Comparative effectiveness of energy doses in critical illness

Short title: The Energy Dose Study

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Abstract:

Protein and/or energy deficits are associated with increased rates of hospital infection, skeletal muscle weakness, impaired wound healing, and prolonged convalescence in ICU patients. To prevent or treat malnutrition, enteral nutrition (EN) and/or parenteral nutrition (PN) are routinely given worldwide to a significant proportion of ICU patients. Optimal caloric requirements in critically ill patients are unknown due to a lack of rigorous randomized clinical trials. The comparative efficacy of energy doses in critically ill patients is unknown and clinical recommendations are conflicting and controversial; this issue is the focus of our study.

We have designed this pilot, single-center Randomized Clinical Trial (RCT) to prospectively compare, for the first time, the clinical efficacy of different energy doses in ICU patients requiring PN due to intestinal failure/dysfunction. A total of 60 patients will be studied (20 per energy dose group) to generate critical preliminary data needed to inform subsequent appropriately powered Phase III multicenter trials.

The primary aim of this study is to perform a controlled, double-blind, prospective, randomized, intent-to-treat Phase II clinical trial to test the efficacy of three specific energy doses [0.6, 1.0 and 1.3 x measured REE (resting energy expenditure), respectively], given for 28 consecutive days during the ICU course, on 28-day total hospital-acquired infections (primary endpoint), Blood Stream Infections (BSI), and other important clinical outcomes in medical/surgical ICU patients requiring specialized parenteral ± enteral feeding. We would also determine, in these subjects A) the impact of cumulative and mean daily 28-day energy deficits [energy intake-measured REE] on clinical outcome endpoints; and B) the practical utility of estimated REE determined by Harris-Benedict equation versus measured REE across different energy doses. We would also like to determine the impact of administered energy dose and energy deficits on global metabolomic patterns over time and their association with key clinical outcomes.

This study will allow us to generate needed data on the effect of energy dose and energy deficits on global metabolomic patterns over time that may be associated with key clinical outcomes in ICU patients. This exploratory research is also needed to develop new methods that evaluate the metabolic responses to nutrition support and their potential relationships to clinical outcomes.

Background:

The increasing rate of hospital-acquired infection and sepsis are major causes of morbidity and mortality in intensive care unit (ICU) patients [1, 2] . The enormous impact of critical care-related illness demands that we find cost-effective therapies to improve outcome that can be rapidly implemented. Malnutrition is also very common in critically ill patients and is associated with increased hospital-acquired infections and mortality in ICU patients [3-13]. Protein and/or energy deficits are associated with increased rates of hospital infection, skeletal muscle weakness, impaired wound healing, and prolonged convalescence in ICU patients [12, 14-17] **[3, 34-37].** To prevent or treat malnutrition, enteral nutrition EN and/or PN are routinely given worldwide to a significant proportion of ICU patients [3-13] . **Unfortunately, very few well-designed RCTs on the clinical efficacy of specialized nutrition support modalities in critical illness have been performed [18, 19].** Observational studies indicate that initial nutritional goals in ICU settings are infrequently achieved; recent surveys show that both underfeeding, and, to a lesser extent, overfeeding, are common in ICU settings [5, 7, 20-22]. Unfortunately, rigorous, prospective studies to determine effects of different energy doses have not been performed [5, 7, 12] . The comparative efficacy of energy doses in critically ill patients is thus unknown and clinical recommendations are conflicting and controversial; this issue is the focus of our application. Given the changes in the methods of ICU nutritional support during the past 5-10 years, including tighter blood glucose control [23, 24] and the use of overall lower caloric loads than in previous decades (when "hyperalimentation" was the norm), there is a clear need for further CER studies of different modalities of nutrition support based on current ICU clinical care practices [25-31]. Energy (calorie) needs in adult patients in the ICU often vary considerably because of day-today changes in clinical conditions [9, 32, 33]. However, optimal caloric requirements in critically ill patients are unknown due to a lack of rigorous RCTs**.** The resting energy expenditure (REE), or basal metabolic rate, is best determined using serial bedside metabolic cart measurements (indirect calorimetry), but technical issues (e.g. air leaks, high inspiratory oxygen concentration) can cause inaccuracies [3, 32, 33] . REE can also be estimated using standard equations, most commonly the Harris-Benedict equation (HBE), which incorporates the patient's age, gender, weight, and height [3, 10, 12]. Unfortunately, the HBE may over- or underestimate REE in critically ill patients [3, 32, 33] .

Current clinical practice guidelines, which are not evidence-based**,** suggest that an adequate energy goal for most adult ICU patients is approximately equivalent to measured or estimated REE x 1.0-1.3, given the minimal physical activity of ICU patients and the known complications of excess caloric doses, including hyperglycemia [8-10, 12] . In observational and retrospective (unblinded) studies, administration of both adequate energy doses (typically via the intravenous route well as "hypocaloric" feeding (typically as enteral feeds given at less than prescribed amounts) have each variously been associated with improved ICU outcomes in prolonged stay (> 4-5/days) ICU patients, particularly with regard to hospital-acquired infections, sepsis, and other complications [6, 11, 34, 35]. Villet et al showed that less negative (i.e. more positive) ICU

energy balance was associated with lower rates of hospital infections, less time on mechanical ventilation, decreased ICU length of stay (LOS) and lower total complication rates [11]. Dvir et al found that daily mean negative ICU energy balance (energy intake – REE by indirect calorimetry) was strongly associated with increased rates of respiratory failure, sepsis, renal failure and pressure ulcers and total ICU complications [6]. In marked contrast, Krishnan et al performed an unblinded descriptive, cohort study in medical ICU patients receiving PN, EN or EN+PN and showed that death rates varied as a function of the mean daily energy intake [34]. The patients in the lowest tertile of mean daily caloric intake in relation to a goal of 25 kcal/kg/day had the lowest mortality rates compared to the higher tertile energy intake groups. Further, Rubinson et al performed a prospective, unblinded cohort study in 138 clinically matched medical ICU patients and found that patients whom received an average of < 6 kcal/kg/day (lowest quartile of intake) had significantly more bloodstream infections (BSI) than patients in the 2nd to 4th quartile groups for energy intake [35]. This study suggested that there might be a threshold of energy intake needed to decrease BSI in medical ICU patients, but that higher levels of caloric intake do not influence this morbidity index.

Unfortunately, in all trials concerning ICU energy dose and outcomes, **a major design flaw is that the amino acid/protein and micronutrient intake has also varied between groups, especially when enteral feeds were employed** [34]. Also, blood glucose levels in the study groups, a known risk factor for ICU morbidity and mortality, were either not reported [6, 11, 35] or were higher than levels now considered appropriate for ICU patients (<150-180 mg/dL) [36]. **Thus, the true impact of energy dose *per se* on clinical outcomes in ICU patients is unknown at the present time.** Studies to help better define energy dosing are also potentially important to guide clinicians to avoid complications related to overfeeding and refeeding syndromes, common in ICU settings [12, 37-39]. **Phase III, adequately powered, double-blind, intent-to-treat RCTs are needed, with design informed by pilot data such as we will generate in this project, to better define calorie requirements associated with improved outcomes in ICU patients.**

Recent discussions have emphasized the utility of metabolomic methods for nutrition research [40, 41]. **Metabolomics analysis represents a novel approach to study integration of metabolic responses to specialized nutrition support** [42, 43]. We, under the direction of Co-I Dr. Dean Jones, have focused on high-resolution liquid chromatography mass spectroscopy (LC-MS) method. Metabolic profiles can be analyzed using our established bioinformatics methods to identify metabolic patterns and potential metabolic biomarkers of energy insufficiency and response to the different ICU energy dose regimens [44]. **The proposed studies will allow us to generate needed data on the effect of energy dose and energy deficits on global metabolomic patterns over time that may be associated with key clinical outcomes in ICU patients.** Future studies could focus on nutritional strategies based on the specific identity of plasma metabolites that may be associated with improved outcomes. The fractal analysis-derived Hurst exponent has recently received attention because it provides a means to interpret biological signals generated by healthy biological systems or pathological states with one numerical value [45-49]. Hurst exponent data is related to a variety of physiologic states and to outcome measures in Parkinson's disease, obstructive sleep apnea and sudden

cardiac death [45-49]. **Fractal analysis of metabolomic data will allow us to test whether the Hurst exponent is related to clinical outcomes and/or response to nutritional interventions in the ICU patients we propose to study.**

Emerging data strongly suggest that microbes of the intestine (the microbiota) play a key role in host metabolism, immune/inflammatory processes and disease pathophysiology [50-52]. The intestinal microbiome (genome of the gut microbiota) is shaped by both environment (e.g. diet) and host genetics; and, vice versa, the gut microbiota itself appears to regulate a wide spectrum of host immune, inflammatory and metabolic responses [53]. **Human data on the impact of nutrition support during critical illness on the gut microbiome are limited.** Despite limited evidence, there is a suspected interplay between critical illness and the gut microbiome, which warrants further exploration in the setting of nutrition support [54-58]. Nelson et al utilized 16S rRNA pyrosequencing to characterize stool of hospitalized patients and observed perturbations in gut microbiota following infection [59]. It is acknowledged that the microbiome plays an integral role in health maintenance, and that modifications of the gut microorganisms may influence outcomes in critically ill patients.

Hypothesis:

1) Adequate energy administration (defined as 1.3 x REE) will result in a lower 28-day total hospital-acquired infection rate compared with ICU patients receiving lower energy doses.
2) Differences in energy intake will result in global changes in metabolic patterns that themselves are associated with clinical outcomes in the ICU patients studied.

Specific Aims:

1) To perform a controlled, double-blind, prospective, randomized, parallel group, intent-to-treat Phase II clinical trial to test the efficacy of three specific energy doses [0.6, 1.0 and 1.3 x measured REE (metabolic cart), respectively], given for 28 consecutive days during the ICU course, on 28-day total hospital-acquired infections (primary endpoint), BSI, and other important clinical outcomes in medical/surgical ICU patients requiring specialized parenteral ± enteral feeding.
2) In Aim 1 subjects to: a) examine the impact of cumulative and mean daily 28-day energy deficits [energy intake-measured REE] on clinical outcome endpoints; and b) to estimate the agreement for REE as determined by the HBEand the measured REE across different energy doses.
3) To determine the impact of administered energy dose and energy deficits on global metabolomic patterns, human microbiome, and handgrip strength over time and their association with key clinical outcomes in Aim 1 subjects.

Study Procedures:

A total of 60 subjects will be enrolled in this intent-to-treat trial at Emory University Hospital (EUH) and EUH-Midtown. Patients admitted to either a medical or surgical (non-neurological)

ICU within the previous 168 hours and who are expected to survive and remain in the ICU for at least 96 hours after entry are eligible. Patients enrolled in this study are also those deemed to likely require central venous parenteral nutrition (PN) for 7+ subsequent days after entry on a clinical basis. Patients who meet the following eligibility criteria will be approached:

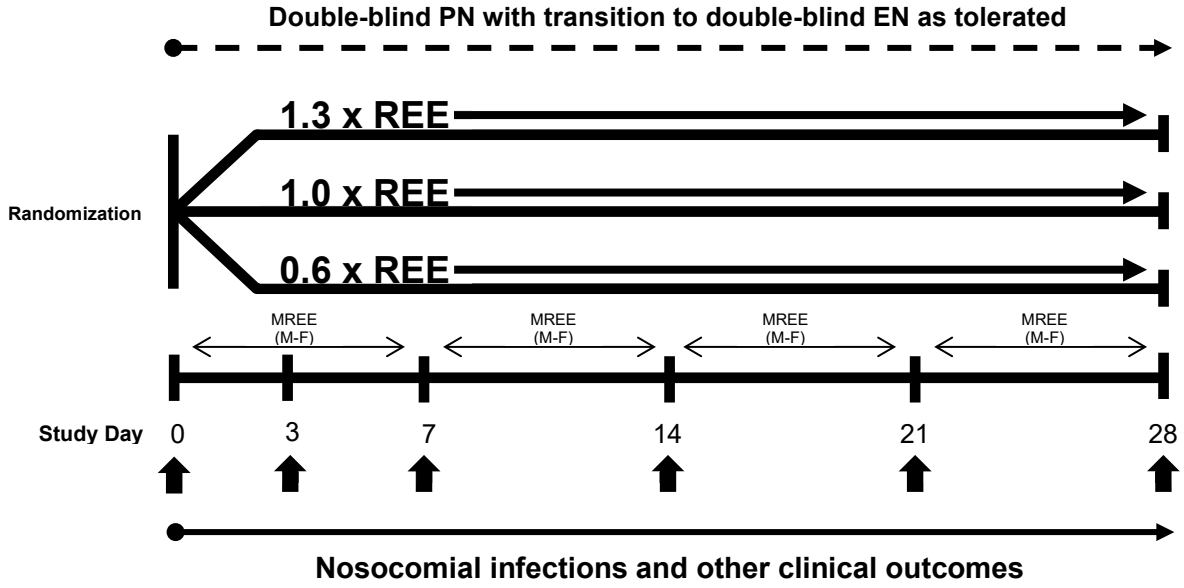
Inclusion criteria: 1) A signed informed consent is in place on the patient's chart; 2) The patient is at least 18 but not more than 90 years of age at time of ICU admission; 3) The patient has a body mass index (BMI) > 40 kg/m^2; 4) The patient has been admitted to either a medical or surgical (non-neurological) ICU and is expected to survive and remain in the ICU for at least 72 hours after entry; 5) There is central venous access for administration of the study PN; 6) The patient's primary physician(s) will allow the investigative team to manage the study PN and enteral feedings during the current hospitalization; and 7) The patient is expected to require total or partial central venous PN for 7 or more subsequent days after entry on a clinical basis (e.g. following massive small bowel ± colonic resection, the presence of high output fistulae or perforated small bowel, with demonstrated intolerance to EN or when EN may be contraindicated, as with severe diarrhea or emesis, partial or complete bowel obstruction, severe gastrointestinal bleeding and severe hemodynamic instability, such as with escalating vasopressor requirements).

Exclusion criteria: 1) The patient is pregnant; 2) The patient has unresuscitated clinical sepsis [defined as unstable blood pressure despite vasopressor support and mean arterial pressure (MAP) < 60 mm Hg on at least 3 consecutive readings within a 3-hour period during the 24 hours prior to study entry; 3); 3) The patient was admitted to the ICU following trauma or burns; 4) The patient has significant renal dysfunction (defined as serum creatinine > 2.5 mg/dL or deemed to have significant acute kidney injury by the primary physicians) and is not receiving continuous renal replacement therapy (CRRT) or intermittent hemodialysis; 5) The patient has previously undergone an organ transplantation; 6) the patient has a current malignancy or is currently receiving an active regimen of chemotherapy and/or radiotherapy to treat a previously diagnosed malignancy$^{\neq}$; 7) the patient has a history of HIV/AIDS; 8) The patient has received any investigational drug within 60 days prior to study entry; and 9) The patient is unable or unwilling to participate in study procedures such as longitudinal blood draws and administration of study nutrient formulations.

≠ † Patients with malignant metastasis and terminal untreatable carcinoma will be excluded.

If the patient meets criteria, we will consent the patient's legally authorized representative (LAR), as potential study subjects will be mechanically ventilated and probably sedated.

Randomization of study subjects: A research team member will calculate the APACHE II score. Treatment assignments will be stratified according to clinical center and on the basis of illness severity (APACHE II score, either ≤ 15 or > 15). The treatment assignments will be generated by the Emory-based DCC with the use of a pseudo-random-number generator with randomly permutated blocks that will be used to ensure balance between the numbers of subjects assigned to the REE x 0.6, 1.0 or 1.3 groups, respectively.



Study Schema: Controlled, randomized, double-blind, prospective, parallel-group, intent-to-treat **Aim 1** clinical trial to test the efficacy of three energy doses [0.6, 1.0, and 1.3 x measured REE (MREE), by metabolic cart, respectively], given for 28 consecutive days during the ICU and post-ICU course in critically ill medical and surgical patients requiring parenteral nutrition (PN) ±enteral nutrition (EN) at Emory University. The primary endpoint is total hospital-acquired infections after entry; secondary endpoints are bloodstream infections, ventilator-free days, and ICU and 28-day hospital length of stay and mortality. **Aim 2** examines the effect of cumulative and mean daily measured 28-day energy deficits after entry on Aim 1 clinical outcome endpoints and relationships between estimated REE (Harris-Benedict equation) at study entry. **Aim 3** explores the impact of energy dose and cumulative and mean daily 28-day energy deficits on global metabolomic patterns, microbiome, and strength over time and in association with key clinical outcomes in Aim 1.

⬆ Plasma (metabolome), microbiome, and strength analysis

Data Collection: Baseline data collected will include APACHE II score, and clinical and demographic data. The following will be collected during the course of the study:

A) Measured resting energy expenditure (MREE; per metabolic cart) will be determined on the baseline day before 2 pm. The goal energy dose composition of PN for all three energy doses for each enrolled subject is calculated by the blinded research team members. The metabolic cart-derived REE will be used to determine basal energy expenditure (REE) and thus the initial energy dosing [5, 10, 12]. MREE will be determined at baseline and 5x weekly (M-F before 2 pm; see Aim 2) using a state-of-the-art metabolic

cart and standard methodologies by a study team respiratory technician with prior experience and expertise in clinical indirect calorimetry. In the event that the metabolic cart is not available, the most recent measurements will be used. The nutrition will not be adjusted if the MREE is <10% changed from the reading the day before.

B) Longitudinal SOFA scores of organ dysfunction/failure: Sequential organ failure assessment (SOFA) scores will be calculated in the morning of each ICU day [60]. The baseline Apache II score on the day of ICU admission will also be calculated in all patients.

C) Blood sampling for metabolomics analysis (Aim 3): Baseline and serial study blood for processing as below will be drawn between 0800 and 1000 h. at the baseline day and study days 3, 7, 14, 21 and 28. A total of 28 mL of blood will be drawn at baseline and days 7, 14, 21 and 28 of study. A total of approximately 150 mL of blood will be obtained for analysis during the first 28 days after entry.

D) Assessment and monitoring of blood glucose concentrations: Three blood glucose data points (the initial AM blood glucose sent to the hospital laboratory, and one representative laboratory or fingerstick blood glucose level obtained at a time point between 1400-1660 h and again between 2200-2400 h) ± 1 hour window.

E) Assessment and monitoring of nosocomial infections: New hospital infections will be diagnosed based on standardized CDC criteria exactly as currently performed in Dr. Ziegler's multicenter NIH U01 study (updated by CDC in 2009). All infections will be adjudicated by review of pertinent data in a blinded fashion by Infectious Disease Co-I Dr. Henry Blumberg.

F) Stool sampling for microbiome analysis (Aim 3): Stool samples, when available, will be collected by the primary nurse at baseline and study days 3, 7, and 14, within 24 hours of the specific study day. The sample will be placed in a vial and frozen for later analysis by colleagues at the University of Chicago. The date, time, and consistency of stool will be collected for each sample.

G) Handgrip strength test for overall strength assessment (Aim 3): If patients are deemed alert and able by study team, a Jamar Hydraulic Hand Dynamometer (Sammons Preston Rolyan, Chicago, IL) will be used to measure handgrip strength on the day subjects leave the ICU, then 3 and 7 days after ICU discharge. If the subject is consented before surgery and subsequent ICU admission, a handgrip measurement will be taken during pre-op. At each time point, the subject will be asked to squeeze the handheld dynamometer as hard as they can for 3 seconds, and they will be asked to repeat this 3 times, with a 1-minute rest between measurements.

Serial data entry via case report forms (CRFs): The investigators will monitor all study subjects daily during the ICU stay and after discharge to the general ward and enter data into

standardized CRFs. The primary endpoint is total nosocomial infection rate during the 28-day study period. Secondary Aim 1 post-entry endpoints to be monitored include: 1) 28-day hospital mortality; 2) Rate of new nosocomial blood stream infections (BSIs) during the initial 28 days; 3) Rate of new nosocomial infections during the initial 28 days; 4) mean ICU SOFA score; 5) ICU ventilator-free days at day 28; 6) ICU and total hospital length of stay.

Hospital nutrition support - Administration of double blind parenteral and enteral nutrition:
Briefly, the nutritional goals are to provide total daily calorie (kcal) intake [from PN + dextrose-containing IV fluids (> 500 mL/day) + propofol/clevidipine + any enteral feedings) at 0.6, 1.0 or 1.3 x baseline MEE (per metabolic cart). We will then serially adjust total PN/EN energy dosing based on real-time serial cart REE measurements during the ICU stay, as indicated by the energy dose group to which the patient is randomized. The total amino acid/protein intake goal is 1.2 g/kg/day. Dextrose will initially comprise 70% of PN non-amino acid kcal and IV fat (30% Intralipid®) 30% of PN non-amino acid kcal daily, adjusted as clinically indicated. The 1.0 x REE energy dose study group will serve as the control group in this CER study. Study PN will be continued only if deemed indicated by the investigators and the primary physicians. Daily intake of study PN volume (mL), PN dextrose (g), PN lipid emulsion (g), intake of study tube feeding volume, and daily PN and enteral (oral or tube feeding) volumes will be calculated and entered into the CRFs daily for the initial 28 days after entry, beginning on day 1.

Study subjects will receive a maximum of 28 days of double blind study PN ± EN. Subjects will be transitioned from PN to enteral feeds as soon as tolerated and clinically indicated. Study PN will be discontinued when enteral intake of ≥ 50% of energy requirements is achieved for ≥ 48 hr. The amount of PN administered will be decreased as a function of actual enteral nutrient intake to maintain daily goals. The 24-hr daily study PN will be prepared under sterile laminar air flow hood conditions by the unblinded research pharmacist and pharmacy technician at each site. The study EN will be made in the Metabolic Kitchen or in the compounding area of the pharmacy.

For study PN, the energy dose will be altered via proportional changes in the administered PN carbohydrate and lipid doses in isovolemic PN using established double-blind methods of our research team in Dr. Ziegler's ongoing Phase III trial U01 DK069322 The method employs use of three written orders by the blinded research team members to account for three possible energy doses per randomization (either 0.6, 1.0 and 1.3 x REE, respectively) given as isovolemic PN solutions. The PN dose will be written for each 24-hr period taking into account calories provided as lipid emulsion in the sedative propofol (1.1 kcal/mL) or clevidipine (2 kcal/mL), and any dextrose-containing IV fluid orders exceeding 500 mL/day. The lipid emulsion will be a conventional soybean-oil based product (30% Intralipid®, Baxter, Deerfield, IL). The PN bag labels will state that study dextrose and study lipids are provided, but will not list the specific amount of each. Labeling of all other PN constituents will be per standard IV Pharmacy procedures. Additional details on study PN and EN nutrient formula preparation by the unblinded pharmacist and technician, blinding procedures, and strategies to prepare PN, EN, and combined PN+EN feeds that a) achieve our planned energy intake goals (0.6, 1.0 and 1.3 x measured REE by metabolic cart) and b) are isonitrogenous.

Clinical management guidelines: The care for the ICU subjects at both EUH and EUH-M hospital study sites are standardized per uniform Emory Healthcare ICU guidelines for ventilator weaning, use of antibiotics, sedation, blood glucose control/insulin administration pathways, etc. The investigators, working closely with the primary clinical teams, will attempt to maintain morning blood glucose levels between 150-180 mg/dL in the ICU and ≤ 200 mg/dL in the less-controlled setting of the general surgical ward [23, 24].

Determination of energy deficit: Energy intake from PN ± EN (tube feeds + any oral diet) will be determined as outlined in Aim 1 each day for the initial 28-days of study. MREE will be determined by metabolic cart 5x week (Monday through Friday) before 2 pm. Any PN or EN being administered will be continued uninterrupted during MREE determinations. The 5 daily cart measurement results (kcal/day) for each study week will be averaged and that mean daily value used as the daily measured energy requirement for all 7 days of that week. The daily energy deficit will be calculated by subtracting the calculated mean daily MREE during each study week from the actual daily energy intake. The cumulative and mean daily ICU and 28-day energy deficits will be determined. Metabolic cart measurements will not be performed on any given day in patients whom are in isolation due to resistant microorganism infection (contraindicated), or if the patient requires a fraction of inspired oxygen of < 60%, has a requirement for positive end-expiratory pressure of > 12 cm H_2O, or has a documented leak in the ventilator or chest tube systems (precluding accurate cart measurements) [32, 33]. The estimated REE by the HBE(outlined in detail in Table 3 of reference 3 [61]) will be calculated on the baseline day using most recent available pre-ICU dry body weight.

High-resolution metabolomics analysis: Plasma samples will be obtained at baseline and on days 3, 7, 14, 21 and 28 after entry (please see study schema above). Metabolomic profiles at the specific analysis time-points will be compared to compare subjects: 1) within the three study energy doses; 2) whom develop a nosocomial infection or a BSI versus those who do not; and 3) who die compared to those who are alive at 28 days. Samples will be analyzed in triplicate with a Thermo LTQ-Velos Orbitrap high-resolution mass spectrometer (≥ 50,000 resolution) following extraction with acetonitrile containing an internal standard mixture and separation on anion exchange and C18 columns. Validated extraction methods (apLCMS and xMSanalyzer) [44, 62] will be used to provide a mass/charge ratio (m/z) feature table of detected ions, characterized by relative retention time and accurate mass. The mass spectrometer will be set to collect data from m/z ratio 85 to 2000 daltons over a 10-minute chromatography period. Internal stable isotopic standards are spiked into every sample so that the relative retention time and accurate mass provide unambiguous denotation of an ion even if the chemical is unidentified [63]; relevant reference samples are included preceding and following each block of 20 samples for long-term quality assurance. Electrospray ionization will be used in the positive ion mode for detection. When necessary, tandem mass spectrometry will be used to positively identify metabolites of interest, with a metabolite defined as any chemical in a biological system.

Stool sampling and gut microbiome analysis: On study days 0, 3, 7, and 14 the primary nurse will collect a small sample of stool (± 24-hr window of each study day). All specimens will be collected into sterile containers, processed, and stored at -80°C as previously outlined [64]. The

gut microbiota in all fecal samples will be identified using established next generation DNA sequencing of 16S rRNA libraries, performed by our collaborator Dr. Eugene Chang of the University of Chicago (6, 18, 66). Briefly, 50 mg of frozen fecal sample will be homogenized in extraction buffer and 0.1-mm-diameter zirconia/silica beads are added prior to lysing of microbial cells on a Mini-Beadbeater-8 cell disrupter. After overnight incubation at 55°C, extraction with phenol: chloroform: isoamyl alcohol, and precipitation with ethanol will be performed. Isolated DNA is dissolved in Tris-EDTA buffer and stored at -80°C. The 16S rRNA gene sequences are amplified from DNA samples using barcode-labeled primers for the V3–V4 region of the 16S rRNA encoding gene (*Escherichia coli* positions 338–806), as outlined [65-67]. The Shannon diversity index and distinctive peak numbers (richness) of the OTUs in the microbiome libraries between baseline (day 0) and serial fecal samples (days 3, 7, and 14) will be compared as previously outlined [65, 67, 68]. These representative sequences are aligned using PyNAST and taxonomy assigned to them using the RDP Classifier. The PyNAST-aligned sequences will also be used to build a phylogenetic tree with FastTree and unweighted UniFrac distances then computed between all samples for additional ecological analyses, including principal coordinates analysis (PCA) [65-67].

Adverse Event (AE) Reporting:

The subjects enrolled in this trial are initially critically ill subjects deemed to require both PN and prolonged ICU care. Such individuals are known to have a high morbidity and mortality rate. No AEs specifically due to the study nutrient formulations are expected. However, we will closely monitor the clinical course of all subjects daily and record conventional serious adverse events (SAEs) and unexpected and expected AEs, as well as note significant clinical or surgical events as narrative data. For the purposes of study reporting, an SAE is any of the events below:

1. Death
2. Re-hospitalization after study PN or tube feeding discontinuation
3. Documented pulmonary aspiration of study tube feeds during study tube feed administration
4. New cancer diagnosis
5. Congenital anomaly/disorder

When any of the above events occur, an SAE form in the subject's CRF binder is filled out and faxed to the DCC. The DCC will in turn report these events to the DSMB and NIDDK. These events will also be reported to each site's IRB per their local reporting requirements.
All other serious, unexpected or unanticipated problems that are potentially related to study PN or tube feeding will be reported to the IRB, per local reporting guidelines, and the DCC. Operationally, this means that if: 1) in the opinion of the PI, was there a causal relationship between the intervention and the serious event (i.e., there is a reasonable possibility that the event may have been caused by the study drug intervention); and 2) the serious event was unexpected (i.e., not identified in nature, severity or frequency in the current IRB approval research protocol or informed consent document). Code responses in terms of relation to study PN will be as: definitely related, possibly related, unsure, probably not related and definitely not related). SAEs and AEs need to be reported up to and including 30 days after study drug

discontinuation. Ongoing SAEs and AEs must be followed until resolved. A 30-day post-study drug follow-up SAE/AE form will be included in subject monitoring.

Expected Adverse Events: There are no expected AEs that are likely to be due to the study PN or tube feeding. **Major AEs common in ICU subjects receiving PN and tube feeding will be identified and reported to the DCC and DSMB.** The following expected AEs would be reported during the 28-day study:

1. Clinically significant pulmonary aspiration
2. Pneumothorax
3. Development of worsening renal function with serum creatinine ≧ 5.0 mg/dL or requiring new initiation of renal replacement therapy
4. Development of severe hepatic dysfunction (serum total serum bilirubin ≧ 15.0 mg/dL)
5. Hyperglycemia > 250 mg/dL
6. Hypoglycemia < 50 mg/dL (from blood draws and laboratory analysis)

The adverse event data will be compiled by the DCC and reported to the DSMB twice a year. This trial will employ an NIH-approved data and safety monitoring board (DSMB) and a comprehensive AEs reporting plan to minimize risks to the subjects.

Potential Risks: A total of approximately 150 mL of blood will be obtained for analysis during the first 28 days after entry. A total of 28 mL of blood will be drawn at each of the baseline and days 3, 7, 14, 21 and 28 of study. Although most of the early blood draws (e.g. baseline and days 3, 7 and 14) are likely to be via an already-indwelling arterial catheter or less likely a central or peripheral venous catheter required for clinical purposes, pain and bruising, and much less likely, infection could occur with venipuncture. Lightheadedness and fainting can also occur with drawing blood.

Indirect calorimetry to measure resting energy expenditure (MREE) is a routine ICU test and performed over a 30-minute period using a metabolic cart (to collect expired air for measurement by the machine), which is connected to the mechanical ventilator tubing in patients on mechanical ventilation. Manipulation of the tubing for this connection on a Monday-to-Friday basis (5x/week) carries an extremely small risk of infection. In patients not on the ventilator, the measurement is done while the patient is lying quietly in bed by placing a large plastic see-through hood over the head of the patient to collect the expired air. This could cause a sense of claustrophobia in some patients, but is typically very well tolerated.

Administration of PN has well-known complications that can be classified as mechanical (e.g. pneumothorax with central line insertion), infectious (e.g. PN-associated infection) and metabolic (e.g. hyperglycemia, electrolyte disturbances, increased serum liver function tests).

Administration of tube feedings can result in nausea, emesis, pulmonary aspiration, pneumonia, diarrhea, a variety of mechanical complications associated with the placement of feeding tubes and metabolic complications similar to that seen with PN. However, these modes of feeding will

be given only as indicated per standard clinical care guidelines and a central venous catheter will be inserted only if indicated per clinical care by the primary physicians.

The only significant difference in nutrient administration is that a different caloric dose will be given to individual patients, depending on the randomization; either 40% below the estimated REE using the standard HBE (i.e. 0.6 x estimated REE), 100% of the REE (i.e. 1.0 x estimated REE), or 30% above the estimated REE (i.e. 1.3 x estimated REE). There is no *a priori* risk to any of these energy doses and the limited data to date are conflicting as to whether one dose confers any risk or morbidity versus another (there are no data to suggest that ICU mortality would be different at any of these three energy doses vs. the others). Further, this range of energy doses is routinely given to adult ICU patients throughout the world. This is due to the lack of consensus on appropriate energy dosing for specialized nutrition support. The total amino acid/protein and fluid amounts in each of the three energy-dose groups is designed to be similar between groups (please see research plan).

Tighter blood glucose control, generally using insulin drip therapy, is the standard of care for ICU subjects as several studies now show that control to levels in the range of < 180 mg/dL decreases ICU morbidity and mortality, although the exact goals for blood glucose remain controversial in surgical ICU patients. Recent data from the NICE-SUGAR trial suggest that maintaining blood glucose levels < 180 mg/dL may be an appropriate goal in ICU patients. However, uncertainty persists with regard to surgical ICU patients, whom may benefit from lower levels, based primarily on earlier data from the large Leuven study (some centers suggest levels lower than 140-150 mg/dL or lower in such patients). Risk of hypoglycemia is theoretically increased using this approach but with careful blood glucose monitoring (routine in ICU subjects on insulin infusion), this rarely occurs. Blood glucose control goals will be liberalized (≤200 mg/dL) when the subject is in the less-controlled environment of the general surgical ward.

Protections Against Risk: Aseptic procedures for central venous catheter (CVC) insertion and line/site maintenance will be followed per identical standard operating procedures (SOPs) in place at the study sites [Emory University Hospital (EUH) and Emory University Hospital-Midtown]. These include a mandatory checklist for physicians or physician assistant placement of CVC that includes documentation of site attempts and type of catheter placed (subclavian vein preferred as first choice of site unless contraindicated). Other required components include use of ultrasound guidance when possible, mandatory performance of CVC placement bundle components (SOPs for optimal hand hygiene, patient positioning, and maximal sterile barriers, including cap, mask and sterile gown and gloves for operators during procedure, large sterile drape and maintenance of sterile field) and notations regarding emergent or elective CVC insertion and tolerance of the procedure. Following line placement, the CVC dressing is changed by primary registered ICU nurses every 7 days or sooner as clinically indicated using chlorhexidine gluconate skin prep and a chlorhexidine gluconate-containing Biopatch (Ethicon, Johnson and Johnson, Somerville, NJ) with an occlusive dressing. The non-genital skin of the ICU patient is also washed every 24 hr with chlorhexidine gluconate skin prep.

We will incorporate conventional ICU insulin administration and tight blood glucose monitoring guidelines. All study personnel will closely communicate with the study patient's primary physicians and ICU care staff regarding nutrition support orders and transition from PN to EN and oral diet. Standard aseptic procedures for all blood drawing will be followed.

Confidentiality: All subject records will be kept in locked file cabinets in the P.I.'s/coordinator's research offices and at the Emory-based Data Coordinating Center (DCC) and will be accessible only to the P.I. and the investigative team. Subject identification numbers will code all data. The master list connecting the codes to identifying information will be secured in the DCC. All data maintained in the computerized database will be accessible only with a login and protected password. After the study is completed, all data will be kept according to NIH and FDA regulations in a locked file.

Data and Safety Monitoring Board (DSMB): The Data and Safety Monitoring Board (DSMB) will act in an advisory capacity to the National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK) to monitor subject safety and evaluate the efficacy of the intervention being studied in this clinical trial funded by the NIDDK. The DSMB will be appointed by the PIs upon study funding, and approved by NIDDK program staff. The DSMB will be composed of at least two experienced clinical nutrition support investigators not otherwise participating in the study and not affiliated with Emory. A biostatistician not otherwise participating in the study will also be named to the DSMB. The DSMB will meet twice yearly, and as needed, and will receive blinded and unblinded reports on study progress prepared by the DCC.

Statistical Analysis:

Continuous variables will be summarized with standard descriptive statistics and represented graphically with displays such as scatterplots, box plots, line plots and histograms, while categorical variables (adverse events) will be described by frequency tables.

For Aim 1: The primary analyses of the data will be performed according to subjects' original treatment assignment (i.e., intention-to-treat analyses) and the inclusion of all data from all subjects randomized in the final analysis. Rates of infection (i.e., BSI and total nosocomial infections) per 1000 hospital days will be estimated overall and for each of the 3 energy doses. Confidence intervals (95%) for the incidence rates will be estimated using an exact method based on the Poisson distribution.

Additionally incidence rates of infection will be estimated by performing a generalized estimating equations (GEE) Poisson regression analysis of the counts, implemented using SAS Proc Genmod, using an exchangeable correlation structure for the repeated counts within subject. The incidence rate ratio (IRR) is the ratio of the incidence density in one group to that of another group. Results by each baseline covariate will be presented as the IRR and the 95% confidence interval (CI). This GEE model can also accommodate time-dependent covariates such as daily blood glucose levels measured before an infection and total 28-day energy (cumulative deficit or as a time-dependent covariate).

Twenty-eight day mortality will be summarized as a proportion plus the 95% confidence interval (overall and for each treatment group). The 28-day mortality will be compared between treatment groups using a chi-square or Fisher's exact test. The potential association of each of the categorical prognostic factors (gender, race, high or low Apache score, clinical center) with mortality will also be evaluated using a Chi-square test or Fisher's exact test. Baseline continuous prognostic factors (age, SOFA score, blood glucose) will be compared by treatment group using the Wilcoxon rank-sum test. Odds ratios will be calculated to measure the degree of association. All statistical tests will be 2-sided. Factors significant to at least a value of $P \leq 0.20$ in the univariable analyses will be used in multivariable logistic regression analyses. Forward and backward stepwise selection will be used if necessary to choose prognostic variables for a multivariable logistic regression model. The odds ratio and its 95% confidence interval will be calculated for each factor in the presence of others in the final model.

Days on mechanical ventilation, length of ICU stay and hospital length of stay will be summarized with percentiles (25th, 50th and 75th) and the median absolute deviation. These secondary outcomes will be compared between the 3 treatment groups using the Kruskal-Wallis test. Repeated-measures analyses of daily SOFA scores and laboratory measures such as blood glucose will be analyzed with a means model with SAS Proc Mixed (version 9, mixed linear models) providing separate estimates of the means by time on study (days 0, 3, 7, 14, 21, and 28) and treatment group (energy dose: 0.6, 1.0 and 1.3 x REE). An unstructured variance-covariance form among the repeated measurements will be assumed for each outcome and estimates of the standard errors of parameters will be used to perform statistical tests and construct 95% confidence intervals. T-tests will be used to compare the pairwise differences between the model-based treatment means (least-squares means) at each time point. Statistical tests will be 2-sided. The model-based means are unbiased with unbalanced and missing data, so long as the missing data are non-informative (missing at random). A dropout process is assumed to be missing at random if; conditional on the observed data, the dropout is independent of the unobserved measurements.

For Aim 2: The agreement between the study entry HBE and the initial REE, as well as the mean daily serial MREE by metabolic cart, will be summarized using graphical methods and by calculating the 95% limits of agreement estimated by the mean difference ± 1.96 standard deviation of the differences. Bland and Altman (1999)[63] extend the limits of agreement approach to data with repeated measurements (i.e., serial REE measurements). To investigate whether the relationship between mean daily serial REE and entry HBE is altered by the mean daily caloric dose administered, regression methods will be used (i.e., analysis of covariance, ANCOVA). ANCOVA analyses will also be stratified by location (ICU versus the floor).

For Aim 3: The data processing will be performed as follows: 1) Feature detection conducted by systematic re-extraction with multiple parameter settings that optimize sensitivity and reliability; 2) Sample quality and feature consistency evaluation; 3) Feature overlap detection between datasets; 4) Statistical analysis includes false discovery rate (FDR; typically with q=0.05) to identify significantly different features between the two groups while correcting for multiple comparisons; hierarchical cluster analysis (HCA) to visualize differences between groups as

well as between time points and to identify clusters of highly correlated features that can be mapped to biological pathways; 5) Characterization of features and biological pathways utilizing multiple chemical databases (KEGG) metabolic pathways (http://www.genome.jp/kegg/tool/color_pathway.html), MetaCore (www.genego.com; Carlsbad, CA), and MetaCyc (http://www.metacyc.org).[69] We will utilize Metaboanalyst 2.0 [70] for the time series analysis to detect metabolic changes throughout the study period and two-way ANOVA to determine the impact of varying energy doses on global metabolomics patterns. Once important features have been identified, we have the ability to positively identify the chemical names of known metabolites by MS/MS analysis.

There is very little data available to inform an adequate power calculation for microbiome analysis; thus a formal power analysis was not completed. Samples will be compared by repeated-measures analyses using a mixed linear model for analysis of specific microbial phyla over time within subjects and for changes from baseline over time within and between the individuals. Both absolute effects and percent (%) change from baseline values will serve to correct for individual heterogeneity. If a specific parameter follows a non-normal distribution, an appropriate transformation will be used to ensure normality of the data. Pearson correlation coefficient will be used for association studies (e.g. SRB microbiota/microbiome changes with *dsr*A gene expression) [67].

References

1. Danai, P. and G.S. Martin, *Epidemiology of sepsis: recent advances.* Curr Infect Dis Rep, 2005. **7**(5): p. 329-34.
2. Milbrandt, E.B., et al., *Growth of intensive care unit resource use and its estimated cost in Medicare.* Crit Care Med, 2008. **36**(9): p. 2504-10.
3. *Guidelines for the use of parenteral and enteral nutrition in adult and pediatric patients.* JPEN J Parenter Enteral Nutr, 2002. **26**(1 Suppl): p. 1sa-138sa.
4. De Jonghe, B., et al., *A prospective survey of nutritional support practices in intensive care unit patients: what is prescribed? What is delivered?* Crit Care Med, 2001. **29**(1): p. 8-12.
5. Debaveye, Y. and G. Van den Berghe, *Risks and benefits of nutritional support during critical illness.* Annu Rev Nutr, 2006. **26**: p. 513-38.
6. Dvir, D., J. Cohen, and P. Singer, *Computerized energy balance and complications in critically ill patients: an observational study.* Clin Nutr, 2006. **25**(1): p. 37-44.
7. Giner, M., et al., *In 1995 a correlation between malnutrition and poor outcome in critically ill patients still exists.* Nutrition, 1996. **12**(1): p. 23-9.
8. Heyland, D.K., et al., *Canadian clinical practice guidelines for nutrition support in mechanically ventilated, critically ill adult patients.* JPEN J Parenter Enteral Nutr, 2003. **27**(5): p. 355-73.
9. Martindale, R.G., et al., *Guidelines for the provision and assessment of nutrition support therapy in the adult critically ill patient: Society of Critical Care Medicine and American Society for Parenteral and Enteral Nutrition: Executive Summary.* Crit Care Med, 2009. **37**(5): p. 1757-61.
10. Singer, P., et al., *ESPEN Guidelines on Parenteral Nutrition: intensive care.* Clin Nutr, 2009. **28**(4): p. 387-400.
11. Villet, S., et al., *Negative impact of hypocaloric feeding and energy balance on clinical outcome in ICU patients.* Clin Nutr, 2005. **24**(4): p. 502-9.
12. Ziegler, T.R., *Parenteral nutrition in the critically ill patient.* N Engl J Med, 2009. **361**(11): p. 1088-97.
13. Lochs, H., L. Valentini, and T. Schutz, *ESPEN Guidelines on Enteral Nutrition.* Clin Nutr, 2006. **25**: p. 177-360.
14. Finfer, S., et al., *Intensive versus conventional glucose control in critically ill patients.* N Engl J Med, 2009. **360**(13): p. 1283-97.
15. O'Brien, J.M., Jr., et al., *Body mass index is independently associated with hospital mortality in mechanically ventilated adults with acute lung injury.* Crit Care Med, 2006. **34**(3): p. 738-44.
16. Schneider, S.M., et al., *Malnutrition is an independent factor associated with nosocomial infections.* Br J Nutr, 2004. **92**(1): p. 105-11.
17. Zaloga, G.P., *Parenteral nutrition in adult inpatients with functioning gastrointestinal tracts: assessment of outcomes.* Lancet, 2006. **367**(9516): p. 1101-11.
18. Doig, G.S., F. Simpson, and A. Delaney, *A review of the true methodological quality of nutritional support trials conducted in the critically ill: Time for improvement.* Anesthesia and Analgesia, 2005. **100**(2): p. 527-533.
19. McClave, S.A., et al., *Are patients fed appropriately according to their caloric requirements?* Journal of Parenteral and Enteral Nutrition, 1998. **22**(6): p. 375-381.

20. Berger, M.M. and R.L. Chiolero, *Hypocaloric feeding: pros and cons.* Curr Opin Crit Care, 2007. **13**(2): p. 180-6.
21. Martin, C.M., et al., *Multicentre, cluster-randomized clinical trial of algorithms for critical-care enteral and parenteral therapy (ACCEPT).* Cmaj, 2004. **170**(2): p. 197-204.
22. Nardo, P., et al., *Clinical relevance of parenteral nutrition prescription and administration in 200 hospitalized patients: a quality control study.* Clin Nutr, 2008. **27**(6): p. 858-64.
23. Elke, G., et al., *Current practice in nutritional support and its association with mortality in septic patients--results from a national, prospective, multicenter study.* Crit Care Med, 2008. **36**(6): p. 1762-7.
24. Van den Berghe, G., et al., *Clinical review: Intensive insulin therapy in critically ill patients: NICE-SUGAR or Leuven blood glucose target?* J Clin Endocrinol Metab, 2009. **94**(9): p. 3163-70.
25. Gramlich, L., et al., *Does enteral nutrition compared to parenteral nutrition result in better outcomes in critically ill adult patients? A systematic review of the literature.* Nutrition, 2004. **20**(10): p. 843-8.
26. Griffiths, R.D., *Is parenteral nutrition really that risky in the intensive care unit?* Curr Opin Clin Nutr Metab Care, 2004. **7**(2): p. 175-81.
27. Marik, P.E., *Death by TPN... the final chapter?* Crit Care Med, 2008. **36**(6): p. 1964-5.
28. Pacelli, F., et al., *Enteral vs parenteral nutrition after major abdominal surgery: an even match.* Arch Surg, 2001. **136**(8): p. 933-6.
29. Peter, J.V., J.L. Woran, and J. Phillips-Hughes, *A metaanalysis of treatment outcomes of early enteral versus early parenteral nutrition in hospitalized patients.* Critical Care Medicine, 2005. **33**(1): p. 213-220.
30. Reid, C.L., *Poor agreement between continuous measurements of energy expenditure and routinely used prediction equations in intensive care unit patients.* Clin Nutr, 2007. **26**(5): p. 649-57.
31. Sena, M.J., et al., *Early supplemental parenteral nutrition is associated with increased infectious complications in critically ill trauma patients.* J Am Coll Surg, 2008. **207**(4): p. 459-67.
32. Anderegg, B.A., et al., *Comparison of resting energy expenditure prediction methods with measured resting energy expenditure in obese, hospitalized adults.* JPEN J Parenter Enteral Nutr, 2009. **33**(2): p. 168-75.
33. Solomon, S.M. and D.F. Kirby, *The refeeding syndrome: a review.* JPEN J Parenter Enteral Nutr, 1990. **14**(1): p. 90-7.
34. Krishnan, J.A., et al., *Caloric intake in medical ICU patients: consistency of care with guidelines and relationship to clinical outcomes.* Chest, 2003. **124**(1): p. 297-305.
35. Rubinson, L., et al., *Low caloric intake is associated with nosocomial bloodstream infections in patients in the medical intensive care unit.* Crit Care Med, 2004. **32**(2): p. 350-7.
36. Holmes, E., et al., *Chemometric models for toxicity classification based on NMR spectra of biofluids.* Chem Res Toxicol, 2000. **13**(6): p. 471-8.
37. German, J.B., et al., *Metabolomics in the opening decade of the 21st century: building the roads to individualized health.* J Nutr, 2004. **134**(10): p. 2729-32.
38. Grau, T., et al., *Liver dysfunction associated with artificial nutrition in critically ill patients.* Crit Care, 2007. **11**(1): p. R10.
39. Stanga, Z., et al., *Nutrition in clinical practice-the refeeding syndrome: illustrative cases and guidelines for prevention and treatment.* Eur J Clin Nutr, 2008. **62**(6): p. 687-94.
40. Brennan, L., *Metabolomics in nutrition research: current status and perspectives.* Biochem Soc Trans, 2013. **41**(2): p. 670-3.
41. Gibney, M.J., et al., *Metabolomics in human nutrition: opportunities and challenges.* Am J Clin Nutr, 2005. **82**(3): p. 497-503.

42. Jones, D.P., Y. Park, and T.R. Ziegler, *Nutritional metabolomics: progress in addressing complexity in diet and health.* Annu Rev Nutr, 2012. **32**: p. 183-202.
43. Llorach, R., et al., *Nutrimetabolomic strategies to develop new biomarkers of intake and health effects.* J Agric Food Chem, 2012. **60**(36): p. 8797-808.
44. Uppal, K., et al., *xMSanalyzer: automated pipeline for improved feature detection and downstream analysis of large-scale, non-targeted metabolomics data.* BMC Bioinformatics, 2013. **14**: p. 15.
45. Goldberger, A.L., et al., *Fractal dynamics in physiology: alterations with disease and aging.* Proc Natl Acad Sci U S A, 2002. **99 Suppl 1**: p. 2466-72.
46. Ivanov, P.C., et al., *Multifractality in human heartbeat dynamics.* Nature, 1999. **399**(6735): p. 461-5.
47. Peng, C.K., et al., *Non-equilibrium dynamics as an indispensable characteristic of a healthy biological system.* Integr Physiol Behav Sci, 1994. **29**(3): p. 283-93.
48. Peng, C.K., et al., *Quantifying fractal dynamics of human respiration: age and gender effects.* Ann Biomed Eng, 2002. **30**(5): p. 683-92.
49. Luo, M., et al., *Metabolic effects of enteral versus parenteral alanyl-glutamine dipeptide administration in critically ill patients receiving enteral feeding: a pilot study.* Clin Nutr, 2008. **27**(2): p. 297-306.
50. *Structure, function and diversity of the healthy human microbiome.* Nature, 2012. **486**(7402): p. 207-14.
51. Lozupone, C.A., et al., *Diversity, stability and resilience of the human gut microbiota.* Nature, 2012. **489**(7415): p. 220-30.
52. Schloissnig, S., et al., *Genomic variation landscape of the human gut microbiome.* Nature, 2013. **493**(7430): p. 45-50.
53. Tremaroli, V. and F. Backhed, *Functional interactions between the gut microbiota and host metabolism.* Nature, 2012. **489**(7415): p. 242-9.
54. Alcock, J., *Dangerous disappearing act: commensal gut microbiota after acute severe insults.* Dig Dis Sci, 2011. **56**(8): p. 2212-4.
55. Alverdy, J.C. and E.B. Chang, *The re-emerging role of the intestinal microflora in critical illness and inflammation: why the gut hypothesis of sepsis syndrome will not go away.* J Leukoc Biol, 2008. **83**(3): p. 461-6.
56. Morowitz, M.J., E.M. Carlisle, and J.C. Alverdy, *Contributions of intestinal bacteria to nutrition and metabolism in the critically ill.* Surg Clin North Am, 2011. **91**(4): p. 771-85, viii.
57. Mittal, R. and C.M. Coopersmith, *Redefining the gut as the motor of critical illness.* Trends Mol Med, 2014. **20**(4): p. 214-23.
58. Oberauner, L., et al., *The ignored diversity: complex bacterial communities in intensive care units revealed by 16S pyrosequencing.* Sci Rep, 2013. **3**: p. 1413.
59. Nelson, A.M., et al., *Disruption of the human gut microbiota following Norovirus infection.* PLoS One, 2012. **7**(10): p. e48224.
60. Vincent, J.L., et al., *The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure.* Intensive Care Medicine, 1996. **22**(7): p. 707-710.
61. Ziegler, T.R., *Parenteral Nutrition in the Critically Ill Patient.* New England Journal of Medicine, 2009. **361**(11): p. 1088-1097.
62. Yu, T., et al., *Hybrid feature detection and information accumulation using high-resolution LC-MS metabolomics data.* J Proteome Res, 2013. **12**(3): p. 1419-27.
63. Jones, D.P., Y. Park, and T.R. Ziegler, *Nutritional metabolomics: progress in addressing complexity in diet and health.* Annual Review of Nutrition, 2012. **32**: p. 183-202.
64. Wu, G.D., et al., *Sampling and pyrosequencing methods for characterizing bacterial communities in the human gut using 16S sequence tags.* BMC Microbiol, 2010. **10**: p. 206.

65. Wang, Y., et al., *16S rRNA gene-based analysis of fecal microbiota from preterm infants with and without necrotizing enterocolitis.* Isme j, 2009. **3**(8): p. 944-54.
66. Harrell, L., et al., *Standard colonic lavage alters the natural state of mucosal-associated microbiota in the human colon.* PLoS One, 2012. **7**(2): p. e32545.
67. Devkota, S., et al., *Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in Il10-/- mice.* Nature, 2012. **487**(7405): p. 104-8.
68. Vermeiren, J., et al., *Decreased colonization of fecal Clostridium coccoides/Eubacterium rectale species from ulcerative colitis patients in an in vitro dynamic gut model with mucin environment.* FEMS Microbiol Ecol, 2012. **79**(3): p. 685-96.
69. Uppal, K., et al., *xMSanalyzer: automated pipeline for improved feature detection and downstream analysis of large-scale, non-targeted metabolomics data.* BMC Bioinformatics, 2013. **14**(15).
70. Xia, J., et al., *MetaboAnalyst 2.0 - a comprehensive server for metabolomic data analysis.* Nucleic Acids Research, 2012. **40**: p. W127-133.