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Pathologic Metabolism: An Exploratory Study of the Plasma Metabolome of Critical Injury

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Abstract

BACKGROUND—Severe trauma is associated with massive alterations in metabolism. Thus far, investigations have relied upon traditional bio-analytical approaches including calorimetry or nuclear magnetic resonance. However, recent strides in the field of mass spectrometry (MS)-based metabolomics present enhanced analytic opportunities to characterize a wide range of metabolites in the critical care setting.

METHODS—MS-based metabolomics analyses were performed on plasma samples from severely injured patients trauma-activation field blood (TAFB) and plasma samples obtained

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Disclosure of Financial Interests and Potential Conflicts of Interest

Dr. Peltz, Dr. D'Alessandro, Dr. Moore, Dr. Chin, Dr. Silliman, Dr. Sauaia, Dr. Hansen and Dr. Banerjee have no reported biomedical financial interests or potential conflicts of interest.

Author's contributions

EP, EEM, CCS, AS, KCH, AB planned the experimental design, patient's enrollment criteria and supervised metabolomics analyses. EP, TC collected the samples. EP, AD, EEM, CCS, KCH and AB analyzed the results, wrote the paper and critically commented on the paper. EP and AD prepared the figures and tables.

during emergency department thoracotomy (EDT). These were compared against the metabolic profiles of healthy controls.

RESULTS—Few significant alterations were observed between TAFB and EDT patients. On the other hand, we identified trauma-dependent metabolic signatures which support a state of hypercatabolism, driven by sugar consumption, lipolysis and fatty acid utilization, accumulation of ketone bodies, proteolysis and nucleoside breakdown which provides carbon and nitrogen sources to compensate for trauma-induced energy consumption and negative nitrogen balance. Unexpectedly, metabolites of bacterial origin (including tricarballoylate and citramalate) were detected in plasma from trauma patients.

CONCLUSIONS—In the future, the correlation between metabolomics adaptation and recovery outcomes could be studied by MS-based approaches and this work can provide a method for assessing the efficacy of alternative resuscitation strategies.

Keywords

metabolomics; trauma; energy metabolism; proteolysis; lipolysis; ketoacidosis

Introduction

Despite significant advances in pre-hospital care, resuscitation strategies, operative techniques, and surgical critical care, trauma remains the leading cause of death for individuals under the age of 40.¹ Uncontrolled hemorrhage and traumatic brain injury are paramount early causes of post-traumatic death (< 24 hours) while MOF remains the leading cause of death for patients surviving the initial 24 hours following injury.²

Severe metabolic changes are a hallmark of major traumatic injury. A complex interplay of altered systems biology contributes to profound inflammatory and immunologic dysfunction, activation of the complement system, induction of the hepatic acute-phase response, altered acid/base metabolism and coagulopathy.³ Concurrent with this pro-inflammatory state, a disequilibrium exists between catabolic and anabolic pathways characterized by hypermetabolism with increased energy expenditure,⁴ enhanced protein catabolism, lipolysis, insulin resistance with associated hyperglycemia, failure to tolerate a high glucose load, high plasma insulin levels ("traumatic diabetes") and hyperlactatemia predisposing to metabolic acidosis.^{5–14} The predominance of a catabolic state in poly-trauma patients leads to increased oxygen demands and corresponds to an enhanced mitochondrial oxygen utilization.¹⁵

It is likely that no single element is independently responsible for the development of posttraumatic SIRS, and that alternatively, elaboration of MOF results from a culmination and interplay of a multiple component, systems biology level pathology. Advances in genomics,¹⁶ proteomics,¹⁷ and pioneering metabolomics investigations¹⁸ provide insight into this deranged pathophysiology. However, the complex biochemistry and metabolism responsible remain largely unknown.

Previous investigations of post-traumatic metabolic changes have been conducted with classic bio-analytical approaches that directly assessed metabolite levels by means of

nuclear magnetic resonance (NMR).^{7, 10–13} These investigations were limited by their ability to resolve the complex metabolites of blood using ¹H-NMR. The paucity of unique hydrogen environments (e.g. CH₃, CH₂) and the overwhelming abundance of H₂O in the samples hinder a broad analytical coverage of the metabolome via NMR. Mass spectrometry-based metabolomics (MS) is a powerful complementary tool in terms of sensitivity and specificity for the unambiguous assignment of a broader range of metabolic species in biological fluids in comparison to NMR.¹⁸ Advantages of the MS-based metabolomics workflow include increased sensitivity and specificity, as it has been extensively reviewed.^{19–22} MS techniques enable detection of thousands of small molecules from key metabolic pathways (e.g. glycolysis, Krebs Cycle, ATP/purine catabolism, etc.) which are intertwined to physiologic modulations in response to trauma (e.g. coagulation cascades, neutrophil priming, inflammatory cascades, gut microbiome translocation).¹⁸

In this study we employed a mass spectrometric analysis of the plasma metabolome of trauma activation patients field blood samples (TAFB) and blood samples obtained during emergency department thoracotomy (EDT) in comparison to fresh frozen plasma (FFP). The EDT population was chosen since the most profound plasma metabolic changes would be anticipated in this severely injured group, which will serve as a reference for extreme metabolic shock in future studies. Specifically, we hypothesize that metabolic signatures will mirror injury-triggered alterations in fuel source utilization to preserve cellular energy production, help identify potential mediators in post-shock pathology (such as metabolites mediating non-lactate metabolic acidosis or those which may impact post-injury coagulopathy) and will further the investigation of early nitrogen imbalance following injury. The precise metabolome following trauma remains incompletely investigated and this exploratory MS study is designed to provide a foundation which will be hypothesis-generating to stimulate further direct investigations of early metabolic shock associated with severe traumatic injury and hypoperfusion.

Materials and methods

Blood samples were collected from 13 critically injured adult trauma patients (age 43±13 years, 62% male, 85% blunt injury, with median ISS 26) presenting to Denver Health Medical Center (Level I trauma center, University of Colorado) in accordance with a Colorado Multiple Institutional Review Board (COMIRB) approved protocol. Patients identified for inclusion were ≥ 18 years of age with an Injury Severity Score (ISS) > 15, who experienced blunt or penetrating injury < 6 hours prior to admission and who were likely to require transfusion of RBC within 6 hours of presentation as indicated by clinical assessment. Patients < 18 years of age, those with known inherited coagulopathy, prisoners, and pregnant females were excluded.

To compare phenotypic changes in the plasma metabolome immediately following injury, and for patients with the most profound injury and shock, we evaluated two subsets of patients meeting the above inclusion criteria in this study: i) seven patients presenting as trauma activations with field blood (TAFB) obtained by paramedics shortly after injury and before crystalloid resuscitation or blood product administration and ii) six patients who required EDT for refractory hypotension secondary to hemorrhage and tissue injury. Patient

blood samples were drawn in citrated blood tubes and compared with control plasma from five healthy volunteers (age 50 ± 15.8 years, 40% male).

Metabolomics analyses

Extended details about the protocols adopted for metabolomics analyses are reported in Supplementary Materials and Methods extended. Briefly, samples were assayed by Gas Chromatography/MS and Liquid Chromatography/MS/MS platforms (Thermo-Finnigan Trace DSQ or Waters ACQUITY UPLC/ Thermo-Finnigan LTQ-FT mass spectrometer). Compounds were identified by comparison to library entries of purified standards within a 5ppm window range. Statistical significance was determined by calculating Welch's two sample t-test ($p < 0.05$).

Results

TAFB and EDT plasma samples differed greatly from control plasma (90 and 106 significantly different features, respectively), but were similar to each other (only 19 significantly different metabolites) (Table 1). Metabolomic analyses highlighted a 97- and 166-fold increase in plasma heme from EDT and TAFB patients, respectively (Figure 1). Consistently, all trauma patients had large (750-fold in EDT, 150-fold in TAFB) increases in mannitol and oxidative stress markers (Figure 1).

Global changes in energy metabolism involved glucose/sugar utilization at the glycolytic and mitochondrial level (Figure 2). Severely injured patients manifest a strong signature of altered central energy metabolism (Figure 2). While pyruvate levels did not increase in response to trauma, lactate levels did with the greatest elevations observed in the most severely injured EDT group (Figure 2). Despite lactate accumulation, fueling of the TCA cycle was also observed through the increase in levels of all Krebs cycle intermediates with the exception of citrate (Figure 2).

Secondly there was a significant increase in fatty acid mobilization and ongoing lipolysis as demonstrated by trauma dependent decrease in the levels of several lipid classes (Supplementary File 2). In parallel, there were increased plasma levels of ketone bodies (3-hydroxybutyrate, acetoacetate and 1,2-propanediol – Figure 3) and the glycogen breakdown metabolite maltotriose (Figure 2), together with the accumulation of citramalate and tricarballic acid (Figure 3). In addition there were significant increases in carnosinase products (1-methylhistidine and β -alanine – Figure 3) and 4-hydroxybutyrate (GHB) for both TAFB and EDT patient plasma samples.

Injured patients also demonstrated significant increases in proteolysis as shown by the accumulation of amino-acids: alanine, aspartate, cysteine, glutamate, glutamine, histidine, lysine and phenylalanine (Figure 3, Supplementary File 1) and cyclic dipeptide cyclo(glutamate) (Supplementary File 1).

In addition, there was significant nucleoside breakdown for metabolic purposes as demonstrated by trauma patients increased levels of purine (xanthine, hypoxanthine and

inosine) and pyrimidine catabolites (uracil, 5,6-dihydrocuracil and 3-aminobutyrate) (Figures 3 and 4).

Lastly, there was metabolic evidence for the activation of coagulation in these injured subjects. Three fibrinogen cleavage peptides accumulated following injury (ADSGEGDFXAEGGGVR - corresponding to fibrinopeptide A, and two other cleavage products, namely DSGEGDFXAEGGGVR, and ADpSGEGDFXAEGGGVR) (Supplementary File 1).

Discussion

Metabolomics describes the complement of metabolites present in a biologic matrix and is reflective of the host's pathologic state or response to stimuli.¹⁹ This pilot study represents the first MS-metabolomic description of profound post-injury metabolic aberration in a critically injured group of trauma patients and serves as the essential foundation for future investigation in post-injury metabolic stress.

Technical issues might affect the metabolomics readouts. In this study, extraction efficiency was tested by the addition of recovery standards and normalizing extraction volumes to protein concentrations and total ion currents. Single platform-associated limitations, such as retention time and chromatographic issues, were controlled by running both LC-MS and GC-MS analyses.²³ MS-resolution issues were addressed by running samples both in low and high-resolution instruments (LTQ-FT and FT-ICR) in both polarity modes, either full MS or MS/MS as to monitor transition fingerprints against validated standards. Biological bias should be considered because the metabolome is influenced by the patients' genetic makeup and is readily responsive to environmental stimuli (e.g. diet, habitudes), including the nature and severity of injury. In this view, it is worth noting that the present study addresses a small heterogeneous group of trauma patients, sharing a comparable elapse time from trauma as a result of different injury mechanisms. Nonetheless, plasma metabolomics provides the opportunity to elucidate pathway-specific responses to injury and develop hypotheses for investigating mechanistic pathology. Future investigations on larger cohorts of injury patients may benefit from incorporating principal component analysis, or other variable reduction techniques (e.g. partial-least square discriminant analysis), to evaluate metabolomic associations to a patient's clinical presentation and hospital course, such as injury severity, transfusion requirement, responsiveness to therapy, risk for morbidity and mortality outcomes.

Critical injury may provoke both complementary and competing physiologic responses in an attempt to maintain homeostasis. Clinical responses include the adrenergic "fight or flight" response which maintains blood pressure, heart rate and critical perfusion, which also mobilizes substrates for energy production (proteolysis, lipolysis, glycogenolysis, and gluconeogenesis) to preserve cellular and tissue metabolic functions^{4, 5, 15}. Adrenergic mobilization of fuel substrates overlaps with hormonal regulation including cortisol release and insulin resistance, which may preserve glucose for sensitive tissues (i.e. brain, heart) thereby potentiating stress hyperglycemia. Other critical response mechanisms include physiologic modulation of the coagulation cascades, endothelium and platelets to achieve

hemostasis at points of bleeding (thrombogenesis and fibrinolysis shut down) while seemingly competing mechanisms exist to prevent systemic clot formation and maintain perfusion to critical tissue beds (fibrinolysis)²⁴. Additionally, the complex interplay of pro- and anti-inflammatory cytokines, growth factors, the complement system and acute hepatic phase response all combine to create the relative anabolic or catabolic state of the system as a whole.

Central to these physiologic adaptations are the multiple inter-related metabolic pathways. Post-injury systemic response mechanisms (i.e. adrenergic response, innate immune response / cytokines / complement, etc.) may influence multiple sub-cellular pathways, whose extended coverage is warranted by the sensitivity of the MS-based analyses.

Hemolysis and hemostasis

Hemorrhagic shock is one of the main features of trauma.³ Plasma from trauma patients was characterized by the accumulation of mannitol, heme, and oxidative stress markers. Mannitol increase secondary to trauma might stem from its use as an osmotherapeutic agent, or rather accumulate upon the administration of blood-derived therapeutics such as packed red blood cells (containing mannitol in the storage solutions). In line with this, EDT patients, receiving blood product resuscitation in the emergency department demonstrated higher levels of plasma mannitol (Figure 1). This finding may convey a potential confounding variable with blood product administration influencing the observed trauma metabolome. However, our data suggest limited transfusion effect, a side from mannitol increase, in this study population. We have recently published several reports and meta-analyses of biochemical and metabolic lesions to stored PRBCs.^{25–28} Specifically, alteration in glutathione and NADPH homeostasis and oxidized pro-inflammatory lipid accumulation by day 42 of storage were identified. This corresponds to a consumption of storage nutritive components (glucose and adenine) and the accumulation of catabolic by products: lactate, carboxylic acids (fumarate, succinate, α -ketoglutarate) and nucleotide metabolism by products (i.e. hypoxanthine). However, although no TAFB patient (0/6) received transfusion prior to sampling by paramedics, there is no difference in the levels of the metabolites mentioned above between EDT patients who received limited transfusion and TAFB patients, in whom blood draws preceded IVF or blood products. Furthermore, no patients in this study received platelets and there was limited FFP administration (2 of 7 EDT patients), which would not be expected to grossly affect the observed metabolic signatures because FFP was used as the baseline control metabolome. Transfusion likely had minimal effect on the global metabolic changes seen in these patients. Moreover, the elevated mannitol in the EDT population also confirms the sensitivity of this technique to detect potential treatment effect. A possible dose response effect of blood product transfusion on the plasma metabolome could be considered for future investigation in populations receiving massive transfusion.

Hemolysis might promote oxidative stress in the form of heme-iron generated reactive oxygen species (ROS). 5-oxoproline significantly increased post injury with a concomitant decrease in the levels of antioxidant vitamins including ascorbate and tocopherol, especially in EDT patients.

Balance of hemostasis and physiologic modulation of coagulation are prominent trauma research interests. Competing mechanisms appear to exist to halt hemorrhage, while preventing systemic thrombosis and preserving microcirculatory flow. Distinct coagulopathy phenotypes in the trauma population have been proposed, including: hypercoagulable (fibrinolysis shutdown), physiologic fibrinolysis and coagulopathic (hyperfibrinolysis).²⁴ While the mechanistic genesis of each remains to be fully elucidated, these coagulation patterns are suggestive of both altered thrombin generation and circulating metabolites, which may contribute to coagulopathy in the injured patient. Fibrinogen cleavage peptides were detected in 60–80% of samples from trauma patients, but not in controls suggesting fibrin cleavage by thrombin to promote clot formation. However, sequelae of this response may exist as evidenced by the identification of a specific fibrin cleavage peptide phosphorylated at Ser3 (Ser22 of fibrinogen alpha chain). This post-translational modification is known to be elevated in acute injury and may play a role in the onset of trauma-induced coagulopathy.²⁹ These important metabolic implications on the coagulation phenotype of the patient could further be explored in controlled animal models of hemorrhagic shock and resuscitation or longitudinal investigation of during massive transfusion with correlation to functional coagulation evaluations (TEG, Platelet mapping, LY-30 etc.)

Glycolysis and TCA

Previous investigations of the metabolic response to trauma have highlighted a trauma-dependent impairment in energy metabolism, resulting in increased rates of glycolysis, through the modulation of hepatic gluconeogenesis and glycogenolysis.^{30, 31} Accumulation of the glycogen catabolite maltotriose was observed in trauma patients, likely due to increased glycogen breakdown and increased consumption of fructose and glucose. Accumulation of lactate in plasma from trauma patients is consistent with metabolic adaptations to poly-trauma, critical illness and isolated injury, such as in the case of concussive brain injury.^{30–32}

Plasma accumulation of TCA cycle intermediates was observed in trauma samples, a phenomenon that can be associated with metabolic acidosis.³³ Succinate accumulation might mediate platelet over-activation and thus potentiate untoward hypercoagulation in trauma patients.³⁴ Concomitant accumulation of lactate and TCA intermediates is suggestive that early metabolic alterations following trauma promote channeling to the TCA cycle of additional carbon substrates other than sugars, such as lipids and proteins.

Lipolysis and ketoacidosis

In healthy subjects, lipolysis is under tight hormonal regulation and altered lipidomic profiles are a hallmark of trauma-induced metabolic adaptation.³⁵ In response to trauma, lipolysis is activated and lipogenesis is blocked facilitating the transition to lipids as a primary fuel substrate for energy production. Consistently, metabolomic analyses hereby highlighted, fatty acid mobilization (accumulation of acylcarnitines), and lipid breakdown (buildup of ketone bodies and breakdown products of fatty acids - e.g. choline, glycerol, ethanolamine, glycerol phosphate and glycerophosphocholine moieties). In addition, elevations in post-injury, pro-inflammatory arachidonate metabolites such as prostaglandin

PGE2 and leukotriene LTB4, observed here, supports the proposed beneficial effect of Ω -3 fatty acid supplementation diets as a measure to inhibit arachidonate mediated leukocyte activation and chemotaxis, and thus attenuate pro-inflammatory gene expression levels.³⁶ Moreover, hyperactivation of lipid metabolism in response to trauma results in the accumulation of anionic compounds such as ketone bodies which in turn promote development of a base deficit and ketoacidosis.³⁷ Organic acidurias, such as ketoacidosis, stem from the accumulation and subsequent excretion of acid metabolites that are typically only present in low abundance, such as ketone bodies.³⁷

Organic Acids

The trauma-dependent accumulation of certain organic acids might underpin both alterations to hemostatic functions and partially explain cross-talk between metabolic adaptations and the neuroendocrine system. Accumulation of 2-hydroxyglutarate in trauma patient samples could be related to platelet activation, as this metabolite has been reported to impair platelet aggregation *in vitro*.³⁸ Elevation of citramalate (methylmalonate) was also observed (Figure 3). This metabolite can accumulate rare pathologies such as methylmalonic aciduria; a metabolic disorder caused by methylmalonyl-CoA mutase deficiency (genetic or B12 deficiency), and might be related to alterations in the gut microbiome.³⁹ *In vivo*, bacterial citramalate is taken up by RBCs and can be released in trauma plasma samples upon hemolysis.⁴⁰ Analogously, tricarballic acid (Figure 3), a magnesium-chelating tricarboxylic organic acid thought to be of bacterial origin, was greatly elevated in trauma patients and may be associated with acidurias.⁴¹

These observations are relevant in that they link the previously reported phenomenon of trauma-dependent gut microbiome effects on the plasma metabolic phenotypes. Additionally they foster translational considerations in the light of the posited role for certain metabolites of microbial origin, rather than bacteria themselves, in tethering neutrophil activation, a known precursor to SIRS/MOF.

Accumulation of organic acids often has a significant negative impact on central nervous system function.⁴² Indeed, one of the largest and statistically significant fold-changes (>500-fold increase, $p < 0.05$) for a post-trauma metabolite was observed in the organic acid 3-hydroxyglutarate (3-HG). This organic acid is a potent neurotoxin contributing to neuropathology in both ketosis and glutaryl-CoA dehydrogenase deficiency. Altered carnosinase activity in trauma patients could change levels of carnosine, a brain anti-oxidant and neuroprotectant. The organic acid 4-hydroxybutyrate (GHB) is a CNS neuromodulator that accumulates with impaired activity of succinate dehydrogenase, an enzyme involved in catabolism of the neurotransmitter GABA. On the other hand, the observed stability of classic neuroendocrine mediators (cortisol, cortisone and epinephrine levels (Supplementary File 1) is suggestive of the presence of a yet unexplored neuroendocrine stress response to trauma. Together these results suggest previously unrecognized potential etiologies for acute mental status change following severe trauma at the metabolic level.

Proteolysis and amino acid accumulation

Accumulation of amino-acids, as observed here, can occur from three principal mechanisms: i) enhanced proteolysis, ii) *de novo* synthesis and altered anabolic reactions using aminoacids as building blocks for larger biomolecules, or iii) arrest of catabolic processes utilizing amino-acids for energy production purposes.

Proteolysis—Trauma recovery is known to involve an initial catabolic phase, often with negative nitrogen balance, followed by an anabolic phase. Proteolysis ensues in response to trauma in an effort to provide carbon backbones for gluconeogenesis in the liver.^{43, 44} In trauma patients, accumulating cyclic dipeptides (diketopiperazines) can be generated as byproducts of protein metabolism and have been reported to have biological activity including an immunomodulatory role in the stimulation of T lymphocytes.

De novo synthesis and anabolic purposes—Aminoacid accumulation (as observed with plasma increases in alanine, aspartate and glutamate) could be secondary to *de novo* synthesis from activation of transaminases for detoxification purposes and redox balance. Glutamate and cysteine accumulation could fuel new reduced glutathione (GSH) synthesis (Figure 2), thereby serving as physiologic protection from the increase in trauma-dependent oxidative stress (see previous paragraphs).

Arrest of catabolic reactions—Alternatively, amino-acid accumulation may result from arrest of metabolic processes during low tissue oxygen conditions or with insufficient phosphate availability to sustain oxidative phosphorylation. These essential criteria are fulfilled in this study population as it is well recognized that trauma patients are characterized by incommensurate oxygen consumption in relation to maximal oxygen availability. This might be associated with mitochondrial uncoupling, or an inefficient electron transport chain. In addition, we found that circulating phosphate levels, representative of high energy phosphate groups, were significantly decreased following injury in both trauma patient populations (Figure 4).

Notably, trauma did not result in the accumulation of glutamine. A potential explanation is that trauma-enhanced consumption of this specific amino-acid for direct cellular energy production or for fueling transamination reactions. However, although glutamine supplementation in ICU patients has been a long-sought after issue, no definitive evidence has been produced to date about the association between patient survival and glutamine supplementation.⁴⁵ Glutamine has been shown to promote neutrophil phagocytic activity and oxidative burst. Additionally, glutamine exerts an important nutritional effect serving as a principal fuel source for enterocytes and intestinal mucosa.

While the levels of most amino-acids were increased in response to trauma, tryptophan and its associated metabolites (tryptophan betaine, N-acetyltryptophan, C-glycosyltryptophan, 3-indoxyl sulfate, indolepropionate) decreased in trauma samples (Supplementary File 1). Altered tryptophan metabolism may be related to oxidative stress in the central nervous system and brain injury.⁴⁶

Nucleoside metabolism as an additional resource to sustain energy and nitrogen metabolism

Nucleosides may also provide additional carbon backbones and act as nitrogen donors to compensate for the negative nitrogen balance in response to trauma. However, increased generation of xanthine and hypoxanthine could also reflect increased xanthine oxidase activity, which would generate ROS and potentiate oxidative stress as has been reported in a rat model of traumatic brain injury.⁴⁷

Altered purinergic metabolism may underlie cellular responses to glucagon stimulation in response to trauma-associated fasting/starvation-like catabolic response. In line with this, the accumulation of nicotinamide (Figure 4), a breakdown product of the purine metabolite NAD as generated by the activity of poly-ADP ribose polymerase (PARP), may have significant clinical impact by contributing to post-traumatic motor, cognitive and histological sequelae.⁴⁸ Indeed, exhaustion of NAD⁺/NADH reservoirs would compromise the activity of many energy and redox metabolism enzymes that are dependent on these cofactors.

Unexpected Non-mammalian Metabolites

We have previously highlighted citramalate (methylmalonate) and tricarballic acid as organic acids contributing to an acidotic milieu and acidurias. The significant elevation in these metabolites shortly after trauma is a unique, unexpected finding as these metabolites have rarely been described in human physiology and are likely of bacterial origin. Investigations of remote organ dysfunction following trauma have implicated post-shock mesenteric lymph (PSML) as the conduit by which etiologic agents are conveyed from the stressed splanchnic beds to the systemic circulation.⁴⁹ Previous investigations have excluded bacterial translocation and endotoxin within PSML or portal venous blood as mediators and though a number of alternative substances have been investigated (cytokines, lipids, proteins, DAMPS) the culprit mediators remain elusive⁵⁰.

Our findings suggest an alternate hypothesis where bacterial metabolites, and not bacteria themselves, may translocate during reperfusion of ischemic splanchnic beds to manifest systemic pathology. Alternatively, bacterial metabolites such as citramalate, may elaborate systemically during RBC lysis associated with trauma and hemorrhagic shock⁴⁰. The precise source of these non-mammalian metabolites, and their role in post-injury pathophysiology, deserve further investigation which could include MS-metabolomic analysis for bacterial metabolites in post-shock mesenteric lymph and blood product cell lysates.

Conclusions

Results from this study expand on existing NMR-based metabolomics investigation for trauma-induced hypercatabolism, driven by sugar consumption, lipolysis and fatty acid utilization, ketone body accumulation, proteolysis and nucleoside breakdown providing substrates, which compensate for increased energy demands and contribute to a negative nitrogen balance. While TAFB and EDT patients were characterized by massive plasma metabolic changes in comparison to controls, minimal alterations were observed between

these two critically injured populations. These similarities suggest that the profound metabolic changes, which ensue immediately after severe traumatic injury (TAFB), persist and may develop into progressive pathologic deterioration as the patient becomes terminal (EDT).

This MS-metabolomic description provides an essential foundation for the development of multiple investigational hypotheses. Cellular energy balance and acute resuscitation are likely to be tied to metabolic endpoints. Resuscitative efforts with crystalloid, colloid, blood products, or the inclusion of alternative therapeutics with acute resuscitation (i.e. anti-oxidants or nutritive support during to balance metabolic deficiencies or meet essential substrate requirements) may be evaluated with respect to normalization of clinical physiology in conjunction with metabolomic endpoints. Specific metabolites identified, such as succinate or modified fibrinogen cleavage products (Ser22 fibrinogen- α), may be evaluated in conjunction with functional coagulation assays to investigate potential mechanistic impact on coagulation phenotypes. Additionally, the unique finding of early elaboration of non-mammalian metabolites in plasma may suggest an etiologic link between the ischemic gut, mesenteric lymph and remote organ injury which could be further elucidated in established animal models with the incorporation of MS-metabolomic analyses.

From this study it emerges that, while initial measures of resuscitation by hemorrhage control and optimizing oxygen balance are aimed at correcting ongoing post-traumatic physiologic aberration, an optimal therapeutic regimen may include the concept of a "metabolic resuscitation" to specific endpoints to maintain or to reinstitute physiologic homeostasis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Heme metabolism and oxidative stress

Figure 1.

An overview of heme metabolism and antioxidant compounds in plasma samples from controls, emergency department thoracotomies (EDT) and trauma-activated field blood (TAFB) patients. Results are graphed as box-plots indicating median values (line), mean values (+) and upper/lower quartile distributions for each group.

Glycolysis and TCA

Figure 2.

An overview of glycolysis and the TCA cycle in plasma samples from controls, emergency department thoracotomies (EDT) and trauma-activated field blood (TAFB) patients. Results are graphed as box-plots indicating median values (line), mean values (+) and upper/lower quartile distributions for each group.

Figure 3.

An overview of protein and lipid metabolism in plasma samples from controls, emergency department thoracotomies (EDT) and trauma-activated field blood (TAFB) patients. Results are graphed as box-plots indicating median values (line), mean values (+) and upper/lower quartile distributions for each group.

Nucleic acid metabolism

Figure 4.

An overview of purine and pyrimidine metabolism in plasma samples from controls, emergency department thoracotomies (EDT) and trauma-activated field blood (TAFB) patients. Results are graphed as box-plots indicating median values (line), mean values (+) and upper/lower quartile distributions for each group.

Figure 5.

A summary of the main metabolic pathways assayed in this study. Mean metabolite quantitative fluctuations were Z-score normalized and graphed as heat maps in GENE-E. For each metabolite, three values are given, indicating values detected, from top to bottom, in TAFB, EDT or control samples, respectively. Quantitative values progressively increase from blue (minimum) to red (maximum), while white indicates normalized values close to 1.

Table 1

Statistical summary

Welch's Two Sample t-Test	EDT/CTRL	TAFB/CTRL	TAFB/EDT
<i>Total number of biochemical with $p < 0.05$</i>	106	90	19
Biochemicals decreasing ↓ or increasing ↑	60 ↓ / 46 ↑	44 ↓ / 44 ↑	6 ↓ / 13 ↑
<i>Total number of biochemical with $0.05 < p < 0.10$</i>	40	43	19
Biochemicals decreasing ↓ or increasing ↑	14 ↓ / 26 ↑	22 ↓ / 21 ↑	6 ↓ / 13 ↑