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ORIGINAL ARTICLE

Carbohydrate fed state alters the metabolomic response to hemorrhagic shock and resuscitation in liver

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Abstract Hemorrhagic shock with injury results in alterations of the metabolic state of an organism, which contribute to organ dysfunction and death. Previous investigations have explored the effects of carbohydrate prefeed in murine models but few in clinically relevant large animal models. We performed carbohydrate prefeed in pigs undergoing simulated polytrauma and hemorrhagic shock with resuscitation to determine if carbohydrate prefeeding if the metabolic response to shock is dependent on fed state. Sixty-four Yorkshire pigs were divided into two experimental groups: fasted (32) and prefed (32). Experimental animals were subjected to a standardized hemorrhagic shock protocol, including pulmonary contusion and liver crush injury. To determine molecular alterations in response to trauma as a result of prefeeding, liver biopsies were obtained at set timepoints throughout the procedure. Fifty-one metabolites were profiled for each sample via proton nuclear magnetic resonance spectroscopy (^{1}H NMR). Partial-Least Squared Discriminant Analysis (PLS-DA) was used to examine clustering of the data with respect to fed state. Cross-validated models separated the fed from fasted animals. Metabolites contributing to the separation have known relationships to alternate carbon energy sources, amino acid metabolism, oxidative stress response, and membrane maintenance. In conclusion, metabolomic techniques allowed identification of an alternate response to shock and resuscitation, dependent upon fed state, through the use of metabolomics.

Keywords Hemorrhagic shock · Porcine · Metabolomics · Critical care · Physiology · Metabolism · Glucose · Pre-condition

1 Introduction

Hemorrhagic shock is a leading cause of trauma-related mortality in both civilian and military settings. Efforts over the years have significantly improved survival in the military sector (Holcomb et al. 2006); however, hemorrhagic shock remains the most common cause of preventable injury in both the civilian and military sectors (Spinella and Holcomb 2009; Holcomb et al. 2007; Dubick 2011; Eastridge et al. 2012). Hemorrhagic shock from traumatic injury results in multiple alterations in the metabolic state of an organism, many of which are not fully elucidated.

Hemorrhagic shock results in inadequate tissue perfusion leading to decreased oxygen availability to mitochondria resulting in a switch towards anaerobic metabolism. The liver serves an important function as a regulator of metabolism during stressed states. Initially, the shift towards anaerobic metabolism stimulates the liver to increase glycogenolysis and process elevated lactate produced in the peripheral tissues. The liver also provides a major site of detoxification and production of alternate metabolic fuel sources including amino acids and lipids. Effects of different fed states on liver metabolic processes following injury and hemorrhagic shock are not well known.

Metabolomics, a high-throughput profiling of all the metabolites within a sample (Nicholson et al. 1999), has

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the ability to identify multiple metabolites simultaneously. This ability potentially permits the elucidation of altered cellular and metabolic pathways in addition to potentially furthering our understanding of the metabolic response to hemorrhagic shock with respect to metabolic state. Practical applications of metabolomics include classification and diagnosis of several forms of cancer and neurodegenerative disorders (Putri et al. 2013). In addition, our laboratory has successfully applied metabolomics tools to a clinically relevant porcine model of hemorrhagic shock in order to identify biomedical pathways and to associate metabolic changes with phase of care (Lusczek et al. 2013; Lexcen et al. 2012; Scribner et al. 2010)

Of interest herein is the impact of fed state upon the response and recovery from hemorrhagic shock and injury. Prior work from our group reported a significant increase in lung injury in animals receiving a carbohydrate prefeeding immediately prior to injury compared to fasted animals (44 vs. 15 %, $p = 0.03$) (Iyegha et al. 2012). This difference in outcome between fed and fasted animals led us to ask the question regarding possible drivers of difference in response. One of the potential mechanisms possible is a difference in metabolism driven by carbohydrate prefeeding. The objective of this study was to determine if the liver responds differently in our clinically relevant model of polytrauma and hemorrhagic shock with respect to fed state with the hypothesis that there would be quantifiable differences in liver metabolites reflecting an altered metabolic response to shock and resuscitation depending upon fed state. The liver is an important regulator during stress and serves as a primary site of gluconeogenesis to meet peripheral glucose requirements thereby making it an ideal location to evaluate alterations in metabolic response. To test this hypothesis, we used our well-established model of hemorrhagic shock and polytrauma comparing the effect of providing a Carbohydrate Prefeed (CPF) versus a Fasted (FS) diet prior to insult. Proton (^{1}H) NMR spectroscopy was used to determine concentrations of metabolites in liver biopsies taken at defined timepoints throughout shock and resuscitation with the goal to identify altered metabolic responses in prefed versus fasted animals so that we may understand potential contributing factors of metabolic state to outcome.

2 Methods

2.1 Animals

Male Yorkshire–Landrace pigs (15–20 kg) were purchased from Manthei Hog Farm, LLC (Elk River, MN) and housed in Research Animal Resources (RAR) at the University of Minnesota. All studies were approved by the Institutional Animal Care and Use Committee. Pigs were fasted overnight prior to surgery, but were allowed water ad libitum.

2.2 Animal preparation and hemorrhagic shock protocol

Sixty four (64) juvenile, male Yorkshire pigs were used in this study. Other results from this group of animals have been previously published (Lusczek et al. 2013; Scribner et al. 2010; Iyegha et al. 2012). Two experimental groups were utilized: Carbohydrate Prefed (CPF, $n = 32$) and Fasted (FS, $n = 32$). CPF animals were given 7 cc/kg bolus of Karo Syrup® (mixture of sugars including ~15 % glucose, maltose, fructose and sucrose) diluted with water 1 h prior to induction of anesthesia. The full experimental polytrauma and shock protocols have been described in detail previously (Lexcen et al. 2012; Scribner et al. 2010). Briefly, animals were instrumented and splenectomized. Polytrauma was induced by a captive bolt device to create a blunt percussive injury to the chest and a liver crush injury using a Holcomb clamp technique (Holcomb et al. 1999). Hemorrhagic shock was then induced by withdrawal of blood from the inferior vena cava until a systolic pressure of 45–55 mmHg was reached for 45 min (S45) to simulate delay prior to medical attention. Typically, this resulted in withdrawal of ~40 % of the pig's blood volume. Blood was placed in an acid-citrate-dextrose bag for later use. Following the shock period, animals received lactated Ringer's fluid given as 20 cc/kg intravenous(IV) boluses to maintain a systolic blood pressure greater than 80 mmHg for 1 h of limited resuscitation to simulate transportation to a medical center; then underwent full resuscitation protocol for the following 24 h (Fig. 1). This resuscitation included fluid, shed blood, and ventilator support in a protocolized fashion. After the resuscitation period, animals were extubated and sent to recovery and subsequently euthanized (Fig. 2).

At several time points throughout the experiment, liver biopsies were taken from the periphery of the liver ranging in weight from 0.2 to 0.6 g. Biopsies were flash frozen in liquid nitrogen and stored at −80 °C until preparation for NMR analysis. Biopsies were taken at the following timepoints: baseline after the animal stabilized from instrumentation (B), 45 min after hemorrhage (S45), 2, 8, and 20 h after full resuscitation (FR2, FR8, FR20). Changes induced by hemorrhagic shock polytrauma and 'early' resuscitation were denoted as the difference between S45 and B (S45-B) and between FR2 and S45 (FR2-S45) respectively.

2.3 Liver metabolite extraction

Stored liver samples were prepared for NMR analysis using a variation of the perchloric acid (PCA) extraction

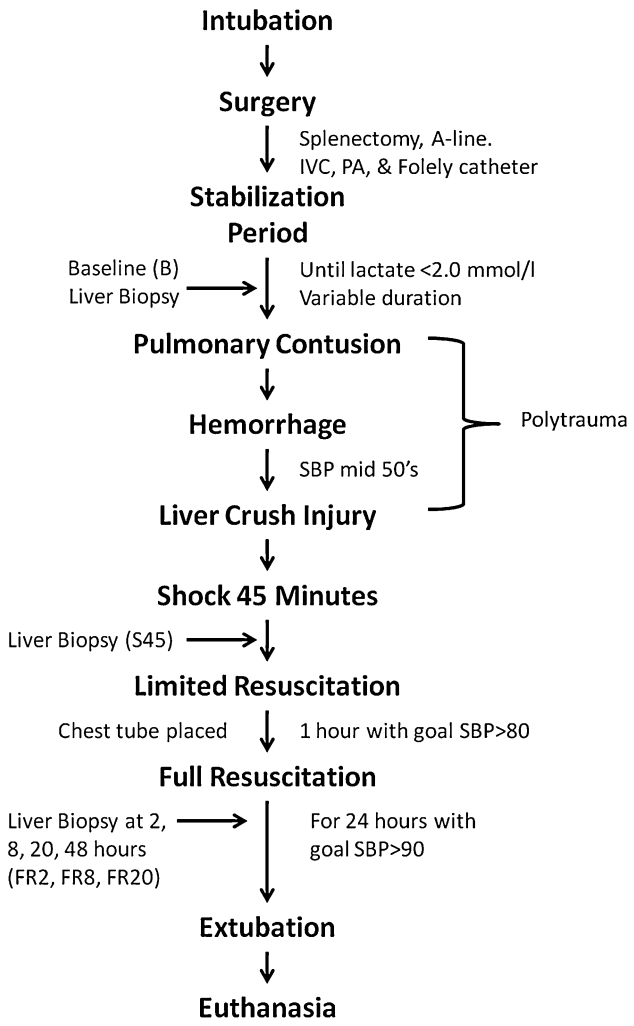

Fig. 1 Graphical representation of the experimental timeline described in Sect. 2

technique (Tyagi et al. 1996). Frozen liver samples were weighed and pulverized into a fine powder using a mortar and pestle in liquid nitrogen, weighed, and kept on ice in eppendorf tubes. Perchloric acid (6 %) was added at 5 mL/g of tissue and vortexed for 30 s. Samples were incubated for 10 min and subsequently centrifuged at 12,000×*g* for 10 min at 4 °C. The supernatant was collected, pH neutralized with 2 M K_2CO_3 to 7.4, incubated on ice for 30 min and centrifuged once more at 12,000×*g* for 10 min at 4 °C. The supernatant was collected, frozen and lyophilized in a LABCONCO Freezone 6 Plus freeze–drier (Kansas City, MO).

2.4 NMR spectroscopy

Lyophilized samples were rehydrated with 500 µL D_2O and 50 µL of internal standard 3 mM DSS (Dimethyl-Silapentane-Sulfonate, Sigma-Aldrich, St. Louis, MO). Solution pH was adjusted with DCl and NaOD to 7.4. The final volume was brought to 600 µL using D_2O and the sample was transferred into a 5 mm tube (Wilmad, Vineland, NJ)

^{1}H NMR spectra were obtained using a Varian 600 MHz spectrometer with a 5 mm HCN triple resonance probe. Spectra were generated from 128 scans with a basic ^{1}H acquisition protocol consisting of a 45° tip angle, a relaxation delay of 1 s and an acquisition time of 1.9 s. All NMR spectra were phase- and baseline-corrected and chemical shifts were referenced to the DSS internal standard.

Chenomx software (Weljie et al. 2006) was used to identify and quantify metabolites present in each liver sample. Fine manual phasing and baseline corrections were applied to each spectrum before targeted profiling was performed. Fifty-one (51) metabolites were fit in each liver sample in this study, resulting in a profile containing the concentration of each identified metabolite in millimoles per liter (mM), as determined by comparison to the internal standard.

2.5 Statistical analysis

A multivariate approach was used to analyze each of the timepoints. To determine the response to shock and resuscitation, changes in concentration between baseline and subsequent timepoints were analyzed. All statistical analysis was conducted using the open source R statistical program (R Core Team 2013). For each timepoint/difference the 51 profiled metabolites were auto-scaled and mean-centered prior to initial Principal Component Analysis (PCA). Samples that fell outside a 95 % Hotelling's ellipse were considered outliers and removed from further analysis. Datasets, with outliers removed, were subsequently analyzed by PLS-DA, a common discrimination technique utilized in metabolomics (Bijlsma et al. 2006; Trygg et al. 2007; Liland 2011) that has been successfully employed in our previous studies (Lusczek et al. 2013; Lexcen et al. 2012). The R packages DiscriMiner (Sanchez 2012) and Permute (Simpson 2012) were used collectively to conduct the PLS-DA model, cross-validation, and permutation tests. PLS-DA models were optimized based on the number of misclassifications (NMC) which has been show to be more powerful than other indicators, such as Q (Spinella and Holcomb 2009), at detecting differences between groups (Szymanska et al. 2012). Cross-validation was conducted via cross-model validation, i.e. nested-CV, 2CV (Westerhuis et al. 2008), where in the dataset is randomly split into training and testing datasets (75, 25 %). PLS-DA models were then generated from the training dataset and a leave-10-out internal cross-validation. Optimized models were used to predict the testing set (outer cross-validation). Prediction accuracy is calculated from

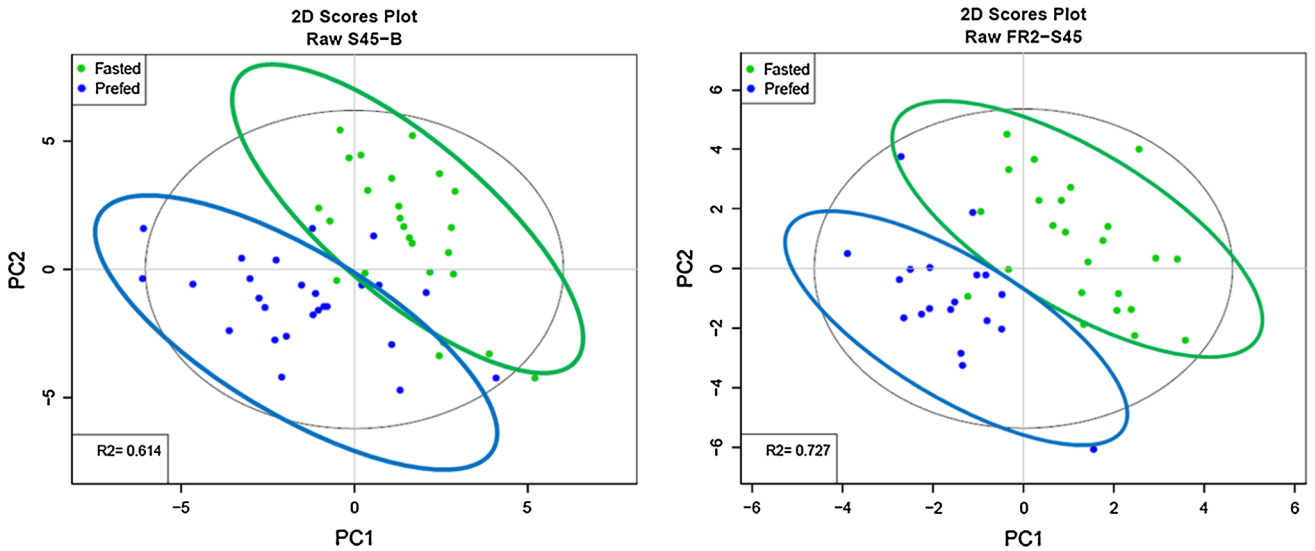

Fig. 2 2D score plot of response to shock (S45-B) and resuscitation (FR2-S45) PLS-DA models visually representing the discrimination between groups. *Green* represents fasted animals and *blue* represents carbohydrate prefed animals

the resulting confusion matrix. Accuracy ≥85 % and R^2 ≥0.500 were considered potential models. Model quality assessment was assessed by random permutation of group class with 1,000 iterations where a low permutation *p* value >0.05 indicated a strong model. Metabolites were subsequently ranked according to their respective variable importance of projection (VIP) score. The highest VIP scores correspond to which metabolites contribute most to discrimination between groups. The top ten metabolites represent the primary drivers of the calculated discrimination.

3 Results

3.1 Partial least squares discriminant analysis (PLS-DA)

PLS-DA allowed discrimination between prefed and fasted state in this hemorrhagic shock model with changes most notable between the fed and fasted state in the period of time between end of shock (S45) and 2 h after full resuscitation (FR2). The final model statistics are reported in Table 1. The majority of models reported accuracy ≥90 % and R^2 ≥0.500 (except FR8-FR2), however, not all models passed the permutation tests (Table 1). The model comparing metabolite changes between baseline (B) and S45 was nearly significant ($p = 0.06$) suggesting an alternate response to shock dependent upon fed state. The model evaluating baseline metabolites provided high accuracy (90 %) and R^2 (0.586) but demonstrated a non-significant permutation ($p = 0.37$).

Table 1 Results of PLS-DA model generation for each timepoint/difference

Model	R2	NMC ± SD	Accuracy (% correct classified	Permutation (*p* -value)
B	.586	2.8 ± 1.5	95	.37
S45-B	.614	2.8 ± 1.3	95	.06
FR2-S45	.727	2.0 ± 1.1	95	.04
FR8-FR2	.495	4.1 ± 1.2	90	.64
FR20-FR8	.540	4.0 ± 1.3	90	.60

NMC number of misclassified, *SD* standard deviation

3.2 Important metabolites

The metabolites identified, by VIP scores, during the response to shock (S45-B) primarily reflected differing energy sources such as glycolysis in CPF animals (carbohydrate sugars, lactate) and amino acid metabolism in FS animals (aspartate, asparagine, 3-hydroxyisovalerate). In addition, some unexpected differences in elevated asparagine and adenosine were observed in CPF animals (Table 2). VIP metabolites identified during the response to resuscitation (FR2-S45) continued to be associated with energy source differences (e.g. branch chain amino acid metabolism). Indications of membrane repair (sn-Glycero-3-phosphocholine) and free radical scavenging (Benzoate) are also reported (Table 3). Lineplots of the highest scoring metabolites are also provided in Fig. 3.

Given the high accuracy of each remaining model, selection of important variables was also performed via ranking metabolites by the VIP statistic (see supplementary

Table 2 Mann–Whitney *U* results of the top 10 VIP (variable importance of projection) metabolites to discriminate Fasted and carbohydrate prefed (CPF) samples during the response to shock (S45-B) via PLS-DA

Metabolite	VIP score	Group	S45 mean ± SE	Difference mean ± SE	Mann–Whitney *U*
Glucose	3.301	CPF	25.69 ± 2.68	191.18 ± 27.83	$p = 5.9 \times 10^{-9}$
		FS	5.62 ± 0.99	−2.27 ± 6.85	
Lactate	2.237	CPF	5.58 ± 0.45	39.81 ± 4.65	$p = 8.1 \times 10^{-4}$
		FS	3.42 ± 0.28	18.14 ± 3.44	
Aspartate	2.042	CPF	1.09 ± 0.07	3.02 ± 0.79	$p = 4.3 \times 10^{-3}$
		FS	0.82 ± 0.05	−0.21 ± 0.56	
Maltose	1.993	CPF	1.30 ± 0.28	−12.31 ± 3.05	$p = 2.1 \times 10^{-5}$
		FS	0.25 ± 0.04	−2.08 ± 0.80	
Sucrose	1.967	CPF	0.76 ± 0.16	−7.96 ± 1.86	$p = 2.8 \times 10^{-3}$
		FS	0.14 ± 0.02	−1.72 ± 0.61	
Glutathione	1.412	CPF	1.74 ± 0.17	3.47 ± 1.71	$p = 0.168$
		FS	1.38 ± 0.12	−1.68 ± 1.61	
Xanthine	1.391	CPF	0.05 ± 0.01	0.08 ± 0.06	$p = 0.109$
		FS	0.06 ± 4 x 10^{-3}	−0.12 ± 0.07	
Asparagine	1.375	CPF	0.35 ± 0.06	1.01 ± 0.47	$p = 0.131$
		FS	0.27 ± 0.03	−0.34 ± 0.43	
Adenosine	1.296	CPF	0.28 ± 0.04	1.11 ± 0.31	$p = 0.032$
		FS	0.21 ± 0.02	0.34 ± 0.23	
3-Hydroxyisovalerate	1.117	CPF	0.07 ± 0.01	0.08 ± 0.04	$p = 0.124$
		FS	0.07 ± 0.01	−0.08 ± 0.07	

Mean concentrations reported as μM ± SE (standard error)

Table 3 Mann–Whitney *U* results of the top 10 VIP (variable importance of projection) metabolites to discriminate Fasted and carbohydrate prefed (CPF) samples during the response to resuscitation (FR2-S45) via PLS-DA

Metabolite	VIP Score	Group	FR2 Mean ± SE	Mean ± SE	Mann–Whitney *U*
Glucose	3.311	CPF	7.40 ± 0.66	−148.38 ± 24.59	$p = 9.5 \times 10^{-6}$
		FS	5.38 ± 0.31	−4.83 ± 11.85	
3-Hydroxyisovalerate	2.083	CPF	0.07 ± 0.01	−0.02 ± 0.03	$p = 2.7 \times 10^{-3}$
		FS	0.08 ± 0.01	0.13 ± 0.04	
Valine	1.659	CPF	0.23 ± 0.02	−1.15 ± 0.31	$p = 0.057$
		FS	0.30 ± 0.02	−2.40 ± 0.43	
Fumarate	1.658	CPF	0.05 ± 0.01	−0.26 ± 0.04	$p = 0.011$
		FS	0.05 ± 3 x 10^{-3}	−0.08 ± 0.08	
Benzoate	1.639	CPF	0.15 ± 0.03	−0.03 ± 0.24	$p = 8.3 \times 10^{-3}$
		FS	0.22 ± 0.03	0.81 ± 0.30	
Leucine	1.590	CPF	0.25 ± 0.03	−1.43 ± 0.32	$p = 0.094$
		FS	0.28 ± 0.02	−2.50 ± 0.36	
Choline	1.367	CPF	0.63 ± 0.16	0.66 ± 1.19	$p = 0.068$
		FS	0.47 ± 0.06	−1.94 ± 0.87	
Isoleucine	1.364	CPF	0.14 ± 0.02	−0.63 ± 0.22	$p = 0.146$
		FS	0.18 ± 0.01	−1.19 ± 0.21	
Lysine	1.356	CPF	1.08 ± 0.12	1.12 ± 1.05	$p = 0.099$
		FS	1.03 ± 0.12	−1.60 ± 1.07	
Sn-Glycero-3-phosphocholine	1.390	CPF	5.20 ± 0.48	−26.06 ± 4.84	$p = 0.080$
		FS	3.46 ± 0.42	−39.36 ± 5.37	

Mean concentrations reported as μM ± SE (standard error)

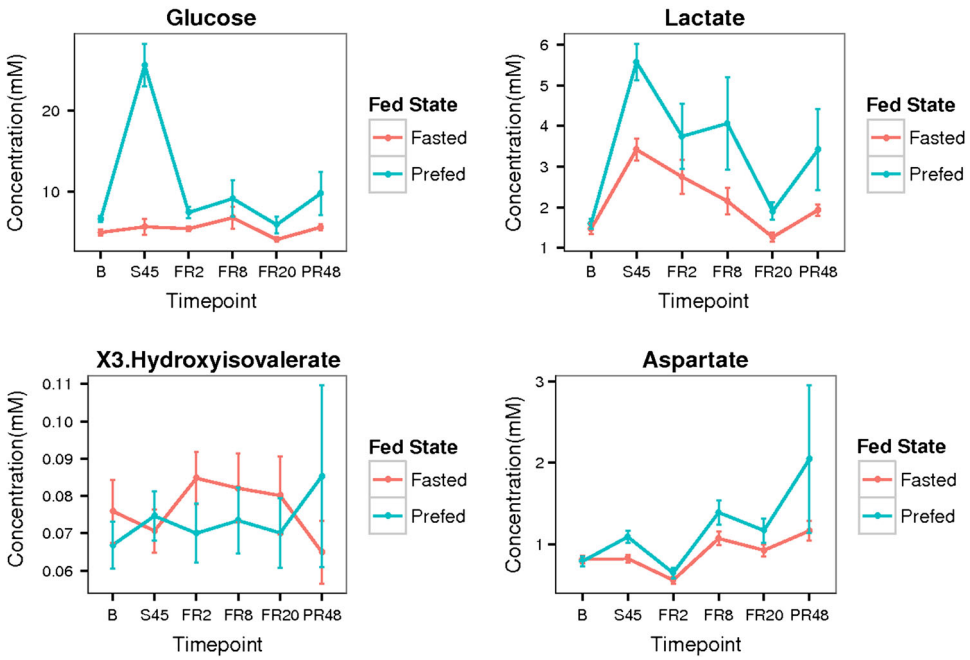

Fig. 3 Lineplots of liver tissue levels of glucose, lactate, 3-hydroxyisovalerate, and aspartate during hemorrhagic shock and resuscitation. Values are represented as mean with standard error

file 1 for full VIP lists); however, strong conclusions could not be drawn from models not passing the permutation test.

4 Discussion

In this article, we identify multiple differences in metabolism during shock and resuscitation associated with preinjury fed state. This discrimination was most notable during the initial response to shock and resuscitation with subsequent loss of discrimination over time suggesting the larger metabolic effect of shock and injury overcame the smaller metabolic effect of fed state.

4.1 Response to shock (S45-B)

As expected, with a carbohydrate diet, immediately preceeding injury, liver tissue demonstrated a predominant glycolytic metabolism during the response to shock (S45-B) with active glucose, maltose and sucrose utilization followed by a dramatic increase in lactate levels. Elevated lactate levels have been previously shown to be associated with poor outcome (McNelis et al. 2001). The changes we observed in glucose metabolism compare to recently described measures of intracellular flux analysis of livers under fed and fasted states which reflect depleted glycogen stores in fasted livers (Orman et al. 2012, 2013). This is most clearly seen when the glucose levels fail to rise following shock (Fig. 3). These reports suggest that maintaining glucose stores would be beneficial; however, these experiments consisted of animals undergoing burn injury and provided no survival measures. Our data suggests that depleted glucose stores as a result of fasting may be protective following polytrauma and hemorrhagic shock.

Aspartate also increases more rapidly in livers of CPF animals suggesting potentially impaired transport of the Malate–Aspartate shuttle leading to elevated aspartate levels and subsequent breakdown in the Urea cycle. The Malate–Aspartate shuttle is required for oxidative phosphorylation and therefore impairment may indicate a more severely stressed oxidative state. The urea cycle enzymes may not be functioning as rapidly as in FS animals and this may be a consequence of the carbohydrate metabolic state, leading to a slower conversion to arginine leading to lower arginine and an accumulation of aspartate in CPF animals. Aspartate can also be converted to asparagine, which also increased in CPF, and either recycled back to aspartate or excreted.

VIP metabolites adenosine and arginine have been reported as protective prior to ischemia–reperfusion associated with Nitric Oxide (NO) metabolism (Voelckel et al. 2003; Zhang et al. 1997). Adenosine is a potent vasodilator released as a response to ischemia–reperfusion (Guinzberg et al. 2006). In addition, adenosine is the product of AMP degradation and is characteristic of an imbalance in the tissue oxygen supply/demand ratio. Elevated levels of adenosine in CPF suggest a greater imbalance of oxygen. Despite having elevated adenosine levels Mean Arterial Pressure (MAP) is higher in CPF animals. This may be explained as baseline levels of adenosine are essentially identical between FS and CPF animals whereas levels of arginine, another important vasodilator, are much higher in FS at S45 (CPF = 1.23 mM, FS = 0.82 mM, p -value = 9.6×10^{-3}). Prior literature reports that arginine is

produced within the urea cycle, which is potentially upregulated by glucagon to handle increased proteolysis (Snodgrass et al. 1978). Our observations of elevated baseline BCAAs and continued decrease of leucine intermediate 3-hydroxyisovalerate in FS animals during the response to shock adds support to this interpretation.

Xanthine and glutathione were also identified as important metabolites. Xanthine is the product of xanthine oxidoreductase and generates Reactive Oxygen Species (ROS). Initially xanthine levels were higher in FS animals, suggesting increased oxidative stress, but decreased to levels similar to those in CPF animals during the response to shock. The increasing xanthine levels in CPF animals is consistent with previous in vitro evidence identifying increased xanthine oxidase activity following prefeeding and ischemia but not following fasting (Kooij et al. 1994). More recently, however, it has been suggested that ROS production by xanthine oxidase has limited importance in practice as the conversion of xanthine dehydrogenase to xanthine oxidase requires long ischemic periods that are rare in the clinical setting (Elias-Miró 2013). This suggests that initial conditions (e.g. fed state) potentially precondition the xanthine oxidase activity prior ischemia. Glutathione, an important antioxidant, also increases within CPF animals to modulate ROS. Measurements of ROS species between fed states are needed to determine possible differences in oxidative stress.

4.2 Response to resuscitation (FR2-S45)

During the response to resuscitation (FR2-S45) we found that fumarate declines sharply in the CPF animals. This reduction is potentially a result of succinate dehydrogenase (i.e. Complex II) of the electron transport chain reducing fumarate to succinate during a period of reduced PaO_2 (Hoberman and Prosky 1967). This mechanism is supported by our data as succinate levels also increase ($+0.05 \pm 0.02$ mM) during the response to resuscitation. Curiously, lysine levels also increase in CPF but decrease in FS animals. One possible explanation is the conversion of lysine to glutamine; an alternative substrate for the Krebs Cycle. Our data reports elevated glutamine levels in CPF animals at FR2 (CPF = 1.44 mM, FS = 1.27 mM, p -value = 0.09) which inhibit the conversion of glutamine synthase, the final step between lysine and glutamine, resulting in elevated lysine levels (Eisenberg et al. 2000).

Metabolism in FS animals continues to reflect BCAA degradation, in which valine, leucine, and isoleucine levels are all decreasing but remain higher indicating continued protein breakdown. However, increasing levels of 3-hydroxyisovalerate (i.e. β-Hydroxy-β-methylbutryate, HMB) in FS animals suggests impaired complete leucine degradation. Prior research on muscle has reported 3-hydroxyisovalerate as a substrate for HMG-CoA reductase, an important enzyme for cholesterol synthesis to stabilize membrane integrity (Nissen et al. 2000). To our knowledge there is no literature exploring 3-hydroxyisovalerate and HMG–CoA in the liver. It is also noted that sn-Glycero-3-phosphocholine (i.e. Glycerophosphocholine, GPC) levels decreased more rapidly in FS relative to CPF animals. GPC is indicative of membrane maintenance and repair (Scribner et al. 2010). This is supported by a slight increase in Choline levels, potentially being quickly incorporated into membranes.

The hypoxic state following hemorrhagic shock and the subsequent reperfusion results in the production of reactive oxygen species (ROS). Interestingly, our data reports higher benzoate concentrations, an oxygen radical scavenger (Weitzman and Stossel 1982; Paulin et al. 1986), in FS animals (Table 3). One potential mechanism is that CPF animals experience greater oxidative stress following anaerobic glycolysis, as noted by prior xanthine levels, where increased antioxidants would prove beneficial; however assays of ROS need to be pursued comparing fed states for definitive conclusions. To our knowledge, no previous research has reported benzoate as a radical scavenger following reperfusion. It is possible that the conversion of benzoate to hippurate for excretion is inhibited resulting in the observed increase, however, further research is needed to determine if this is an alternate antioxidant following reperfusion.

Although this study measured a large number of metabolites, this study is limited by the both the extraction method and tissue utilized. The PCA method only extracts water soluble metabolites and there may be other compounds that could reflect other altered metabolic processes. An investigation using alternate extraction techniques may prove valuable. The liver is only one component of the systemic response to hemorrhagic shock and resuscitation, and analysis of other compartments such as serum, urine and muscle would be beneficial.

5 Conclusions

The liver metabolic response to shock is initially significantly different depending upon fed state. This difference dissipates over time as the effects of injury overwhelm the effects of prefeeding. Our identification of VIP metabolites validated previously expected changes in metabolism between CPF and FS animals such as alternate carbon energy sources. In addition, metabolites associated with NO production, branched chain amino acid metabolism, oxidative stress, and membrane maintenance were significantly different between CPF and FS animals during the response to shock and response to resuscitation. This analysis reveals that fed state has defined implications on the

response to hemorrhagic shock and injury and thereby provides some clues about how future therapeutics should be modified. Exploring other prefeeding methods, such as protein or lipid, would provide further useful information concerning the response to shock.

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