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Early Metabolic Support for Critically Ill Trauma Patients – A Prospective Randomized Controlled Trial

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Background

The role of nutritional support and its benefits to patient outcomes are well established for surgical patients who present with malnutrition or who are at high risk of developing malnutrition during their illness. However, there is a lack of consensus regarding the optimal timing and components of nutritional support, particularly for critically ill patients after significant trauma. Despite a bimodal distribution in age of trauma patients, a majority of trauma victims are generally younger than other hospitalized patients, often have fewer co-morbidities, and are usually well nourished at the time of their injury. (1-3) Of trauma patients that survive the initial post-injury period, those that ultimately die do not succumb to their initial injury per se, but rather from the later complications of injury. (3)

The post-injury response has been well described beginning with the work of Cuthbertson in the 1930's. (4) Nutrition support post injury is crucial in supporting and modulating these metabolic and immunologic responses initially, and in limiting the likelihood of severe malnutrition and its associated complications subsequently. (5-7) Attempts at early enteral nutrition in the first week rarely meets energy and protein requirements due to the frequent pausing of feedings for interventions and procedures early in the hospitalization and/or feeding intolerance, routinely providing less than 50% of caloric and protein needs. (5, 6) Furthermore when energy requirements are seriously underprovided, protein needs are increased due to diminished protein utilization. The suggested protein requirements for critically ill patients are approximately 1.5g protein/day with suggested higher intakes (2-2.5g protein/day) in some patients, such as with severe burns. (7, 8) These intakes are rarely achieved in the first week post injury. (9-12) There is compelling evidence that early enteral feeding leads to significant

improvements in outcomes for patients during critical illness. (13) It remains unclear however, whether, and if so why, generally well-nourished trauma patients benefit from early nutrition support and which components of nutrition are primarily responsible for these benefits.

Due to the lack of expert consensus on the best way to feed critically ill trauma patients in the first week post injury, we chose to focus on providing supplemental protein within 24-hours of admission to the ICU for the first 5 days post injury, when these patients are believed to be the most catabolic.(14) It is our hypothesis that early immediate post injury metabolic support with a focus primarily on adequate protein, in the form of intravenous amino acids, would have a unique effect on the metabolic environment leading to nontoxic changes in substrate utilization with substantial alterations in metabolic pathways. This early provision of high protein in the form of parenteral amino acids to injured patients should favorably modulate their systemic inflammatory response, which could ultimately have a significant impact on patient outcomes.

Methods

We conducted an IRB-approved, prospective randomized controlled trial at our high-volume level-one trauma center in patients 18 to 65 years-old admitted to the surgical intensive care unit (SICU) post-injury from either the emergency department or the operating room. Study designed in accordance with CONSORT guidelines. To be recruited, potential patients were expected to survive a minimum of 72-hours and then to remain in the hospital for at least seven days. Patients were excluded from the study if they had: pre-existing malnutrition as defined by a body mass index (BMI) of less than 18 kg/m^2, Class II obesity (BMI>35 kg/m^2), on active immunosuppression, with type-1 or type-2 diabetes mellitus, were clinically determined to not be

appropriate candidates for enteral feedings upon admission (i.e. requiring parenteral nutrition), were pregnant, or had pre-existing renal dysfunction. (Supplemental 1, http://links.lww.com/TA/C179).

Individuals admitted to the SICU were nutritionally assessed within 24-hours to determine energy and protein requirements. Full energy requirements were set within a range of 18-25 kcals/kg current body weight. Current body weight was used, with an estimate of dry weight if subjects had been vigorously resuscitated. Protein requirements were set within a range of 1.5-2.0 grams protein/kg of body weight/day in the critically ill, depending upon organ function, route of feeding, and other possible protein losses. (7, 8, 11, 15) Protein from enteral nutrition was included in this calculation. Other electrolytes and micronutrients were supplied via enteral feedings or administered parenterally based on plasma levels, which were checked in the SICU. The primary outcome of interest was observed differences in metabolomic profiles between both groups. Secondary outcomes included differences in: total nitrogen balance, urea nitrogen change, length of stay, as well as changes in pro-inflammatory cytokines and soluble-receptors.

Randomization and Blinding: In this open-label, randomized controlled trial, all eligible patients were randomized within the first 24-hours of admission to the SICU by the Investigational Pharmacy Service into either *early metabolic support* (EMS) or *standard of care nutrition support* (standard). Randomization was performed stratified by type of injury (blunt, head or penetrating injury) using a block size of two.

***Standard of Care Nutritional Support (Standard)*:** Among those randomized to the standard group, enteral nutrition was initiated as soon as clinically feasible as determined by the SICU team. Only if and when enteral feedings failed after 7 days, consistent with the standard-of-care and published ASPEN/SCCM guidelines (7), were parenteral feeds started. Enteral nutrition utilized in the SICU varied based on the needs of the patient and were typically either an immune-enhancing formula or fish-oil- enhanced predigested formula; this was consistent for both study groups.

***Early Metabolic Support (EMS)*:** Subjects randomized to the EMS group began peripheral infusion of amino acids within 24-hours of admission to SICU. The initial intravenous nutrition solution used as our intervention consisted of approximately 27 grams dextrose and 65 grams of a mixed standard parenteral amino acid solution per liter (65 grams of 15% amino acids, 70 mEq NaCl, mixed with D5W to provide 27.4 grams dextrose per liter, with a total osmolarity of 877.8 mOsm). The solution provided approximately 353 kcals per liter, primarily as protein (65 grams of protein/L). The solutions were infused at a constant rate over 24-hours and titrated to provide the patient with at approximately 1.5 grams of protein/kg/day. Similar to the Standard group, enteral feedings were initiated when clinically appropriate and advanced as tolerated as per standard-of-care in both groups. As protein delivery via enteral feeds increased, the quantity of infused amino acids was adjusted to maintain within the study goals of 1.5-2.0 g/kg/day of protein delivery.

After 5 days, the parenteral amino acids were discontinued in the EMS group. Acute

Physiology and Chronic Health Evaluation Score II (APACHE II), Injury Severity Scores (ISS), Glascow Coma Scale (GCS), and sequential organ failure assessment (SOFA) scores were measured on admission to the SICU and on day-5.

Two time points were used to assess for metabolic status: Baseline is defined as the period within 24-hours of admission to the SICU and day-5 is defined as five days after admission to the SICU. Twenty-four-hour urine collections were performed for measurement of catabolic index to assess the level of catabolism and urine urea nitrogen to estimate clinical nitrogen balance within 24-hours of admission (baseline) and again on day-5 of the study. Nitrogen balance in the intervention group included approximately 12-hours of amino acid infusions. Nitrogen balance was calculated by the total nitrogen intake, minus urea nitrogen output, plus 20% for other urine nitrogen losses, and 2 grams nitrogen for other insensible losses.(16) Insensible losses include sweat, respiratory losses, and gastrointestinal losses, etc.(16)

Blood draws occurred at baseline prior to intervention initiation and again on day-5 to profile subjects' inflammatory and metabolomics status as well as: white blood cell count (WBC), creatinine, albumin, and blood urea nitrogen level (BUN).

Metabolomics: Metabolomics of each group were assessed from blood samples taken at baseline and again on day-5 by a commercially available company, (Metabolon, Morrisville, NC). Full reports and data from metabolomics are available for review on request. Given the exploratory nature of metabolomics profiling, a vast number of potential endpoints (in the form

of spectral bins or metabolite concentrations) are acquired for each sample analyzed. In order to manage and analyze the large volume of data points obtained for each patient sample, dimension reduction via techniques such as Principal Component Analysis (PCA) was utilized. Significant metabolomics differences are not attributed to variation in single dimensions, but rather to variation in clusters of metabolites. Consequently, there is no one accepted way to do a power analysis for a metabolomics study, however our sample size was modeled after similar successful metabolomic studies in sepsis patients. (17, 18).

Cytokine Assessment: A total of 27 cytokines and soluble receptors were assessed at baseline and again on day-5 as part of the multiplex assay (Eve Technologies, Calgary, Canada). (Supplemental 2, http://links.lww.com/TA/C180).

Statistical Analysis: All statistical analysis was performed in by a blinded statistician. Summary statistics presented include the median and interquartile range (IQR) for measured variable and N (%) for categorical variables. Differences in baseline characteristics between the EMS and standard groups are compared using the Wilcoxon rank sum test for measured characteristics, and Fisher's exact test for categorical characteristics. Comparisons of change from baseline to day-5 between groups, including cytokines, was assessed using the Wilcoxon rank sum test. Mean daily nutritional parameters from baseline to day-5 were compared between groups using a linear mixed effects model with a random intercept to account for correlation within measurements from the same patient. Analyses were performed using SAS v9.4 with $p<0.05$ considered statistically significant. No adjustment for multiple testing was performed in this pilot study so that results should be considered exploratory in nature.

In addition to the above analyses, an evaluation of biochemical markers was performed by Metabolon. Changes over time in metabolites were compared between groups using two-way ANOVA. PCA was used to visually examine how samples with similar biochemical profiles cluster together and samples with different profiles tend to segregate from each other. Random Forest analysis was used to classify markers that best distinguished changes within groups and differences between the intervention and control groups, while providing a measure of predictive accuracy. Boxplots of biochemical markers by group and time are also presented.

Results

A total of 42 patients were randomized into well balanced groups with similar presenting factors and baseline demographics as shown in Tables 1a and 1b. The distribution of mechanism of injury for each group was similar.

Median baseline urea nitrogen excretion was similar between both the EMS and standard groups (11.5 vs. 9.0 grams over 24-hours; p=0.1191) respectively. The median change in urea nitrogen excretion over the five days of the study (as assessed by 24-hour urine collections) was significantly higher in the EMS group (13.5g/24-hours) compared to the standard of care group (3.5g/24-hours; p=0.0031). (Table 2).

The baseline nitrogen balance in the EMS group was collected approximately 12-hours after the start of amino acid infusion (24-hour urine collection) so may not reflect true day one nitrogen economy, as it takes approximately four days to see the full impact of an alteration in nitrogen balance in response to dietary changes in protein. In this trial, we provided a daily

average over 5 days of 1053 kcals (12.6 kcals/kg/d) with 122 grams protein (1.43 g/kg/d) in the EMS group, and 596 kcals (7.5 kcals/kg/d) with 31g protein (0.35 gm/kg/d) in the standard of care group. (Supplemental 3, http://links.lww.com/TA/C181). Despite this, we found that day-5 nitrogen balance was significantly more negative (-16.3g, IQR -26.6, -8.5) in the EMS group as compared to the standard group (-5.3g IQR -15, 0.56), p=0.0276. (Table 2).

To assess the metabolic profile of each group, a total of 834 biochemicals were analyzed. We found that providing EMS had a significant effect on 67 biochemicals (8%) over the intervention period as compared to the standard group. When we analyzed the impact of treatment versus time using a PCA, we found that the greatest difference occurred over time as opposed to across treatment groups as seen in Figure 1.

The baseline metabolic profiles of both the EMS and standard of care groups were similar; (predictive accuracy of a random forest confusion matrix = 31%) indicating successful randomization. At day-5 after intervention, a clear distinction emerged between the intervention group and standard group as demonstrated by a predictive value of 77% by random forest confusion matrices, indicating an ability to predict group membership.

Assessing changes within each group over time, the biochemical profile of patients who received 5 days of EMS was defined by greater declines in circulating levels of stress hormone precursors and increased levels of amino acids. (Figure 2). Whereas the metabolic profile for patients who received the standard of care during the study period was defined by two biochemicals consistent with food metabolites (homostachydrine; 4-vinylphenol sulfate) and a

marker of skeletal muscle protein catabolism (3-methylhistidine). (Figure 3).

Comparing day-5 values between the EMS group and standard group, the five metabolites defining the greatest difference between the groups at day-5 (in order of impact) were histidine, 2-aminoheptanoate, isobutyrlcarnitine, methionine, and proline. (Figure 4). Both the standard and EMS groups experienced an increase in levels of circulating amino acids between baseline and day-5; however, patients that received EMS experienced greater increases in levels of circulating levels of amino acids. Similarly, both groups experienced increased levels of arginine, ornithine, and urea between baseline and day-5 with the EMS group experiencing the greatest increase. (Supplemental 4, http://links.lww.com/TA/C182). Additionally, both groups experienced an increase in tryptophan metabolism between baseline and day-5 as evident by an increase in tryptophan metabolites. (Supplemental 5, http://links.lww.com/TA/C183). However, patients in the EMS group saw a significant increase in tryptophan levels over the course of the intervention (ANOVA Contrast baseline/day-5 = 1.16) as compared to the standard of care group. Patients who received supplemental amino acids also had greater increases in branched chain amino acids levels and their metabolites over time as compared to the standard of care group. (Supplemental 6, http://links.lww.com/TA/C184). The EMS group experienced an increase in methionine and glutathione metabolism and a significantly greater decrease in glucose metabolism as demonstrated by a decrease in both pyruvate (ANOVA contrast 0.67 vs. control of 0.55) and lactate (ANOVA contrast 0.71 vs. control of 0.55) between baseline and day-5. (Supplemental 7, http://links.lww.com/TA/C185).

Cytokine levels between EMS and standard groups were similar at baseline. Alterations

in the inflammatory response following EMS resulted in a greater decrease in IL-1B (p=0.02) and increase in sIL-6-receptor (p=0.01) between baseline and day-5 as compared to the standard of care. (Supplemental 1, http://links.lww.com/TA/C179).

Furthermore, we found that the EMS group had significantly greater increase in BUN and significantly smaller decline in albumin over the course of the intervention as compared to the standard group. (Supplemental 8, http://links.lww.com/TA/C186). After adjustment for ISS, there was no significant difference in median length of stay (LOS) or SICU LOS between both groups.

Discussion

In the setting of a randomized controlled pilot study, we found that early metabolic support (in the form of parenteral amino acid supplementation) administered to critically ill trauma patients, is not harmful, substantially modulates many beneficial aspects of amino acid metabolism, and may both decrease the inflammatory stimulus and lower the stress response. However, EMS did not improve net-protein balance in the first week post injury. Although steady state was not likely achieved in this setting, there was an apparent greater net catabolism with the higher protein intake. Given the improvement in some other markers of protein metabolism and immune responsiveness (enhanced amino acid metabolism, higher albumin and sIL-6 receptor levels, reduced glucose metabolism, and enhanced antioxidant production despite lower IL-1B levels) in the EMS group, this does suggest a beneficial modulation and perhaps decrease of the systemic inflammatory response by the increased amino acid therapy with limited carbohydrate intake. Critically ill trauma patients are unique, as represented in this study, being

young and well-nourished at baseline which is consistent with the literature.(1, 2, 19-21) The significant stress from trauma in these patients, as reflected in their elevated ISS and SOFA scores on presentation, carry high metabolic demands.

It is well established that adequate feeding with protein in a carbohydrate-based formula in other critically ill patients can improve nitrogen balance by improving protein synthesis, but has limited effect on protein catabolism.(14, 22, 23) It has also been shown in trauma patients that a protein dosage of 2g/kg per day or greater may be necessary to achieve nitrogen equilibrium.(24, 25) In this trial, the average caloric intake of 1053 kcals was provided to the EMS group and approximately 50% of these kcals were from the amino acids. Although hypocaloric, it was close to 50% of the energy requirements in the first five days post-trauma. Average protein intake in the EMS group over the first 5 days was 1.43 g protein/kg, close to recommended levels and substantially greater than the standard group which was undernourished with an average of 0.35g protein/kg. This highlights how little ICU patients are typically fed. The non-protein calories provided to the EMS group were predominantly fat as propofol. Minimal carbohydrate (in the form of dextrose) was administered as part of their nutrition or resuscitation fluid. Additional protein sparing over that expected due to protein intake alone when provided at 1.0-1.5 g/kg in the critically ill state occurs when at least 50% of energy expenditure is provided as a combination of protein and carbohydrate.(9, 26) This requires approximately 150-200 grams of carbohydrate to reach the 50% energy expenditure for one to begin to improve protein balance.(26) This may be one important explanation as to why we did not see a protein-sparing effect with EMS despite its other likely benefits as described.

Nitrogen balance defined as the difference between nitrogen intake and nitrogen output (urea production, urine, and insensible losses). (24, 25) Interestingly, we found that early metabolic support with protein results in a significantly more negative nitrogen balance at day-5 of the intervention. The initial interpretation, considering only day-5 nitrogen balance between both groups, is that amino acids, without sufficient carbohydrates, will be almost completely oxidized and used as energy. The lack of improvement of nitrogen balance in this study with a substantially greater nitrogen intake in the EMS group is markedly different from previous studies and is perhaps related to the limited amounts (certainly less than 150 grams) of carbohydrate administration during the first 5 days of hospitalization.(9-11, 24, 25, 27, 28).

Although we did not directly measure protein flux (Q) in this trial, one can speculate based on published data that during severe stress an increase in protein flux results from both an increase in protein intake and in protein breakdown on one side of the flux equation and an increase in synthesis and oxidation on the other side.(14, 22, 29, 30). The majority of the additional amino acids in the EMS group were predominately oxidized via traditional pathways including the tricarboxylic acid cycle (TCA), gluconeogenesis, ketogenesis and then excretion in the urine as urea. This is well supported by metabolomics and our data that shows a significantly greater increase in urinary urea nitrogen and total nitrogen excretion in the EMS group. Furthermore, the EMS group had significantly higher BUN levels at the end of the intervention as compared to standard of care. One can speculate that to avoid toxicity and maintain a balance of protein flux, that there may have been a compensatory reduction in the intensity of the systemic inflammatory response to limit the increase in flux so as to avoid even greater amino acid appearance which might stress the system. One can further speculate that the modulation of

the increase in flux can also accommodate the increase in acute phase protein and other visceral protein synthesis inherent after a significant traumatic injury.

In our study we demonstrated a greater decline in pro-inflammatory IL-1B with a simultaneous increase in the sIL-6R after amino acid supplementation. The interplay between pro-inflammatory cytokines and soluble receptors is complex leading us to suspect that supplemental amino acids may be able to alter favorably the inflammatory response following trauma. However, it remains unclear if the inflammatory response is as a whole down regulated given the putative benefits shown in this study. Thus, this may represent a novel mechanism for modifying the short-term inflammatory response to significant trauma with this form of nutritional therapy.

To assess the impact of assessing such a large number of cytokines in a relatively small population, which is a limitation of this study, we performed a Bonferroni correction that reveals a level of significance of 0.0019 (0.05/27) would have been needed to be reached to formally declare significance given the broad array of cytokines assessed in this pilot study. A better powered, larger randomized controlled trial further investigating the impact of early supplemental amino acids will be required to determine if these alterations in the cytokine inflammatory response network are statistically and clinically significant.

Tryptophan-derived metabolites are downstream products of pro-inflammatory cytokine regulated enzyme indoleamine 2,3-dioxygenase (IDO), which plays an important role in the inflammatory response. We found that tryptophan-derived metabolites xanthurenate and

picolinate levels were higher in the EMS group at day-5 potentially suggesting an altered inflammatory response after supplementation with amino acids which would appear to conflict with the previous suggestion of reduced inflammation with the higher amino acid intake. However, a larger study is needed to differentiate if this trend is a true change in inflammatory protein synthesis or if it is merely secondary to higher amino acid levels in the EMS group at day-5.

Consistent with the supplementation of amino acids, the top biomarkers separating the EMS and Standard of Care groups at day-5 consisted almost exclusively of amino acids and related derivatives. The increase in amino acid availability, in turn, appeared to have impacts on several amino acid-dependent pathways including the urea cycle, the branched chain amino acid catabolic and trans-sulfuration pathways, glutathione synthesis, and the kynurenine pathway. Many of these metabolic pathways were also significantly altered in the absence of amino acid supplementation when looking at the change over time, suggesting that the effects of EMS are relatively minor and that the driving force is the injury response which is not reversed with nutritional interventions.

We demonstrate that early supplementation of amino acids results in non-toxic alterations in the metabolic and inflammatory state of critically ill trauma patients. The higher amount of protein intake observed in the EMS group, in addition to the increase in amino acid levels observed over the course of the trial, indicate that the additional amino acids were completely oxidized but did not exceed the maximal oxidative capacity of 3.8 g protein/kg.(31) There was also no clinical or metabolic evidence for protein intolerance in the EMS group, and lack of

serious liver injury or severe sepsis in the study population which might impact amino acid tolerance.(9)

Supplementation with amino acids with limited carbohydrate intake also resulted in a decrease in circulating metabolites of stress hormone precursors implying a downregulated stress response. However, there are several other possible explanations for altered progesterone metabolism after EMS which may be further elucidated with a larger randomized trial.

Consistent with all pilot randomized controlled trials, this study's primary limitation is the relatively small sample size, particularly in regards to the large volume of data collected to develop a metabolomic profile for each patient. We attempted to concurrently minimize this limitation while analyzing all of the generated data with dimension reduction techniques such as principal component analysis. To eliminate the limitation of sample size, adaptation of this pilot study to a large multi-institutional randomized controlled trial should be considered. A larger study may wish to focus on the key findings outlined above and conduct quantitative assays as this exploratory pilot study was limited by metabolomic profiles defined by "relative changes" as opposed to precise quantitative metabolite levels. Additionally, the low enteral intake across both groups limits the studies applicability as other centers may be more aggressive with early nutrition following significant trauma.

Conclusion

Early metabolic support with amino acids is safe, modifies protein metabolism, and may down regulate the inflammatory state associated with significant trauma. However, to assess

whether the apparent modulation of the inflammatory state by EMS is real and is truly beneficial will require a larger study perhaps using tracer technology and as well focused on patient-centered clinical outcomes. Furthermore, we suggest early and aggressive nutrition should be considered to narrow the significant discordance in nutritional demands and administration in the early post-injury period for critically ill trauma patients."

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Author Contributions:

Allan E. Stolarski, Lorrie Young, and Peter Burke contributed to data collection, data processing, analysis and writing of the manuscript. Jiyoun Kim, Beth Lusczek, Daniel G. Remick, and Bruce Bistrian contributed to the data processing, data analysis, and writing of the manuscript.

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References:

1. Meagher AD, Zar zaur BL. Epidemiology. In: Feliciano DV, Mattox KL, Moore EE, editors. Trauma, 9e. New York, NY: McGraw Hill; 2020.

2. Centers for Disease Control and Prevention. Key injury and violence data https://www.cdc.gov/injury/wisqars/overview/key_data.html. Accessed August 2021.

3. Demetriades D, Kimbrell B, Salim A, Velmahos G, Rhee P, Preston C, Gruzinski G, Chan L. Trauma deaths in a mature urban trauma system: is "trimodal" distribution a valid concept? *J Am Coll Surg*. 2005;201(3).

4. Cuthbertson DP. The disturbance of metabolism produced by bony and non-bony injury, with notes on certain abnormal conditions of bone. *Biochem J*. 1930;24(4):1244-63.

5. Casaer MP, Mesotten D, Hermans G, Wouters PJ, Schetz M, Meyfroidt G, Van Cromphaut S, Ingels C, Meersseman P, Muller J, et al. Early versus late parenteral nutrition in critically ill adults. *N Engl J Med*. 2011;365(6):506-17.

6. Cahill NE, Dhaliwal R, Day AG, Jiang X, Heyland DK. Nutrition therapy in the critical care setting: what is "best achievable" practice? An international multicenter observational study. *Crit Care Med.* 2010;38(2):395-401.

7. McClave SA, Taylor BE, Martindale RG, Warren MM, Johnson DR, Braunschweig C, McCarthy MS, Davanos E, Rice TW, Cresci GA, et al. Guidelines for the Provision and Assessment of Nutrition Support Therapy in the Adult Critically Ill Patient: Society of Critical Care Medicine (SCCM) and American Society for Parenteral and Enteral Nutrition (A.S.P.E.N.). *JPEN J Parenter Enteral Nutr*. 2016;40(2):159-211.

8. De Waele E, Jakubowski JR, Stocker R, Wischmeyer PE. Review of evolution and

current status of protein requirements and provision in acute illness and critical care. *Clin Nutr*. 2020.

9. Hoffer LJ, Bistrian BR. Appropriate protein provision in critical illness: a systematic and narrative review. *Am J Clin Nutr*. 2012;96(3):591-600.

10. Hoffer LJ. Protein and energy provision in critical illness. *Am J Clin Nutr*. 2003;78(5):906-11.

11. Hoffer LJ, Bistrian BR. Why critically ill patients are protein deprived. *JPEN J Parenter Enteral Nutr*. 2013;37(4):441.

12. Hoffer LJ, Bistrian BR. Nutrition in critical illness: a current conundrum. F1000Res. 2016;5:2531.

13. Krishnan JA, Parce PB, Martinez A, Diette GB, Brower RG. Caloric intake in medical ICU patients: consistency of care with guidelines and relationship to clinical outcomes. *Chest*. 2003;124(1):297-305.

14. Sundström Rehal M, Liebau F, Wernerman J, Rooyackers O. Whole-body protein kinetics in critically ill patients during 50 or 100% energy provision by enteral nutrition: A randomized cross-over study. *PLoS One*. 2020;15(10):e0240045.

15. Ishibashi N, Plank LD, Sando K, Hill GL. Optimal protein requirements during the first 2 weeks after the onset of critical illness. *Crit Care Med.* 1998;26(9):1529-35.

16. Wilmore D. The Metabolic Management of the Critically Ill. NY: Plenum Publishing; 1977. 193-4 p.

17. Cohen MJ, Serkova NJ, Wiener-Kronish J, Pittet J-F, Niemann CU. 1H-NMR-based metabolic signatures of clinical outcomes in trauma patients--beyond lactate and base deficit. *J. Trauma Acute Care Surg*. 2010;69(1).

18. Mao H, Wang H, Wang B, Liu X, Gao H, Xu M, Zhao H, Deng X, Lin D. Systemic metabolic changes of traumatic critically ill patients revealed by an NMR-based metabonomic approach. *J Proteome Res*. 2009;8(12).

19. Genton L, Romand JA, Pichard C. Basics in Clinical Nutrition: Nutritional support in trauma. *Eur J Clin Nutr*. 2009;5:107-9.

20. Chuntrasakul C, Siltharm S, Chinswangwatanakul V, Pongprasobchai T, Chockvivatanavanit S, Bunnak A. Early nutritional support in severe traumatic patients. *J Med Assoc Thai*. 1996;79(1):21-6.

21. Li PF, Wang YL, Fang YL, Nan L, Zhou J, Zhang D. Effect of early enteral nutrition on outcomes of trauma patients requiring intensive care. *Chin J Traumatol.* 2020;23(3):163-7.

22. Berg A, Rooyackers O, Bellander BM, Wernerman J. Whole body protein kinetics during hypocaloric and normocaloric feeding in critically ill patients. *Crit Care*. 2013;17(4):R158.

23. LJ H. Protein requirement in critical illness. Appl. Physiol. Nut. Metab. 2016;41(5).

24. Dickerson RN. Hypocaloric feeding of obese patients in the intensive care unit. *Curr Opin Clin Nutr Metab Care.* 2005;8(2):189-96.

25. Dickerson RN. Optimal caloric intake for critically ill patients: first, do no harm. Nutrition in clinical practice. *ASPEN*. 2011;26(1).

26. Greenberg GR, Marliss EB, Anderson GH, Langer B, Spence W, Tovee EB, Jeejeebhoy KN. Protein-sparing therapy in postoperative patients. Effects of added hypocaloric glucose or lipid. *N Engl J Med*. 1976;294(26):1411-6.

27. Hoffer LJ. High-Protein Hypocaloric Nutrition for Non-Obese Critically Ill Patients. *Nutr Clin Pract*. 2018;33(3):325-32.

28. Hurt RT, McClave SA, Martindale RG, Ochoa Gautier JB, Coss-Bu JA, Dickerson RN,

Heyland DK, Hoffer LJ, Moore FA, Morris CR, et al. Summary Points and Consensus Recommendations From the International Protein Summit. *Nutr Clin Pract.* 2017;32(1_suppl):142S-51S.

29. Prelack K, Yu YM, Dylewski M, Lydon M, Sheridan RL, Tompkins RG. The contribution of muscle to whole-body protein turnover throughout the course of burn injury in children. *J. Burn Care Res.* 2010;31(6).

30. Malagaris I, Herndon DN, Polychronopoulou E, Rontoyanni VG, Andersen CR, Suman OE, Porter C, Sidossis LS. Determinants of skeletal muscle protein turnover following severe burn trauma in children. *Clin Nutr.* 2019;38(3):1348-54.

31. Rudman D, DiFulco TJ, Galambos JT, Smith RB, Salam AA, Warren WD. Maximal rates of excretion and synthesis of urea in normal and cirrhotic subjects. *J Clin Invest.* 1973;52(9):2241-9.

Figure Legend:

Table 1: Demographic and Baseline Comparisons

a: Categorical Variables

b: Continuous Variables

Table 2: Urea Change and Nitrogen Balance

Figure 1: Principal Component Analysis of EMS vs. Standard Groups Over Time.

Figure 2: Random Forest – Biochemical Importance Over Time: EMS Group

Figure 3: Random Forest – Biochemical Importance Over Time: Standard Group

Figure 4: Random Forest – Biochemical Importance of EMS vs. Standard at Day 5

Supplemental Digital Content:

S1: Flow Diagram of Inclusion and Exclusion Criteria

S2: Comparison of Cytokine Levels by Intervention and Time

S3: Nutritional Intake

S4: Alterations in Circulating Amino Acid Levels with Time and Treatment

S5: Tryptophan Metabolism

S6: Branched Chain Amino Acid Catabolism

S7: Methionine and Glutathione Metabolism

S8: Change from baseline to day 5 of assorted Laboratory Values

Figure 1: Principal Component Analysis of EMS vs. Standard Groups Over Time.

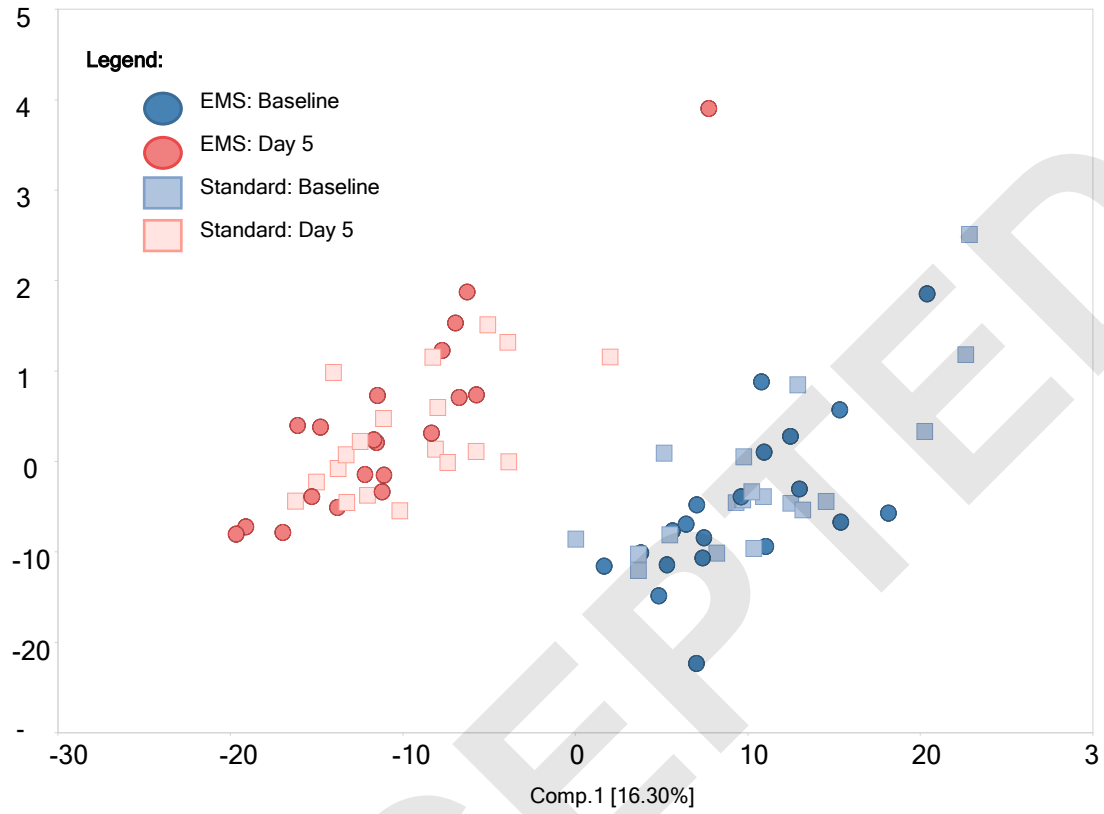


Figure 2: Random Forest – Biochemical Importance Over Time: EMS Group

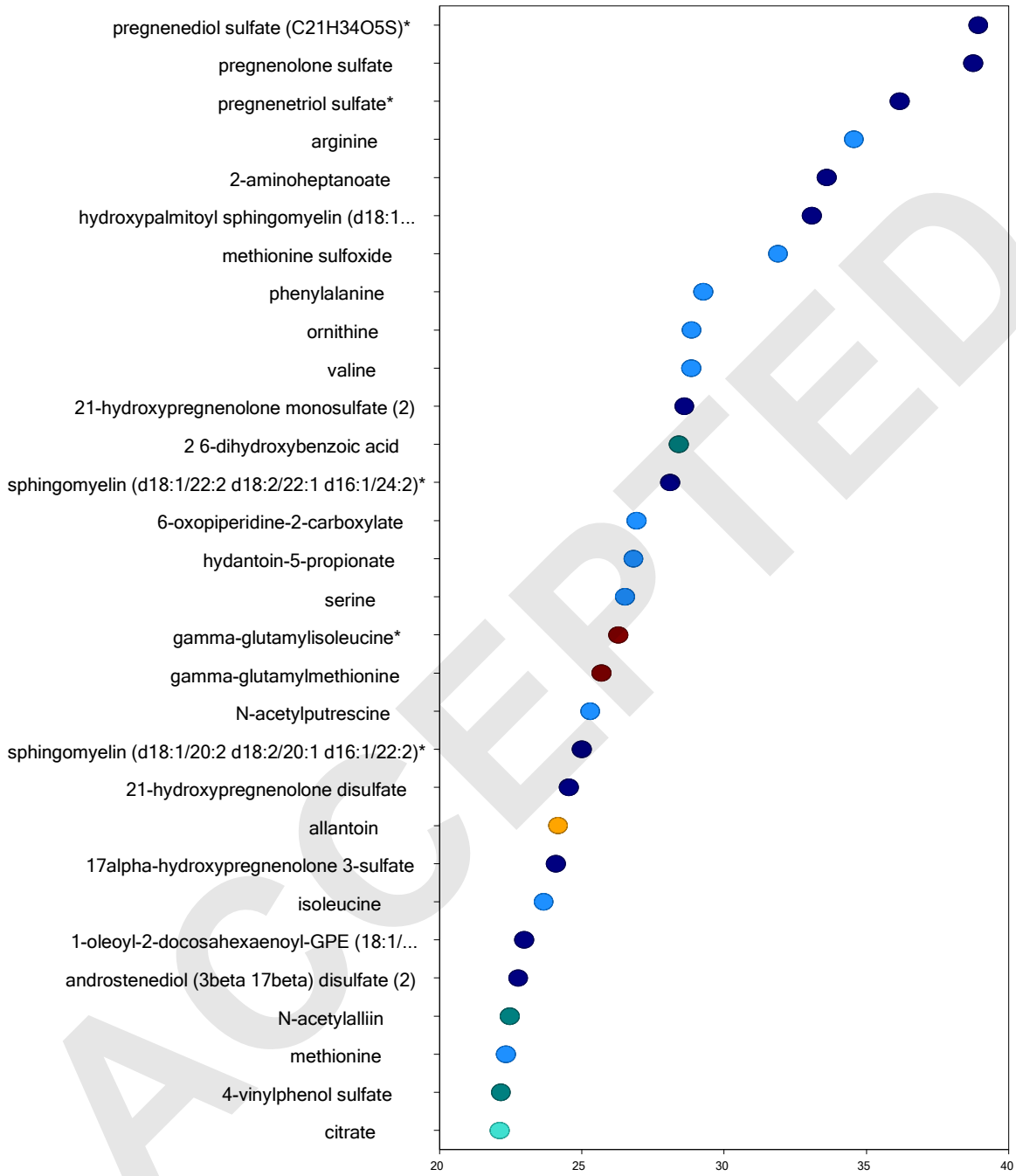


Figure 3: Random Forest – Biochemical Importance Over Time: Standard Group

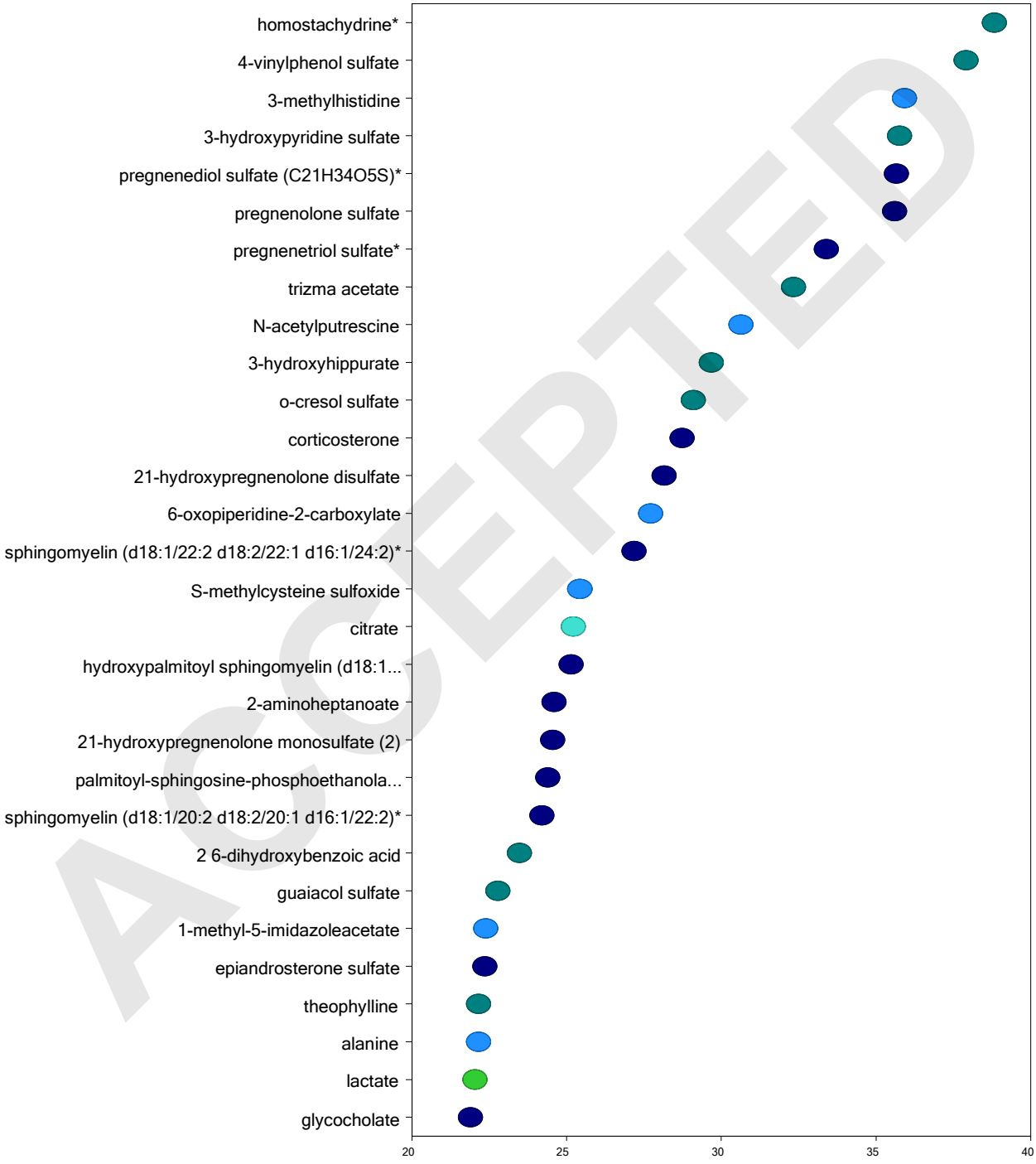


Table 1a: Demographic and Baseline Comparisons – Categorical Variables

		TOTAL (N=42)	EMS (N=21)	STANDARD (N=21)	P-VALUE*
		n (%)	n (%)	n (%)	
SEX	**Female**	5 (11.9)	1 (4.8)	4 (19.1)	0.3433
	Male	37 (88.1)	20 (95.2)	17 (81.0)	
RACE	**Black**	12 (28.6)	8 (38.1)	4 (19.1)	0.4144
	White	23 (54.8)	10 (47.6)	13 (61.9)	
	Other	7 (16.7)	3 (14.3)	4 (19.1)	
ETHNICITY	**Hispanic/Latino**	4 (9.5)	1 (4.8)	3 (14.3)	0.3343
	Not Hispanic/Latino	37 (88.1)	20 (95.2)	17 (81.0)	
	Unknown	1 (2.4)	0 (0.0)	1 (4.8)	
INJURY	**Blunt**	17 (40.5)	9 (42.9)	8 (38.1)	0.2963
	Penetrating	12 (28.6)	6 (28.6)	6 (28.6)	
	Head Alone	6 (14.3)	1 (4.8)	5 (23.8)	
	Blunt + Head	7 (16.7)	5 (23.8)	2 (9.5)	

* Fischer's Exact test.

Table 1b: Demographic and Baseline Comparisons – Continuous Variables

	N	TOTAL MEDIAN (IQR)	N	EMS MEDIAN (IQR)	N	STANDARD MEDIAN (IQR)	P-VALUE*
AGE	42	31.9 (24.8,50.7)	21	31.0 (26.3, 44.1)	21	33.6 (23.6, 52.3)	0.9198
BMI	42	27.4 (23.9, 30.3)	21	28.7 (23.9, 30.9)	21	26.6 (23.8, 30.2)	0.4562
BASELINE CRP	32	103 (56, 203)	17	103 (55, 210)	15	103 (64, 197)	0.8449
BASELINE CATABOLIC INDEX	35	3.8 (0.2, 7.5)	18	3.0 (-2.1, 7.5)	17	5.0 (1.7, 7.0)	0.2283
APACHE	42	10.5 (5, 14)	21	10 (5, 14)	21	11 (5, 13)	0.8400
GCS	42	9.5 (7, 14)	21	9.0 (7, 14)	21	10 (8, 11)	0.8392
ISS	42	25 (17, 35)	21	22 (17, 35)	21	26 (17, 34)	0.7051
SOFA	42	4 (3, 7)	21	4 (2, 7)	21	4 (3, 6)	0.4853

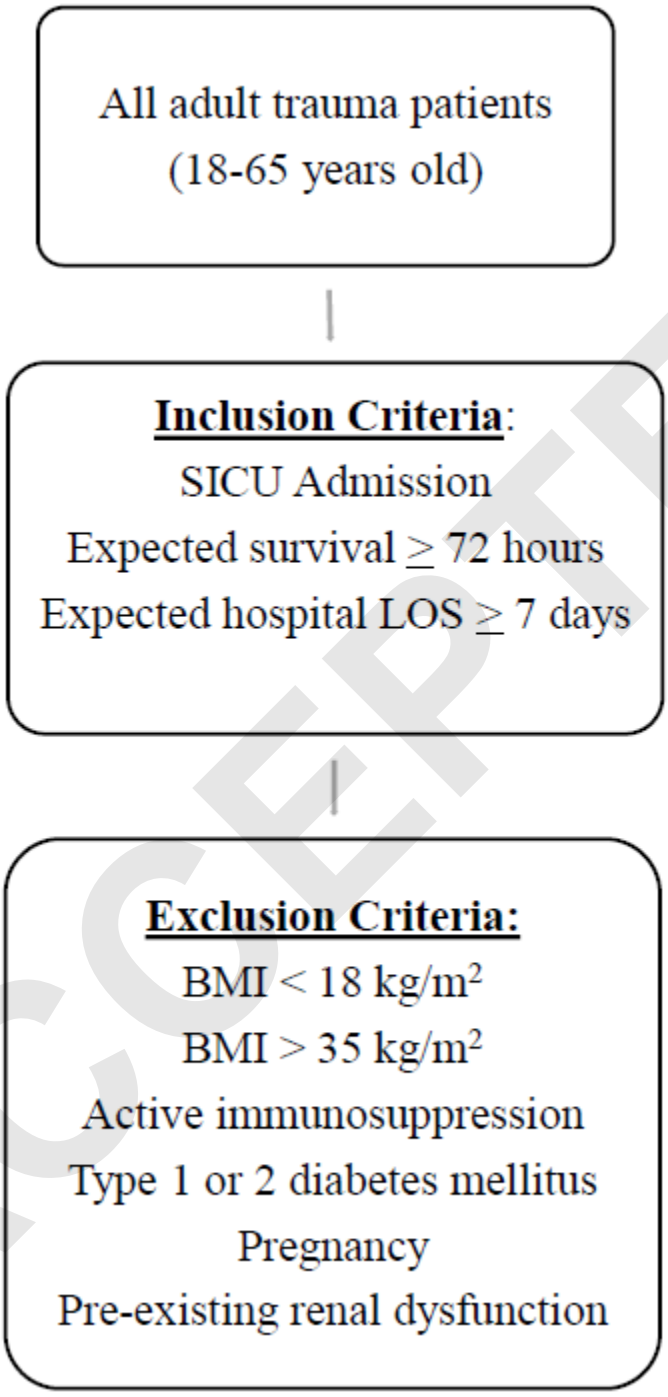
* Wilcoxon rank sum test

Table 2: Urea Change and Nitrogen Balance

	EMS		Standard		
	n	**Median (IQR)**	**n**	**Median (IQR)**	**P-value***
Baseline Urea Nitrogen	20	11.5 (7, 16)	17	9 (7, 10)	0.1191
Day 5 Urea Nitrogen	19	24 (21, 31)	20	11.5 (8.0, 15.5)	<0.0001
Urea Nitrogen Change	18	13.5 (9.0, 17.0)	16	3.5 (1.0, 7.0)	0.0031
Baseline Total Nitrogen Balance	18	-0.5 (-7.2, 1.4)	17	-11.3 (-12.8, -5.8)	0.0006
Day 5 Total Nitrogen Balance	19	-16.3 (-26.6, -8.5)	18	-5.3 (-14, 0.56)	0.0276
Total Nitrogen Balance Change	17	-15.6 (-24, -10.5)	15	5.8 (-1, 10.3)	0.0004

*Wilcoxon rank sum test.

S1: Flow Diagram of Inclusion and Exclusion Criteria



S2: Comparison of Cytokine Levels by Intervention and Time

	Baseline		***Day 5***		
Proinflammatory CKS	**EMS**	**Standard**	**EMS**	**Standard**	**p-value (Change in Intervention vs. Change in Control)**
	Median (pg/ml)	Median (pg/ml)	Median (pg/ml)	Median (pg/ml)	
GM-CSF (20)	1.02	2.58	1.21	2.58	0.3327
IFNy (25)	2.00	2.18	1.33	1.32	0.4011
IL-1B (46)	1.90	0.78	0.64	0.50	**0.02***
IL-2 (48)	0.67	0.53	0.63	0.75	0.153
IL-4 (53)	1.14	2.04	2.86	2.86	0.9495
IL-5 (55)	0.41	0.33	0.70	0.48	0.8247
IL-6 (57)	103.41	85.68	9.25	21.36	0.0967
IL-8 (63)	29.25	35.09	14.81	17.31	0.6239
IL-10 (27)	46.29	33.77	14.81	8.53	0.6016
IL-12(p70) (33)	0.64	1.38	0.64	1.04	0.6909
IL-13 (35)	0.92	0.77	0.67	0.58	0.1065
MCP-1 (67)	479.06	447.02	393.14	321.91	0.2114
TNFa (75)	14.02	13.02	23.18	14.58	0.4765
Soluble Receptors	**EMS**	**Standard**	**EMS**	**Standard**	**p-value (Change in EMS vs. Change in Standard)**
sCD30 (12)	46.6	67.6	41.1	62.3	0.6464
sEGFR (14)	29112.5	28133.7	27057.1	24636.2	0.2001
sgp130 (18)	35195.7	79043.3	34113.8	48955.8	0.319
sIL-1RI (20)	50.3	48.6	55.0	73.1	0.5582
sIL-1RII (22)	9240.6	11454.3	9623.8	9778.7	0.2892
sIL-2Ra (33)	893.8	1118.4	1080.1	923.2	0.0556
sIL-4R (35)	220.4	224.0	216.4	216.3	0.9243
sIL-6R (37)	10120.3	18432.1	100796.9	10840.3	**0.0099***
sRAGE (39)	59.5	53.1	38.2	49.7	0.704
sTNFRI (51)	1900.8	1950.6	1695.3	1906.3	0.6693
sTNFRII (53)	7610.6	9217.5	7213.0	7069.9	0.319
sVEGFR1 (55)	217.8	252.2	223.2	160.9	0.1101
sVEGFR2 (57)	8357.8	8156.0	8395.8	7807.5	0.275
sVEGFR3 (61)	2535.1	1929.7	1803.7	1738.6	0.0642

*p<0.05 by Wilcoxon Rank Sum Test.

S3: Nutritional Intake

Nutritional Intake*

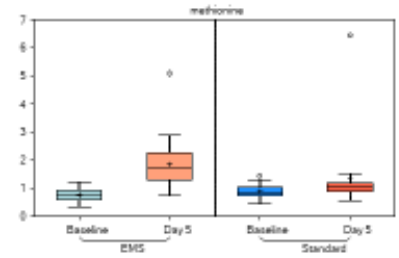
	EMS**	Standard**	Difference (95% CI)	P-value
Total calories (Kcals/kg/24hr)	1053	596	457 (195, 720)	0.0007
Kcals/kg/24hr	12.6	7.5	5.1 (2.1, 8.2)	0.0012
Protein/24hr	122	31	91 (75, 108)	<0.0001
Protein/kg/24hr	1.43	0.35	1.08 (0.91, 1.25)	<0.0001

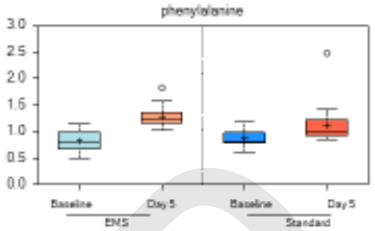
**Random intercept model incorporating all available values*

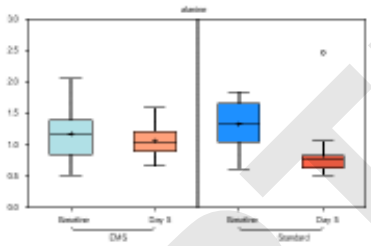
*** Estimated model means*

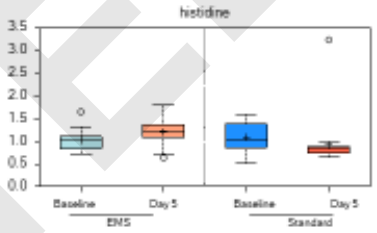
S4: Alterations in Circulating Amino Acid Levels with Time and Treatment

	Merged Dataset			
	Fold of Change			
	ANOVA Contrasts			
	Day 5 Baseline1		EMS Standard	
Biochemical Name	EMS	Standard	Baseline	Day 5
alanine	0.94	0.6	0.87	1.36
asparagine	1.29	1.23	0.91	0.96
aspartate	2.38	1.41	0.97	1.63
cysteine	0.81	0.95	1.12	0.96
glutamine	0.97	0.89	1	1.09
methionine	2.34	1.31	0.85	1.51
serine	2.49	1.99	0.97	1.22
threonine	1.88	1.3	0.91	1.32
tryptophan	1.16	1.07	0.96	1.05
tyrosine	1.08	1.15	0.93	0.87
valine	1.97	1.49	0.96	1.27
arginine	2.34	1.55	0.81	1.22
glutamate	0.89	0.91	1.13	1.1
glycine	1.25	0.88	0.96	1.36
histidine	1.17	0.82	0.96	1.37
isoleucine	2.31	1.78	0.9	1.18
leucine	1.7	1.56	0.94	1.02
lysine	1.58	1.26	0.84	1.06
phenylalanine	1.6	1.25	0.92	1.17
proline	1.38	0.95	0.96	1.38





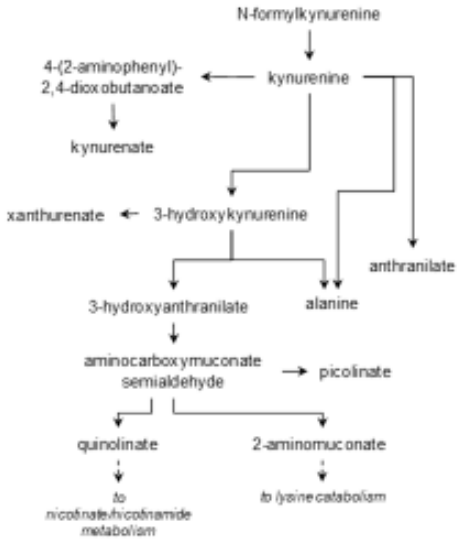


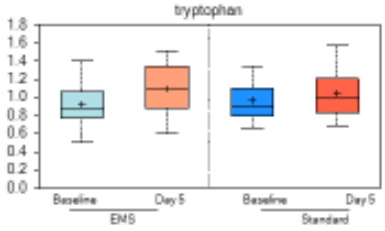


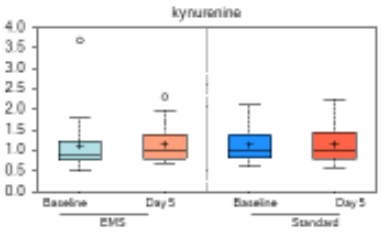
Red cell color represents relative increases. Dark red significant ($p<0.05$). Light red approaches significance ($0.05p<0.10$).

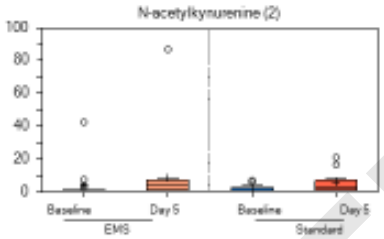
Green cell color represents relative decreases. Dark green significant ($p<0.05$). Light green approaches significance ($0.05p<0.10$).

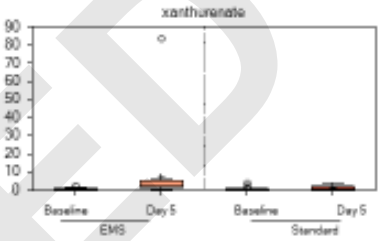
S5: Tryptophan Metabolism

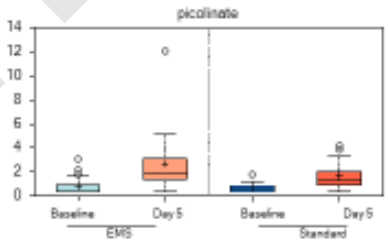










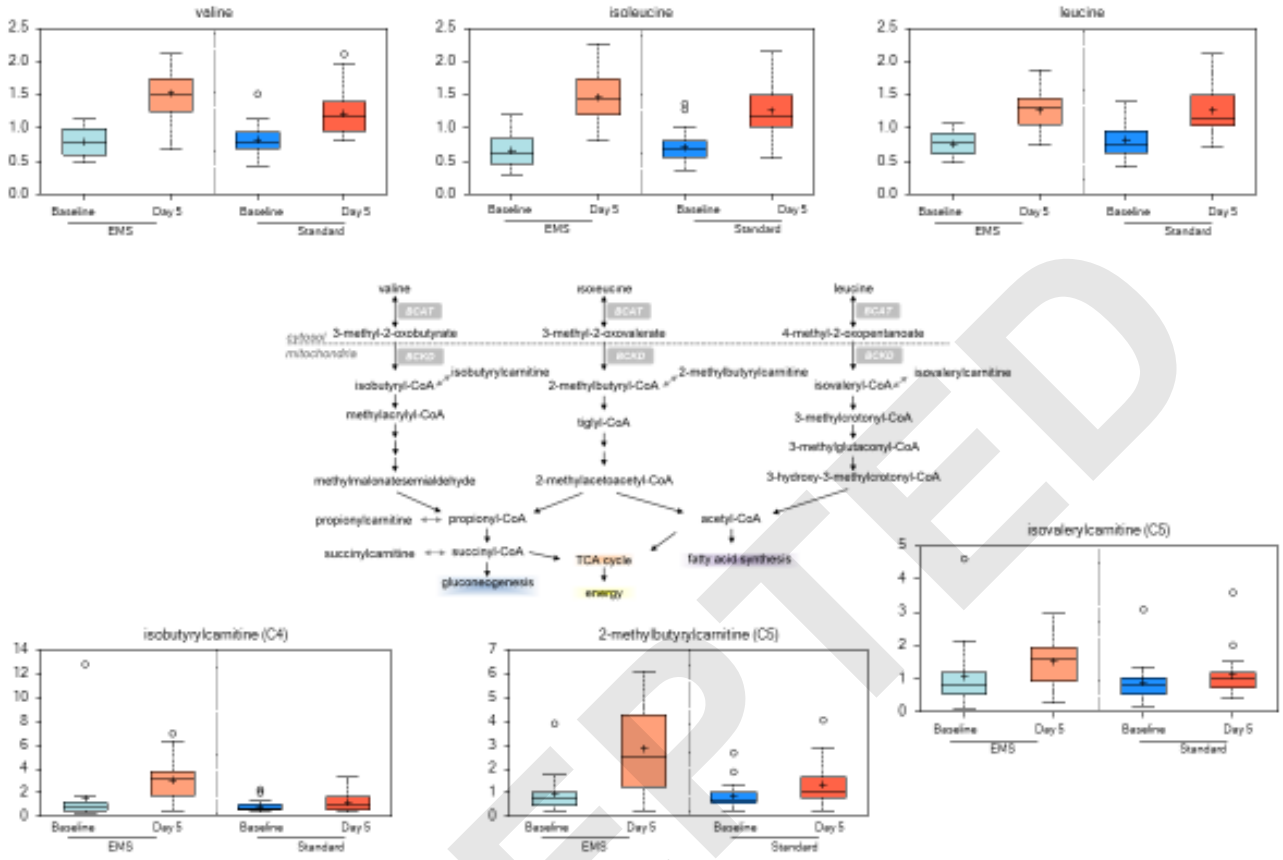


		Merged Dataset ANOVA Contrasts			
		Day 5 / Baseline		EMS / Standard	
Sub Pathway	Biochemical Name	A	B	Baseline	Day 5
Tryptophan Metabolism	tryptophan	1.16	1.07	0.96	1.05
	kynurenine	1.08	1.01	0.94	1.01
	N-acetylkynurenine (2)	2.55	1.86	0.98	1.35
	kynurenate	1.45	0.83	0.85	1.49
	N-formylanthranilic acid	1.84	1.56	0.74	0.93
	anthranilate	0.97	0.99	0.78	0.77
	xanthurenate	4.13	1.57	0.99	2.60
	picolinate	3.47	2.14	1.01	1.84

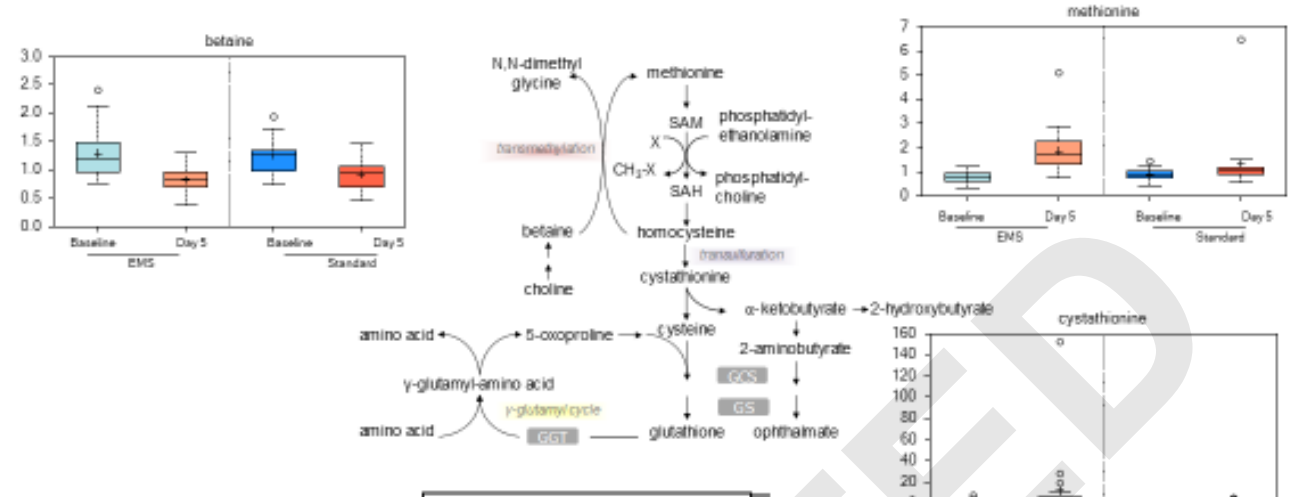
Red cell color represents relative increases. Dark red significant ($p<0.05$). Light red approaches significance ($0.05p<0.10$).

Green cell color represents relative decreases. Dark green significant ($p<0.05$). Light green approaches significance ($0.05p<0.10$).

S6: Branched Chain Amino Acid Catabolism



S7: Methionine and Glutathione Metabolism



		Merged Dataset			
		ANOVA Contrasts			
		Last / Day 1		A / B	
Sub Pathway	Biochemical Name	A	B	Day 1	Last
γ-glutamyl Amino Acid	gamma-glutamylalanine	0.86	0.48	0.86	1.52
	gamma-glutamylglutamate	0.40	0.38	1.01	1.07
	gamma-glutamylglutamine	0.94	0.84	0.91	1.02
	gamma-glutamylglycine	1.37	1.04	1.00	1.32
	gamma-glutamylhistidine	0.96	0.83	1.04	1.21
	gamma-glutamylisoleucine*	3.03	2.19	0.92	1.28
	gamma-glutamylleucine	2.08	1.98	0.97	1.01
	gamma-glutamyl-alpha-lysine	2.01	1.55	0.84	1.08
	gamma-glutamyl-epsilon-lysine	1.49	1.14	1.03	1.35
	gamma-glutamylmethionine	3.49	1.57	0.73	1.62
	gamma-glutamylphenylalanine	2.10	1.58	0.89	1.18
	gamma-glutamylthreonine	1.16	1.08	0.95	1.02
	gamma-glutamyltryptophan	1.31	1.04	0.82	1.03
	gamma-glutamyltyrosine	1.28	1.42	0.92	0.83
	gamma-glutamylvaline	2.49	1.77	0.99	1.39
	gamma-glutamylcitrulline*	0.75	0.59	0.95	1.20
	gamma-glutamyl-2-aminobutyrate	2.05	1.41	0.87	1.26

Red cell color represents relative increases. Dark red significant ($p<0.05$). Light red approaches significance ($0.05p<0.10$).

Green cell color represents relative decreases. Dark green significant ($p<0.05$). Light green approaches significance ($0.05p<0.10$).

S8: Change from baseline to day 5 of assorted Laboratory Values

	EMS		*Standard*		
	n	**Median (IQR)**	**n**	**Median (IQR)**	**P-value***
WBC Day 5	19	9.5 (7.0, 16.0)	20	8.3 (6.2, 11.4)	0.1866
WBC Change	19	-1.5 (-3.8, 2.1)	20	-5.2 (-8.0, -1.3)	0.1156
BUN Day 5	20	20.0 (15.0, 26.0)	20	14.0 (12.0, 19.5)	**0.0333***
BUN Change	20	6.5 (4.0, 11.0)	20	-0.5 (-3.0, 5.5)	0.0024
Albumin Day 5	18	2.9 (2.6, 3.1)	19	2.8 (2.5, 3.0)	0.4361
Albumin Change	18	-0.3 (-0.5, -0.1)	19	-0.6 (-1.0, -0.2)	**0.0384***
Creatinine Day 5	20	0.73 (0.68, 0.88)	20	0.69 (0.66, 0.80)	0.4482
Creatinine Change	20	-0.31 (-0.43, -0.08)	20	-0.24 (-0.50, -0.13)	0.7454

**Wilcoxon rank sum test*

Changes are calculated as the Day 5 value minus the Day 1 value