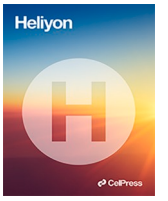
Heliyon 10 (2024) e35239

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Non-invasive ventilation restores the gut microbiota in rats with acute heart failure

He Jiang [a,1], Shan Liu [b,1], Chao Chang [a], Yanwen Shang [a], Jie Geng [a,c,*], Qingliang Chen [a,c,**]

[a] Department of Cardiology, Clinical School of Thoracic, Tianjin Medical University, Tianjin, 300051, China
[b] Institute of Cardiology, Clinical School of Thoracic, Tianjin Medical University, Tianjin, 300222, China
[c] Tianjin Key Laboratory of Cardiovascular Emergency and Critical Care, Tianjin Municipal Science and Technology Bureau, Tianjin, 300051, China

ARTICLE INFO

Keywords:
Heart failure
Non-invasive ventilation
Gut microbiota dysbiosis
Co-occurrence network
Community assembly

ABSTRACT

Heart failure (HF) is an increasingly prevalent disease in humans; it induces multiple symptoms and damages health. The animal gut microbiota has critical roles in host health, which might be related to HF symptoms. Currently, several options are used to treat HF, including non-invasive ventilation (NIV). However, studies on gut microbiota responses to acute HF and associated treatments effects on gut communities in patients are scarce. Here, short-term (1 week after treatments) and long-term (3 months after treatment) variations in gut microbiota variations in rats with acute HF treated were examined NIV through high-throughput sequencing of the bacterial 16S rRNA gene. Through comparison of gut microbiota alpha diversity, it was observed lower gut microbiota richness and diversity in animals with acute HF than in normal animals. Additionally, beta-diversity analysis revealed significant alterations in the gut microbiota composition induced by acute HF, as reflected by increased Firmicutes/Bacteroidetes (F/B) ratios and Proteobacteria enrichment. When network analysis results were combined with the null model, decreased stability and elevated deterministic gut microbiota assemblies were observed in animals with acute HF. Importantly, in both short- and long-term periods, NIV was found to restore gut microbiota dysbiosis to normal states in acute HF rats. Finally, it was shown that considerable gut microbiota variations existed in rats with acute HF, that underlying microbiota mechanisms regulated these changes, and confirmed that NIV is suitable for HF treatment. In future studies, these findings should be validated with different model systems or clinical samples.

1. Introduction

Heart failure (HF) is a global and increasingly prevalent epidemic disease, which affects more than 26 million people [1]. The condition occurs when the heart cannot deliver sufficient blood and oxygen to the organs. While this is not the same as cardiac arrest, it does mean that the heart is failing to pump blood effectively. The condition also induces multiple symptoms, including shortness of

* Corresponding author. Department of Cardiology, Clinical School of Thoracic, Tianjin Medical University, Tianjin, 300051, China.
** Corresponding author. Department of Cardiology, Clinical School of Thoracic, Tianjin Medical University, Tianjin, 300051, China.
E-mail addresses: gengjie1886@126.com (J. Geng), qingliang1971@126.com (Q. Chen).
[1] These two authors are co-first authors.

https://doi.org/10.1016/j.heliyon.2024.e35239
Received 26 November 2023; Received in revised form 24 July 2024; Accepted 25 July 2024
Available online 26 July 2024

breath, fatigue or weakness (even after rest), persistent coughing or wheezing, swelling in the legs and feet, and abdominal pain or nausea [2]. Coronary heart disease, a previous heart attack, or high blood pressure are the most common causes of HF, as they limit the supply of oxygen-rich blood to the heart [3]. In the USA, HF has an estimated prevalence of 18–21/1000 individuals and caused 1.25 million hospitalizations in 2018 [4]. In China, HF prevalence estimates are approximately 0.9 %, but increase significantly to 23.5%–30.8 % in patients 60 years of age or older [5]. HF mortality rates are approximately 25 % after 1 year following diagnosis but increase to more than 50 % at 5 years [6]. Therefore, further research is required to control and treat this disease.

The gut microbiome is an extremely complex microbial community in the intestinal tract and is critical for immunoregulation, nutrition absorption, physiological responses, and overall host development [7]. Gut microbiota homeostasis is closely related to host physiology and health [8]. Many studies have confirmed and identified the relationships between the gut microbiota and host disease, including those not occurring in the intestines, such as cardiovascular disease [9]. Dysbiotic gut microbiota are reportedly implicated in HF as they affect cells by using primary and secondary bile acids, short-chain fatty acids, N-oxides, and trimethylamine [10]. Reduced intestinal wall integrity, due to altered microbiota, may also be associated with HF [11]. Specifically, a study found a diminished percentage of Bacteroides in fecal samples from patients with ischemic and dilated cardiomyopathic HF [12]. Another study has revealed diminished *Faecalibacterium prausnitzii* and elevated *Ruminococcus gnavus* in the gut microbiota in patients with HF [13]. These previous investigations have suggested a correlation between gut microbiota dysbiosis and HF progression. Importantly, the relationship between HF and the gut microbiota is mutual. Dysbiotic gut microbiota are not only a cause of HF, but also a possible consequence of it. When the intestinal microbiome composition is significantly altered, bacterial richness can become reduced in chronic HF patients when compared with healthy individuals [14]. In contrast with chronic HF, acute HF is a sudden, life-threatening condition where the heart cannot deliver enough oxygen to meet bodily needs [15]. In this situation, intestinal flora changes are more likely a consequence of HF. However, studies have rarely focused on gut microbiota responses to acute HF and examined associated treatment effects on patient gut microbiota.

HF can be divided into two categories based on timing and severity: acute and chronic. Treatment options for chronic HF include medication, lifestyle changes, and surgery [16], whereas therapeutics for acute HF include vasodilators, diuretics, and aldosterone inhibitors [17]. Surgical options for underlying HF include heart valve repair and coronary artery bypass grafting [18]. In contrast to chronic HF, acute HF may cause significant respiratory failure, and the main curative modalities include non-invasive ventilation (NIV), high-flow nasal cannulation, and continuous positive airway pressure [18]. NIV, a method for delivering ventilatory support without the use of an invasive airway, is a highly valuable tool for the management of acute HF [19]. Several treatments have been explored to restore gut microbiota balance in human or annimal models with HF; these treatments include fecal microbiota transplantation [20], administration of probiotics [10], and targeted therapy with trimethylamine N-oxide inhibitors [21]. However, these options often require complex procedures or long treatment period, and thus are suitable for the treatment of only chronic HF [22]. To date, no research has examined methods to rapidly treat acute HF while restoring the gut microbiota in patients.

To address this, acute HF, NIV treatment effects, and the gut microbiota in a rat model system (Fig. 1) were examined. Feces samples were collected from normal, HF, and NIV-treated HF rats in daily and monthly schedules to reflect short- and long-term effects. Gut microbiota dynamics were tracked using high-throughput bacterial *16S rRNA* gene sequencing. Microbiota diversity, composition,

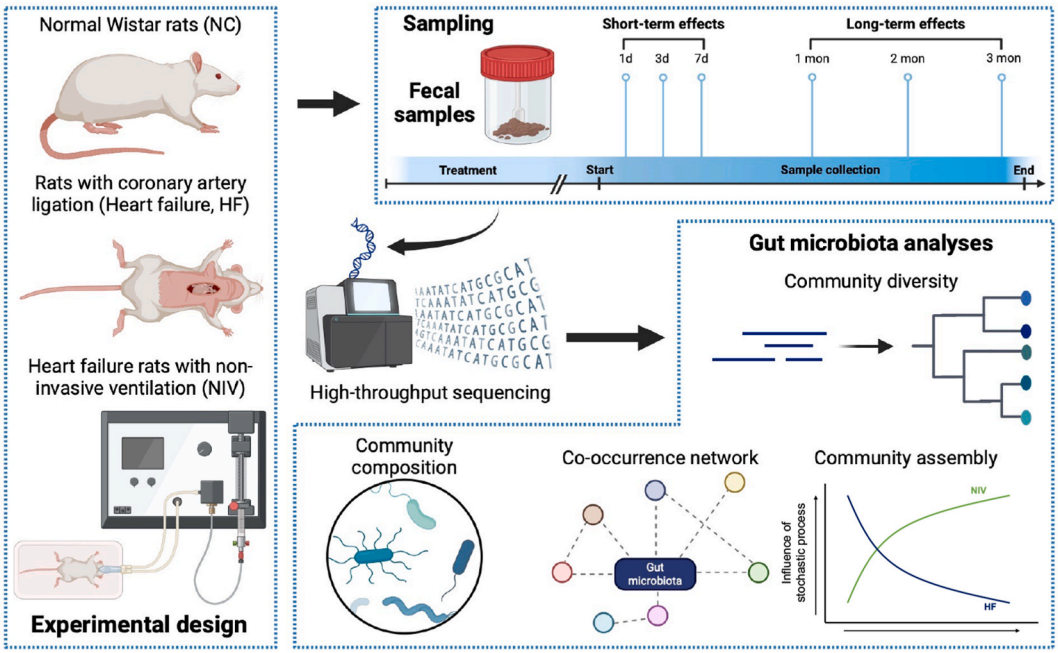


Fig. 1. Flow chart of experimental design, sample collection, and data analysis.

and functional changes were analyzed in rats. From co-occurrence network analyses, complexity and stability variations in microbiota were also uncovered. Additionally, microbiota assembly mechanisms were determined using neutral community models. The following questions were addressed: (i) How does acute HF affect microbiota diversity, composition, and assembly in the studied rat model? (ii) Does NIV treatment eliminate gut microbiota dysbiosis caused by acute HF? and (iii) Are NIV treatment effects maintained for long periods? The findings of present study provide a deeper understanding of the adverse effects of acute HF and beneficial effects of NIV on the microbiota.

2. Materials and methods

2.1. Animals, treatments, and sample collection

This study was approved by the local Ethics Committee. Wistar rats (220 ± 20 g adult males) were purchased from the Animal Experimental Center of the Academy of Military Medical Sciences. In total, 120 rats were used and assigned to three groups: 1) rats undergoing coronary artery ligation were assigned to the HF group (n = 40); 2) rats undergoing NIV treatment after coronary artery ligation were assigned to the NIV group (n = 40); and 3) rats undergoing thoracotomy and ligation were assigned to the Normal Control (NC) group (n = 40). Treatments across groups were consistent with those in a previous study [23]. To examine short-term effects, feces were collected from five individuals/group at 3, 5, and 7 days post-surgery, while for long-term effects, samples were collected at 1, 2, and 3 months. Fecal samples were stored at −80 °C for DNA extraction.

2.2. Histopathology

At 3, 5, and 7 days post-surgery, rats were humanely culled. After opening the heart along the longitudinal axis, ventricles were fixed in 10 % formalin solution for 24 h. After fixing in 4 % paraformaldehyde, heart tissue sections were paraffinized and stained in hematoxylin and eosin (H&E) (Leagene Biotechnology). All detailed procedures were consistent with those described in a previous study [24].

2.3. DNA extraction

Using the QIAamp Power Fecal DNA Kit (QIAGEN, CA, USA), total microbial DNA was extracted from feces samples according to manufacturer's instructions. Successful DNA extraction was ascertained using agarose gel electrophoresis (2 % agarose). Then, Nanodrop 2000 (ThermoFisher, CA, USA) instrumentation was used to estimate DNA purity and concentrations. DNA samples were stored at −20 °C for further applications.

2.4. High-throughput sequencing and data processing

The V3–V4 region of the bacterial *16S rRNA* gene were sequenced using 341F and 806R primers [25]. Polymerase chain reaction (PCR) amplifications, PCR product gel extractions, and sequencing library construction were performed according to a previous study [26]. Sample libraries were finally sequenced at BIOZERON Biotech. Co., Ltd. (Shanghai, China) using the Illumina Novaseq 6000 platform based on a 250 bp paired-end strategy. Raw reads were quality-assessed using previously published thresholds [27]. High-quality reads were then assigned to samples and tags assembled and clustered into operational taxonomic units (OTUs) using QIIME v1.9.1 software [28]. Tags with the highest number of OTUs were selected as representative sequences and annotated to a taxonomy using the SILVA database (Release 138) [29]. Finally, OTU abundance data were normalized using lowest read numbers in samples (31,224).

2.5. Statistical analysis

Statistical analyses were performed using the R 4.2.2 platform. Two α-diversity indices, Chao1 and Shannon, were computed for gut microbiota according to the OTU abundance table obtained from high-throughput sequencing ("vegan" package). In addition, according to the taxonomy of OTUs and their abundance data, the relative abundances of gut microbiota were calculated from the phylum to genus levels, respectively. Tukey's honest significant difference (HSD) tests were used to confirm dominant bacteria abundance variations and α-diversity indices in microbiota across treatments ("multcomp" package). Additionally, principal coordinate analysis (PCoA) and adonis tests for Bray-Curtis distances were also executed ("vegan" package) to evaluate HF and NIV influences on gut microbiota compositional structures. Ternary plots were also generated to identify gut bacterial relative abundance differences across treatments ("ggtern" package). To visualize co-occurrence networks in gut microbiota, Spearman's rank correlations among OTUs in samples were calculated ("WGCNA" package). Only OTUs detected in at least 9/15 samples across treatments were used for correlation analyses. If the Spearman correlation coefficient was >0.8 and the Benjamini-Hochberg adjusted P value < 0.05, the correlation between two OTUs was considered statistically robust. Network graphs were visualized using the Gephi interactive platform [30] and topological network parameters were also calculated ("igraph"). To assess gut microbiota stability, co-occurrence network cohesion was calculated according to a previous study [31]. Cohesion index changes across different treatments were analyzed using Tukey's HSD tests. Finally, stochastic processes and their importance to gut microbiota assemblies were investigated using a neutral community model [32].

3. Results

3.1. Pathological examinations

From histopathology results (Fig. 2), NC rats showed distinct nuclei and regular myocardium without neutrophils and low lymphocyte numbers. Additionally, distinct cardiomyocyte alterations were observed during the experiment operation. In contrast, on day 3 in the HF group, muscle cross-striation loss as well as neutrophil and nuclear enlargement had occurred. The myocardium was replaced by fibroblasts and aggregated lymphocytes appeared on day 5. The epicardium was dominated by lymphocyte infiltration and necrotic myocardium was replaced by inflammatory granulation on day 7. In the NIV group on day 3, disordered muscle cross-striations and scattered neutrophils could be observed. Fibroblasts subsequently appeared, and muscle cross-striations disappeared on day 5. On day 7, lymphocytes were the major inflammatory cell group and connective tissue with focal hyperplasia replaced the myocardium. These findings suggested distinct myocardial degeneration and necrosis in HF rats when compared with NC counterparts, while NIV showed effective HF symptom remission.

3.2. Gut microbiota diversity and variations

Regardless of the time period, significantly lower Chao1 and Shannon indices were observed in the gut microbiota of rats with HF when compared with NC animals (Tukey's HSD test, $p < 0.05$, Fig. 3). More importantly, gut microbiota α-diversity in HF rats after NIV treatment returned to normal levels after a short time and were maintained for a long period (Tukey's HSD test, $p < 0.05$, Fig. 3). Additionally, the range of α-diversity changes in gut microbiota was shorter in the long term than short term, and was possibly related to natural recovery rates in HF rats over these periods (Fig. 3). In the short-term periods, gut microbiota α-diversity levels in NC and HF groups were stable, but levels in NIV-treated samples gradually increased in line with study duration (Fig. S3). Also, α-diversity indices in NIV-treated rats were stable over the long-term periods (Fig. S3).

3.3. Variations in gut microbiota composition

PCoA based on Bray-Curtis distances identified distinct and separated gut microbiota distribution across different treatments, in both the short- and long-term periods (Fig. 4a and b). Adonis tests also confirmed significant variations in microbiota composition

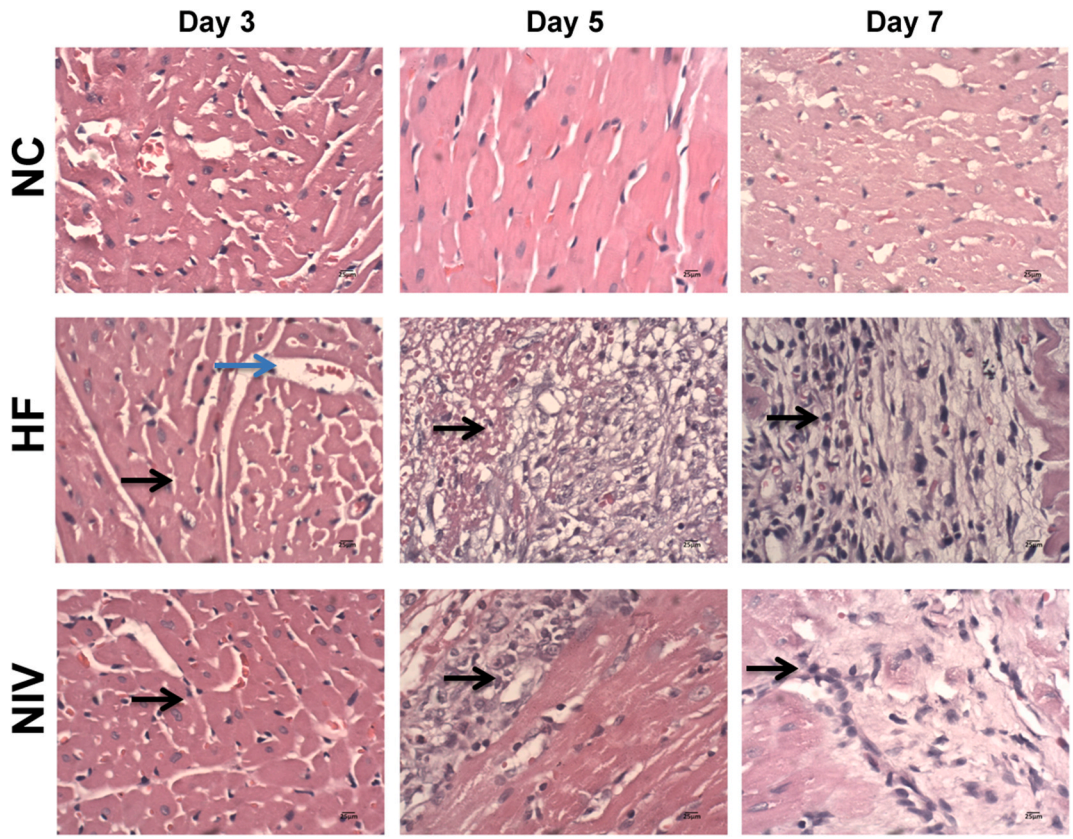


Fig. 2. Hematoxylin and eosin-stained cardiomyocytes in three rats from each group at indicated time points.

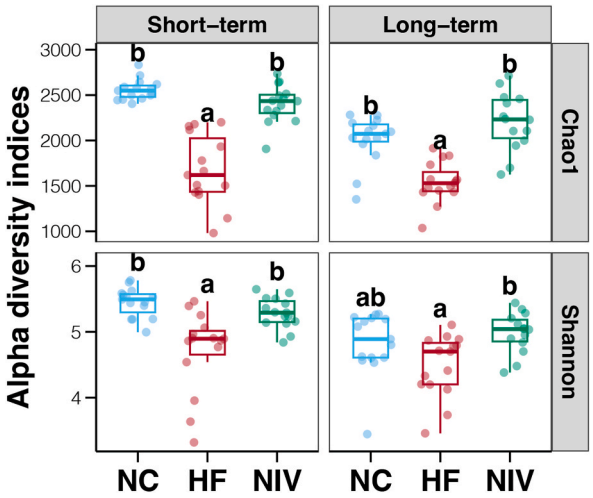


Fig. 3. Alpha diversity index variations in gut microbiota across different treatments. Significant differences across samples are indicated by different lowercases letters above boxes in the same sub-figure (Tukey's HSD test, $p < 0.05$).

structures across different treatments ($p < 0.05$). Regardless of the period, gut microbiota in HF animals showed remarkable differences when compared with NC individuals, but NIV reversed these phenomena (Fig. 4a and b). HF also increased inter-individual gut microbiota differences (Tukey's HSD test, $p < 0.05$), while NIV restored them to normal levels (Fig. 4c and d). Also, amplitude changes in gut microbiota composition across different treatments in the long-term periods were weaker when compared with the short-term periods, consistent with α-diversity results.

3.4. Variations in the relative abundance of gut bacteria

Firmicutes was the most dominant bacterial phylum in the gut microbiota, followed by Bacteroidota, while the relative abundance of other bacterial phyla was relatively low (Fig. S4). In the short-term periods, six bacterial phyla were identified as connected to HF; the relative abundance of Firmicutes, Proteobacteria, Actinobacteriota, and Desulfobacterota was significantly increased, while the relative abundance of Bacteroidota and Verrucomicrobiota was significantly decreased in rats with HF compared with NC individuals (Tukey's HSD test, $p < 0.05$, Fig. 5a). Critically, any changes in the relative abundance of phyla were practically eliminated after NIV treatment (Fig. 5a). Ternary plots indicated shared bacterial OTUs and abundance comparisons between NC, HF, and NIV groups. These results showed that multiple OTUs belonged to Firmicutes, which were enriched in HF rat gut microbiota in the short-term periods (Fig. 5b). However, multiple OTUs belonging to Bacteroidota were identified at the bottom of the plot, suggesting gut microbiota similarities between NC and NIV-treated rats (Fig. 5b). In the long-term periods, the most altered and abundant phyla from the short-term periods had not changed significantly, except for Proteobacteria (Fig. 5c). Additionally, in the long-term, most bacterial OTUs in the center and bottom of the ternary plot were enriched in HF rats, with only a few low-abundance bacterial OTUs (Fig. 5d).

3.5. Gut microbiota co-occurrence networks

Gut microbiota co-occurrence networks across different treatments for the short- and long-term periods were constructed (Fig. 6a and b, respectively). Based on topological parameters, all networks followed non-random distribution patterns and exhibited small-world characteristics (Table 1). In both periods, network nodes were lower for HF rats than NC rats but returned to normal in NIV networks (Table 1). In contrast, the change trend in edge number was completely opposite to the node number, indicating a higher degree in HF rats but a lower degree in NIV rats when compared with NC animals (Table 1). Moreover, network modularity, from high to low, was NIV, NC, and HF (Table 1). These results identified more complex co-occurrence patterns in HF gut microbiota, while changes reverted to normal levels after NIV treatment. Moreover, cohesion was compared to assess network stability alterations in gut microbiota across different treatments. Significantly lower network cohesion was identified in the HF group when compared with NC and NIV groups in the short-term period (Tukey's HSD test, $p < 0.05$, Fig. 6c). In contrast, no significant variations were identified in cohesion networks across different treatments in long-term periods (Tukey's HSD test, $p > 0.05$, Fig. 6d).

3.6. Assembly mechanisms in gut microbiota

The relative importance of niches and neutral processes in community assemblies was analyzed to explore mechanisms underlying observed spatial-temporal patterns. From the neutral community model results, 78.1 %, 59.0 %, and 74.4 % of the gut microbiota in rats conformed to the neutral-based theory in rats in the short term (Fig. 7a). In the long-term periods, many of the relationships between frequently occurring OTUs and associated relative abundance variations were successfully estimated using the neutral

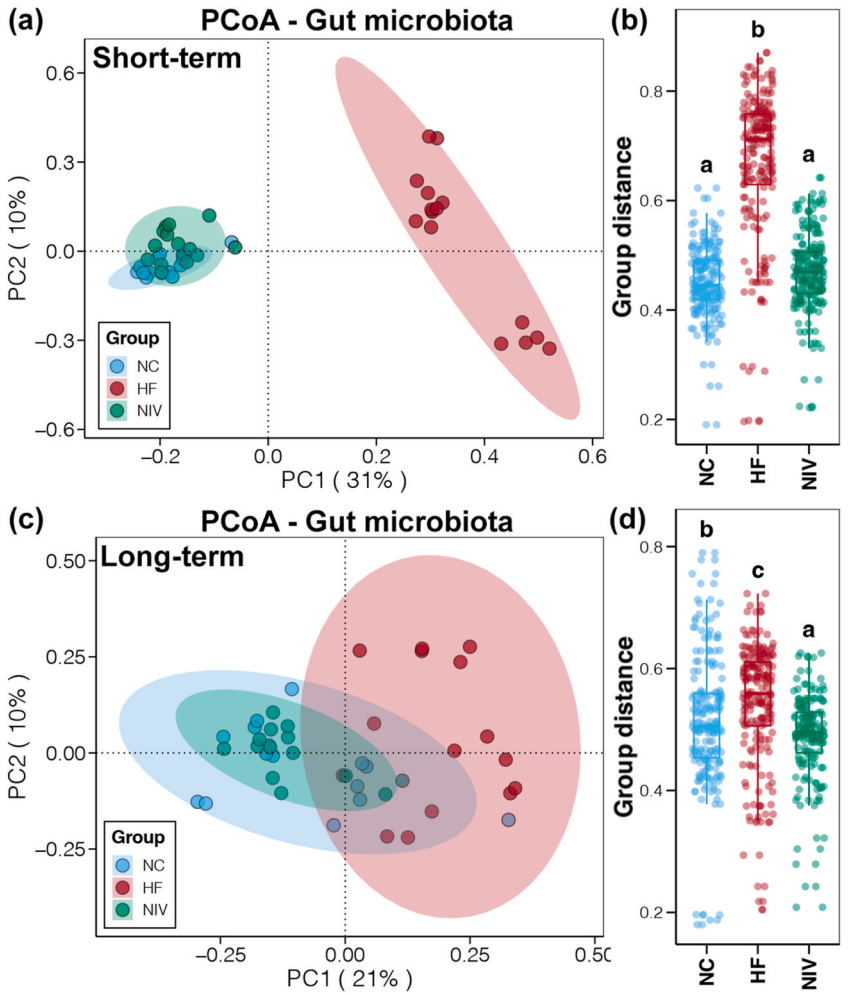


Fig. 4. Principal coordinate analysis (PCoA) based on Bray-Curtis distances in gut microbiota across different treatments for the short- (a) and long- (b) term periods, respectively. Bray-Curtis distance differences in gut microbiota across different treatments in the short- (c) and long- (d) term periods, respectively. Significant differences in samples across treatments are indicated by different lowercases letters above boxes in the same sub-figure (Tukey's HSD test, $p < 0.05$).

community model. Of these, the explained proportion of the neutral community model was highest in NIV, followed by NC, and lowest for HF samples (Fig. 7b). Thus, HF reduced the contribution of stochastic processes on rat gut microbiota assembly, while NIV reverted assemblies back to normal levels.

4. Discussion

4.1. Gut microbiota dysbiosis in rats with acute HF

In animals, changes in gut microbiota communities can indicate a wide variety of health conditions. Gut microbiota α-diversity reductions often cause a poor host-health status and are manifested as weaker resistance to potentially harmful factors [33]. Previous studies reported decreased gut microbiota diversity in patients with diverse diseases [34–36], including those with HF [37], in agreement with the results of this study. Additionally, in the present study, the abundance of some major bacterial taxa in rat gut microbiota was altered by acute HF. It is widely accepted that host intestinal homeostasis is highly influenced by Firmicutes/Bacteroidetes (F/B) ratios in gut microbiota [38]. Increased F/B ratios in the gut microbiota of rats with acute HF were identified in this study. Similar result have also been found in patients with cardiovascular disease [39]. Increased F/B ratios are usually observed in obese individuals and reflects lower acetate and propionate, and higher butyrate production levels [40]. In recent years, the relationship between obesity and the risk of cardiovascular disease has been recognized [41]. The results of this study further suggest that cardiovascular disease may also be a possible cause of obesity. In addition to Firmicutes, specific gut microbial species belonging to Proteobacteria were reportedly associated with HF [42]. In this study, more abundant Proteobacteria levels are detected in HF rats

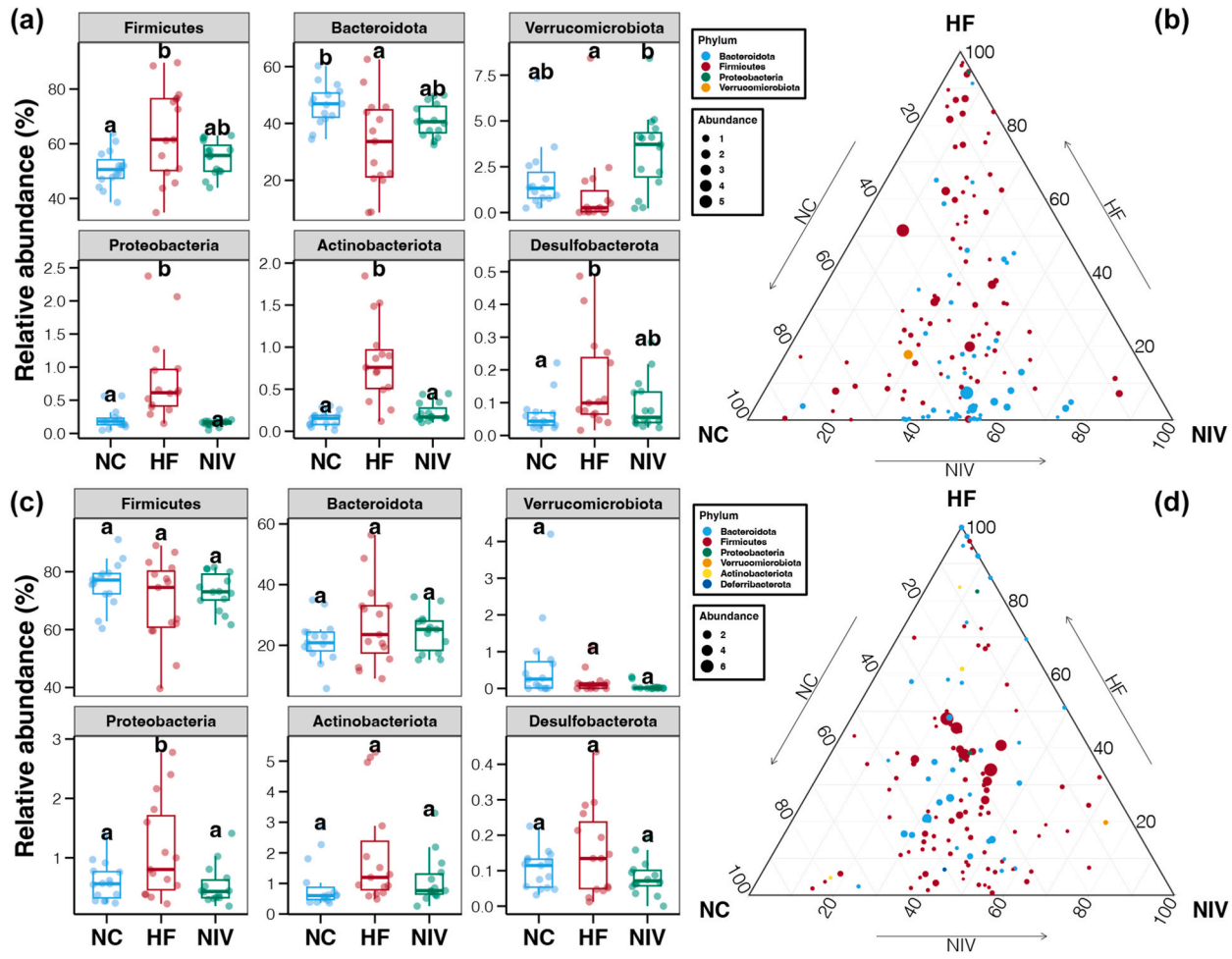


Fig. 5. Variations in relative phyla abundance in the gut microbiota across different treatments in the short- (a) and long- (c) term periods, respectively. Significant differences in samples across different treatments are indicated by different lowercases letters above boxes in the same sub-figure (Tukey's HSD test, $p < 0.05$). Ternary plots show the relative occurrence of individual OTU's in gut microbiota across different treatments over the short- (b) and long- (d) term periods.

when compared with NC animals. Proteobacteria contain multiple pathogens (e.g., *Escherichia*), while Proteobacteria blooms are often linked to inflammatory bowel disease, metabolic syndrome, and some extraintestinal diseases [43,44]. Taken together, the results of this study show that acute HF induces gut microbiota dysbiosis in rats.

4.2. *Determinism and unstable gut microbiota in rats with acute HF*

The examination of co-occurrence networks yielded several insights into the effects of acute HF in mediating complexity and stability in the gut microbiota of rats. Acute HF improved complexity and reduced gut microbiota stability, which suggests that HF acts as a deterministic filtering factor selecting certain bacteria in rat intestinal tracts [45]. More complex co-occurrence pattern have also been found in the gut microbiota of mice with increased risk of cardiovascular disease [46] and Tibetan patients with coronary heart disease [47]. As a result, in individuals with acute HF, taxa that are more interrelated will dominate the gut microbiota, while the proportion of neutral, unrelated taxa will decrease. This shift will lead to the development of more complex network structures. This inference is consistent with the results of neutral community models in the present study, suggesting that many non-neutral taxa accounted for the majority of the gut microbiota in rats with acute HF. Stability is more critical than complexity in the gut microbiota for host health and well-being as it ensures that beneficial symbionts and associated functions are maintained over time [48]. In the present study, network stability clearly decreased with increased network complexity in the gut microbiota of rats with acute HF. Acute HF induced the gut microbiota to form cooperating networks with more links between limited taxa, which may be more efficient in resisting HF stress but may be unstable [49]. In normal individuals, higher gut microbiota diversity introduces species which enhance competition and could stabilize such networks [50]. Moreover, greater stability in the gut microbiota of normal rats could be due to stochastic contributions to gut microbiota assembly [51]. Stochastic gut microbiota assembly could represent a more balanced state,

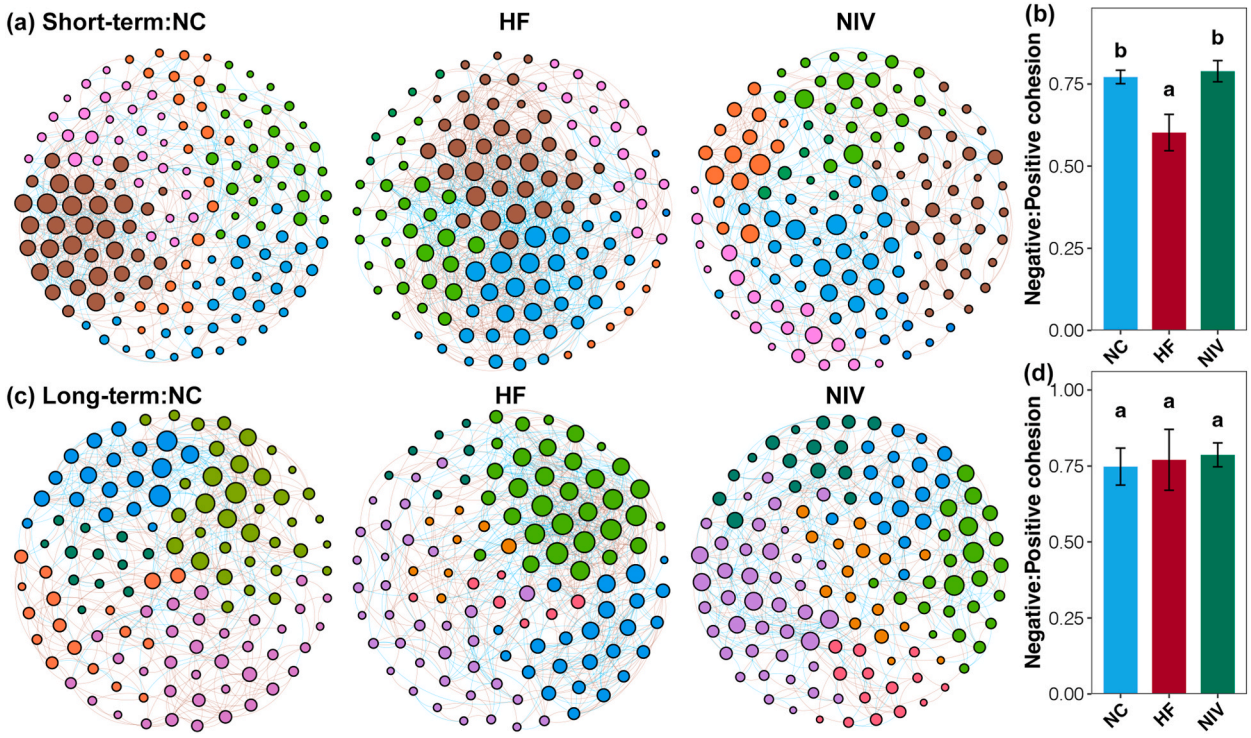


Fig. 6. Co-occurrence networks in gut microbiota across different treatments in the short- (a) and long- (b) term periods, respectively. Nodes belonging to different modules are in different colors. Negative: positive cohesion differences among networks across different treatments in the short- (c) and long- (d) term periods, respectively. Significant differences in samples across different treatments are indicated by different lowercases letters above boxes in the same sub-figure (Tukey's HSD test, $p < 0.05$).

Table 1
Topological parameters of co-occurrence networks of gut microbiota for rats among different treatments in short- and long-term experiments.

	Noses	Edges	Modularity	Average degree	Diameter	Density	Average path length	Clustering coefficient	Power-law model	Small world coefficient
S-NC	149	766	0.442	10.282	6	0.069	2.930	0.518	0.805	3.744
S–HF	127	1038	0.284	16.346	6	0.130	2.248	0.509	0.890	2.156
S–NIV	134	547	0.477	8.164	8	0.061	3.283	0.424	0.818	3.800
L-NC	120	604	0.424	10.067	6	0.085	2.772	0.453	0.837	3.416
L-HF	114	812	0.360	14.247	6	0.126	2.589	0.586	0.822	2.562
L-NIV	134	547	0.477	8.164	8	0.061	3.283	0.424	0.818	3.800

which may enhance gut microbiota resistance to external stresses [52].

4.3. NIV treatment ameliorates gut microbiota dysbiosis in rats with HF

In healthy individuals, the gut microbiota tends to form a fixed and stable state for physiological function [53], whereas a transient perturbation can push the gut microbiota to a transient alternative state [54]. When perturbations disappear, two possible outcomes may occur. Microbial communities in some ecosystems may return to their initial state after a few days, however, incomplete recovery may lead to alternative stable states [55]. Such alternative states are frequently observed in gut microbiota after pathogen invasion [56], antibiotic treatments [57], and dietary interventions [58]. However, in the present study, after 3 days, the gut microbiota reverted to an NC-like state after NIV. For longer periods, HF symptoms were alleviated due to the establishment of collateral circulation. The gut microbiota in HF rats also gradually changed to an NC state, while NIV-treated gut microbiota remained stable for a long time in the NC-like state. This finding suggested that NIV treatment reversed the gut microbiota dysbiosis induced by acute HF in rats and showed long-term effectiveness. Resilience, the ability of the gut microbiota to withstand and recover from perturbations, is crucial for gut health and overall well-being in hosts [59]. The diverse and stable microbial communities in a resilient microbiota can outcompete pathogens and maintain a protective barrier against infections [60]. In addition, by maintaining a stable and diverse microbial composition, a resilient gut microbiota can support various physiological processes, including nutrient metabolism, immune regulation, and gut barrier function [61]. As a strategy to promote gut microbiota resilience, NIV treatment may have broad clinical

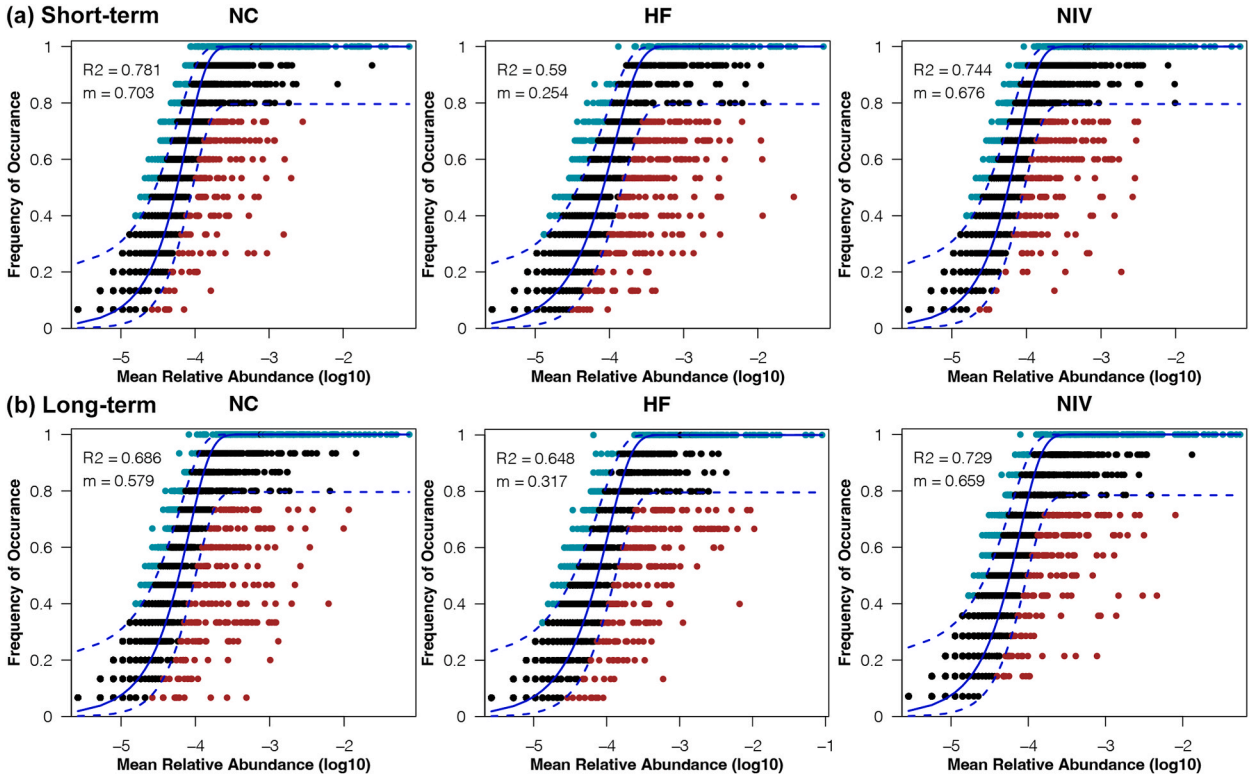


Fig. 7. Neutral community rat gut microbiota models across different treatments in the short- (a) and long- (b) term periods, respectively.

and nursing applicability.

4.4. Limitations and strengths

The experimental setup is critical factor directly affecting study scope and applicability. In this study, variations in the gut microbiota of rats over short- (1 week) and long-term periods (3 months) were examined in three sampling points per each period. The results indicated rapid and significant changes in the gut microbiota of rats, which were subsequently stably maintained over time. In future studies, examining more time points at the beginning of acute HF and NIV treatment, such as change within 24 h, might provide a more detailed view. In this study, acute HF was a strong selective factor, and rat gut microbiota variations were determined by this. Additionally, the present study involved feeding rats in a laboratory, with study individuals having the same genetic background and survival conditions. This may be why good resilience was observed in rat gut microbiota after NIV treatment. However, in clinical environments, responses to perturbations are very different from one person to the next [62] Thus, incomplete recovery may be observed in the gut microbiota of patients with acute HF after NIV, similar to the results from patients with HF treated with left ventricular assist devices or heart transplantation [63]. In addition, although some valuable insights were obtained in this study, the methods allowed us to analysis only the diversity and composition of the gut microbiota [64]. To examine the functional implications of the gut microbiota and potential interaction mechanisms in rats with acute HF and treated with NIV, further investigations integrating metagenomic or metatranscriptomic analyses will be necessary [65]. Moreover, a coronary artery ligation model in Wistar rats was used in this study; however, studies using other models might yield different results. Validating the findings of this study in different model systems or clinical samples in the future could increase the generalizability and applicability of these findings.

4.5. Significance and implications

The gut microbiota plays a major role in the pathogenesis and progression of acute HF. In patients with acute HF, splanchnic hypoperfusion causes intestinal ischemia and edema, thus increasing intestinal permeability [66]. Consequently, bacterial translocation and entry of bacterial metabolites into the bloodstream result in local and systemic inflammatory responses that exacerbate HF [67]. Investigating the gut microbiota in rats with acute HF may reveal its crucial role in disease pathogenesis, such as decreased diversity, increased F/B ratios, and decreased stability. Moreover, targeting the gut microbiota may serve as a potential therapeutic strategy for acute HF. Approaches including dietary interventions, prebiotics, probiotics, and fecal microbiota transplantation may help restore a healthy gut microbiome and decrease the production of harmful metabolites [68]. Thus, the results of present study may aid in the development of both microbiome-based diagnostics and therapeutics for acute HF. Additionally, the findings of this study

further expand the implications of NIV in treating acute HF. NIV, delivered as non-invasive pressure support ventilation, compared with conventional oxygen therapy, can rapidly ameliorate respiratory distress and decrease the need for endotracheal intubation [18]. Previous studies have explored several advantages of NIV, including amelioration of respiratory distress, decreased intubation rates, and potentially decrease mortality [69]. In this study, the previously poorly understood effects of NIV on restoring gut microbiota dysbiosis were revealed, which further supporting NIV as a valuable non-invasive tool in the management of acute HF.

5. Conclusions

This study examined the efficacy of NIV in treating HF and provided perspectives on the roles of acute HF and NIV in shaping the gut microbiota in rats. Gut microbiota richness and diversity were significantly decreased under acute HF stress, while composition varied significantly and was represented by increased F/B ratios and Proteobacteria levels. Additionally, animals with acute HF were shown to have a less stable and deterministic dominant gut microbiota. More importantly, acute HF was the direct cause of gut microbiota dysbiosis in rats, while NIV restored dysbiosis to a normal state. These findings contribute key insights into the implications of HF in gut microbiota dysbiosis, and highlights the use of NIV for treating acute HF.

Abbreviations

Abbreviations	Full name
HF	Heart failure
NIV	Non-invasive ventilation
F/B	Firmicutes/Bacteroidetes
NC	Normal control
H&E	Hematoxylin and eosin
PCR	Polymerase chain reaction
OTU	Operational taxonomic units
HSD	Honest significant difference
PCoA	Principal coordinate analysis

Funding

This research was funded by the Self-Funded Project by Tianjin Municipal Health Commission, grant number ZC20127 and the Key Projects of Tianjin Science and Technology Bureau, grant number 22JCZDJC00580.

Institutional review board statement

The animal study protocol was approved by the Ethics Committee of Affiliated Chest Hospital of Tianjin University (protocol code TJCH-2023-022 and 2023-5-4 of approval).

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories of NCBI SRA and accession number (PRJNA1084188) can be found below: https://www.ncbi.nlm.nih.gov/bioproject.

CRediT authorship contribution statement

He Jiang: Writing – review & editing, Validation, Investigation, Conceptualization. **Shan Liu:** Writing – original draft, Validation, Software, Formal analysis. **Chao Chang:** Validation. **Yanwen Shang:** Resources. **Jie Geng:** Visualization, Conceptualization. **Qingliang Chen:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We thank Zelong Zhao (Biozeron Co. Ltd, Shanghai, China) for the high-throughput sequencing and the support in data analysis.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e35239.

References

[1] G. Savarese, L.H. Lund, Global public health burden of heart failure. Cardiac Fail, Rev 3 (1) (2017) 7.
[2] I. Ekman, J.G. Cleland, B. Andersson, K. Swedberg, Exploring symptoms in chronic heart failure, Eur. J. Heart Fail. 7 (5) (2005) 699–703.
[3] A.J. Coats, What causes the symptoms of heart failure? Heart 86 (5) (2001) 574–578.
[4] C.W. Tsao, A.W. Aday, Z.I. Almarzooq, et al., Heart disease and stroke statistics—2022 update: a report from the American Heart Association, Circulation 145 (8) (2022) e153–e639.
[5] Y. Guo, G.Y. Lip, A. Banerjee, Heart failure in east asia, Curr. Card. Rev. 9 (2) (2013) 112–122.
[6] C.J. Taylor, J.M. Ordóñez-Mena, A.K. Roalfe, et al., Trends in survival after a diagnosis of heart failure in the United Kingdom 2000-2017: population based cohort study, BMJ (2019) 364.
[7] Y. Fan, O. Pedersen, Gut microbiota in human metabolic health and disease, Nat. Rev. Microbiol. 19 (1) (2021) 55–71.
[8] E. Rinninella, P. Raoul, M. Cintoni, et al., What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet, and diseases, Microorganisms 7 (1) (2019) 14.
[9] M. Witkowski, T.L. Weeks, S.L. Hazen, Gut microbiota and cardiovascular disease, Circ. Res. 127 (4) (2020) 553–570.
[10] M. Rahman, F. Islam, M. Or-Rashid, et al., The gut microbiota (microbiome) in cardiovascular disease and its therapeutic regulation, Front. Cell. Infect. Microbiol. 713 (2022) 903570.
[11] A.L. Beale, J.A. O'Donnell, M.E. Nakai, et al., The gut microbiome of heart failure with preserved ejection fraction, J. Am. Heart Assoc. 10 (13) (2021) e020654.
[12] J. Qin, Y. Li, Z. Cai, et al., A metagenome-wide association study of gut microbiota in type 2 diabetes, Nature 490 (7418) (2012) 55–60.
[13] X. Cui, L. Ye, J. Li, et al., Metagenomic and metabolomic analyses unveil dysbiosis of gut microbiota in chronic heart failure patients, Sci. Rep. 8 (1) (2018) 635.
[14] L. Chen, S. Li, L. Ai, et al., The correlation between heart failure and gut microbiome metabolites, Infect. Microb. Dis. 2 (4) (2020) 136.
[15] M. Arrigo, M. Jessup, W. Mullens, et al., Acute heart failure, Nat. Rev. Dis. Primers 6 (1) (2020) 16.
[16] P. Abete, G. Testa, D. Della-Morte, et al., Treatment for chronic heart failure in the elderly: current practice and problems, Heart Fail. Rev. 18 (2013) 529–551.
[17] P. Rossignol, A.F. Hernandez, S.D. Solomon, F. Zannad, Heart failure drug treatment, Lancet 393 (10175) (2019) 1034–1044.
[18] J. Masip, Noninvasive ventilation in acute heart failure, Curr. Heart Fail. Rep. 16 (2019) 89–97.
[19] R. Boldrini, L. Fasano, S. Nava, Noninvasive mechanical ventilation, Curr. Opin. Crit. Care 18 (1) (2012) 48–53.
[20] V.V. Lupu, A. Raileanu, C.M. Mihai, et al., The implication of the gut microbiome in heart failure, Cells 12 (8) (2023) 1158.
[21] A.T. Chen, J. Zhang, Y. Zhang, Gut microbiota in heart failure and related interventions, iMeta 2 (3) (2023) e125.
[22] M. Trøseid, G.Ø. Andersen, K. Broch, J.R. Hov, The gut microbiome in coronary artery disease and heart failure: current knowledge and future directions, EBioMedicine (2020) 52.
[23] S. Liu, H. Jiang, C. Chang, et al., Effects and mechanism of noninvasive positive-pressure ventilation in a rat model of heart failure due to myocardial infarction, Med. Sci. Monit. 27 (2021) e928476, 1.
[24] H. Jang, S. Liu, C. Chang, J. Geng, Evolution of intestinal flora in naturally aging rats, Chinese J. Front. Med. Sci. 15 (3) (2023) 46–52.
[25] J. Berg, K.K. Brandt, W.A. Al-Soud, et al., Selection for Cu-tolerant bacterial communities with altered composition, but unaltered richness, via long-term Cu exposure, Appl. Environ. Microbiol. 78 (2012) 7438–7446.
[26] Y. Hou, R. Jia, B. Li, J. Zhu, Apex predators enhance environmental adaptation but reduce community stability of bacterio-plankton in crustacean aquaculture ponds, Int. J. Mol. Sci. 23 (2022) 10785.
[27] N.A. Bokulich, S. Subramanian, J.J. Faith, et al., Quality-filtering vastly improves diversity estimates from illumina amplicon sequencing, Nat. Methods 10 (2013) 57–59.
[28] J.G. Caporaso, J. Kuczynski, J. Stombaugh, et al., QIIME allows analysis of high-throughput community sequencing data, Nat. Methods 7 (5) (2010) 335–336.
[29] P. Yilmaz, L.W. Parfrey, P. Yarza, et al., The SILVA and "all-species living tree project (LTP)" taxonomic frameworks, Nucleic Acids Res. 42 (2014) D643–D648.
[30] M. Bastian, M. Jacomy, Gephi: an Open Source Software for Exploring and Manipulating Networks, International AAAI Conference on Weblogs and Social Media, San Jose, CA, USA, 2009.
[31] M.M. Yuan, X. Guo, L. Wu, et al., Climate warming enhances microbial network complexity and stability, Nat. Clim. Change 11 (4) (2021) 343–348.
[32] W.T. Sloan, M. Lunn, S. Woodcock, I.M. Head, S. Nee, T.P. Curtis, Quantifying the roles of immigration and chance in shaping prokaryote community structure, Environ. Microbiol. 8 (2006) 732–740.
[33] T.D. Lawley, A.W. Walker, Intestinal colonization resistance, Immunology 138 (1) (2013) 1–11.
[34] J.U. Scher, C. Ubeda, A. Artacho, et al., Decreased bacterial diversity characterizes the altered gut microbiota in patients with psoriatic arthritis, resembling dysbiosis in inflammatory bowel disease, Arthritis Rheumatol. 67 (1) (2015) 128–139.
[35] S.R. Sharpton, V. Ajmera, R. Loomba, Emerging role of the gut microbiome in nonalcoholic fatty liver disease: from composition to function, Clin. Gastroenterol. H. 17 (2) (2019) 296–306.
[36] S. Gu, Y. Chen, Z. Wu, et al., Alterations of the gut microbiota in patients with coronavirus disease 2019 or H1N1 influenza, Clin. Infect. Dis. 71 (10) (2020) 2669–2678.
[37] D.M. Simadibrata, S. Auliani, P.A. Widyastuti, et al., The gut microbiota profile in heart failure patients: a systematic review, J. Gastrointestin. Liver Dis. 32 (3) (2023) 393.
[38] S. Stojanov, A. Berlec, B. Štrukelj, The influence of probiotics on the Firmicutes/Bacteroidetes ratio in the treatment of obesity and inflammatory bowel disease, Microorganisms 8 (11) (2020) 1715.
[39] H.J. Tsai, W.C. Tsai, W.C. Hung, et al., Gut microbiota and subclinical cardiovascular disease in patients with type 2 diabetes mellitus, Nutrients 13 (8) (2021) 2679.
[40] F. Magne, M. Gotteland, L. Gauthier, et al., The Firmicutes/Bacteroidetes ratio: a relevant marker of gut dysbiosis in obese patients? Nutrients 12 (5) (2020) 1474.
[41] K.C. Zalesin, B.A. Franklin, W.M. Miller, E.D. Peterson, P.A. McCullough, Impact of obesity on cardiovascular disease, Endocrinol. Metabol. Clin 37 (3) (2008) 663–684.
[42] N. Kazemian, M. Mahmoudi, F. Halperin, J.C. Wu, S. Pakpour, Gut microbiota and cardiovascular disease: opportunities and challenges, Microbiome 8 (1) (2020) 1–17.
[43] P.H. Bradley, K.S. Pollard, Proteobacteria explain significant functional variability in the human gut microbiome, Microbiome 5 (1) (2017) 1–23.
[44] G. Rizzatti, L.R. Lopetuso, G. Gibiino, C. Binda, A. Gasbarrini, Proteobacteria: a common factor in human diseases, BioMed Res. Int. 2017 (2017) 9351507.
[45] M.I. McBurney, C. Davis, C.M. Fraser, et al., Establishing what constitutes a healthy human gut microbiome: state of the science, regulatory considerations, and future directions, J. Nutr. 149 (11) (2019) 1882–1895.
[46] F. Liu, Z. Li, X. Wang, C. Xue, Q. Tang, R.W. Li, Microbial co-occurrence patterns and keystone species in the gut microbial community of mice in response to stress and chondroitin Sulfate disaccharide, Int. J. Mol. Sci. 20 (9) (2019) 2130.
[47] F. Liu, C. Fan, L. Zhang, Alterations of gut microbiome in Tibetan patients with coronary heart disease, Front. Cell. Infect. Microbiol. 10 (2020) 373.
[48] C.A. Lozupone, J.I. Stombaugh, J.I. Gordon, J.K. Jansson, R. Knight, Diversity, stability and resilience of the human gut microbiota, Nature 489 (7415) (2012) 220–230.
[49] K.Z. Coyte, J. Schluter, K.R. Foster, The ecology of the microbiome: networks, competition, and stability, Science 350 (6261) (2015) 663–666.
[50] F. Kokou, G. Sasson, J. Friedman, et al., Core gut microbial communities are maintained by beneficial interactions and strain variability in fish, Nat. Microbiol. 4 (12) (2019) 2456–2465.
[51] J.M. Chase, Stochastic community assembly causes higher biodiversity in more productive environments, Science 328 (5984) (2010) 1388–1391.
[52] Z. Zhao, J. Jiang, J. Zheng, et al., Exploiting the gut microbiota to predict the origins and quality traits of cultured sea cucumbers, Environ. Microbiol. 24 (9) (2022) 3882, 2897.

[53] M. Fassarella, E.E. Blaak, J. Penders, Gut microbiome stability and resilience: elucidating the response to perturbations in order to modulate gut health, Gut 70 (3) (2021) 595–605.
[54] E.K. Costello, K. Stagaman, L. Dethlefsen, B.J.M. Bohannan, D.A. Relman, The application of ecological theory toward an understanding of the human microbiome, Science 336 (2012) 6086.
[55] K. Faust, L. Lahti, D. Gonze, W.M. De Vos, J. Raes, Metagenomics meets time series analysis: unraveling microbial community dynamics, Curr. Opin. Microbiol. 25 (2015) 56–66.
[56] L.A. David, A.C. Materna, J. Friedman, et al., Host lifestyle affects human microbiota on daily timescales, Genome Biol. 15 (2014) R89.
[57] L. Dethlefsen, D.A. Relman, Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 4554–4561.
[58] L.A. David, C.F. Maurice, R.N. Carmody, et al., Diet rapidly and reproducibly alters the human gut microbiome, Nature 505 (2013) 559–563.
[59] F. Sommer, J.M. Anderson, R. Bharti, J. Raes, P. Rosenstiel, The resilience of the intestinal microbiota influences health and disease, Nat. Rev. Microbiol. 15 (2017) 630–638.
[60] M. Cipelli, E.M. da Silva, N.O.S. Câmara, Gut microbiota resilience mechanisms against pathogen infection and its role in inflammatory bowel disease, Curr. Clin. Microbiol. Rep. 10 (2023) 187–197.
[61] S.K. Dogra, J. Doré, S. Damak, Gut microbiota resilience: definition, link to health and strategies for intervention, Front. Microbiol. 11 (2020) 572921.
[62] A. Salonen, L. Lahti, J. Salojarvi, et al., Impact of diet and individual variation on intestinal microbiota composition and fermentation products in obese men, ISME J. 8 (2014) 2218–2230.
[63] M. Yuzefpolskaya, B. Bohn, M. Nasiri, et al., Gut microbiota, endotoxemia, inflammation, and oxidative stress in patients with heart failure, left ventricular assist device, and transplant, J. Heart Lung Transpl 39 (9) (2020) 880–890.
[64] F. Finotello, E. Mastrorilli, B. Di Camillo, Measuring the diversity of the human microbiota with targeted next-generation sequencing, Brief. Bioinform. 19 (4) (2018) 679–692.
[65] E.A. Franzosa, X.C. Morgan, N. Segata, et al., Relating the metatranscriptome and metagenome of the human gut, Proc. Natl. Acad. Sci. U. S. A. 111 (22) (2014) E2329–E2338.
[66] Y. Zhang, Y. Wang, B. Ke, J. Du, TMAO: how gut microbiota contributes to heart failure, Transl. Res. 228 (2021) 109–125.
[67] W.W. Tang, S.L. Hazen, Dietary metabolism, gut microbiota and acute heart failure, Heart 102 (2016) 813–814.
[68] V.V. Lupu, A. Adam Raileanu, C.M. Mihai, et al., The implication of the gut microbiome in heart failure, Cells 12 (2023) 1158.
[69] B. Deniau, M.R. Costanzo, K. Sliwa, A. Asakage, W. Mullens, A. Mebazaa, Acute heart failure: current pharmacological treatment and perspectives, Eur. Heart J. 44 (2023) 4634–4649.