Study Protocol:

Supplemental enteral protein in critically ill trauma and surgical patients: A randomized clinical trial

NCT03170401

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This document provides a description of the study protocol and analysis plan for this clinical trial.

1. Overall design and conduct of this single center clinical trial.

2. Data safety and monitoring plan.

3. Modification to the study protocol specifically regarding pauses in enrollment and additional evaluation of safety and adverse events.

4. Names and responsibilities of the study team members.

5. Study sponsorship.

6. Citations for study description and protocol.

1 Overall design and conduct of the clinical trial

This is a single center, randomized clinical trial in which critically ill trauma victims and surgical patients are identified, recruited, and randomized to either standard enteral nutritional support or to treatment with early supplemental enteral protein to at target amount of 2gm/kg/day. The treatment protocol/intervention was designed, tested and reported in a prospective, observational cohort study of 53 critically ill surgical patients.[1]

1.1 Background and rationale

Observational studies suggest that higher amounts of protein are associated with better outcomes in critically ill patients. However, clinical trials addressing the question of protein dosing in critically ill trauma patients have not been performed. Based upon expert opinion in addition to observational data, the 2016 ASPEN guidelines recommended that critically ill patients should receive 1.2 – 2.0 gm/kg/day of protein and potentially higher amounts in critically ill trauma and burn patients.[2] Our trial is designed to test this hypothesis in trauma and surgical patients by randomizing to early supplemental enteral protein or to standard enteral nutritional support.

1.2 Primary Research Question

In critically ill trauma and surgical patients does receiving a higher amount (≥2.0 grams/kg/day) of protein compared to usual care (~1.0 gram/kg/day) improves biomarkers of nutritional status (serum transthyretin concentration) and short-term clinical outcomes (ventilator-free days and ventilator-associated pneumonia).

1.3 Study hypotheses

Compared to receiving standard enteral nutritional therapy, the administration supplemental enteral protein to target a total protein intake of 2 gm/kg/day leads to higher serum transthyretin

concentrations during the 3rd week after admission to the intensive care unit and increase the number of ventilator-free days.

1.4 Trial design

A single center, randomized, open-label, clinical trial including subjects admitted to the trauma/surgical intensive care unit at Harborview Medical Center in Seattle, Washington, USA. A CONSORT style flow diagram will present the numbers of patients screened and all reasons excluded prior to randomization. This figure will also present the number randomized to each arm and the numbers included in the assessment of outcomes.

1.5 Eligibility criteria:

Published at https://clinicaltrials.gov/ct2 Study Details | Supplemental Enteral Protein in Critical Illness | ClinicalTrials.gov/show/NCT03160547.

1.6 Study procedures

Screening of all potential subjects will be done by the Registered Dietitian who is assigned to the Critical Care team and is a member of the research team. Potential study subjects will be identified upon admission to the critical care unit and considered for enrollment if they meet inclusion criteria. Once a subject has been identified, we will randomize that subject into one of the two treatment arms. We will randomly assign subjects to receive either additional enteral protein to achieve a total of 2g/kg/day of protein or to standard enteral nutritional support. Due to the limited published information and the variation across hospitals and within our own practice, both treatment groups can be considered standard care. At Harborview, there is no protocol utilizing one method of protein dosing vs the other. Both are approaches used in our critically ill surgical and trauma patients. The University of Washington Institutional Review Board has approved this study with a waiver of documented consent as it is considered minimal risk (.

We will use a ***randomized design*** to test whether supplementing enteral protein improves patient care and outcomes. Subjects will be followed until discharged from the hospital and clinical data will be obtained from the electronic medical record. The randomization will be done by previously sealed envelopes that contain an equal number of each treatment assignment. Subjects are randomized 1:1 to either no or additional enteral protein.

Standard of care at our institution includes obtaining nutrition-related labs. Vitamin C, Vitamin D, Zinc and other vitamins and micronutrients are typically measured within 24 hours of ICU admission and used to guide supplementation and treat any deficiencies. Circulating transthyretin (TTHY), C-reactive protein (C-RP) are measured approximately weekly in patients requiring greater than 7 – 10 days of nutritional support. Similarly, we often obtain urine for 24-hour total urinary nitrogen (TUN) measurements, and indirect calorimetry (this is a measure, not a lab procedure) after 7 – 10 days of support. Indirect calorimetry is used to adjust caloric intake and to better understand a patient's general use of energy sources. In this trial the dietitian and

clinical team may adjust caloric goals based upon indirect calorimetry. We will not adjust protein intake based upon TUN or TTHY measurements.

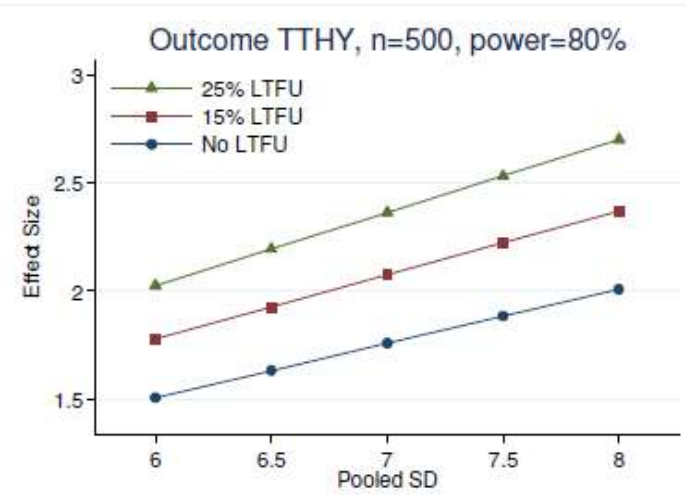
In a subgroup of study subjects, we will also obtain serial blood and urine samples for analyses of metabolites. Each blood sample collected will be 5ml and each urine sample will be l00ml. The samples will be obtained on Day 0 (defined as the day nutritional support is initiated), Day 1 (24 hours later), Day 3 (72 hours later +/- 24 hours), Day 7-13, Day 14-20, Day 21-27, Day 28-34. If the subject is discharged prior to any of these timepoints, we would obtain the final sample on the day of discharge. Only one sample will be collected during the above timepoints.

Urine will be collected from the subject's indwelling catheter, if they do not have an indwelling catheter, no urine samples will be obtained. Blood samples will be obtained through an existing IV catheter if possible. If the subject does not have an existing IV catheter, we will obtain the sample by venipuncture by either the bedside RN or the research RN. We have found that without the ability to obtain the samples by venipuncture (with the subject's permission), we are not able to collect the later samples, and this is affecting the data analysis. We will stop all urine collection when the indwelling catheter has been removed. The blood and urine samples will be analyzed for approximately 200 metabolites as well as inflammatory biomarkers.

Patients will be screened, evaluated, and randomized within 96 hours of admission to the ICU by the study dietitian with guidance from the principal investigator in the case of uncertainty whether the patient qualifies. Subjects will be randomized 1:1 to either supplemental enteral protein or standard enteral nutritional support. Randomization is performed using sealed envelopes with the treatment assignment.

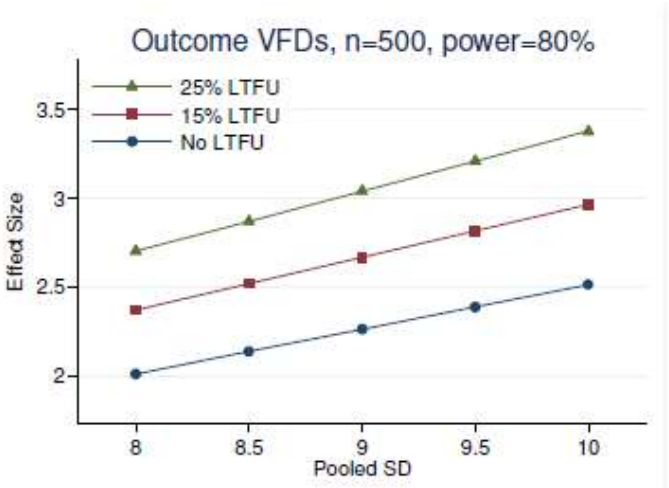
1.7 Sample Size Considerations

Sample size calculation and statistical analysis of circulating transthyretin concentrations (Primary hypothesis): **With 500 participants** this study has 80% power to detect a difference of 2.4 mg/dl in average circulating transthyretin levels during the third week between the protein supplementation group (assumed average circulating transthyretin level of 14.4, and the standard of care group (assumed average circulating transthyretin level of 12.0, with a pooled standard deviation of 7.0). This conservatively assumes that there will be 25% of the patients for whom the protein supplementation has no effect in that they are either well enough to have high circulating transthyretin levels or die prior to obtaining the primary outcome. Because the



primary outcome is a surrogate marker, we do not plan for any formal interim analyses to evaluate efficacy or futility. Harborview Medical Center typically admits about 300 - 350 trauma victims per year who would meet eligibility criteria. With enrollment over 4 - 5 years, we anticipate that ~1500 patients will be eligible. Therefore, the rate of recruitment among eligible patients needs to be ~33%. The statistical analysis of the primary hypothesis will use a two-sided t-test with alpha of 0.05. We expect that less than 10% of patients will die prior to day 21. For these and for patients who are lost-to-follow-up prior to day 21, we will multiply impute the primary outcome separately for each group and incorporate previously observed circulating transthyretin levels (if available) and patient age information.

Power calculation and statistical analyses of ventilator-free days (VFDs; secondary hypothesis): VFD is a relevant endpoint in critical care and critical care research. We will use this endpoint as our main clinical outcome as it generally reflects the development of complications and provides and objective estimate of the rate of recovery. VFD could be expected to be impacted by nutritional support in several important ways. The adjacent figure represents the difference in VFD between the groups that we have 80% power to detect for a range of pooled VFD standard deviations and assuming no loss to follow-up (LTFU), 15% LTFU and 25% LTFU, based upon the sample size selected for the primary hypothesis (n = 500). For patients who die while in the hospital, VFDs will be set to zero. For patients who are lost to follow-up (unlikely), VFDs will be multiply imputed separately by treatment group, using patient age and measures of injury severity (injury severity score, AIS scores).



1.8 Interim analyses

There are no planned interim analyses for this study and no early stopping rules were designed.

1.9 Timing of final analysis

All outcomes will be analyzed by arm once all data is collected, data cleaning is complete and after the statistical analysis plan (SAP) is finalized. See modifications to the study protocol for details regarding the final timing of data analysis that was based upon additional collection of data related to potential complications of the treatment intervention.

1.10 Timing of outcome assessments and recording of nutritional intake

All outcome assessments are based upon data from the index hospitalization. Daily nutrition intake was assessed only for up to a maximum of 28 days in the ICU. This included daily enteral formula type and volume, daily enteral caloric and protein intake, and daily intravenous fat (as propofol) and glucose calories administered.

1.11 Confidence intervals and p-values

95% confidence will be presented for selected key outcomes. P-values will be two-sided without adjustment for multiplicity. We will use the traditional two-sided $p \leq 0.05$ to indicate statistical significance for the primary and other prespecified outcomes. For subgroup analyses we will report only confidence intervals.

1.12 Description of general nutritional support for trial subjects

Our approach to nutritional support and monitoring for subjects in this trial will follow the general approach that has been used in our intensive care unit and reported previously.[3-5] Once deemed ready to receive enteral nutrition, the patient is prescribed an isotonic formula with 1 – 1.5 kcal/ml. Continuous infusion starts at 20 ml/hour and the rate is advanced over 24 hours to an initial goal of 25 kcal/kg/day delivered by continuous infusion via an orogastric or nasogastric tube. Subsequent modifications of caloric and other nutritional prescriptions are performed by the ICU dietitian and include use of the Harris-Benedict equation and indirect calorimetry in appropriate patients after 5 – 7 days of mechanical ventilation. Monitoring for intolerance is primarily by clinical examination. Diarrhea is managed first but modifying the enteral formula to include higher fiber and testing for *C. difficile* infection.

1.13 Description of enteral protein supplementation treatment

The dietitian will calculate energy and protein needs as indicated in *1.11* above. For subjects randomized to the supplemental protein, arm 2 gm/kg/day of Prosource (Medtrition, Lancaster, PA) will be administered via the nasal/oral feeding tube in 60 – 180 ml bolus infusions 2 – 4 times per day, independently of the enteral formula received. That is, the daily target amount of protein will be administered as supplement boluses beginning on the day the patient is enrolled. Once the enteral formula infusion approaches the target rate then the amount of supplemental protein will be gradually decreased. Supplemental protein will be reduced by 50% once the patient received 75% of targeted caloric intake over the previous day (0700 – 0700). Once the patient reaches the target caloric intake for 48 continuous hours, the amount of supplemental protein will be decreased in order that the total protein prescribed equals 2 gm/kg/day.

2 Data safety monitoring plan

Safety of our intervention will be reviewed by an independent Data Safety Monitoring Board (DSMB) after at 2 timepoints during the trial. The committee will include surgical intensivists who also have experience with clinical and translational research. Data for review will be

prepared by the study analyst (Qian Qui) and study biostatistician (Susanne May). The review will be iterative. After review of the summary information, additional data from the EMR can be added at the request of the members. Members will also have access to individual patient/study subject records as needed for additional details.

3 Modifications to the study from initial protocol

3.1 Enrollment pauses

There were 3 pauses in subject enrollment over the course of the clinical trial. The first pause was from August 8th – 27th, 2019. This pause was necessary while waiting for approval from the IRB for an updated blood sampling strategy that included more frequent blood sampling. The second pause was due to the COVID-19 pandemic, and we were required to stop non-critical activities at our hospital for 2 ½ months (3/10/2020 – 5/24/2020). Enrollment was also paused for 3 ½ month period while the hospital transitioned to a new electronic health record (EHR). This new EHR included a mechanism to record patient enrollment into clinical trials and we paused enrollment until that system was functional.

3.2 Update to Institutional Review Board approval – August 2019

On August 23, 2019, the protocol was updated following review by the full IRB committee. The IRB had initially provided this approval using the process of "expedited review". When the study approval was migrated from the original "paper"-based format to the new Zipline, electronic format the IRB determined that review by the entire committee was required to permit waiver of documented consent for the frequency of blood sampling that we requested. This oversight on the part of the IRB led to a pause in enrollment from August 6th – 27th, 2019 (first pause described in 3.1).

3.3 Expansion of data collection procedures

In 2021, as we were approaching complete enrollment, we obtained approval from the University of Washington IRB to obtain data from the electronic medical record and from the institutional quality improvement databases to provide additional information regarding hospital acquired infections and other complications for study subjects. At this time, the IRB also approved our request to obtain a minimal dataset for patients who were potentially eligible but who were not enrolled in the trial. We included the following request in our modification: "We are requesting permission to retrospectively and prospectively enroll subjects who meet inclusion/exclusion criteria but are not planned for randomization. We are requesting permission to obtain this data from the retrospective group to the start of this study. We will identify patients who met enrollment criteria, but were not randomized, beginning on 11/17/2016 (date first randomized subject was enrolled) through the present date. For these retrospectively identified subjects, we will obtain data from the EMR. Prospective: Beginning with approval of this MOD, we will

collect data from the EMR from patients who met enrollment criteria, but not randomized. This group will be identified prospectively, and data collected upon discharge from the hospital. The purpose of obtaining information for these eligible, but not randomized subjects is to be able to create an accurate CONSORT diagram, which will be required for reporting/publication of any data from this trial. This information will also be helpful for future grant submissions."

3.4 Additional assessments of adverse events and complications

Following the completion of enrollment, initial data collection and analyses, we observed more respiratory adverse events in the supplemental protein treatment arm than the control arm (see manuscript Table 3: Safety, complications, and adverse events). Both reintubation rates and possible aspiration rates were higher. To better understand whether subjects had aspirated gastric contents, the timing of this aspiration, whether it caused deterioration in gas exchange or lead to pneumonitis or pneumonia, we reviewed the medical records directly. Briefly, 2 co-investigators independently reviewed medical records and were asked to "adjudicate" whether a subject had clear evidence of tracheobronchial aspiration of gastric contents. All instances of reintubation or possible aspiration (see eMethods for definitions) were reviewed. Adjudicated aspiration was assigned if there was consensus assignment by the 2 reviewers. If there was disagreement, a third, independent reviewer made the assignment. All reviewers were blinded to the treatment assignment. These reviews were conducted from 1/4/2023 – 4/1/2023. The data were then merged with the research database and submitted to a study statistician (Siobhan Brown) for analysis.

4. Names and responsibilities of the study team members

Grant E. O'Keefe, MD. Professor of Surgery, University of Washington. Lead investigator. Obtained funding from NIGMS, Designed the trial and co-designed the treatment intervention with Marilyn Shelton. Oversaw study subject enrollment and assisted with determination of potential study subject eligibility. Responsible for reviewing all data analyses and drafting the manuscript.

Siobhan P. Brown, PhD. Senior Research Scientist. Department of Biostatistics. University of Washington. Created the statistical analysis plan in consultation with Grant O'Keefe and Susanne May. Conducted the statistical analyses for the manuscript and prepared the summary tables based upon these analyses. Also performed critical review of the manuscript.

Qian Qui, MBA. Harborview Injury Prevention and Research Center, University of Washington. Responsible for overall data management over the course of the clinical trial. This included extraction of data from the EMR databases, cleaning the data and generating scores (APACHE, SOFA, etc.) from the raw data in the EMR. Also linked data from the various data sources (EMR derived data, trauma registry and other hospital registries and the nutritional data collected during the trial) to create the dataset used for the final analyses. Prepared data for the DSMB. Critical review and revision of the manuscript.

Marilyn Shelton, RD. Clinical Dietitian, Harborview Medical Center. Co-designed the treatment intervention with Grant O'Keefe. Identified potential study subjects for inclusion. Determined nutritional intake goals (calories and protein) for enrolled subjects. Recorded daily nutritional intake while subjects were receiving care in the intensive care unit up to a maximum of 28 days. Critical review and revision of the manuscript.

Erika K Bisgaard, MD, Assistant Professor. Department of Surgery. University of Washington. Responsible for health record review and adjudication of aspiration. Critical review of the manuscript.

Alex Malloy, MD. Clinical Instructor. Department of Surgery. Responsible for health record review and adjudication of aspiration. Critical review of the manuscript.

Jamie Robinson, MD. Clinical Instructor. Department of Surgery. University of Washington. Responsible for health record review and adjudication of aspiration. Critical review of the manuscript.

Dan Robiuk, MD. Clinical Instructor. Department of Surgery. University of Washington. Responsible for health record review and adjudication of aspiration. Critical review of the manuscript.

Ida Wilson, MD. Clinical Instructor. Department of Surgery. University of Washington. Responsible for health record review and adjudication of aspiration. Critical review of the manuscript.

Susanne May, PhD. Professor, Biostatistics. University of Washington. Initial design of the clinical trial, including sample size estimates and power analyses for secondary outcomes. Assisted with the completion of progress reports and with the preparation of data and consultation as part of the data safety reviews. Critical review of the manuscript.

5. Study sponsorship

This study was supported, in part, but National Institutes of Health Grant 1RO1GM127790 to the University of Washington (PI: Grant O'Keefe). The funding agency had no role in any aspects of the design of the trial or the analyses of data.

6. Citations for study description and protocol

1. O'Keefe GE, Shelton M, Qiu Q, Araujo-Lino JC. Increasing Enteral Protein Intake in Critically Ill Trauma and Surgical Patients. *Nutr Clin Pract*. Oct 2019;34(5):751-759. doi:10.1002/ncp.10256
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5. Chung CK, Whitney R, Thompson CM, Pham TN, Maier RV, O'Keefe GE. Experience with an enteral-based nutritional support regimen in critically ill trauma patients. *J Am Coll Surg*. Dec 2013;217(6):1108-17. doi:10.1016/j.jamcollsurg.2013.08.006