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Effect of Hypobaria After Polytrauma on Gut Function and Its Microbiota

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University of Maryland School of Medicine

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Final Report
for March 2016 to November 2017

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14. ABSTRACT

In the current study, we hypothesized that hypobaria would worsen polytrauma-induced gut dysfunction and lead to pathologic alterations in the gut microbiota. This was tested in an established model of controlled cortical impact-induced traumatic brain injury and hemorrhagic shock. We found that gut inflammation and injury were most significantly worsened in animals exposed to hypobaria at 100% acutely but also at 30 days after hypobaria and polytrauma. Changes in gut pathology were accompanied by changes in the gut microbiota, again most marked in the hypobaria 100% group at 30 days, with an increase in diversity and a significant increase in abundance of Bacteroides. Metatranscriptome data suggested that this technique might be able to detect changes early after polytrauma and hypobaria and warrants investigation in future studies.

15. SUBJECT TERMS
Gut dysfunction, microbiota, hypobaria, polytrauma

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TABLE OF CONTENTS

Section **Page**

LIST OF FIGURES

Page

LIST OF TABLES

Page

1.0 SUMMARY

In the current study, we hypothesized that hypobaria will worsen polytrauma-induced gut dysfunction and lead to pathologic alterations in the gut microbiota. This hypothesis was tested in an established model of controlled cortical impact-induced traumatic brain injury in combination with hemorrhagic shock by testing the following specific aims:

SA1. Investigate gut epithelial and endothelial barrier integrity after polytrauma and hypobaria.
SA2. Characterize changes in the gut microbiota after polytrauma and hypobaria.
SA3. Correlate changes in gut barrier integrity and gut microbiota after polytrauma with neurologic outcome and distant organ injury

2.0 INTRODUCTION

Air Force-funded research by Dr. Fiskum at our center (FA8650-11-2-6D04) has shown that exposure of rats to 6 hours of hypobaria (approximately 8000 feet altitude) after experimentally induced traumatic brain injury (TBI) worsened neurologic and neuropathologic outcomes [1,2]. His current Air Force-funded project, "Effects of Hypobaria on Brain Injury and Mortality Following Head Trauma Combined with Hemorrhagic Shock," is expanding his studies to include the effects of aeromedical evacuation-relevant hypobaria on neurologic outcomes and survival in a polytrauma model consisting of controlled cortical impact (CCI)-induced TBI, in combination with hemorrhagic shock (HS). There is nothing known about the effects of hypobaria in polytrauma models, specifically, HS and TBI. Dr. Fiskum has shown that hypobaria worsens TBI and hypothesizes that hypobaria will worsen neurologic outcome, and possibly increase mortality, in his polytrauma model. One important organ that was not included in this study is the gut. Gut function can be adversely affected by trauma and HS [3], TBI [4], and hypobaria [5]. Xu et al. demonstrated that prolonged hypobaria (5 days) alone leads to intestinal injury and associated systemic inflammation and cytokine release [5]. Therefore, in the current study, we proposed to expand Dr. Fiskum's study of hypobaria and polytrauma to include the gut. We used gut samples from his study and performed additional studies and time points relevant to the gut.

3.0 BACKGROUND

3.1 Important Role of the Gut after Trauma and Hemorrhagic Shock

Early traumatic deaths both on the battlefield and in civilian settings result from uncontrolled hemorrhage and/or brain injury, while multiple organ dysfunction syndrome (MODS) remains the most common cause of late deaths in severely injured patients. The mortality of MODS remains high, in part due to the *lack of early therapeutic interventions to mitigate shock-induced gut dysfunction.* Gut-derived factors from the shocked gut can enter the mesenteric lymph, activate neutrophils, and potentiate the development of distant organ failure. Additionally, bacterial translocation occurs from loss of gut barrier integrity and similarly results in systemic inflammation and distant organ injury.

3.2 Important Role of the Gut after Traumatic Brain Injury

Bidirectional signaling between the central nervous system and the enteric nervous system has been increasingly recognized as an important pathway with both pathologic and therapeutic implications. Gastrointestinal (GI) dysfunction frequently occurs after TBI through the brain-gut axis. Borovikova et al. were the first to describe the important role of the vagus nerve in this pathway [6]. TBI-induced gut dysfunction, similar to shock-induced gut dysfunction, can contribute to systemic inflammation and distant organ injury. Alternatively, the gut can have neurologic implications. Qi et al. recently demonstrated that the GI hormone ghrelin attenuated brain injury and facilitated functional recovery in a rodent model of combined HS and TBI [7]. Thus, the gut may be used as a therapeutic modality after TBI.

3.3 The Gut Microbiota and the Brain

The gut microbiota is a complex community of microorganisms that plays a key role in numerous metabolic, physiological, nutritional, and immunological processes in intimate association with its host [8]. Recent estimates using molecular approaches suggest that there may be up to 15,000 different species in the human GI tract. The gut microbiota is an important factor in determining the health status of the host and has been implicated in both GI and extra-intestinal disorders [9]. It is positioned to impact the homeostatic response of the host through multiple mechanisms, including actions of microbial metabolites (e.g., sugars, amino acids, short chain fatty acids) on nutrient-sensing cells of the GI tract and through modulation of inflammation, gut barrier function, and bile acid synthesis [10]. Braniste et al. reported that the gut microbiota influenced blood brain barrier permeability [11], which has therapeutic inflammations to reduce brain edema

3.4 Knowledge Gap and Scope of the Problem

On the battlefield and in the civilian setting, TBI often occurs concomitantly with HS. Little is known about the combined effect of TBI and HS (polytrauma) on the gut and how this may impact outcomes. There are no published reports, to our knowledge, on the gut microbiota and TBI or polytrauma; thus, there is a critical gap in our knowledge that can have important therapeutic implications for the injured service member.

We recently found that animals subjected to impact-induced moderate TBI or blast-induced mild TBI exhibit greater histologic and neurobehavioral evidence of brain injury when exposed to 100% as compared to 28% oxygen (O_2) during hypobaria. One clinical study performed at our Shock Trauma Center in Baltimore found that the occurrence of hyperoxemia within the first 24 hours of hospitalization is associated with worse short-term functional outcomes and higher mortality after TBI [12]. In models of septic shock, hyperoxia was shown by Gennari and Alexander to preserve gut barrier function [13]. In a model of combined cortical impact and HS, Blasiole et al. found that hyperoxia improved brain oxygen tension and hippocampal neuronal survival but increased neuroinflammation and oxidative stress [14]. Similarly, Proctor et al., in the polytrauma model proposed in the current study, found that survival was greater with hyperoxic resuscitation, but neurologic outcomes were improved with normoxic resuscitation [15,16]. *The effect of hypobaria on hyperoxia after polytrauma is not known.* Since most polytrauma victims who are flown long distances receive 70-100% inspired

or ventilator O_2 during these flights, it is extremely important to determine whether exposure to such high O_2 levels is beneficial or deleterious to the gut and brain in an animal model of polytrauma.

4.0 METHODS

4.1 Polytrauma Model

The study protocol was reviewed and approved by the University of Maryland Institutional Animal Care and Use Committee and Air Force Medical Support Agency. Animals were handled and studies were conducted under a program of animal care accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International and in accordance with the National Research Council's 2011 *Guide for the Care and Use of Laboratory Animals* (in compliance with Department of Defense Instruction 3216.1).

Male Sprague Dawley rats were used in our polytrauma model of TBI and HS [17]. There were three groups of animals: the 3-hour polytrauma alone group (xx rats), the second group (xx rats) of polytrauma then hypobaria with euthanasia at 30 hours, and the third group (xx rats), which was poytrauma and hypobaria with euthanasia at 30 days. The polytrauma model consists of TBI induced by CCI to a depth of 2.0 mm followed by 30 minutes of HS (mean arterial pressure = 35-40 mmHg). To mimic the clinical scenario, resuscitation was with Hextend (pre-hospital) 1 mL and then an additional 0.5 mL to maintain mean arterial pressure/55 mmHg, then shed blood at 1 mL/min (in-hospital). Hypobaria experiments were performed using Dr. Fiskum's animal-dedicated altitude chamber. The vacuum pump was set to reach hypobaric conditions over 20 minutes (576 mmHg or 0.75 atmosphere absolute), approximating those found in military transport planes. The chamber was maintained at either 28% O_2 or 100% O_2. At 20 minutes prior to the end of the 6-hour hypobaria period, the vacuum pump was adjusted until sea level altitude was reached. Shams underwent craniotomy but not cortical impact and placement of femoral lines but not HS. Animals subjected to sham hypobaria were placed in the chamber but with normobaria. At the time of euthanasia, distal small bowel was harvested for the following assays: gut injury by histology using the fold change in injured villi, gut myeloperoxidase (MPO) as a measure of neutrophil infiltration using a commercially available kit, activated caspase by enzyme-linked immunosorbent assay (ELISA), edema as measured by wet:dry ratio, and a variety of junctional proteins by ELISA (E-cadherin) or western blot (occludin, cleaved E-cadherin, claudin).

4.1.1 Three Hours. The first group of animals compared polytrauma to shams without hypobaria. Animals underwent CCI and HS then 3 hours later were euthanized and compared to shams, which were anesthetized but did not undergo CCI or HS. Gut samples were collected for functional endpoints and analysis of the microbiome.

4.1.2 Thirty Hours. The second group of animals underwent polytrauma as described above and then 24 hours later were subjected to hypobaria for 6 hours at either 28% or 100% O_2 and compared to animals placed in the chamber at normobaria with room air (21%) and shams. Animals were euthanized 3 hours after the completion of hypobaria, which was 30 hours after polytrauma. Groups are referred to as 30-hour normobaria at 21% O_2 (30hNB21), 30-hour hypobaria at 28% O_2 (30hHB28), or 30-hour hypobaria at 100% O_2 (30hHB100).

4.1.3 Thirty Days. The third group of animals were Dr Fiskum's animals that underwent polytrauma and then 24 hours later were subjected to hypobaria for 6 hours at either 28% or 100% O_2 and compared to animals placed in the chamber at normobaria with room air (21%) or 100% fraction of inspired oxygen (FIO_2) and compared to shams (in some figures referred to as "controls" [CTRL]). Animals were euthanized at 30 days.

4.2 Microbiota

4.2.1 16S rRNA Gene Amplification, Sequencing, and Raw 16S rRNA Data Processing. The gut microbiota was characterized by sequencing bacterial 16S rRNA gene amplicons from stool samples. Enterotype classification was carried out using the methods of Arumugan, as modified by the Fraser laboratory.

4.2.2 Statistical Analyses of 16S rRNA Data. The first hypothesis that polytrauma and hypobaria are associated with a change in the relative abundance of known species or operational taxonomic units in the gut was tested. Recognizing the exploratory nature of current generation microbiome studies, associated shifts in relative abundance of specific taxa were tested in an agnostic fashion by examining whether shifts in the dominant taxa are associated with this intervention. Shannon and Simpson measures were carried out to assess for alpha diversity and the Chao 1 index was used to examine species richness. Normal distributions of the data were checked with the D'Agostino-Pearson test and homoscedasticity of variances was analyzed using Bartlett's test. Significant differences in the variance of parameters were evaluated, depending on the distribution of the estimated parameters, either with analysis of variance or the Kruskal-Wallis rank sum test. Post hoc comparisons were conducted by either Tukey's honest significant differences tests or pairwise Wilcoxon rank sum tests.

4.2.3 Metatranscriptomics. 16S rRNA gene sequencing provides an inventory of community members present in the GI tract, whereas the metatranscriptomic approach provides an actual snapshot of the expressed genes. RNA was isolated from the same samples as those used for 16S rRNA profiling. For metatranscriptomics, total RNA was extracted from chyme and stool samples using an acid-phenol protocol, then to efficiently sequence mRNA, rRNA molecules were depleted using the Ribo-Zero® Human/Mouse/Rat rRNA Removal Kit and the Ribo-Zero® Meta-Bacteria (Gram-negative and Gram-positive) rRNA Removal Kit (Illumina, San Diego, CA). The abundances of individual transcripts were determined based on the depth of read coverage.

4.2.4 Metatranscriptome Sequence Read Mapping and de novo Assembly. Functions and pathways were established for entire communities and for dominant individual members of the communities. The bacterial species identified through bacterial community profiling (16S rRNA gene sequencing) were used to focus the metatranscriptome sequence read mapping strategy. Analyses were carried out to look specifically at genes involved in polytrauma and hypobaria (polytrauma alone, then the combination) and also to look for differential gene expression in an agnostic manner.

5.0 RESULTS

5.1 Gut Analysis

5.1.1 Polytrauma Alone. Animals subjected to polytrauma alone and euthanized 3 hours after polytrauma (CCI+HS 3h) had a significant increase in gut inflammation as measured by both MPO and active caspase-3 and a significant increase in gut histopathologic injury (Figure 1). This was accompanied by an increase in gut permeability as measured by wet:dry ratios and the junctional protein occludin (Figure 2), but not the junctional proteins E-cadherin or cleaved E-cadherin (Figure 3) or claudin 2 or claudin 4 (Figure 4). We also examined gut syndecan-1 as a measure of endothelial activation, and there was no significant difference between sham and polytrauma animals.

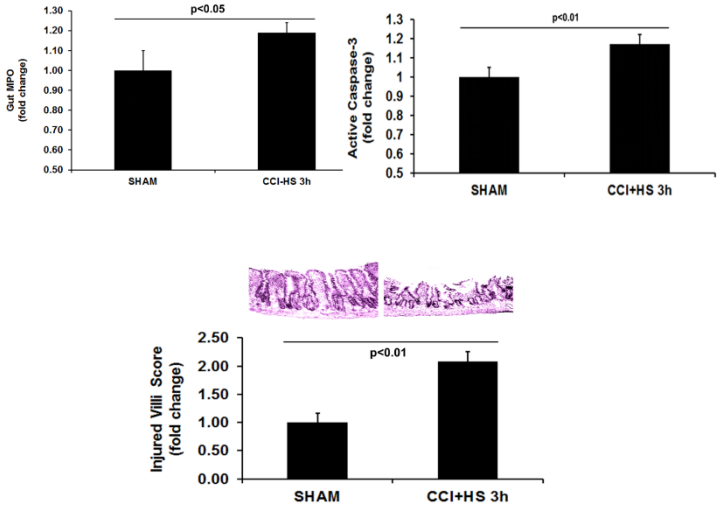

Figure 1. Three-hour gut inflammation and injury.

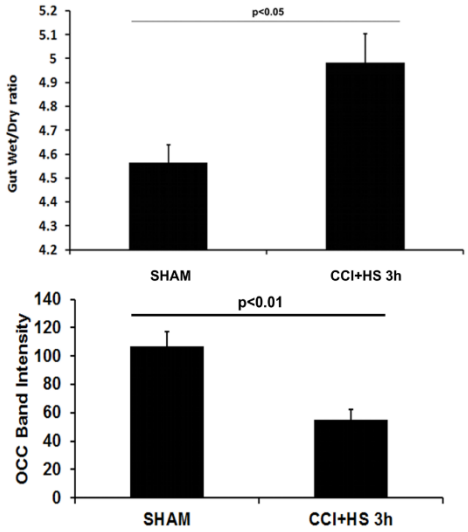

Figure 2. Three-hour gut edema and occluding.

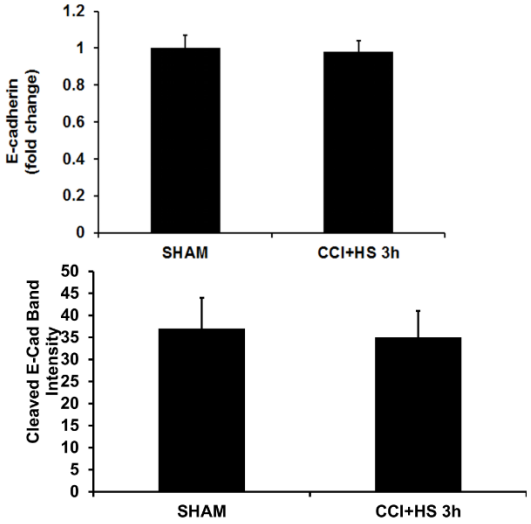

Figure 3. Three-hour gut E-cadherin and cleaved E-cadherin.

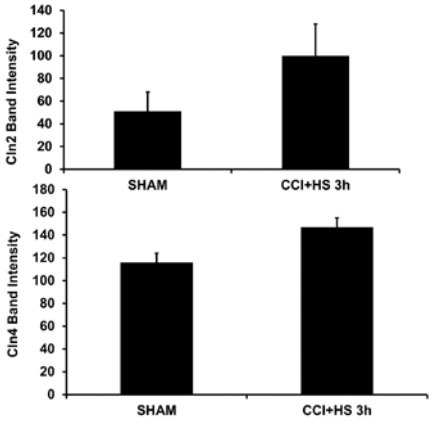

Figure 4. Three-hour gut claudin 2 and claudin 4.

5.1.2 Polytrauma and Hypobaria after 30 Hours. Animals subjected to normobaria at either 21% or 100% or hypobaria at either 28% or 100% all had a significant increase in gut inflammation as assessed by MPO or active caspase-3 and a significant increase in gut injury compared to shams (Figure 5), but there was no differential changes between experimental groups. Although there was a trend toward an increase in gut edema, particularly in the hypobaria at 28% O_2, this did not reach significance (Figure 6). Similar to polytrauma, there was a significant decrease in the junctional protein occludin after polytrauma (Figure 6), but there was no differential effect between animals subjected to normobaria or hypobaria at either O_2 concentration. Additionally, there was no difference between any of the groups for E-cadherin or cleaved E-cadherin (Figure 7) or between claudin 2 or claudin 4 (Figure 8).

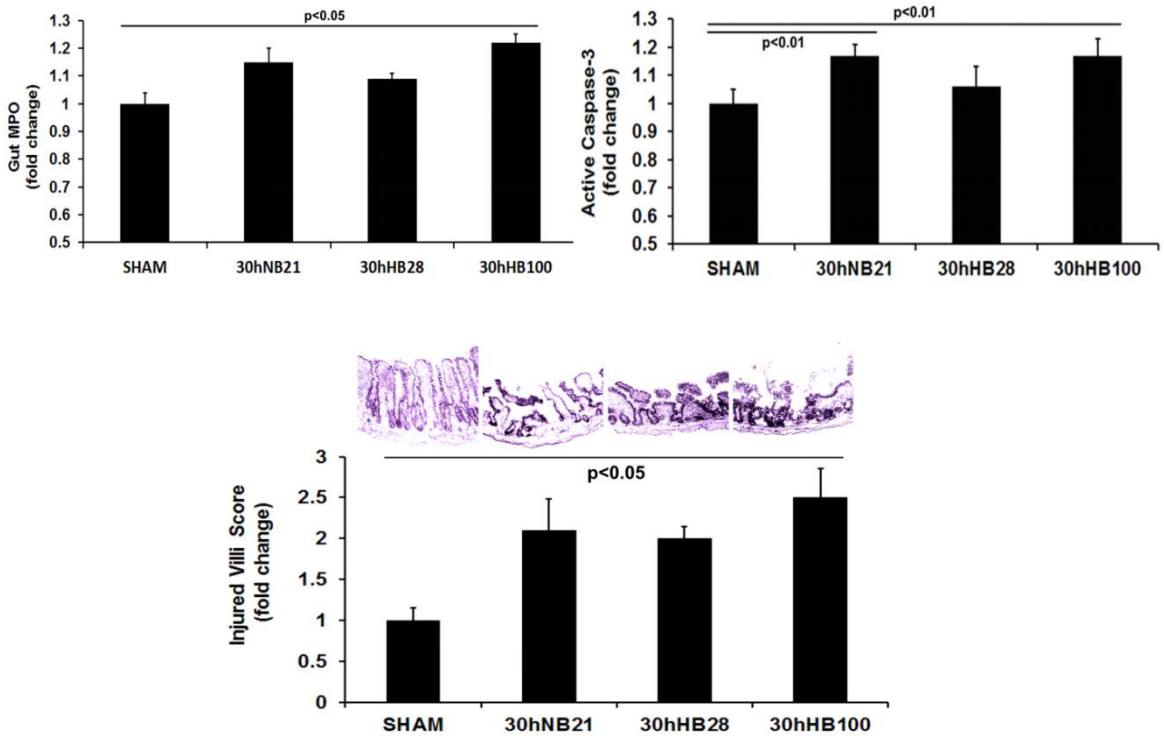

Figure 5. Thirty-hour gut injury and inflammation.

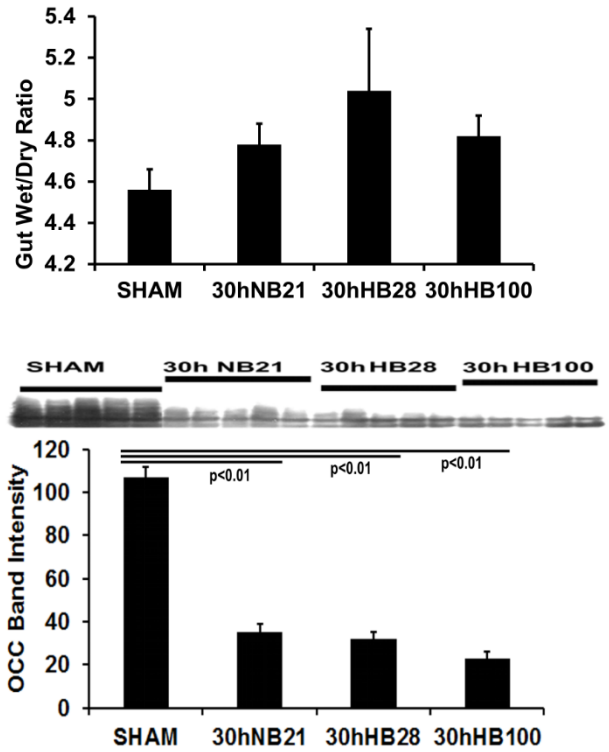

Figure 6. Thirty-hour gut edema and occludin.

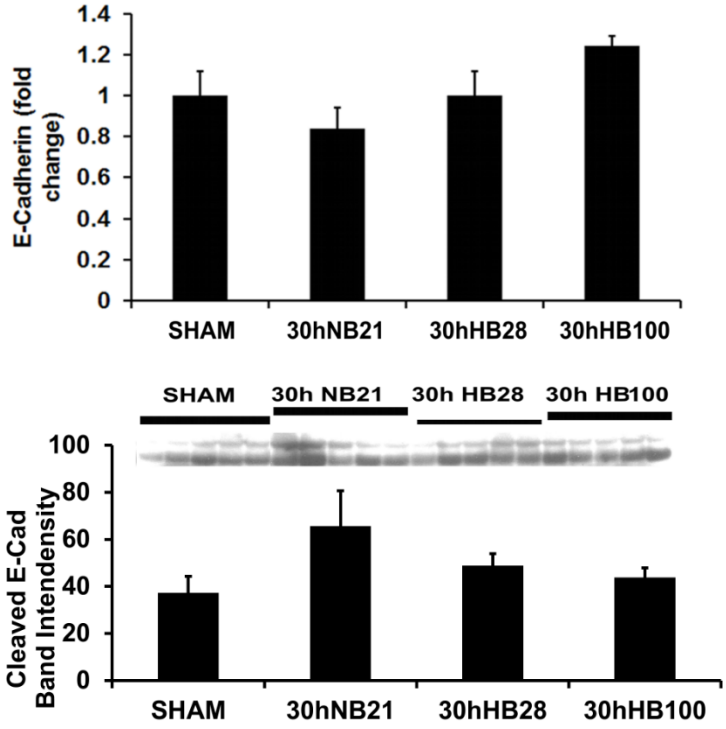

Figure 7. Thirty-hour gut E-cadherin and cleaved E-cadherin.

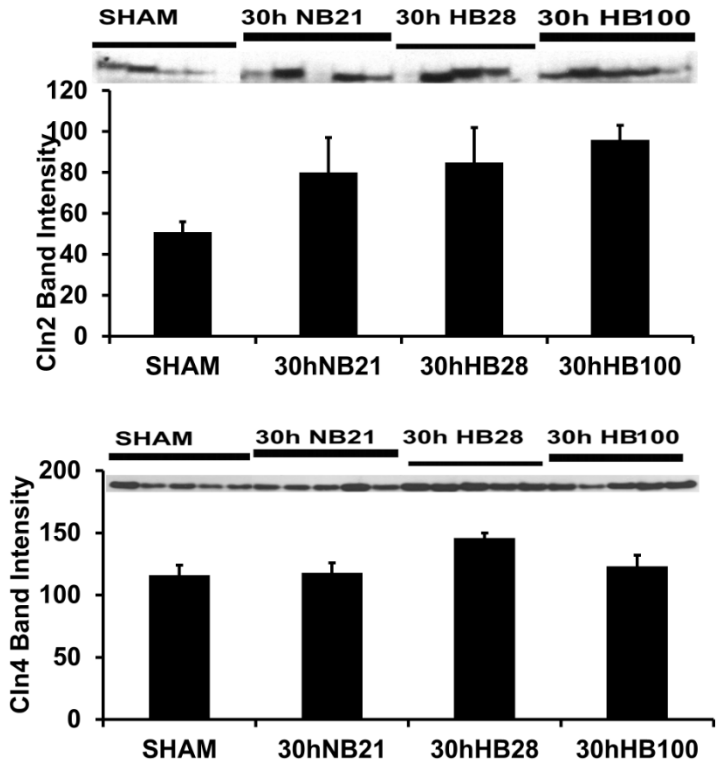

Figure 8. Thirty-hour gut claudin 2 and claudin 4.

5.1.3 Polytrauma and Hypobaria after 30 Days. Animals subjected to normobaria at 21% but not 100% had a significant increase in gut inflammation compared to controls, as assessed by MPO. Additionally, there was a significant increase in MPO in hypobaria at 28% and 100% compared to normobaria at either concentration, and hypobaria at 100% resulted in greater inflammation than hypobaria at 28% (Figure 9). Active caspase-3 was also increased in normobaria animals at 21% but not 100%, but there was no increase in hypobaria animals at either concentration (Figure 9). For both MPO and active caspase, hypobaria at either concentration increased inflammation compared to normobaric groups. All groups had a similar increase in gut histopathologic injury compared to shams, but there was no differential increase among normobaric or hypobaric animals (Figure 10). Results for gut edema demonstrated no discernable changes among groups (Figure 11). Like for the 3-hour and 30-hour groups, changes were seen in occludin (Figure 11). There was a significant decrease in occludin in animals undergoing hypobaria at either concentration and interestingly in normobaric 100% but not 28% O_2. As with the other time points, there was no change in E-cadherin or cleaved E-cadherin (Figure 12) nor any change in claudin 2 or claudin 4 (Figure 13).

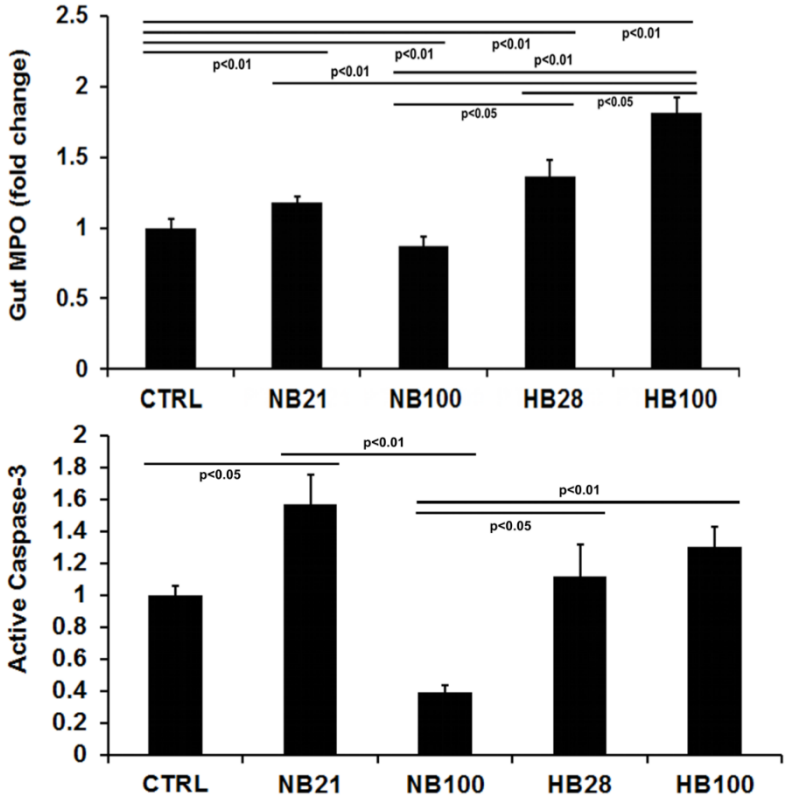

Figure 9. Thirty-day gut inflammation.

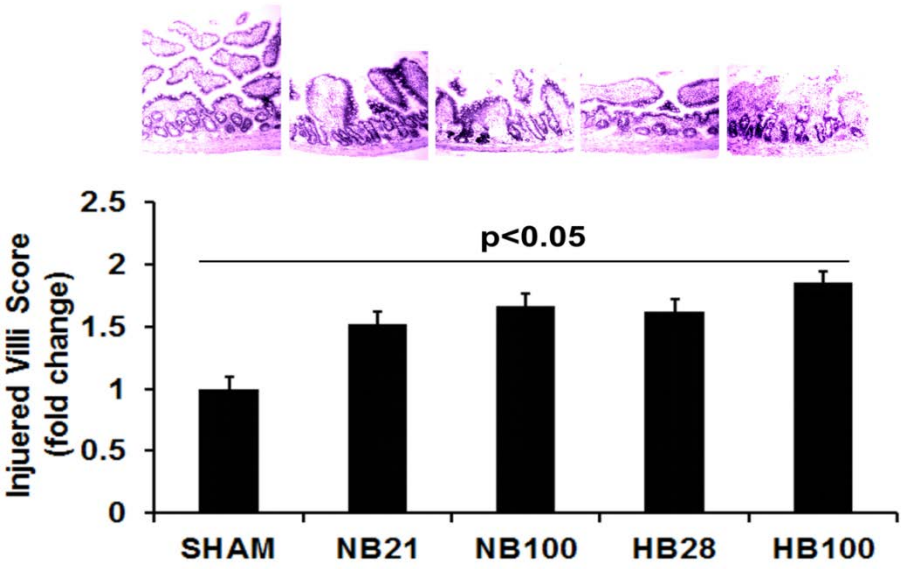

Figure 10. Thirty-day gut injury.

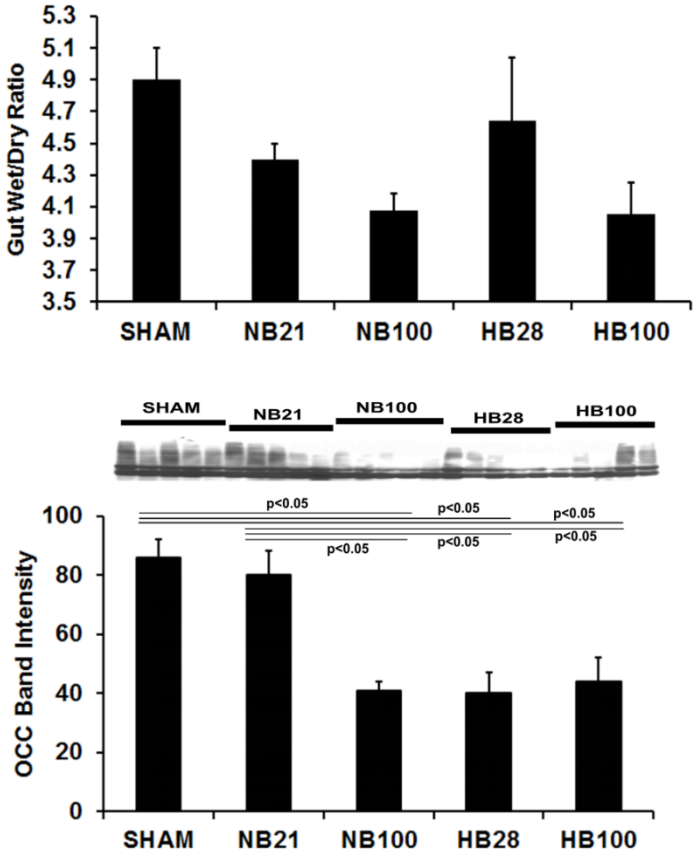

Figure 11. Thirty-day gut edema and occludin.

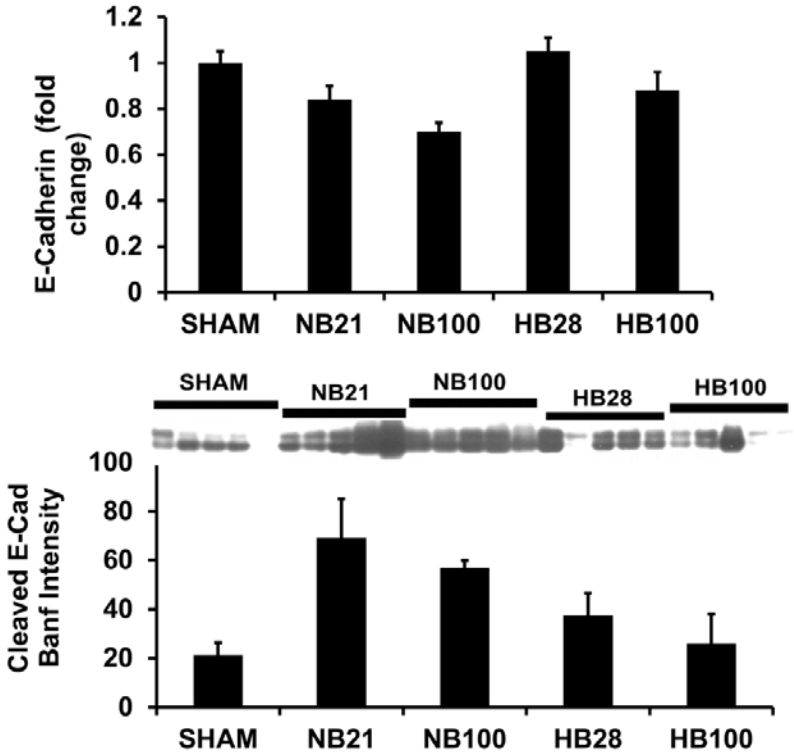

Figure 12. Thirty-day gut E-cadherin and cleaved E-cadherin.

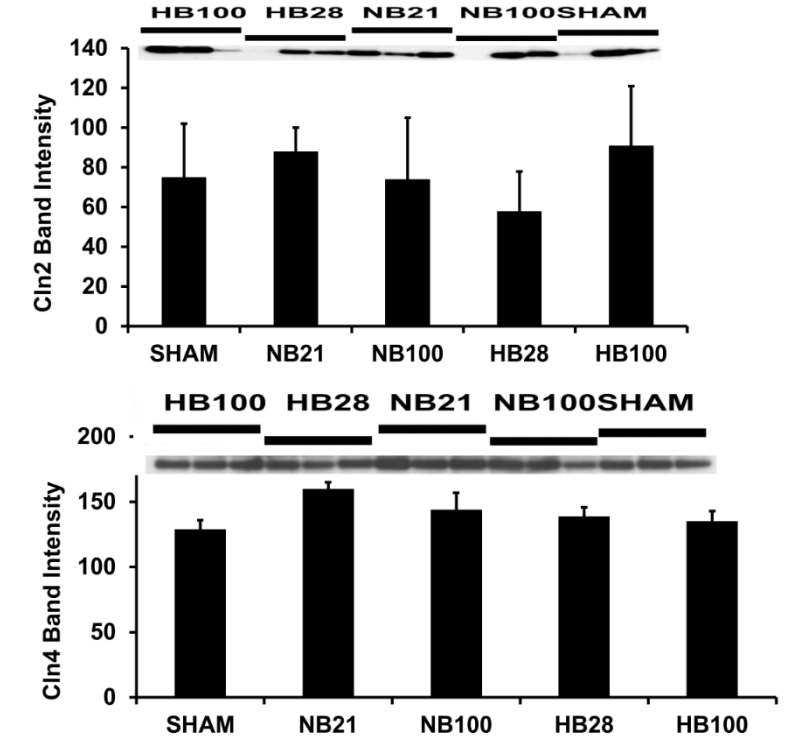

Figure 13. Thirty-day gut claudin 2 and claudin 4.

5.2 Gut Microbiota

To first evaluate the quality of the data, the following HiSeq run statistics were reviewed:

- Input read pairs: 151,633,757
- Surviving reads: 118,622,759 (78.23%)
- Dropped reads: 17,154,824 (11.31%)
- Combined pairs: 115,680,664 (97.52%)
- Uncombined pairs: 2,942,095

As shown above, surviving reads and combined pairs were high, and dropped reads low, confirming the quality of the data. As shown in Figure 14, the sample read counts approximate a bell curve.

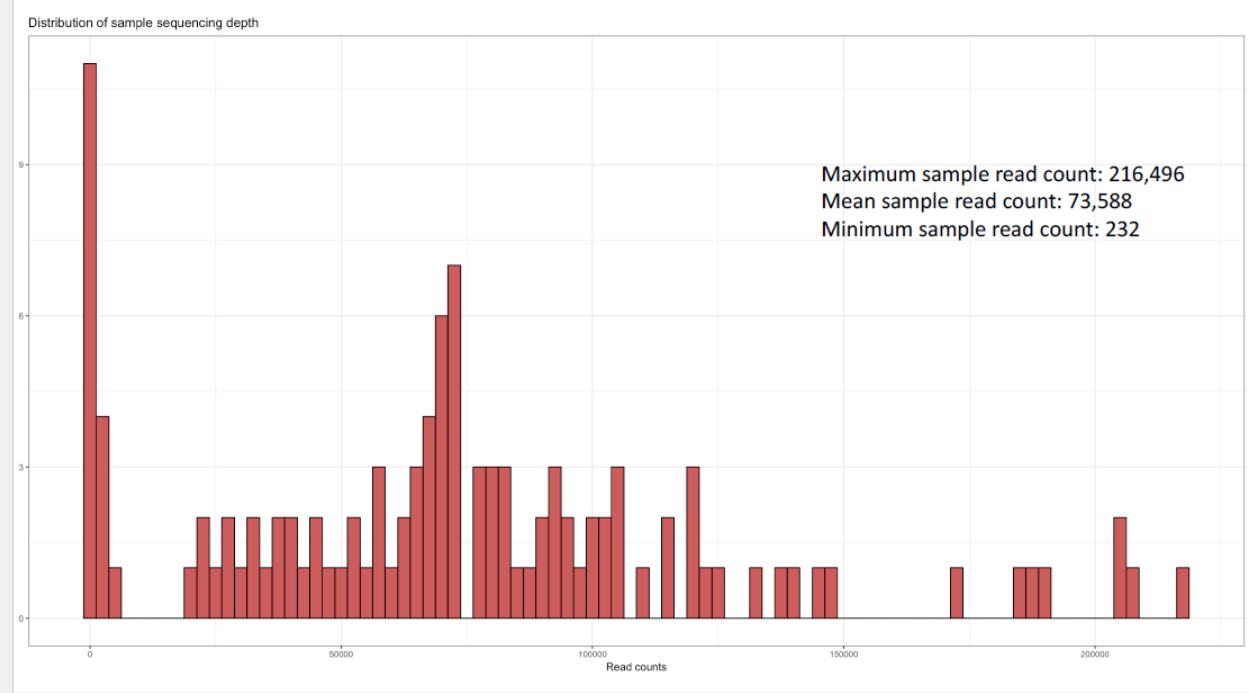

Figure 14. Distribution of sample sequencing depth.

5.21.16S RNA Analysis at 30 Days. A number of different alpha diversity measures were carried out on animals comparing their baseline (prior to any intervention, day 0) to their 30-day stool sample and are shown in Table 1. Shannon and Simpson look at diversity and Chao (Chao 1 index) at richness. Shannon's diversity function quantifies the uncertainty in the species identity of an individual that is taken at random from the dataset. Simpson's diversity index quantifies the probability that two individuals taken at random from the dataset represent the same species. Chao1 estimates the total species richness (i.e., number of different species). As shown in Table 1, there was a significant increase in diversity, as measured by both Shannon's diversity function and Simpson's diversity index, in the polytrauma animals subjected to

polytrauma and hypobaria at 100% (group 8). There was a significant increase in species richness in animals in all groups except shams (group 10). The differences in group 8, polytrauma and 100% FIO_2, are illustrated in Figure 15. The red boxes highlight the changes in Chao1, Shannon, and Simpson. In summary:

- Polytrauma resulted in a significant increase in species richness in all polytrauma groups at 30 days, regardless of hypobaria or FIO2 concentration.
- Hyperoxia at 100% alone (sham animals) caused a significant increase in species richness.
- Polytrauma and hypobaria at 100% FIO2 resulted in a significant increase in diversity.

Table 1. Alpha Diversity Measures

Group[a]			Comparison	Test	*U* value	*p*-value
No.	**Pressure**	**O_2 (%)**				
Shannon's Diversity Function						
6	Normobaria	21	Baseline vs. 30 dpi	Mann-Whitney	33	0.2176
7	Hypobaria	28			15	0.2593
8	Hypobaria	100			0	**0.0022**
9	Normobaria	100			16	0.1049
10	Normobaria	21			2	0.8000
11	Normobaria	100			3	0.1111
Simpson's Diversity Index						
6	Normobaria	21	Baseline vs. 30 dpi	Mann-Whitney	42	0.5787
7	Hypobaria	28			16	0.3176
8	Hypobaria	100			1	**0.004**
9	Normobaria	100			24	0.4418
10	Normobaria	21			2	0.8000
11	Normobaria	100			5	0.2857
Chao1 Total Species Richness						
6	Normobaria	21	Baseline vs. 30 dpi	Mann-Whitney	2	**<0.0001**
7	Hypobaria	28			0	**0.0006**
8	Hypobaria	100			0	**0.0022**
9	Normobaria	100			4	**0.0019**
10	Normobaria	21			2	0.8000
11	Normobaria	100			0	**0.0159**

[a]Group 6: polytrauma normobaria 21% FIO_2; Group 7: polytrauma hypobaria 28% FIO_2; Group 8: polytrauma hypobaria 100% FIO_2; Group 9: polytrauma normobaria 100% FIO_2; Group 10: sham normobaria 21% FIO_2; Group 11: sham normobaria 100% FIO_2.

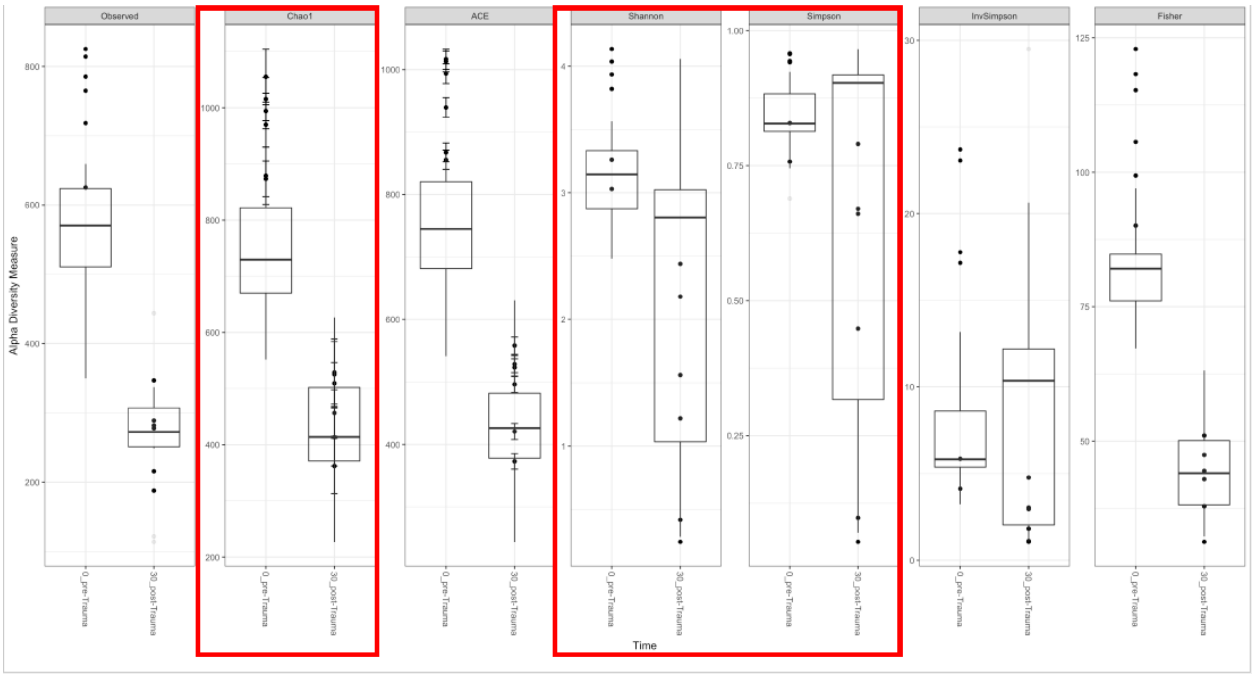

Figure 15. Alpha diversity changes for polytrauma hypobaria 100% FIO_2 animals.

5.2.2 Bacteria Analysis. The relative abundance of bacteria was analyzed. In the hierarchy of bacteria, phyla is above family. The relative abundance of bacterial phyla is shown in Figure 16 and bacterial family is shown in Figure 17. For both figures, the baseline samples are shown in the lower panel and the 30-day samples are in the upper panel.

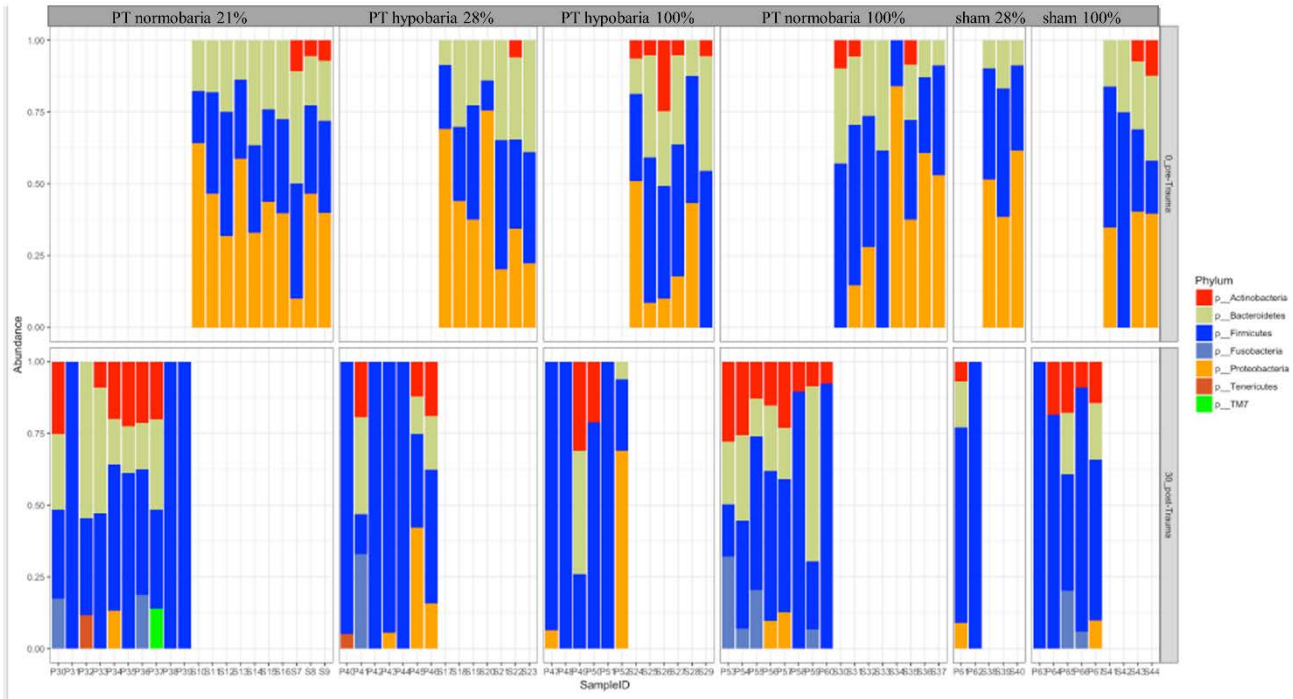

Figure 16. Relative abundance of bacterial phyla.

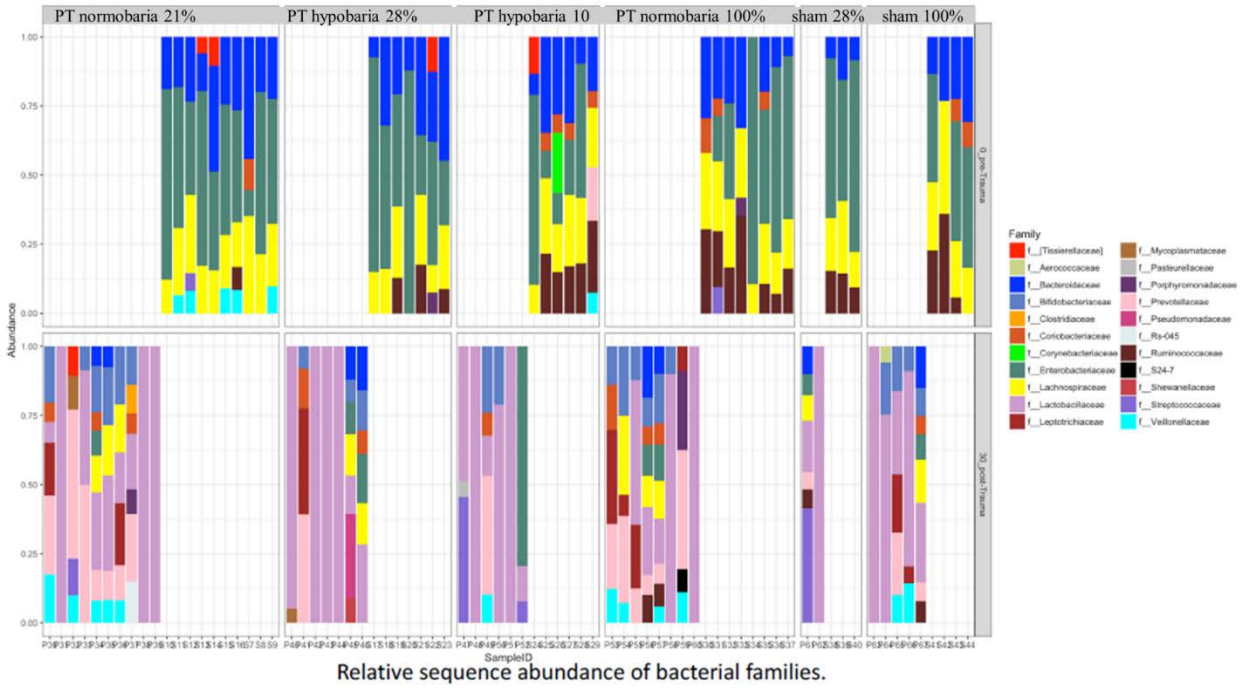

Figure 17. Relative abundance of bacterial families.

To further illustrate the changes in bacterial families for the polytrauma 100% FIO_2 group, Figure 18 is just that group. The baseline analysis is shown on the left and the 30 days on the right. There is clear change in the bacteria over time. The data for all groups were subjected to statistical analysis, revealing a significant reduction in relative abundance of Firmicutes after poltrauma, a significant increase in Proteobacteria after polytrauma, and a significant increase in Bacteroidetes after polytrauma and hypobaria.

Overall similarities in bacterial community structures are displayed as principal component analysis (PCoA). PCoA revealed strong clustering of bacterial communities at baseline and at 30 days. There was tight clustering of baseline samples suggesting lower variance of bacterial communities, whereas, not unexpectedly, the 30-day samples demonstrated wider spread, i.e., higher variance. This is shown in Figure 19.

5.2.3 Microbiome Data Analysis. Microbiome data were analyzed for the 3-hour (polytrauma alone) and 30-hour (polytrauma then 24 hours later hypobaria) groups. As shown in Figures 20 and 21, changes were not as marked as in the 30-day animals. Groups for these figures are as follows: group 1 shams, group 2 polytrauma alone 3 hours, group 3 polytrauma then normobaria 21%, group 4 polytrauma then hypobaria 28%, group 5 polytrauma then hypobaria 100%.

As we hypothesized that 3 or 30 hours may not be sufficient to see major changes in the microbiota, samples were also subjected to metatranscriptome analysis (Figure 22). This involves an actual snapshot of the expressed genes and represents state-of-the-art techniques. Due to the cost, only a subset of samples was analyzed. Although there is considerable variability between samples, changes do appear to be occurring.

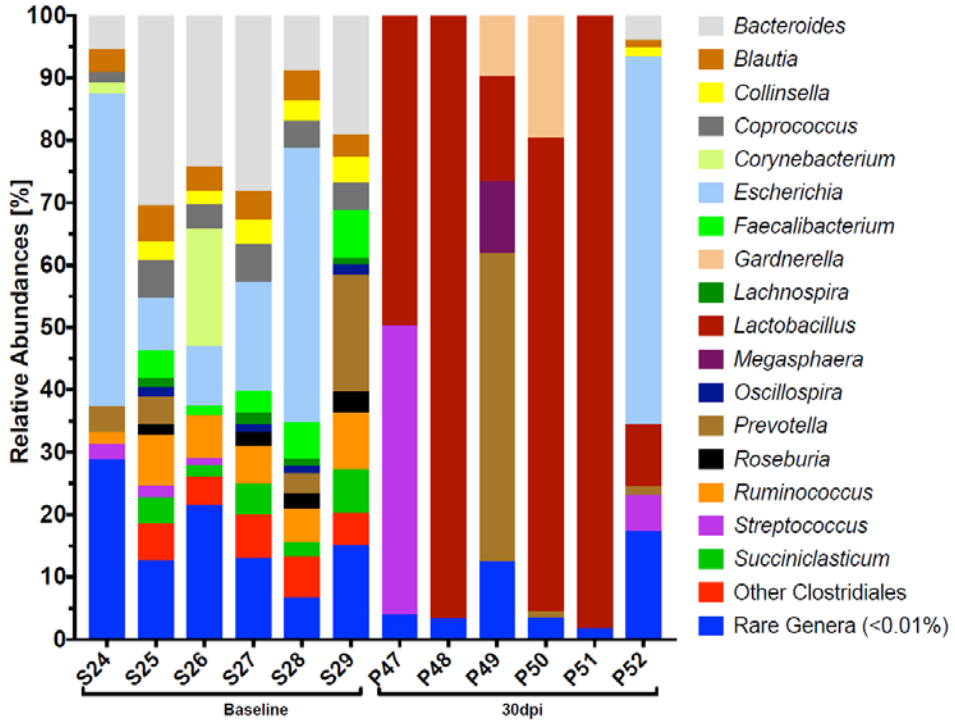

Figure 18. Relative abundance in bacterial species in the polytrauma hypobaria 100% animals.

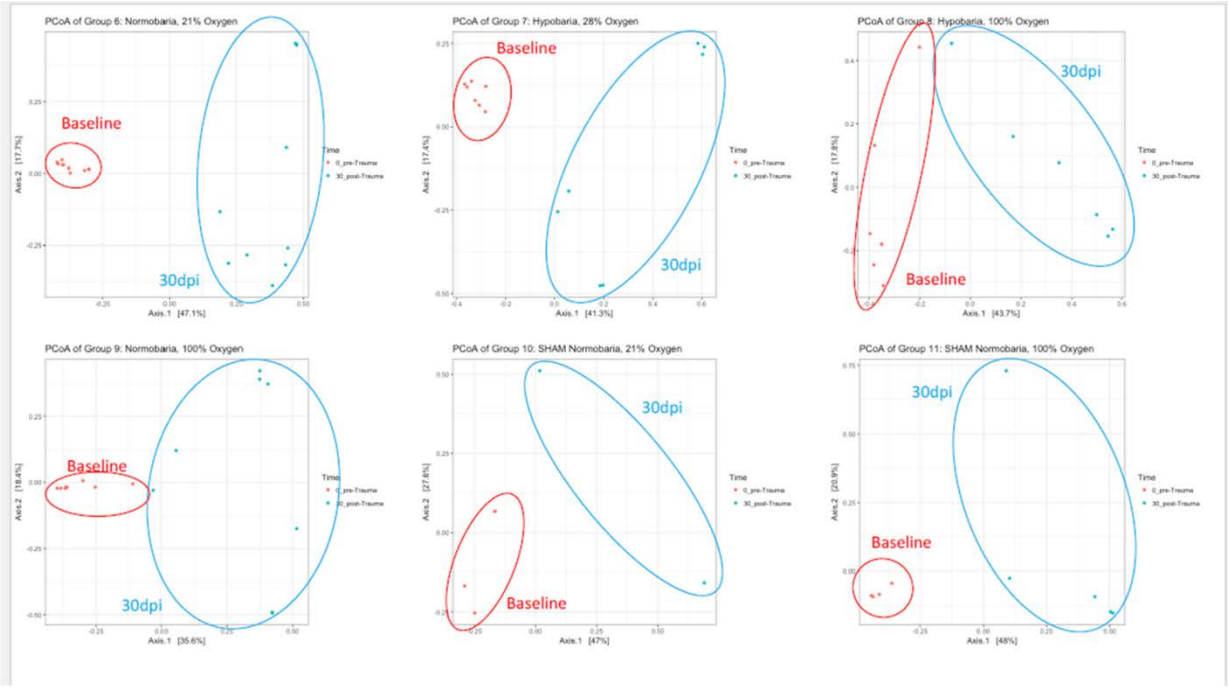

Figure 19. PCoA of baseline and 30-day samples by group.

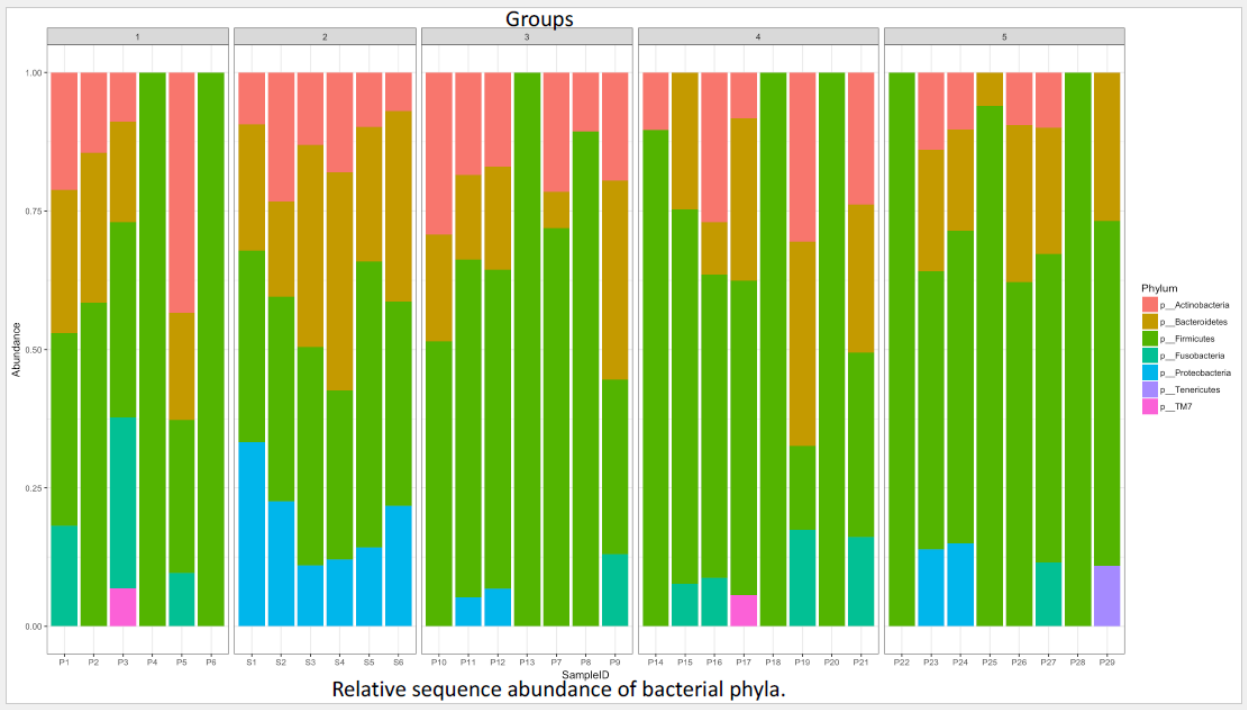

Figure 20. Relative abundance of bacterial phyla after 3 and 30 hours.

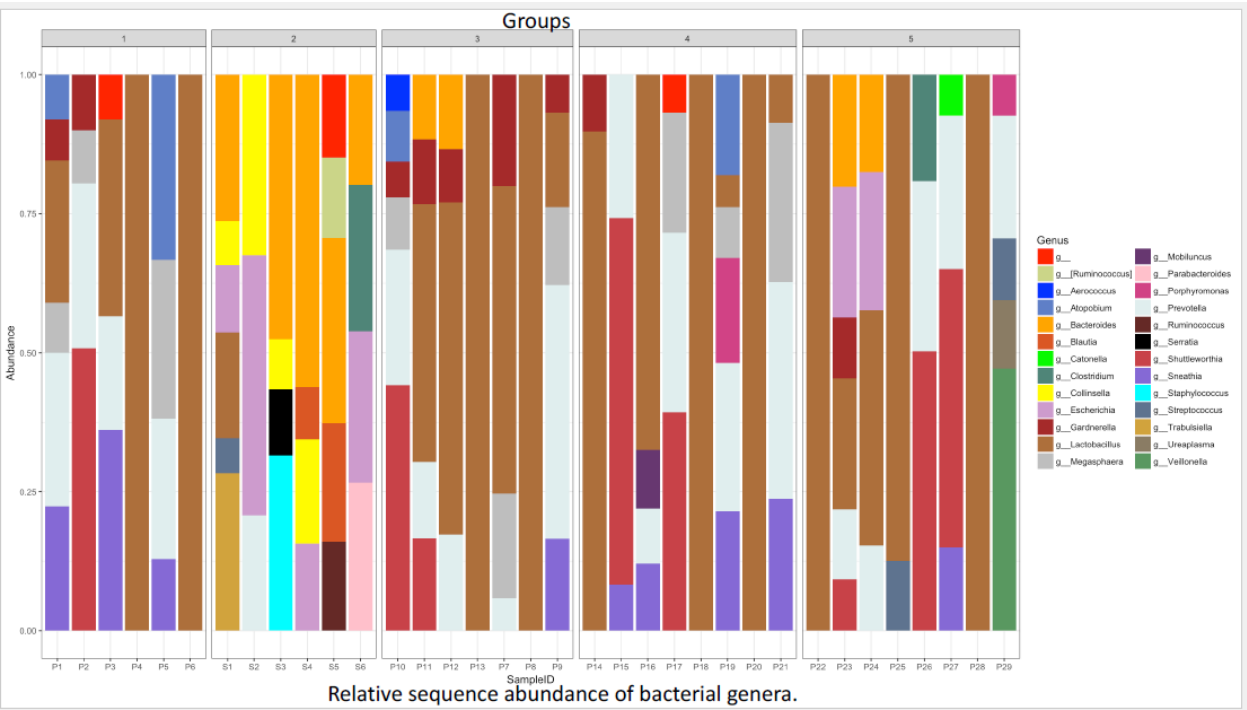

Figure 21. Relative abundance of bacterial families after 3 and 30 hours.

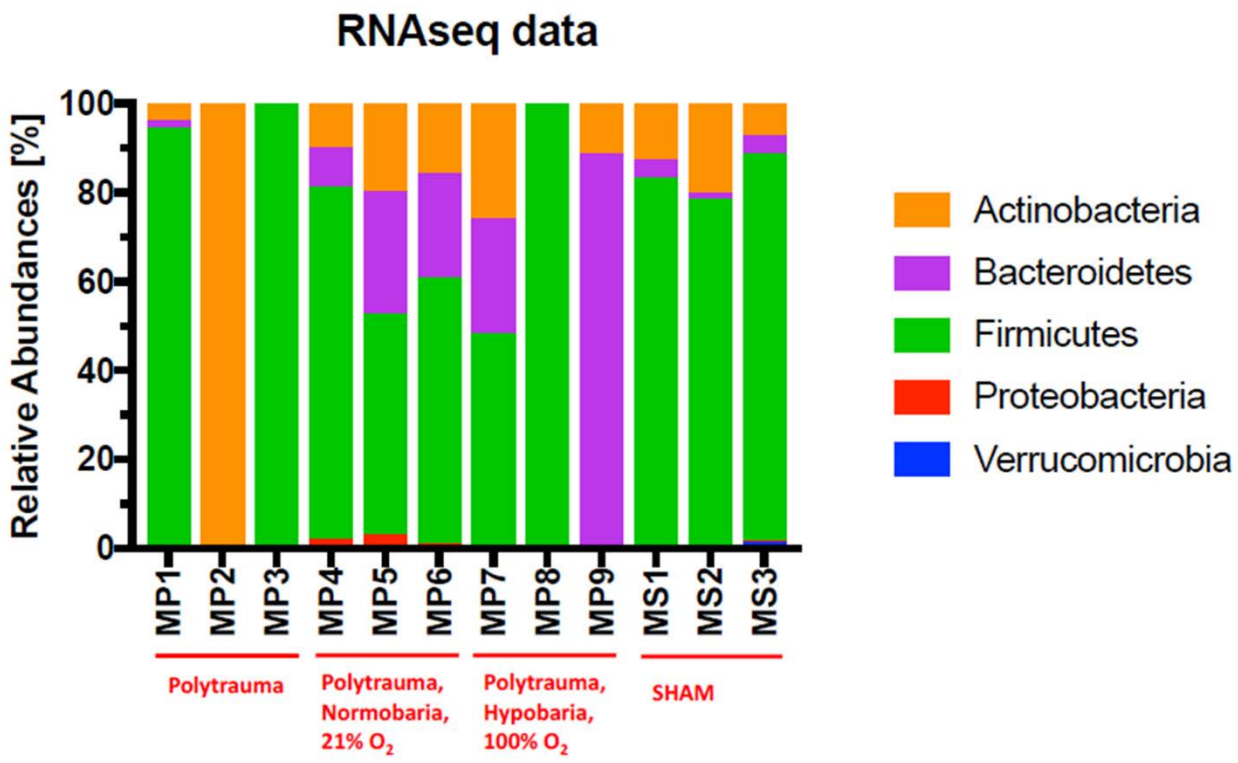

Figure 22. Metatransciptome analysis on 3-hour and 30-hour gut samples.

5.3 Correlation of Gut Data with Dr. Fiskum's Lung and Brain Data

5.3.1 Gut and Lung. Using the same methodology by the same animal surgeon, we compared gut data from polytrauma animals obtained 6 hours after hypobaria and lung data from polytrauma animals 24 hours after hypobaria. Although the times were not perfectly matched, there was a significant increase in lung and gut histopathologic injury in the polytrauma hypobaria group that received 100% O_2.

5.3.2 Gut and Brain. Again, using the same methodology by the same animal surgeon, we compared outcomes at 30 days. Dr. Fiskum's 30-day animals undergoing hypobaria (with any O_2 concentration) had a significant increase in mortality compared to normobaria animals and animals receiving hyperoxia (100%) regardless if normobaria or hypobaria had an increased mortality. The following data are from animals that survived to 30 days. While Dr. Fiskum's brain data showed that neither lesion volume nor behavior outcomes were different at 30 days in survivors, there was a significant increase in gut inflammation and injury in animals undergoing hypobaria at 100% even at 30 days.

6.0 DISCUSSION

We have shown significant changes in the gut at 3 hours in a polytrauma alone model, at 30 hours in a polytrauma and hypobaria model, and at 30 days in a polytrauma and hypobaria model. This is actually quite surprising, as the gut has a very rapid turnover. The principal

investigator (Kozar) has extensive experience in the gut in both ischemia/reperfusion and HS models. In these models, despite significant injury early after the insult, the gut was essentially repaired by 12-24 hours. The prolonged changes that were demonstrated in the 30-day animals suggest that it is the TBI that is responsible for the ongoing injury. Consistent among the time points were increases in gut inflammation and injury and a decrease in the junctional protein occludin, but not the junctional proteins E-cadherin, cleaved E-cadherin, claudin 2, or claudin 4.

Pathologic changes in the polytrauma hypobaria 100% FIO_2 group started to emerge at 30 hours with a significant increase in gut inflammation (both MPO and caspase-3) and injury. All polytrauma groups had a decrease in occludin. We compared these data to Dr. Fiskum's lung data, which were not at precisely the same time but close, and lungs also demonstrated an increase in inflammation in the polytrauma hypobaria group that received 100% O_2.

By 30 days, the polytrauma hypobaria 100% group continued to demonstrate ongoing inflammation and injury. Occludin was decreased in the polytrauma and hypobaria groups at either FIO_2 but also in the normobaria 100% group, suggesting that even in the absence of hypobaria, 100% FIO_2 may be detrimental. Dr. Fiskum has previously demonstrated in a TBI alone model that hypobaria at 100% FIO_2 worsened neurologic and neuropathologic outcomes. With the addition of HS in his ongoing studies, his group showed hypobaria (with any O_2 concentration) had a significant increase in mortality compared to normobaria animals and that animals receiving hyperoxia (100%) regardless if normobaria or hypobaria had an increased mortality. However, in those animals that survived polytrauma to 30 days, neither lesion volume nor behavior outcomes were different. It is likely the additional mortality associated with polytrauma allowed for survivors to have less injury.

Interestingly, our microbiota data reflected the gut data. Even at 30 days, there was an increase in species richness in the polytrauma animals and in hyperoxia alone animals, while the polytrauma and hypobaria animals had an increase in diversity. Additionally, there was a significant reduction in relative abundance of Firmicutes after poltrauma, a significant increase in Proteobacteria after polytrauma, and a significant increase in Bacteroidetes after polytrauma and hypobaria 100%. We also looked at shorter times, which did not reveal as clear changes, but using metatranscriptome analysis we were able to see trends of changes even at very short times including 3 hours and 30 hours. This is a state-of-the-art technique but very expensive, so as a pilot we only did a few animals from some of the groups.

7.0 CONCLUSIONS

In this study, we found in a rodent model of polytrauma that the gut is affected by polytrauma in an adverse manner that is further worsened by hypobaria, in particular hypobaria 100%, and most marked at 30 days. This was true for gut pathology and pathologic changes in the gut microbiota.

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LIST OF ABBREVIATIONS AND ACRONYMS

30hHB28	30 hours hypobaria 28%
30hHB100	30 hours hypobaria 100%
30hNB21	30 hours normobaria 21%
CCI	controlled cortical impact
CTRL	control
ELISA	enzyme-linked immunosorbent assay
FIO_2	fraction of inspired oxygen
GI	gastrointestinal
HS	hemorrhagic shock
MODS	multiple organ dysfunction syndrome
MPO	myeloperoxidase
O_2	oxygen
PCoA	principal component analysis
TBI	traumatic brain injury