Acute exposure to simulated high-altitude hypoxia alters gut microbiota in mice

Feng Wang · Han Zhang · Tong Xu · Youchun Hu · Yugang Jiang

Received: 18 September 2021 / Revised: 30 May 2022 / Accepted: 1 June 2022 / Published online: 22 June 2022
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2022

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 h 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.

Keywords High altitude · Hypoxia · Gut microbiota

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 decreases. At an altitude of 4,000 m, oxygen content was only ≈ 60% of sea level (Beall 2007).

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 (Zhang et al. 2015). Acute hypoxia environment increased bacterial translocation and decreased protein expression of occluding, facilitating the entry of lipopolysaccharide into the blood (Luo et al. 2017). 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 to 4500 m, Lan et al. (2017) observed that altitude had a positive correlation to Faecalibacterium, Bacteroides and Bifidobacterium, but negative correlation to Ruminococcaceae, Prevotella and Lachnospiraceae. 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 (Li et al. 2016). In wild house mice, Suzuki et al (2019) 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 (Zhao et al. 2018). 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.

**Materials and methods**

**Animals and experimental design**

Twelve-week-old male C57BL/6 J 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 h 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-93 M 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.

**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™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.

**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.

**Results**

**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 (Fig. 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.

**Microbiota diversity**

Shannon and Simpson were significantly higher in the hypoxia group than those in the normoxia group (Fig. 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.
Analysis of similarities showed there was a significant difference between the two groups (Fig. 3B).

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 (Fig. 4A). *Firmicutes*-to-*Bacteroidetes* ratio was significantly lower in the hypoxia group than that in the normoxia group (Fig. 4B). At genus level, *Dubosiella*, *Faecalibacterium* and *Bifidobacterium* were significantly decreased and *unidentified_Lachnospiraceae*, *Akkermansia*, *Parabacteroides* and *Bacteroides* were significantly increased in the hypoxia group relative to the normoxia group (Fig. 4C).

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 (Fig. 5). No significant difference was observed in aerobic, anaerobic and facultatively anaerobic between the two groups.
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 (Fig. 6).

Discussion

Nowadays, with a growing number of people from low altitude come to high altitude for traveling, 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 (Karl et al. 2018). Moreover, Jiang et al. (2019) 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 (Reese and Dunn 2018). Indeed, alpha diversity estimation revealed higher microbiota diversity in patients with colorectal adenomas (Lu et al. 2016). For men who were HIV-infected, the alpha diversity of the Bacteroidetes phylum was positively correlated with viral load (Nowak et al. 2017). 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. (2019) found that maximal oxygen consumption was positively associated with Firmicutes-to-Bacteroidetes ratio. Voluntary exercise increased proportionally to the ΔCt ratio of Firmicutes:Bacteroidetes (Evans et al. 2014). On the other hand, relatively high ratio of Firmicutes to Bacteroidetes is associated with highly efficient energy harvest (Turnbaugh et al. 2006). In youth, Firmicutes-to-Bacteroidetes ratio was positively associated with body mass index, visceral and hepatic fat (Goffredo et al. 2016). Both Tibetans and Chinese Han living at high altitude had a high abundance of Firmicutes and a low abundance of Bacteroidete (Li and Zhao 2015). Similarly, animals living in high altitude, such as Tibetan antelope (Ma et al. 2019), European mouflon and blue sheep (Sun et al. 2019), 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 (Bai et al. 2019). In addition, Bacteroides and Parabacteroides were negatively associated with obesity (Gong et al. 2019). 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 (Butterfield et al. 1992). Kong et al. (2019) 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

![Fig. 6 Effect of hypoxia on microbiota function in mice. N, normoxia; H, hypoxia](image-url)
et al. (2005) 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 (Lizko 1991). Consistent 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. (2018) 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* (Liu et al. 2019). Likewise, *A. muciniphila* was significantly increased in IFNγ-deficient mice and restoration of IFNγ level decreased *A. muciniphila* (Greer et al. 2016).

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 (Adak et al. 2014) and small intestine (Adak and Ghosh 2014) 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 increased cellular processes and organismal systems. The underlying mechanisms include promotion of glycolytic capacity and suppression of oxidative metabolism (Murray et al. 2018). 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 variables, 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.

**Conclusion**

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**

**Funding** This research was supported by the National Natural Science Foundation of China (No. 81903303) and China Postdoctoral Science Foundation (No. 2018M643901).

**Conflicts of Interest** The authors declare no conflict of interest.

**References**

Adak A, Ghosh MKC (2014) Modulation of small intestinal homeostasis along with its microflora during acclimatization at simulated hypobaric hypoxia. Indian J Exp Biol 52:1098–1105

Adak A, Maity C, Ghosh K, Mondal KC (2014) Alteration of predominant gastrointestinal flora and oxidative damage of large intestine under simulated hypobaric hypoxia. Z Gastroenterol 52:180–186. [https://doi.org/10.1038/s41387-019-0097-6](https://doi.org/10.1038/s41387-019-0097-6)

Bai YF, Wang SW, Wang XX, Weng YY, Fan XY, Sheng H, Zhu XT, Lou LJ, Zhang F (2019) The flavonoid-rich Quzhou Fructus Auranti extract modulates gut microbiota and prevents obesity in high-fat diet-fed mice. Nutr Diabetes 9:30. [https://doi.org/10.1038/s41387-019-0097-6](https://doi.org/10.1038/s41387-019-0097-6)

Beall CM (2007) Two routes to functional adaptation: Tibetan and Andean high-altitude natives. Proc Natl Acad Sci U S A 104(Suppl 1):8655–8660. [https://doi.org/10.1073/pnas.0701985104](https://doi.org/10.1073/pnas.0701985104)

Butterfield GE, Gates J, Fleming S, Brooks GA, Sutton JR, Reeves JT (1992) Increased energy intake minimizes weight loss in men at high altitude. J Appl Physiol 72:1741–1748. [https://doi.org/10.1152/jappl.1992.72.5.1741](https://doi.org/10.1152/jappl.1992.72.5.1741)

Derk RP, Castillo E, Marquez-Magana L, Grosicki GJ, Bolter ND, Lee CM, Bagley JR (2019) Gut microbiota composition is related to cardiorespiratory fitness in healthy young adults. Int J Sport Nutr Exerc Metab 29:249–253. [https://doi.org/10.1123/ijsnem.2018-0024](https://doi.org/10.1123/ijsnem.2018-0024)

Evans CC, LePard KJ, Kwak JW, Stancuscas MC, Laskowski S, Dougherty J, Moulton L, Glawe A, Wang Y, Leone V, Antonopoulos DA, Smith D, Chang EB, Ciancio MJ (2014) Exercise prevents weight gain and alters the gut microbiota in a mouse model of high-fat diet-induced obesity. PLoS ONE 9:e92193. [https://doi.org/10.1371/journal.pone.0092193](https://doi.org/10.1371/journal.pone.0092193)

Gao X, Xie Q, Kong P, Liu L, Sun S, Xiong B, Huang B, Yan L, Sheng J, Xiang H (2018) Polyphenol- and caffeine-rich post-fermented pu-erh tea improves diet-induced metabolic syndrome by remodeling intestinal homeostasis in mice. Infect Immun 86:e00601-e617. [https://doi.org/10.1128/iai.00601-17](https://doi.org/10.1128/iai.00601-17)

Goffredo M, Mass K, Parks EJ, Wagner DA, McClure EA, Graf J, Savoye M, Pierpont B, Cline G, Santoro N (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. [https://doi.org/10.1210/jc.2016-1797](https://doi.org/10.1210/jc.2016-1797)

Gong L, Wang T, Sun C, Wang J, Sun B (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. [https://doi.org/10.1039/c9ff01268k](https://doi.org/10.1039/c9ff01268k)

Greer RL, Dong X, Moraes AC, Zielke RA, Fernandes GR, Peremyslova E, Vasquez-Perez S, Schoenborn AA, Gomes EP, Pereira AC, Ferreira SR, Yao M, Fuss IJ, Strober W, Sikora AE, Taylor GA, Galuti AS, Morgun A, Shulzenko N (2016) *Akkermansia muciniphila* mediates negative effects of IFNGamma on glucose
metabolism. Nat Commun 7:13329. https://doi.org/10.1038/s41467-019-0724-4
Jiang P, Green SJ, Chipala GE, Turek FW, Vitaterna MH (2019) Reproducible changes in the gut microbiome suggest a shift in microbial and host metabolism during spaceflight. Microbiome 7:113. https://doi.org/10.1186/s40168-019-0724-4
Karl JP, Berryman CE, Young AJ, Radcliffe PN, Brancik TA, Pantoja-Kleessen B, Schroedl W, Stueck M, Richter A, Rieck O, Krueger J, Jiang P, Green SJ, Chlipala GE, Turek FW, Vitaterna MH (2019) Gut microbiota adaptation to high altitude in indigenous animals. Biochem Biophys Res Commun 516:120–126. https://doi.org/10.1016/j.bbrc.2019.05.085
Murray AJ, Montgomery HE, Feelisch M, Grocott MPW, Martin DS (2018) Metabolic adjustment to high-altitude hypoxia: from genetic signals to physiological implications. Biochem Soc Trans 46:599–607. https://doi.org/10.1042/bst20170502
Nowak RG, Bentzen SM, Ravel J, Crowell TA, Dauda W, Ma B, Liu H, Blattner WA, Baral SD, Charurat ME (2017) Rectal microbiota among HIV-uninfected, untreated HIV, and treated HIV-infected in Nigeria. AIDS 31:857–862. https://doi.org/10.1097/qad.0000000000001409
Reese AT, Dunn RR (2018) Drivers of microbiome biodiversity: a review of general rules, feces, and ignorance. Mbio 9:e01294-e1318. https://doi.org/10.1128/mBio.01294-18
Sun G, Zhang H, Wei Q, Zhao C, Yang X, Wu X, Xia T, Liu G, Zhang L, Gao Y, Sha W, Li Y (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. https://doi.org/10.3389/fmicb.2019.01735
Suzuki TA, Martins FM, Nachman MW (2019) Altitudinal variation of the gut microbiota in wild house mice. Mol Ecol 28:2378–2390. https://doi.org/10.1111/mec.14905
Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI (2006) An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 444:1027–1031. https://doi.org/10.1038/nature05414
Zheng F, Wu W, Deng Z, Zheng X, Zhang J, Deng S, Chen J, Ma Q, Wang Y, Yu X, Kang S, Wang X (2015) High altitude increases the expression of hypoxia-inducible factor 1alpha and inducible nitric oxide synthase with intestinal mucosal barrier failure in rats. Int J Clin Exp Pathol 8:5189–5195
Zhao J, Yao Y, Li D, Xu H, Wu J, Wen A, Xie M, Ni Q, Zhang M, Peng G, Xu H (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. https://doi.org/10.1007/s00248-018-1146-8

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.