Early metabolic support for critically ill trauma patients: A prospective randomized controlled trial

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AUTHORS/CONTRIBUTORS

Allan E. Stolarski, Journal of the American Heart Association/Janssen Pharma, Consulting Fee, Consultant. Lorraine Young, Janice Weinberg, Jiyoun Kim, Elizabeth Lusczek, DanielG. Remick, Bruce Bistrian, Peter Burke have nothing to disclose.

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BACKGROUND: There is a lack of consensus regarding the optimal nutritional support for trauma patients. We hypothesize that early postinjury metabolic support focusing on adequate protein would modify the metabolic signature and alter the inflammatory environment for critically ill trauma patients.

METHODS: We conducted a prospective randomized controlled pilot trial for adult patients admitted to the surgical intensive care unit following traumatic injury. Patients were randomized to receive early metabolic support (EMS) (peripheral amino acid infusions) or standard of care (enteral nutrition as soon as feasible). Routine laboratory assessments, nitrogen balance, cytokines, and metabolomic analyses were assessed at baseline and day 5 after intervention.

RESULTS: A total of 42 trauma patients were randomized into well-balanced groups with similar age (32 years), Injury Severity Score (25), and body mass index (27.4 kg/m^2). Early metabolic support provided significantly more protein (1.43 g/kg vs. 0.35 g/kg; $p < 0.0001$) and more calories (12.6 kcal/kg vs. 7.5 g/kg; $p = 0.0012$) over the first 5 days as compared with the standard of care. Early metabolic support modified protein catabolism and synthesis as demonstrated by a larger median negative nitrogen balance (−16.3 g vs. −5.3 g; $p = 0.03$) and a unique metabolomic profile at day 5. The biochemical profile of patients who received EMS was defined by greater declines in circulating levels of stress hormone precursors and increased levels of amino acids. The inflammatory response following EMS resulted in a greater decrease in interleukin-1B ($p = 0.02$) and increase in soluble interleukin-6 receptor ($p = 0.01$) between baseline and day 5 as compared with the standard of care. The EMS group had a decreased length of stay (15 vs. 22 days) and decreased surgical intensive care unit length of stay (8 vs. 9 days); however, this disappeared after adjustment for Injury Severity Score in this small population.

CONCLUSIONS: Early metabolic support with amino acid is safe, modifies metabolism, and may downregulate the inflammatory state associated with significant trauma, warranting a larger trial to assess for improved outcomes. (*J Trauma Acute Care Surg.* 2022;92: 255–265. Copyright © 2021 American Association for the Surgery of Trauma.)

LEVEL OF EVIDENCE: Therapeutic/Care Management; Level II.

KEY WORDS: Trauma; nutrition; inflammation; cytokines; amino acid.

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 comorbidities, and are usually well nourished at the time of their injury.[1–3] Of trauma patients who survive the initial postinjury period, those who ultimately die do not succumb to their initial injury per se but rather from the later complications of injury.[3]

The postinjury response has been well described beginning with the work of Cuthbertson[4] in the 1930s. Nutrition support postinjury 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 meet energy and protein requirements because of 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 because of diminished protein utilization. The suggested protein requirements for critically ill patients are approximately 1.5 g protein/kg/day with suggested higher intakes (2-2.5 g protein/kg/day) in some patients, such as with severe burns.[7,8] These intakes are rarely achieved in the first week postinjury.[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.

Because of the lack of expert consensus on the best way to feed critically ill trauma patients in the first week postinjury, we chose to focus on providing supplemental protein within 24 hours of admission to the intensive care unit for the first 5 days postinjury, when these patients are believed to be the most catabolic.[14] It is our hypothesis that early immediate postinjury 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.

PATIENTS AND METHODS

We conducted an institutional review board–approved, prospective randomized controlled trial at our high-volume level 1

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trauma center in patients 18 to 65 years old admitted to the surgical intensive care unit (SICU) postinjury from either the emergency department or the operating room. The study was designed in accordance with Consolidated Standards of Reporting Trials 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 7 days. Patients were excluded from the study if they had preexisting malnutrition as defined by a body mass index of less than 18 kg/m^2, class II obesity (body mass index, >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 preexisting renal dysfunction (Supplementary Fig. 1, http://links.lww.com/TA/C221).

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 to 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 to 2.0 g protein/kg of body weight per 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, and changes in proinflammatory 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 used 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

Subjects randomized to the EMS group began peripheral infusion of amino acids within 24 hours of admission to the SICU. The initial intravenous nutrition solution used as our intervention consisted of approximately 27 g of dextrose and 65 g of a mixed standard parenteral amino acid solution per liter (65 g of 15% amino acids, 70 mEq NaCl, mixed with D5W to provide 27.4 g of dextrose per liter, with a total osmolarity of 877.8 mOsm). The solution provided approximately 353 kcals/L, primarily as protein (65 g of protein/L). The solutions were infused at a constant rate during 24 hours and titrated to provide the patient with at approximately 1.5 g of protein/kg per 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 to 2.0 g/kg per 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, Injury Severity Scores (ISSs), Glasgow Coma Scale scores, and sequential organ failure assessment 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 5 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 g of nitrogen for other insensible losses.[16] Insensible losses include sweat, respiratory losses, and gastrointestinal losses, etc.[16]

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

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. To manage and analyze the large volume of data points obtained for each patient sample, dimension reduction via techniques such as principal component analysis was used. 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) (Supplementary Table 1, http://links.lww.com/TA/C222).

Statistical Analysis

All statistical analyses were performed 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

TABLE 1. Demographic and Baseline Comparisons—Categorical Variables

		Total (N = 42)	EMS (n = 21)	Standard (n = 21)	*p**
		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 (28.12 (28.6)	8 (38.1)	4 (19.1)	0.4144
	White	23 (54.23 (54.8)	10 (47.6)	13 (61.9)	
	Other	7 (16.77 (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)	

*Fisher's exact test.

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, were 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 version 9.4 (SAS, Cary, NC) 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 aforementioned analyses, an evaluation of biochemical markers was performed by Metabolon. Changes over time in metabolites were compared between groups using two-way analysis of variance (ANOVA). Principal component analysis 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 1 and 2. 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 g during 24 hours; $p = 0.1191$), respectively. The median change in urea nitrogen excretion over the 5 days of the study (as assessed by 24-hour urine collections) was significantly higher in the EMS group (13.5 g/24 hours) compared with the standard of care group (3.5 g/24 hours; $p = 0.0031$) (Table 3).

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-1 nitrogen economy, as it takes approximately 4 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 during 5 days of 1,053 kcals (12.6 kcals/kg per day) with 122 g of protein (1.43 g/kg per day) in the EMS group, and 596 kcals (7.5 kcals/kg per day) with 31 g protein (0.35 g/kg per day) in the standard of care group (Supplementary Table 2, http://links.lww.com/TA/C223). Despite this, we found that day-5 nitrogen balance was significantly more negative (−16.3 g; IQR, −26.6 to −8.5) in the EMS group as compared with the standard group (−5.3 g; IQR, −15 to 0.56) ($p = 0.0276$; Table 3).

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 with the standard group. When we analyzed the impact of treatment versus time using a principal component analysis, 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

TABLE 2. Demographic and Baseline Comparisons—Continuous Variables

	n	Total Median (IQR)	n	EMS Median (IQR)	n	Standard Median (IQR)	*p**
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 to 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.
APACHE, Acute Physiology and Chronic Health Evaluation Score; BMI, body mass index; CRP, C-reactive protein; GCS, Glasgow Coma Scale; SOFA, sequential organ failure assessment.

TABLE 3. Urea Change and Nitrogen Balance

	EMS		Standard		
	n	Median (IQR)	n	Median (IQR)	*p**
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 to 1.4)	17	−11.3 (−12.8 to −5.8)	0.0006
Day 5 total nitrogen balance	19	−16.3 (−26.6 to −8.5)	18	−5.3 (−14 to 0.56)	0.0276
Total nitrogen balance change	17	−15.6 (−24 to −10.5)	15	5.8 (−1 to 10.3)	0.0004

*Wilcoxon rank sum test.

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 (Fig. 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) (Fig. 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 (Fig. 4). Both the standard and EMS groups experienced an increase in levels of circulating amino acids between baseline and day 5; however, patients who 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 (Supplementary Fig. 2, http://links.lww.com/TA/C224). In addition, both groups experienced an increase in tryptophan metabolism between baseline and day 5 as evident by an increase in tryptophan metabolites (Supplementary Fig. 3, http://links.lww.com/TA/C225). 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 with 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 with the standard of care group (Supplementary Fig. 4, http://links.lww.com/TA/C226). 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 of 0.67 vs. control of 0.55) and lactate (ANOVA contrast of 0.71 vs. control of 0.55) between baseline and day 5 (Supplementary Fig. 5, http://links.lww.com/TA/C227).

Cytokine levels between EMS and standard groups were similar at baseline. Alterations in the inflammatory response following EMS resulted in a greater decrease in interleukin 1B (IL-1B) ($p = 0.02$) and increase in soluble interleukin 6 (sIL-6) receptor ($p = 0.01$) between baseline and day 5 as compared with the standard of care (Supplementary Table 1, http://links.lww.com/TA/C222).

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 with

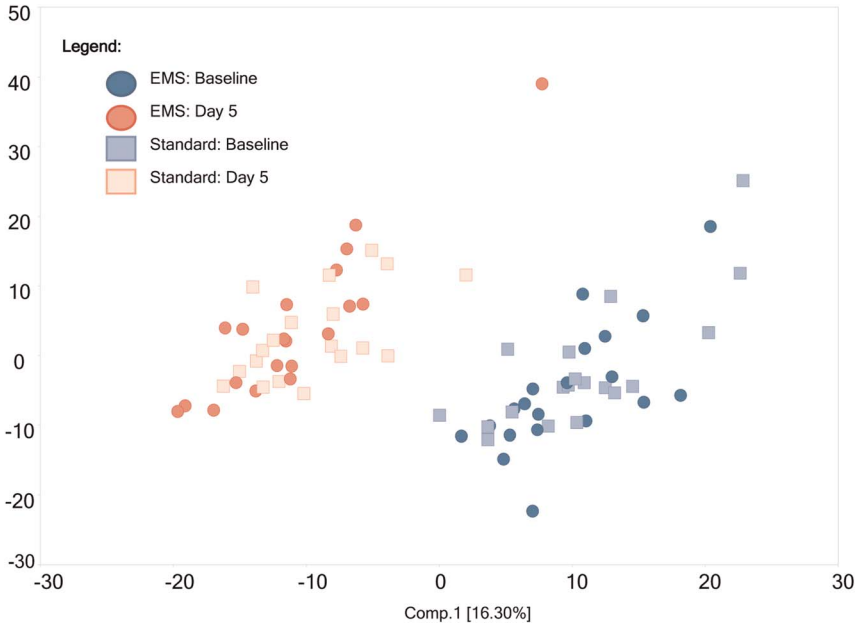

Figure 1. Principal component analysis of EMS versus standard groups over time.

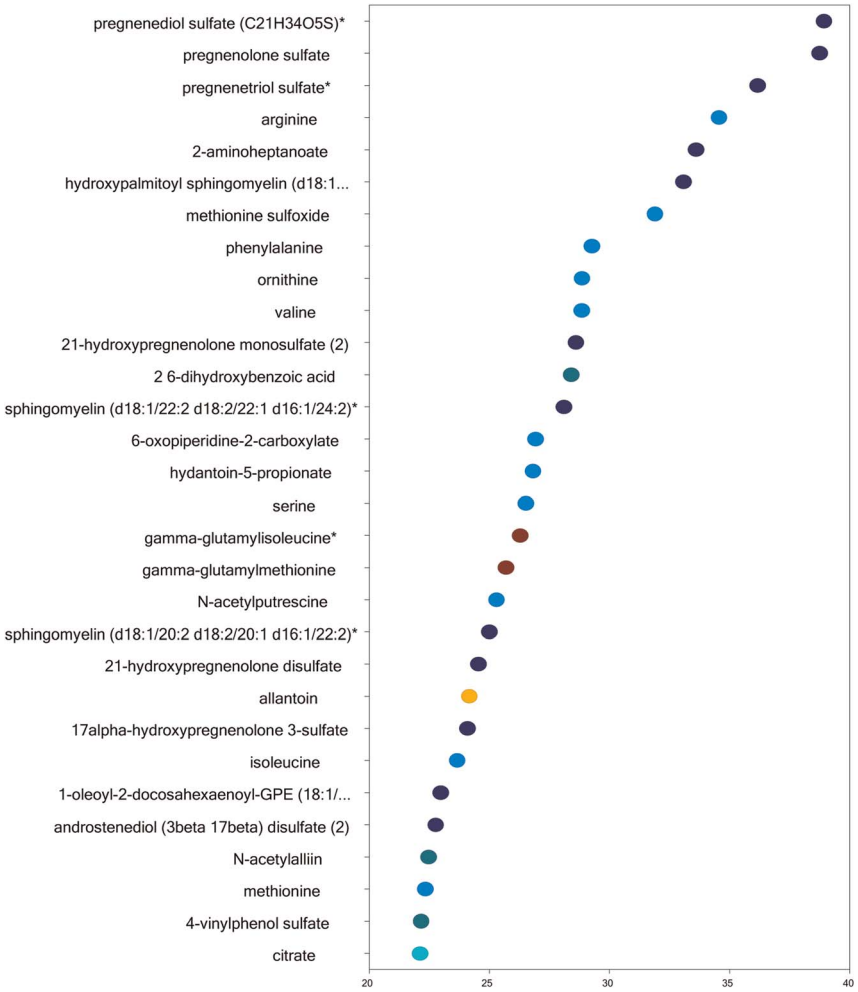

Figure 2. Random forest—biochemical importance over time: EMS group.

the standard group (Supplementary Table 3, http://links.lww.com/TA/C228). After adjustment for ISS, there was no significant difference in median length of stay or SICU length of stay between both groups.

DISCUSSION

In the setting of a randomized controlled pilot study, we found that EMS (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 postinjury. 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 sequential organ failure assessment scores on presentation, carries 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 2 g/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 kilocalories were from the amino acids. Although hypocaloric, it was close to 50% of the energy requirements in the first 5 days posttrauma. 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

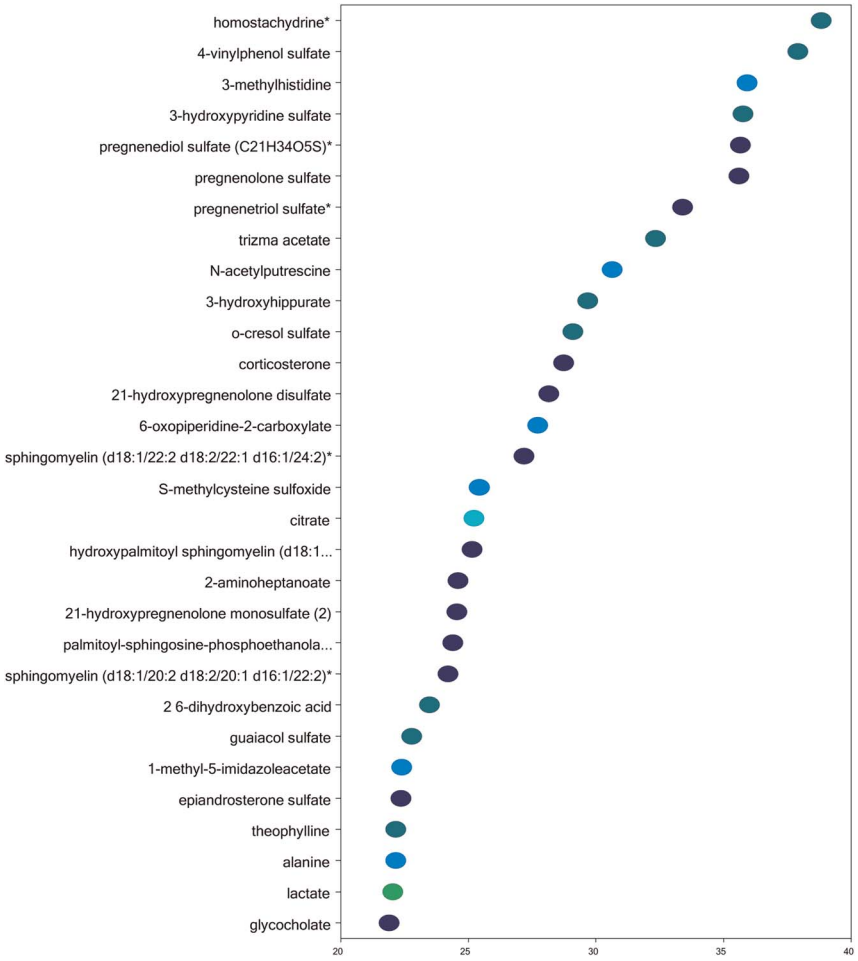

Figure 3. Random forest—biochemical importance over time: standard group.

an average of 0.35 g protein/kg. This highlights how little intensive care unit patients are typically fed. The nonprotein 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

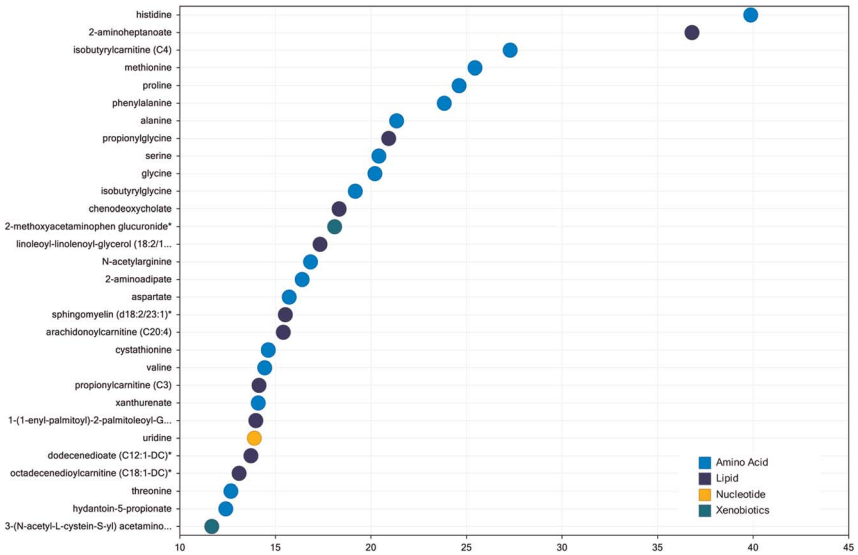

Figure 4. Random forest—biochemical importance of EMS versus standard at day 5.

provided at 1.0 to 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 to 200 g 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 is defined as the difference between nitrogen intake and nitrogen output (urea production, urine, and insensible losses).[24,25] Interestingly, we found that EMS 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 g) 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, gluconeogenesis, ketogenesis, and then excretion in the urine as urea. This is well supported by metabolomics and our data that show 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 with standard of care. One can speculate that, to avoid toxicity and maintain a balance of protein flux, 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 proinflammatory IL-1B with a simultaneous increase in the sIL-6 receptor after amino acid supplementation. The interplay between proinflammatory 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 downregulated 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 that 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 proinflammatory cytokine regulated enzyme indoleamine 2,3-dioxygenase, 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 transsulfuration 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 nontoxic 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, indicates 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 a 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 regard 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. In addition, the low enteral intake across both groups limits the studies' applicability because 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 focused on patient-centered clinical outcomes. Furthermore, we suggest that early and aggressive nutrition should be considered to narrow the significant discordance in nutritional demands and administration in the early postinjury period for critically ill trauma patients.

AUTHORSHIP

A.E.S., L.Y., and P.B. contributed in the data collection, data processing, analysis, and writing of the manuscript. J.W., J.K., E.L., D.G.R., and B.B. contributed in the data processing, data analysis, and writing of the manuscript.

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DISCLOSURE

The authors declare no conflicts of interest.

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DISCUSSION

ALICIA MOHR, M.D. (Gainesville, Florida): Good morning, Dr. Spain, Dr. Reilly. I'd like to thank the AAST for the privilege of the podium and inviting me to discuss this manuscript.

And I'd like to congratulate the authors on their work. They presented a large amount of data which always leads to innumerable questions. The authors have added to the published studies regarding protein intake during the early phase of critical illness.

Recent studies have focused on high protein intake early in the ICU course and initial enthusiasm came from studies examining ICU-acquired muscle weakness.

In addition to the benefits of early mobilization and ambulation, high-protein intake was expected to limit the loss of muscle mass and function. And some studies demonstrated improved outcomes.

However, there still remains controversy as other studies have raised safety concerns due to the increased urea generation and increased glucagon production.

In your study you compared the use of early and adequate metabolic support with peripheral amino acids compared to enteral nutrition, alone, as standard of care for five days.

In your early metabolic support group clearly the amount of protein given was significantly higher than the group that received enteral nutrition, alone. In addition to this benefit of increased protein received, you were able to show that there was a decrease in the inflammatory state; however, there were no differences in your length of stay outcomes that you measured.

I have the following questions and one comment for the authors.

What was the difference in your enteral feeding tolerance in each group? Did the group that received early amino acids also receive enteral nutrition? And what was the volume of enteral nutrition received during this time period? What was the enteral feeding tolerance rate in each group?

Why was the decision to give amino acid support to a target of 1.5 to 2 grams of protein per kilogram? And why did you choose intravenous amino acids as opposed to enteral?

You explained why the urea nitrogen excretion was so much higher in the EMS group. Is it possible that too many amino acids were provided and not incorporated into protein and they were just metabolized to the urea?

Were the differences in your metabolite concentration seen due to the changes in the utilization or the changes in the production of these metabolites?

And did you examine any other outcomes? Difference in infectious outcomes? Do you know if the decreased inflammatory state was sustained?

While the provision of an appropriate balance of amino acids may reduce the net muscle catabolism, improvement in clinical outcomes should be the primary focus of future trials so it would be important to measure muscle mass, mobility status, the incidence of ICU-acquired weakness, as well as other important outcomes such as your infectious outcomes to see what the long-term sequela of the benefit of reducing that stress response would be.

Thank you.

AJAI K. MALHOTRA, M.D., (Burlington, Vermont): Very intriguing study. But if I understand your data correctly, there was a greater negative nitrogen balance in your intervention group. So which is the chicken and which is the egg?

I mean the amino acid might be driving inflammation – I know you showed one inflammatory marker to be different and be lower in the non-intervention group, but one does not mean anything. It was not a panel that was changing towards inflammation or away from inflammation. So I don't know if one answered the question.

But the amino acid infusion, itself, bypassing the liver, changes metabolism in very different ways and I think this limited study is very, very premature.

ALLAN E. STOLARSKI, M.D. (Boston, Massachusetts): Thank you for the questions. I'll first address Dr. Mohr's. Thank you for taking the time to review our paper.

In regards to safety concerns with peripheral amino acids, we chose the concentration of 1.5 to 2 grams because we felt it was going to be within the published and accepted ASPEN and SCCM guidelines. Additionally, we demonstrated that the infusion is at an osmolarity that is safe and consistent with peripheral infusions prior to implementation and we did not see any harm in the EMS population

In regards to differences in the enteral feeding tolerance, this is an excellent question that we continue to discuss and warrants further investigation in a larger, randomized trial.

Both groups had the option of starting enteral feeds if the patients qualified for it early during the study period. However, the number of EMS patients that crossed over and sustained early enteral feeds were very low.

Enteral feeding intolerance is high due to frequent takebacks to the OR and the need for procedures necessitating frequent pausing. Consequently, none of these patients ever reached anywhere near goal for enteral feeds.

But I do want to highlight that the amount of protein and energy that was given in the parenteral infusions was appropriately adjusted for whatever minute enteral feeds were started. The balance between early enteral nutrition and parenteral nutrition is something that may be flushed out in a larger trial.

Regarding the question about why parenteral not enteral nutrition was the focus of the study early in critical illness, is simple. It was because enteral nutrition, as we saw in this study, was frequently interrupted and these patients in the standard care group received less than 600 kilocalories a day on average in that first week. So we clearly demonstrated that enteral nutrition alone following significant trauma is not sufficient in the first week.

Furthermore, providing parenteral infusions of amino acids alone allowed us to do a pure experiment, looking at just protein, and in the setting of protein alone we studied the impact on inflammation and metabolism.

Regarding the question about the difference in metabolism due to, the difference in amino acid infusions, as we demonstrated in the various random forest plots and principal component analysis for the extensive metabolic profilse, we identified several differences in the metabolism including methionine, glutathione, tryptophan, as well as stress hormone precursors.

The response to inflammation after injury is a complex interplay of various pro- and anti-inflammatory mediators and there is not a distinct pathway where one cytokine release leads to another and so forth in a step-wise fashion. Instead there is a milieu of regulatory and counter-regulatory hormones, proteins, chemokines, etc. We hope by using Metabolon to develop metabolic profiles for every patient and assessing these metabolites at baseline and day 5 we would get a good sense of the changes in metabolism not only due to the injury effect but also how protein alone modifies this highly catabolic pathway as a result of significant injury.

You discussed, as well, looking at additional outcomes such as infections, muscle mass, and hospital length of stay and ICU length of stay.

These are all outcomes that we hope to flush out in a larger clinical trial, as well. In this study there was no significant difference in these secondary outcomes. We acknowledge our pilot population was 42 and the primary outcome of this study was to assess differences in metabolism and metabolic profiles as measured by Metabolon.

Then Dr. Malhotra, thank you for your insight and your questions, as well. The greater nitrogen balance was something that really stumped us at first.

Negative 16, despite giving lots of substrate very early in the trauma period, highlights that all these patients are started on this tremendous catabolic cascade that we cannot reverse by adding extra amino acids upstream.

Consequently, all those amino acids, or a majority of them are oxidized through traditional cycles, such as the TCA cycle, etc. We believe that all of the oxidation of the upstream substrate in the resultant highly catabolic state associated with trauma, we saw significantly higher or more negative nitrogen balance in the EMS group.

We do not believe that by giving additional parenteral amino acids we further increase catabolism, we just think we provided more substrate for that already active catabolic pathway.

You bring up a really good question regarding if we really did alter inflammation with finding a decrease in IL-1 beta and an increase in soluble IL-6 receptor. This is something that needs to be flushed out in a larger trial.

And looking back at a Bonferroni correction, assessing 27 cytokines levels, the level to determine significance would be 0.0019 instead of 0.05. However, with more patients we hope to better elicit this.

The metabolic and inflammatory response after signficiant trauma is a complex system so a decrease in IL-1 beta alone doesn't necessarily imply a decrease in IL-6, as they all have different pathways and may be interchangeable in this very intricate web of inflammatory response.

Thank you.