained when clinical labs are being obtained. SUPPER Sample Kits are labeled for each sample collection and have either a 4mL purple top EDTA tube or a sterile double swab. For each blood collection tube, there are 5 red-capped aliquots.

Blood Samples

- From the patient's indwelling catheter, draw 2mLs of blood per unit protocol and place into the provided EDTA tube.
- Gentle invert EDTA tube 3 times to mix preservative.
- Place sample tube on ice after collection or store at 4$^{o}$C.

- Within 4 hours of collection, spin blood samples at 3600 RPM for 15 minutes in 4$^{o}$C refrigerated centrifuge (if available). The specific technique standard for separating plasma by a site's central laboratory is also acceptable.
- Using a micropipette, pipette 250µL of plasma into each of the 5 red capped aliquots.
- Complete labels with patient Study ID, date, and time of collection and place labels on collection tube and aliquots.
- Freeze collection tube and 5 aliquots upright at -80$^{o}$C in provided boxes. Use Box 1 for serum and blood pellet. Label each box with the patient Study ID.

Stool samples

- Collect stool samples using provided double swap sample container (preservative free) from the patients diaper, or from bedpan. It is acceptable to have urine in the same diaper.
- Place sample tube on ice after collection or store at 4$^{o}$C.
- Freeze at -80$^{o}$C within 4 hours of collection. Use Box 2 for stool samples tubes.

All samples must remain frozen until ready for shipping.

Shipping

Timing of shipping is to be quarterly or after collection of all samples for 4 patients, whichever is first. Contact the site PI or research coordinator the day prior to shipping to obtain FedEX label and to notify that shipment is coming.

Directions for Shipping Samples to University of Arizona

- Place collection boxes in large biohazard bags. All bags should fit in one large shipping box.
- Label the bag with Study ID.
- Obtain dry ice and package these samples in the large insulated shipping box.
- Using provided shipping labels, ship these samples to:
  Katri Typpo, MD
  1501 N. Campbell Ave.
  Pediatric Critical Care, Room 3354
  Tucson, Arizona 85724-5073

Protocol Deviations

In the event of protocol deviations, deviations must be recorded on the SUPPER Protocol Deviation Log and on the SUPPER Daily Data Collection form for the day the protocol deviation took place. There are six protocol deviation categories.

1. EN Feeds paused for >2 hours; one exception is if feeds are stopped for 4hr pre and post extubation.
2. PN not administered per protocol
3. Metabolic cart not done per protocol (Not a violation at CHOP)
4. 2 Sugar Test not done per protocol (Not a violation at CHOP)
5. Blood sample collection not done per protocol
6. Other

Describe the deviation with as much detail as possible on the SUPPER Protocol Deviation Log. Protocol deviations that result in the harm of the patient, others, or indicate that the patient or others may be at increased risk of harm must be reported to study PI within 24 hours of knowledge of the deviation.

**Serious, Unexpected, and Expected Adverse Events Definitions**

The study subjects are mechanically ventilated, critically ill infants and children with acute respiratory failure and as such are known to have mortality rates of 8-22%. We do not anticipate any serious adverse events specifically related to the short-term use of study PN formulations, performance of the metabolic cart, or to blood and urine sampling, but will report standard adverse events. All serious, unexpected, or unanticipated problems that are potentially related to study procedures will be reported to the PI, local IRB, the medical monitor, he DSMB and study sponsor within 24 hours. SAE's and AE's will be reported up to and including 14 days after study enrollment. Ongoing SAE's and AE's will be followed until resolved. A 28-day AE, SAE follow up form will be included in subject monitoring for 28-day vital status. The medical monitor will determine if the serious adverse event is potentially related to study procedures.

For safety monitoring, all adverse and serious adverse events are to be recorded and tracked. In addition to local site reporting for adverse events and serious adverse events, following guidelines are to be followed for this study.

**Serious Adverse Event is any of the events listed below:**

1. Death within 28 days of enrollment
2. Cardiopulmonary arrest
3. Operation or re-operation
4. Any other event that is not listed in the Expected Adverse Events, that increased risk and/or harm to the patient, may increase hospital length of stay, or require treatment.

If one of these events occurs, the medical monitor and PI within 24 hours of knowledge of the event. These events are also recorded on the SUPPER Adverse Event Log. A chart review of events leading up to the SAE will be required to determine relatedness to the study.

**Expected Adverse Events**

1. Aspiration of tube feeding
2. Unplanned extubation or need for reintubation
3. Pneumothorax
4. Desaturation < 85%
5. Pulmonary embolus
6. Catheter associated thrombosis
7. Healthcare acquired infections (Catheter associated blood stream infection, ventilator associated pneumonia, catheter associated urinary tract infection) as defined by the hospital infection prevention
8. Readmission to the pediatric intensive care unit
9. Cholestatic jaundice
10. Hyperglycemia (blood glucose >250 mg/dL)
11. Hypoglycemia (blood glucose <60 mg/dL)
12. Hyperammonemia
13. Acute Kidney Failure

If an adverse event occurs, it is to be noted on the SUPPER Adverse Event Log and the SUPPER Daily Data Collection form for the day the event occurred. All adverse events will be reviewed weekly by Dr. Katri Typpo to ensure study patient safety.

Report A/E and SAE per local IRB regulations as needed and required.

**Data and Safety Monitoring Plan**

Should study subjects have adverse events related to study interventions in Aim 1 or Aim 2 we will report these to the local and primary IRB and the Safety Officer (K Typpo). The DCMC study coordinator will produce Administrative Reports quarterly to describe study progress to include: accrual, subject demographics, and subject status. Reports will also describe adherence to inclusion/exclusion criteria, and protocol deviations. Safety reports will also be generated quarterly, or if enrollment exceeds expectations, at least with every 10 patients enrolled, list frequencies of adverse events, serious adverse events, safety outcomes, deaths, and disease or treatment specific events in blinded groups for review. These reports will be reviewed internally for ongoing quality control and then presented to the Safety Officer, DSMB, and IRB. The Safety Officer will review these quarterly reports to ensure good clinical care and identify any potential trends. The Safety Officer may request interim reports and analysis between meetings or prior to the scheduled interim analysis when necessary. Clinicians will be blinded to the safety monitoring data, as exposure to emerging trends may influence enrollment and care, biasing the study. This is designed as a single-blinded study so that the treating physician and investigative team are aware of group assignment in the event of electrolyte abnormalities, loss of central venous access, and related to feasibility of blinding PN. Our safety officer will also review charts for randomized subjects to ensure that no adverse events are attributable to the study intervention, this is in addition to review by the study PI.

Interim Safety Analysis

After 40 patients are enrolled, the Safety Officer and biostatistician blinded to group intervention will examine the safety endpoints stratified by TPN group, blinded into group A and group B. By utilizing the REDCap database, we are able to lock the database during this safety evaluation. We will trigger additional safety evaluations (e.g., more frequent or using an independent clinical panel) analysis if mortality rates (or other safety outcomes) in either arm are statistically different based on the previously mentioned safety reports, or statistically different from historical controls. Furthermore, since safety outcomes are not always powered for "statistical significance", these safety evaluations will also be examined in a more qualitative fashion, with error being on the side of overly conservative (e.g., any possible difference in safety outcomes). If there is any doubt then an independent panel of clinicians will be convened; if this group decides that there is an unacceptable difference in the two treatment arms then Aim 1 data collection will terminate prior to completing enrollment. Specifically, if either treatment group has significantly increased incidence of hospital acquired infections, increased utilization of dialysis, increased duration of mechanical ventilation, or mortality, then the independent panel can ask for unblinded data. If after data are presented data in an unblinded manner, the independent panel believes that safety concerns are unbalanced between the two arms then the study described in Aim 1 will be discontinued. Aim 2 data and sample collection will continue as planned but in patients on usual care for timing of EN and PN.
A DSMB was convened for this study as part of the Data Safety Monitoring Plan:

**DSMB responsibilities**
The first responsibility of the DSMB will be to approve the final protocol of the clinical study so that the study/studies can begin enrolling patients. In this case, the Supper study is ongoing and the DSMB will provide approval for continuing enrollment. After initial review of the study protocol and consent documents, and at periodic intervals during the course of the study, the DSMB responsibilities are to:

- Provide input to assist the investigators in protecting the safety of study participants;
- Provide input to the investigators on major changes to the research protocol, informed consent documents and plans for data and safety monitoring;

- Provide input to the investigators on the progress of the study, including periodic assessments of the data quality and timeliness, participant recruitment, accrual and retention, participant risk vs benefit, performance of the study sites, and other factors that may affect study outcomes;
- Review areas of concern regarding the performance of individual sites and provide comment to the investigators on actions to be considered regarding sites that perform unsatisfactorily;
- Consider factors external to the study when relevant information becomes available, such as scientific or therapeutic developments that may have an impact on the safety of the participants or the ethics of the study;
- Provide input to the investigators on modification of the study protocol or possible early termination of the study because of attainment of study objectives, safety concerns, low likelihood of showing a benefit of the intervention, or inadequate performance (such as enrollment and retention problems);
- If appropriate, review the interim analysis of efficacy in accordance with stopping rules which are clearly defined in the protocol and have the concurrence of the DSMB;

**Statistical Analysis Plan:**

Aim 1. We will assess differences between cumulative percent of goal energy and protein by study arm. We will report means and standard deviations as standard descriptive statistics. We will compare means between study arms with the Student's t tests as we expect cumulative percent of goal energy and protein to have normal distributions. Results will be considered significant at $p<0.05$. Relationships between cumulative energy and protein delivered with clinical characteristics, mid upper arm circumference changes, and weight for age z scores will be reported by Pearson's correlation coefficient.

Aim 2. We will assess differences between FABP2, claudin 3, citrulline, lactulose/mannitol ratios, and by study arm. Descriptive plots: serum biomarker (y-variable) over time (x–variable) will be fit, with linear and smoothing splines. Plots are qualitative. Splines allow examination of possible nonlinear relationships and can be used to specify nonlinear or change-point models. We utilize one-way repeated measures ANOVA with the intestinal barrier assays as continuously measured outcomes and time (1,2,3,5, and 7 days) as explanatory variables. If the slope of the serum biomarkers is linear, then change in the slope over time will be calculated using a linear mixed effects model with an AR(1) variance-covariance structure. The variance-covariance structure allows for measurements taken closer together to be more similar than those farther apart in time, and adjusts the standard errors for this correlation. If the relationship between the serum biomarker is not linear in time, then change-point or nonlinear mixed effects models will be used. Aim Multivariable regression analysis will evaluate possible effect modification, or mediation, for non-invasive measures of intestinal epithelial barrier function. Effect modifiers are the interactions with PN group; mediators are those variables that could have a potential additive effect with the PN group. Additionally we will assess any potential confounding that might be present by assessing the change in the parameter estimate for the PN group effect. Potential modifiers, mediators, and confounders, include clinical variables, center effects, and severity of illness. We will test associations between measures of intestinal function and clinical variables, severity of illness, as well as dose and route of nutrition. With 40 subjects total, we will be able to include up to 4 possible predictors; therefore, if effect modifiers are warranted, model simplification will be necessary. If the study is stopped early the analysis plan may be modified due to small sample size.