Acute Exposure to Simulated High-altitude Hypoxia Alters Gut Microbiota in Mice

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Abstract

Gut microbiota bears adaptive potential to different environments, but little is known regarding its responses to acute high-altitude exposure. This study aimed to evaluate the microbial changes after acute exposure to simulated high-altitude hypoxia. C57BL/6J mice were divided into hypoxia and normoxia groups. The hypoxia group was exposed to a simulated altitude of 5500 m for 24 hours above sea level. The normoxia group was maintained in low-altitude of 10 m above sea level. Colonic microbiota was analyzed using 16S rRNA V4 gene sequencing. Compared with the normoxia group, shannon, simpson and *Akkermansia* were significantly increased, while *Firmicutes* to *Bacteroidetes* ratio and *Bifidobacterium* were significantly decreased in the hypoxia group. The hypoxia group exhibited lower mobile element containing and higher potentially pathogenic and stress tolerant phenotypes than those in the normoxia group. Functional analysis indicated that environmental information processing was significantly lower, metabolism, cellular processes and organismal systems were significantly higher in the hypoxia group than those in the normoxia group. In conclusion, acute exposure to simulated high-altitude hypoxia alters gut microbiota diversity and composition, which may provide a potential target to alleviate acute high-altitude diseases.

1. Introduction

High-altitude has substantial meaning not only to the abundant resource, but to the military significance. Hypoxia is one of the main characteristics of high-altitude. With increased elevation above sea level, air pressure as well as oxygen pressure decrease. At an altitude of 4,000 meters, oxygen content was only ≈60% of sea level[1].

The oxygen homeostasis is vital for maintaining gastrointestinal health. With altitude increasing, morphologic injuries of villous height, crypt depth, mucosal wall thickness and villous surface area were aggravated[2]. Acute hypoxia environment increased bacterial translocation and decreased protein expression of occluding, facilitating the entry of lipopolysaccharide into the blood[3]. Recent studies highlight that gut microbiota bears adaptive potential to high-altitude in human and animals. In a survey of gut microbiota of Tibetans from six regions with altitudes ranging from 2800 m to 4500 m, Lan et al[4] observed that altitude had a positive correlation to *Faecalibacterium*, *Bacteroides* and *Bifidobacterium*, but negative correlation to *Ruminococcaceae*, *Prevotella* and *Lachnospiraseae*. Comparing the microbiome of Han population living in Chengdu (500 m) and the immigrant Han population living in Lhasa (3600 m) revealed that the latter had a more energy efficient flora[5]. In wild house mice, Suzuki et al[6] found that anaerobic bacteria were positively correlated with altitude, while facultative anaerobes, microaerophiles and aerotolerant bacteria were negatively correlated with altitude. Chinese Rhesus Macaques living in Tibet had higher environmental information processing and organismal systems than those in the other geographical populations[7]. However, human investigations and animal experiments conducted in long-term exposure to high-altitude environments are hard to separate effects of hypoxia from potentially confounding factors such as dietary habit, and little is known about the intestinal flora changes that occur with acute exposure to high-altitude.

Therefore, the aims of this study were to evaluate the microbial responses to acute high-altitude hypoxia exposure, and provide basis for future efforts to develop microbiota-based countermeasures that alleviate acute high-altitude diseases.

2. Materials And Methods

2.1 Animals and experimental design

Twelve-week-old male C57BL/6J mice were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd (Beijing, China). With 1 week of acclimatization, mice were randomly divided into 2 groups (ten mice per group): hypoxia group and normoxia group. For the hypoxia group, mice were exposed to a simulated altitude of 5500 m for 24 hours above sea level in a hypobaric chamber (Yantai Hongyuan Oxygen Industrial Inc, China). For the normoxia group, mice were maintained in low-altitude of 10 m above sea level. Both groups of mice were kept under stable conditions with controlled humidity(40%-60%), a temperature range of 22°C ± 2°C, and a 12 h light/dark cycle. AIN-93M diet and water were given *ad libitum*. Colonic contents were collected after scarification. The experimental protocols were approved by the Institutional Animal Care and Use Committee of Tianjin Institute of Environmental and Operational Medicine.

2.2 Microbiome analysis

Genomic DNA from the colonic contents was extracted using cetyltrimethyl ammonium bromide method. The V4 region of 16S rRNA gene was amplified using primers 515 F and 806R. Sequencing libraries were generated using Ion Plus Fragment Library Kit 48 rxns (Thermofisher, USA). The library was sequenced by Ion S5^{TM}XL (Thermofisher, USA). Operational taxonomic units that reached 97% similarity were used for alpha diversity estimation. Cluster analysis was preceded by principal component analysis. Linear discriminant analysis effect size was performed to identify specific bacteria. BugBase was used to infer microbiological phenotype. Phylogenetic investigation of communities by reconstruction of unobserved states was used to predict functional change.

2.3 Statistical analysis

Data are represented as mean ± standard deviation. Differences between groups were evaluated by Student's *t*-test (normally distributed) and Mann–Whitney U test (non-normally distributed). Statistical analysis was conducted using PASW statistics 18.0 (SPSS Inc, USA). Significance was defined as $P < 0.05$.

3. Results

3.1 Body weight, food and water intake, behavioral performance

Body weight, food and water intake were significantly decreased in the hypoxia group than those in the normoxia group (Figure 1). Physical activities such as standing and grooming were reduced gradually as the hypoxic time extended in the hypoxia group relative to the normoxia group.

3.2 Microbiota diversity

Shannon and simpson were significantly higher in the hypoxia group than those in the normoxia group (Figure 2). No significant difference was observed in chao1 and abundance-based coverage estimator between the two groups. In the principal component analysis plot, a separation between the hypoxia group and the normoxia group was observed (Figure 3A). Analysis of similarities showed there was a significant difference between the two groups (Figure 3B).

3.3 Microbiota composition

At phyla level, *Firmicutes* (42.8% in the hypoxia group versus 66.0% in the normoxia group) and *Bacteroidetes* (42.8% in the hypoxia group versus 13.3% in the normoxia group) were the two most abundant bacterial phyla in both groups (Figure 4A), *Firmicutes* to *Bacteroidetes* ratio was significantly lower in the hypoxia group than that in the normoxia group (Figure 4B). At genus level, *Dubosiella*, *Faecalibaculum* and *Bifidobacterium* were significantly decreased and *unidentified_Lachnospiraceae*, *Akkermansia*, *Parabacteroides* and *Bacteroides* were significantly increased in the hypoxia group relative to the normoxia group (Figure 4C).

3.4 Microbiota phenotype

Mobile element containing was significantly decreased, whereas potentially pathogenic and stress tolerant were significantly increased in the hypoxia group relative to the normoxia group (Figure 5). No significant difference was observed in aerobic, anaerobic and facultatively anaerobic between the two groups.

3.5 Microbiota function

Metabolism, cellular processes and organismal systems were significantly increased, whereas environmental information processing was significantly decreased in the hypoxia group relative to the normoxia group (Figure 6).

4. Discussion

Nowadays, with a growing number of people from low-altitude come to high-altitude for travelling, mountaineering or strategic reasons, acute high-altitude reaction has become particularly prominent. Although much attention has been paid to respiratory, cardiac and neurological symptoms in acute high-altitude exposure, there is scarcity of reports on gut microflora. In this study, acute exposure to simulated high-altitude hypoxia altered gut microbiota in mice, as indicated by the increase of shannon, simpson and *Akkermansia*, and the decrease of *Firmicutes* to *Bacteroidetes* ratio and *Bifidobacterium*.

Among the numerous studies describing disease-associated microbiota, loss of microbiota diversity is a general feature of most dysbiosis. The increase in community diversity such as shannon and simpson in mice under acute hypoxia exposure observed in our study could reflect a beneficial response to environmental stress. This finding is consistent with individuals who experienced acute mountain sickness[8]. Moreover, Jiang et al[9] reported an elevated microbiome alpha diversity in mice during spaceflight. However, attention should be given to the new perspective that more diversity is not always better[10]. Indeed, alpha diversity estimation revealed higher microbiota diversity in patients with colorectal adenomas[11]. For men who were HIV-infected, the alpha diversity of the *Bacteroidetes* phylum was positively correlated with viral load[12]. Therefore, the real drivers of microbiome biodiversity in host are worth investigating.

More than 75% gut bacteria are in 1 of 2 phyla: *Firmicutes* and *Bacteroidetes*. On the one hand, *Firmicutes* to *Bacteroidetes* ratio can influence cardiorespiratory fitness. In healthy young adults, Durk et al[13] found that maximal oxygen consumption was positively associated with *Firmicutes* to *Bacteroidetes* ratio. Voluntary exercise increased proportionally to the ΔCt ratio of *Firmicutes*:*Bacteroidetes*[14]. On the other hand, relatively high ratio of *Firmicutes* to *Bacteroidetes* is associated with highly efficient energy harvest[15]. In youth, *Firmicutes* to *Bacteroidetes* ratio was positively associated with body mass index, visceral and hepatic fat[16]. Both Tibetans and Chinese Han living at high-altitude had a high abundance of *Firmicutes* and a low abundance of *Bacteroidete*[17]. Similarly, animals living in high-altitude such as Tibetan antelopes[18], European mouflon and blue sheep[19] had higher *Firmicutes* to *Bacteroidetes* ratio than their counterparts living in low-altitude. Unlike these residents and animals at high-altitude, our results showed a decrease in *Firmicutes* and an increase in *Bacteroidetes* after acute hypoxic exposure. This contradiction indicated that *Firmicutes* to *Bacteroidetes* ratio may be involved in the process of long-term high-altitude adaptation.

At genus level, the lower abundance of *Firmicutes* was mainly caused by a significant decrease in *Dubosiella* and *Faecalibaculum*. The higher abundance of *Bacteroidetes* was mainly caused by a significant increase in *Bacteroides* and *Parabacteroides*. A recent study showed that high-fat diet enlarged *Dubosiella* and *Faecalibaculum*[20]. In addition, *Bacteroides* and *Parabacteroides* were negatively associated with obesity[21]. Our functional analysis also indicated that acute exposure to simulated high-altitude hypoxia upregulated metabolism. In fact, basal metabolic rate was 27% greater than at sea level in men at high-altitude[22]. Kong et al[23] indicated that the acute phase response signaling, liver X receptor/retinoid X receptor and farnesoid X receptor/retinoid X receptor pathways were activated in Holstein dairy cows exposed to high-altitude hypoxia.

Bifidobacterium and *Akkermansia* are considered to be beneficial to the host. In seven mountaineers who took part in German expedition to the Nepalese Himalayas, Kleessen et al[24] observed a significant decrease in *Bifidobacterium* at the high camp. Furthermore, *Bifidobacteria* deficiency has been identified as a disorder of the ecological barrier after flights in astronauts[25]. Consist with these studies, our result also showed a decrease in *Bifidobacterium*. Unexpectedly, *Akkermansia* was increased in the hypoxia group. It may be a protective reaction against acute hypoxia exposure. *Akkermansia* is known to play a

vital role in the regulation of energy homeostasis. Gao et al[26] found that *A. muciniphila* treatment promoted the browning of inguinal fat pad, reduced energy efficiency and improved metabolic disorders in the high fat diet-fed mice. Besides, multiple sclerosis patients also had a higher *Akkermansia*, and transfer from their fecal microbiota ameliorated disease in recipients by expanding *Akkermansia*[27]. Likewise, *A. muciniphila* was significantly increased in IFNγ-deficient mice and restoration of IFNγ level decreased *A. muciniphila*[28].

Intriguingly, acute exposure to simulated high-altitude hypoxia did not result in phenotypic variation in oxygen utilizing, including aerobic, anaerobic and facultatively anaerobic. On the contrary, it was found that the strict anaerobes and obligate anaerobes were increased in large intestine[29] and small intestine[30] under simulated hypobaric hypoxia for 30 days. The difference of exposure time may lead to such discrepancy. In addition, the decrease of mobile element containing and the increase of potentially pathogenic and stress tolerant shed light on the harmful effects of acute hypoxia. It is consistent with the results obtained from functional analysis, including decreased environmental information processing and increased cellular processes and organismal systems. The underlying mechanisms include promotion of glycolytic capacity and suppression of oxidative metabolism[31]. Future studies are needed to confirm the phenotypic and functional prediction spectrum of the flora. Moreover, exact details of physiological adaptability in the high-altitude environment remain to be resolved.

A limitation of the study is that only one option for exposure time and altitude was administrated, so the time-dependent and altitude-dependent intestinal flora changes have yet to be described. In addition, besides hypoxia other high-altitude environment variable such as cold, wind and ultraviolet radiation were not fully considered. It is an important developing direction to investigate how the gut microbiota respond to high-altitude environment.

5. Conclusions

In conclusion, acute exposure to simulated high-altitude hypoxia alters gut microbiota diversity and composition. Our findings provide a potential microbiota-based target to alleviate acute high-altitude diseases.

Declarations

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Conflicts of Interest:

The authors declare no conflict of interest.

References

1. Beall CM (2007) Two routes to functional adaptation: Tibetan and Andean high-altitude natives. Proc Natl Acad Sci USA 104(Suppl 1):8655–8660
2. Zhang F, Wu W, Deng Z et al (2015) High altitude increases the expression of hypoxia-inducible factor 1alpha and inducible nitric oxide synthase with intest-inal mucosal barrier failure in rats. Int J Clin Exp Pathol 8:5189–5195
3. Luo H, Zhou DJ, Chen Z et al (2017) Establishment and evaluation of an experimental rat model for high-altitude intestinal barrier injury. Experimental and therapeutic medicine 13:475–482
4. Lan D, Ji W, Lin B et al (2017) Correlations between gut microbiota community structures of Tibetans and geography. Sci Rep 7:16982
5. Li K, Dan Z, Gesang L et al (2016) Comparative analysis of gut microbiota of native Tibetan and Han populations living at different altitudes. PLoS ONE 11:e0155863
6. Suzuki TA, Martins FM, Nachman MW (2019) Altitudinal variation of the gut microbiota in wild house mice. Mol Ecol 28:2378–2390
7. Zhao J, Yao Y, Li D et al (2018) Characterization of the gut microbiota in six geographical populations of Chinese Rhesus Macaques (Macaca mulatta), implying an adaptation to high-altitude environment. Microb Ecol 76:565–577
8. Karl JP, Berryman CE, Young AJ et al (2018) Associations between the gut microbiota and host responses to high altitude. American journal of physiology-gastrointestinal and liver physiology 315:G1003–G1015
9. Jiang P, Green SJ, Chlipala GE et al (2019) Reproducible changes in the gut microbiome suggest a shift in microbial and host metabolism during spaceflight. Microbiome 7:113
10. Reese AT, Dunn RR (2018) Drivers of microbiome biodiversity: a review of general rules, feces, and ignorance. mBio 9:e01294–e01218
11. Lu Y, Chen J, Zheng J et al (2016) Mucosal adherent bacterial dysbiosis in patients with colorectal adenomas. Sci Rep 6:26337
12. Nowak RG, Bentzen SM, Ravel J et al (2017) Rectal microbiota among HIV-uninfected, untreated HIV, and treated HIV-infected in Nigeria. AIDS 31:857–862
13. Durk RP, Castillo E, Marquez-Magana L et al (2019) Gut microbiota composition is related to cardiorespiratory fitness in healthy young adults. Int J Sport Nutr Exerc Metab 29:249–253
14. Evans CC, LePard KJ, Kwak JW et al (2014) Exercise prevents weight gain and alters the gut microbiota in a mouse model of high fat diet-induced obesity. PLoS ONE 9:e92193
15. Turnbaugh PJ, Ley RE, Mahowald MA et al (2006) An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 444:1027–1031
16. Goffredo M, Mass K, Parks EJ et al (2016) Role of gut microbiota and short chain fatty acids in modulating energy harvest and fat partitioning in youth. J Clin Endocrinol Metab 101:4367–4376

17. Li L, Zhao X (2015) Comparative analyses of fecal microbiota in Tibetan and Chinese Han living at low or high altitude by barcoded 454 pyrosequencing. Sci Rep 5:14682
18. Ma Y, Ma S, Chang L et al (2019) Gut microbiota adaptation to high altitude in indigenous animals. Biochem Biophys Res Commun 516:120–126
19. Sun G, Zhang H, Wei Q et al (2019) Comparative analyses of fecal microbiota in European mouflon (Ovis orientalis musimon) and blue sheep (Pseudois nayaur) living at low or high altitudes. Front Microbiol 10:1735
20. Bai YF, Wang SW, Wang XX et al (2019) The flavonoid-rich Quzhou Fructus Aurantii extract modulates gut microbiota and prevents obesity in high-fat diet-fed mice. Nutrition & diabetes 9:30
21. Gong L, Wang T, Sun C et al (2019) Whole barley prevents obesity and dyslipidemia without the involvement of the gut microbiota in germ free C57BL/6J obese mice. Food Funct 10:7498–7508
22. Butterfield GE, Gates J, Fleming S et al (1992) Increased energy intake minimizes weight loss in men at high altitude. J Appl Physiol 72:1741–1748
23. Kong Z, Zhou C, Chen L et al (2019) Multi-omics analysis reveals up-regulation of APR signaling, LXR/RXR and FXR/RXR activation pathways in Holstein dairy cows exposed to high-altitude hypoxia. Animals 9:406
24. Kleessen B, Schroedl W, Stueck M et al (2005) Microbial and immunological responses relative to high-altitude exposure in mountaineers. Med Sci Sports Exerc 37:1313–1318
25. Lizko NN (1991) Problems of microbial ecology in man space mission. Acta Astronaut 23:163–169
26. Gao X, Xie Q, Kong P et al (2018) Polyphenol- and caffeine-rich postfermented pu-erh tea improves diet-induced metabolic syndrome by remodeling intestinal homeostasis in mice. Infect Immun 86:e00601–e00617
27. Liu S, Rezende RM, Moreira TG et al (2019) Oral administration of miR-30d from feces of MS patients suppresses MS-like symptoms in mice by expanding Akkermansia muciniphila. Cell Host Microbe 26:779–794
28. Greer RL, Dong X, Moraes AC et al (2016) Akkermansia muciniphila mediates negative effects of IFNgamma on glucose metabolism. Nat Commun 7:13329
29. Adak A, Maity C, Ghosh K et al (2014) Alteration of predominant gastrointestinal flora and oxidative damage of large intestine under simulated hypobaric hypoxia. Z Gastroenterol 52:180–186
30. Adak A, Ghosh, Mondal KC (2014) Modulation of small intestinal homeostasis along with its microflora during acclimatization at simulated hypobaric hypoxia. Indian J Exp Biol 52:1098–1105
31. Murray AJ, Montgomery HE, Feelisch M et al (2018) Metabolic adjustment to high-altitude hypoxia: from genetic signals to physiological implications. Biochem Soc Trans 46:599–607

Figures

Figure 1

Effect of hypoxia on body weight, food and water intake. (A), Body weight; (B), Food intake; (C), Water intake. Data are expressed as mean ± standard deviation. $^{*}P < 0.05$ versus normoxia. N, normoxia; H, hypoxia.

Figure 2

Effect of hypoxia on community richness and diversity in mice. (A), Shannon; (B), Simpson; (C), Chao1; (D), ACE. Data are expressed as mean ± standard deviation. $^{*}P < 0.05$ versus normoxia. N, normoxia; H, hypoxia; ACE, abundance-based coverage estimator.

Figure 3

Effect of hypoxia on overall structural of microbiota in mice. (A), Principal component analysis; (B), Analysis of similarities. N, normoxia; H, hypoxia.

Figure 4

Effect of hypoxia on microbiota composition in mice. (A), Relative abundance of top 10 phylum; (B), *Firmicutes* to *Bacteroidetes* ratio, data are expressed as mean ± standard deviation, $^{*}P < 0.05$ versus normoxia; (C), LDA score. N, normoxia; H, hypoxia; LDA, linear discriminant analysis.

Figure 5

Effect of hypoxia on microbiota phenotype in mice. Data are expressed as mean ± standard deviation. $^{*}P < 0.05$ versus normoxia. N, normoxia; H, hypoxia.

Figure 6

Effect of hypoxia on microbiota function in mice. N, normoxia; H, hypoxia.