Study on the Impact of a Hot and Humid Environment on Gut Microbiota in Mice

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Abstract

[Objectives]

This study explores the impact of high-temperature and high-humidity (HTH) environments on the gut microbiota of mice, aiming to understand the potential mechanisms through hot and humid climate conditions affect intestinal microbiota. As global warming leads to increased temperatures and humidity, these factors pose significant health risks, and gut microbiota may be notably impacted by these environments. The study provides scientific insight into how hot and humid climates might affect gut microbiota and offers a potential framework for addressing gut health issues related to climate changes and individual migration to such environments.

[Methods]

By using 16S rRNA gene sequencing data from publicly available databases, we analyzed differences in gut microbiota composition in mice under normal and HTH conditions, revealing structural changes in microbial communities and their dependence on exposure duration.

[Results]

Compared to normal conditions, mice exposed to HTH environments experienced notable changes in gut microbiota by week 8, particularly in their relative abundances. Although HTH exposure led to noteworthy compositional changes, it did not significantly reduce gut microbiota diversity, suggesting that the primary impact of HTH conditions was on microbiota composition without compromising overall microbial health.

Keywords: HTH environment; Gut microbiota; Microbiota diversity; Host health

Research Background

In recent years, the impact of environmental factors on human health has received increasing attention. Common sense indicates that high-temperature and high-humidity (HTH) environments can cause significant discomfort. Studies have shown a notable association between long-term exposure to high humidity and high temperatures and increased rates of morbidity and mortality. Specifically, temperature and humidity are key meteorological factors affecting heat-related health outcomes, and the rise in frequency and intensity of compound heat events, including compound heat-dry events (CHDEs) and compound heat-wet events (CHWEs), which poses serious health threats [1].

Through global climate warming, the frequency and intensity of high-temperature weather have been gradually increasing. Early studies on heat effects on health mainly focused on temperature alone, yet relative humidity is crucial in affecting health in high-temperature conditions due to its relationship with body heat exchange. Recent studies suggest that the risk associated with high

temperatures in humid conditions may exceed that of high temperatures alone, intensifying the health impact of hot environments [2]. Elongated exposure to HTH environments may lead to various health issues. Increased relative humidity reduces the efficiency of sweat cooling, increasing the body's heat load and thus leading to higher morbidity and mortality.

The relationship between gut microbiota and host health is multi-faceted, significantly influencing human health. Some studies specify that gut microbiota consists of "keystone functional groups" and "pathogenic functional groups" that interact like a "seesaw" pattern, with fluctuations affecting human health. When keystone functional groups dominate, the gut microbiota is in a healthy state; on the contrary, pathogenic groups' dominance signals potential health issues [3]. The gut microbiota is closely associated with processes like food digestion, nutrient absorption, and metabolic processes. Normal gut microbes, such as Bifidobacterium and Lactobacillus acidophilus, synthesize essential vitamins for human growth and development, which play a crucial role in health [4]. Additionally, the gut contains beneficial bacteria that produce organic acids and hydrogen peroxide to inhibit pathogens from invading the intestinal mucosa. The gut microbiota acts as an antigenic stimulus, promoting immune system development and function, thus enhancing the host's immunity [5].

The study focuses on the impact of high-temperature and high-humidity environments on the gut microbiota, exploring how changes in environmental conditions alter the composition and function of gut microorganisms and indirectly affect host physiological responses. By analyzing gut microbiota gene sequences from public databases, this research aims to understand the role of microbial communities in environmental adaptation and physiological regulation in hosts under normal and HTH conditions and further explores how targeting gut microbiota may help address health issues arising from climate change and relocation to hot and humid climates.

Research Methods

Data Collection

Gut microbiota 16S rRNA sequencing data from mice raised under normal and HTH conditions were collected from the classified SRA database.

Sequence Quality Control

Sequences were segregated by sample, allowing precise extraction of each experimental group's data. Low-quality sequences were removed, providing reliable microbiota data, which helps accurately observe the impact of HTH conditions on gut microbiota composition.

Sample Taxonomic Composition Analysis

A pre-trained Naive Bayes Classifier and the q2-feature-classifier plugin were used to preprocess raw sequences. The classifier was trained on the 99% OTUs in the Greengenes 13_8 database, pruning sequences to retain only the valid 16S region (V4 region restricted by 515F/806R primers) with 250 base pairs. This classifier was applied to raw sequences to generate visual mappings of sequences and classifications.

Alpha and Beta Diversity Analysis

Interactive visualization charts were generated by calculating alpha and beta diversity indices and applying related statistical tests. The core-metrics-phylogenetic tool was used to rarefy a FeatureTable[Frequency] to a specific depth and calculate several alpha and beta diversity indices. Principal Coordinate Analysis (PCoA) plots were produced for each beta diversity index using the Emperor tool. In this case, rarefaction depth was set to 500, conducting non-repeated sampling within each sample to achieve a total count of 500 per sample in the table, maximizing sequence retention while minimizing excluded samples.

Research Results

1. Data Quality Suitability for This Study

In microbial community studies, the precise segregation of sequences and separation by sample are essential initial steps. This approach enables precise extraction of each group's experimental data, avoiding errors from data mixing. Further removal of low-quality sequences using tools like Trimmomatic or FastQC is critical for enhancing data reliability by eliminating sequences with sequencing errors, contaminants, or low-quality regions. These quality control measures provide more reliable microbial community data [6]. This quality-controlled data allows a more accurate assessment of environmental factors in gut microbiota structure and function, contributing valuable scientific evidence to understanding environmental changes' health impacts. Experimental data included 16S rRNA gene sequencing data of mice from normal and HTH conditions at weeks 1, 2, 4, and 8, with six mice per group, suitable for subsequent analysis.

2. Gut Microbiota Composition in Mice Exposed to HTH Changed

Sample composition was analyzed within the metadata classification framework using Permutational Multivariate Analysis of Variance (PERMANOVA) to determine whether samples within the same group were more similar in distance than those from different groups. Pairwise comparisons were conducted with the p-pairwise parameter to identify significant differences between specific groups. Using Bray-Curtis distance-based Principal Component Analysis (PCoA) plots, significant differences were observed between the two sample groups at week 8, confirmed by PERMANOVA. Non-metric multidimensional scaling (NMDS) further revealed differences in beta diversity between the two groups, showing clear separation. Unweighted PCoA analysis also indicates significant differences at week 8 compared to weeks 1, 2, and 4. These findings suggest that HTH conditions significantly affect the composition of gut microbial communities.

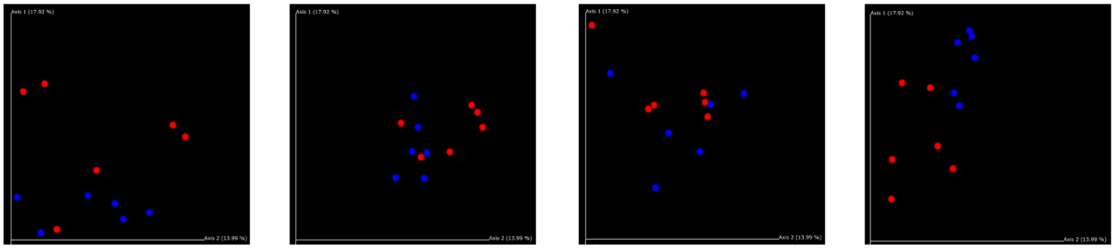

Figure 1: PCoA plot based on unweighted UniFrac distances of OTU relative abundance.

3. Proportion Changes in Gut Microbiota Composition under HTH Conditions

An in-depth taxonomic annotation of 16S rRNA sequencing data is carried out, which accurately classifies each feature sequence to corresponding taxonomic units. 16S rRNA gene sequence analysis provides detailed information on microbial community structure and composition, allowing identification and distinction of different microbial species. Bar plots were drawn to visually display the relative abundance of different taxa within each sample. These results indicate that HTH conditions affect the gut microbiota composition. The dominant taxa's relative proportions were evaluated at genus and species levels for the two groups, showing notable changes in gut microbiota of DH and NC groups. At week 8, Bacteroides dominated the gut bacterial communities in both DH and NC mice, with an average relative abundance of 46.4% and 61.2%, respectively, followed by Firmicutes, constituting 39.9% and 32.7% in DH and NC groups, respectively.

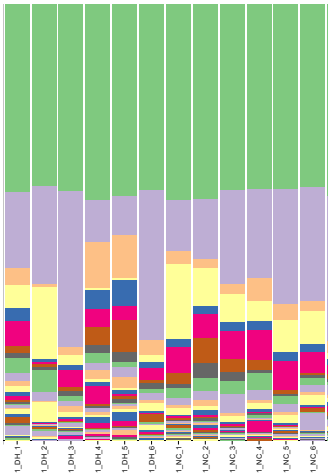

Figure 2: Bacterial composition proportions from fecal 16S rRNA sequencing data between the DH group and NC group at Week 1.

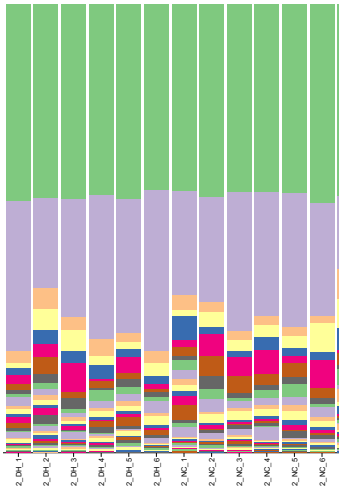

Figure 3: Bacterial composition proportions from fecal 16S rRNA sequencing data between the DH group and NC group at Week 8.

4. Time-Dependent Changes in Gut Microbiota Composition under HTH Conditions

The relative proportions of dominant taxa were evaluated for both groups over different weeks under HTH conditions. As exposure time to HTH increased, bacterial composition in the mice gut

microbiota gradually changed. The relative abundance of Firmicutes showed an upward trend with prolonged humid-heat exposure, though no statistically significant difference was observed in comparison to the NC group. On the other hand, the relative abundance of Bacteroides showed a downward trend, reaching statistical significance compared to the NC group. These findings suggest that the humid-heat environment may influence gut microbial community structure, particularly in the relative abundance of Bacteroides and Firmicutes.

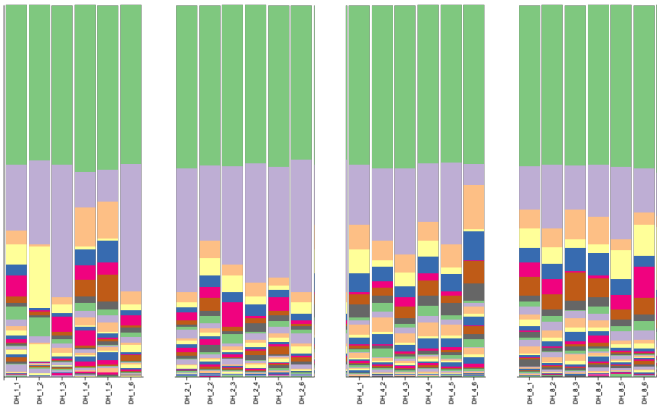

Figure 4: Bacterial composition proportions from fecal 16S rRNA sequencing data of mice raised in the HTH environment across different weeks.

5. No Significant Differences in Species Richness of Gut Microbiota Exposed to HTH Conditions

Alpha and beta diversity indices were calculated to evaluate the bacterial diversity between the two groups. The Shannon diversity index reflects gut microbial community complexity, including species diversity and evenness. High Shannon index values indicate abundant and evenly distributed gut microbiota, typically associated with a healthy gut environment. Conversely, a low Shannon index may indicate low microbial diversity, possibly linked to certain disease states. No significant differences were found between DH and NC groups for the Shannon index.

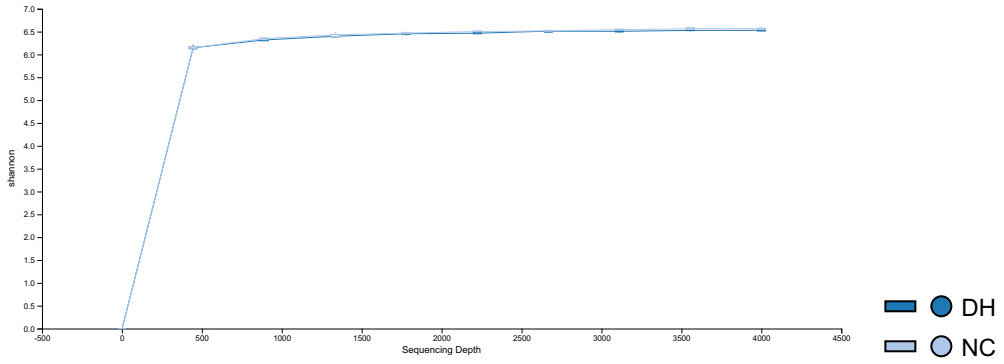

Figure 5: Shannon diversity index indicating species diversity differences between the DH and NC groups.

Observed Features were also calculated, showing no significant differences between DH and NC groups. This indicates that HTH exposure did not fundamentally disrupt gut microbiota structure, with the gut microbiota maintaining a relatively healthy diversity status before and after exposure.

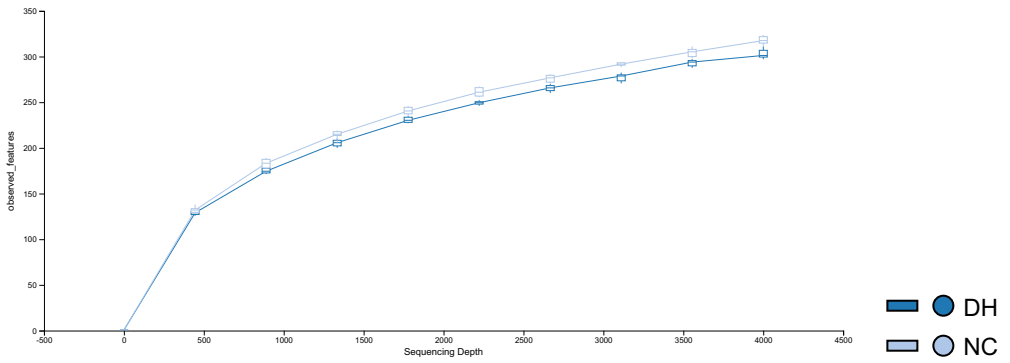

Figure 6: Feature counts indicating species diversity differences between the DH and NC groups.

Research Conclusions

1. Exposure to HTH conditions alters gut microbiota composition in mice, with the changes becoming more evident over time.
2. HTH exposure increases Bacteroides and decreases Firmicutes in the gut microbiota of mice.
3. HTH exposure does not change gut microbiota diversity, indicating that environmental changes impact composition but not the overall health state of the microbiota.

Innovations

1. This study explores the effects of temperature and humidity on gut microbiota in mice, providing insights into health impacts related to acclimatization, climate change, and climatic differences.
2. The combined effect of temperature and humidity on gut microbiota is examined, simulating humid-heat conditions more accurately.

References

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