ORIGINAL ARTICLE

Changes in the gut microbiota during and after commercial helium–oxygen saturation diving in China

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ABSTRACT

Objectives The influence of commercial helium–oxygen saturation diving on divers' gut microbiotas was assessed to provide dietary suggestion.

Methods Faecal samples of 47 divers working offshore were collected before (T1), during (T2) and after (T3) saturation diving. Their living and excursion depths were 55–134 metres underwater with a saturation duration of 12–31 days and PaO_2 of 38–65 kPa. The faecal samples were examined through 16S ribosomal DNA amplicon sequencing based on the Illumina sequencing platform to analyse changes in the bacteria composition in the divers' guts.

Results Although the α and β diversity of the gut microbiota did not change significantly, we found that living in a hyperbaric environment of helium–oxygen saturation decreased the abundance of the genus *Bifidobacterium*, an obligate anaerobe, from 2.43%±3.83% at T1 to 0.79%±1.23% at T2 and 0.59%±0.79% at T3. Additionally, the abundance of some short-chain fatty acid (SCFA)-producing bacteria, such as *Fusicatenibacter, Faecalibacterium*, rectale group and *Anaerostipes*, showed a decreased trend in the order of before, during and after diving. On the contrary, the abundance of species, such as *Lactococcus garvieae*, *Actinomyces odontolyticus*, *Peptoclostridium difficile*, *Butyricimonas virosa*, *Streptococcus mutans*, *Porphyromonas asaccharolytica* and *A. graevenitzii*, showed an increasing trend, but most of them were pathogens.

Conclusions Occupational exposure to high pressure in a helium–oxygen saturation environment decreased the abundance of *Bifidobacterium* and some SCFA-producing bacteria, and increased the risk of pathogenic bacterial infection. Supplementation of the diver diet with probiotics or prebiotics during saturation diving might prevent these undesirable changes.

Key messages

What is already known about this subject?

- Saturation divers are exposed to a confined, hyperoxic and hyperbaric environment for a relatively long time, which is found to disrupt physiological and metabolic homoeostasis, and may impact human health and performance.
- The appropriate diet has the potential to alleviate many of these physiological and metabolic concerns, and enhance the health and performance of saturation divers.

What are the new findings?

- Helium–oxygen saturation diving at up to 134 metres of depth had little effect on the α and β diversity of the divers' gut microbiotas. However, the abundance of the genus *Bifidobacterium* and some short-chain fatty acid (SCFA)-producing bacteria decreased, and that of some pathogenic bacteria increased during and after diving, suggested that saturation diving may affect the gut microbiota homoeostasis and increased the risk of pathogen infection.

How might this impact on policy or clinical practice in the foreseeable future?

- A diet supplemented with probiotics, such as species from *Bifidobacterium*, or prebiotics that can stimulate gut *Bifidobacterium* and SCFA-producing bacteria might promote the health of saturation divers.

INTRODUCTION

Saturation diving, a form of diving that allows greater depths to be attained for longer periods than in other forms of diving, is currently widely used to accomplish long-term subsea work. During saturation diving assignments, divers remain within a pressurised chamber filled with mixed inert gas (helium is often used) and oxygen for extended periods (usually up to 28 days). Chronic exposure to this confined, hyperoxic and hyperbaric environment appears to disturb physiological and metabolic homoeostasis and has been associated with a range of adverse health and performance-related outcomes, including disturbing fluid balance, redox homoeostasis, immunological function and haematological variables.[1–6] Furthermore, saturation diving raises the energy demand, thus leading to a significant reduction in body mass (especially muscle mass). Appropriate dietary strategies have the potential to alleviate many of these physiological concerns. However, the current dietary guidelines only consider energy balance, macronutrient composition, fluid and micronutrient requirements,[7] and the influence of saturation diving on divers' health requires further study to provide more information about the ideal dietary standards.

The human gut is a complicated ecosystem comprised of a diverse and abundant (10^{14} cells) microbial community, including bacteria, archaea and eukaryotes, that live in an intimate relationship with the host.[8] Over the last 15 years, our understanding of the composition and functions of the human gut microbiota has greatly increased,

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largely driven by the rapid improvement and wide availability of next-generation sequencing-based analysis techniques. An abnormal gut microbiota profoundly influences many aspects of host health beyond classical infectious diseases and may induce metabolic syndrome, obesity-related disease, liver disease, inflammatory bowel disease and colorectal cancer.[8–12] Neurodegenerative disease and neuropsychiatric disorders are also linked to gut microbiota likely through microbiota-derived metabolite signals.[13 14] However, the influence of saturation diving on the diver gut microbiota and the related health concerns are still unknown.

The human gut microbiota is composed of a core and a variable commensal community, which is modified by recent diet and environmental exposure.[15] Many microorganisms that inhabit the gastrointestinal tract are anaerobic bacteria. During a deep saturation dive, divers have to live in a hyperoxia environment for up to about a month, where the PaO_2 is usually 40–60 kPa, double to triple of that in normal conditions, which may affect the composition of the diver's gut microbiota. Therefore, the present study was designed to assess the influence of commercial helium–oxygen saturation diving on the diver gut microbiota. Since diet can rapidly and reproducibly alter gut microbiota,[16 17] unwanted changes may be counteracted by modifying divers' diets in the future.

METHODS

Study population

A total of 24 divers were recruited from Shanghai Salvage Ministry of Transport and 23 divers were from Shenzhen DIV Diving Engineering Co. Ltd. They took part in helium–oxygen saturation diving during offshore work from May to August 2018. All the divers were informed about the nature of the study. The divers who could not collect samples at any time point following the instructions were excluded from the study. Information about diver age, height, weight, living place, dietary preference, alcohol drinking habits and health conditions were collected through questionnaires. The basic information about the divers and saturation diving is presented in table 1. Detailed information is presented in online supplementary table 1.

Sample collection

Faecal samples were collected at three time points for each diver: T1, 10 days to 1 day before pressurisation; T2, 10–14 days after pressurisation; and T3, the first defecation after decompression was completed. Stool samples were obtained with a sterilised pipette tip, put into sterilised cryogenic vials and then were quick-frozen and stored in a –80°C freezer within 5 min. At the end of the operation, a batch of stool samples was transported on dry ice to the lab for further processing. The data producing and processing were performed by Gene Denovo Co. Ltd.

Table 1 Basic information about divers and saturation diving

		Shanghai	Shenzhen
Age (years)	Mean	32	34
	Range	26–42	23–46
BMI (kg/m^2)	Mean	25.41	25.39
	Range	22.22–28.08	21.05–29.41
Total diving (years)	Mean	10.88	9.48
	Range	6–21	5–20
Saturation duration (days)	Mean	27.13	23.86
	Range	21–31	12–31
Living	Depth (metres)	82–107	55–114
	PaO_2 (kPa)	38–42	44–48
Excursion	Depth (metres)	92–117	70–134
	PaO_2 (kPa)	58–62	50–65

BMI, body mass index.

Genomic DNA extraction, library construction and sequencing

Genomic DNA from stool samples was extracted using HiPure Stool DNA Kits (Magen, Guangzhou, China) according to the manual instructions. The 16S ribosomal DNA V3–V4 region of the ribosomal RNA gene was amplified by PCR, using primers 341F: CCTACGGGNGGCWGCAG and 806R: GGACTACHVGGGTATCTAAT, where the eight-base barcode was unique to each sample. PCR reactions were performed using high-fidelity KOD polymerase (Toyobo, Osaka, Japan). Purified amplicons were pooled in equimolar and sequenced using the paired-end strategy (2×250) on the Illumina HiSeq 2500 platform following the standard protocols. The raw reads were deposited into the NCBI Sequence Read Archive database (SRP200145).

Bioinformatics and community comparisons

Quality control and reads assembly

To get high-quality clean reads, raw reads containing more than 10% of unknown nucleotides (N), or containing less than 80% of bases with quality (Q-value) >20, were removed using FASTP (https://github.com/OpenGene/fastp). Paired-end clean reads were merged as raw tags using FLSAH[18] (V.1.2.11) with a minimum overlap of 10 bp and mismatch error rates of 2%. Noisy sequences of raw tags were filtered by QIIME[19] (V.1.9.1) pipeline under specific filtering conditions[20] to obtain the high-quality clean tags. Then, clean tags were searched against the reference database (http://drive5.com/uchime/uchime_download.html) to perform reference-based chimaera checking using the UCHIME algorithm (http://www.drive5.com/usearch/manual/uchime_algo.html). All chimaeric tags were removed to obtain effective tags for further analysis.

Operational taxonomic unit cluster and taxonomy classification

The effective tags were clustered into operational taxonomic units (OTUs) of ≥97% similarity using UPARSE[21] pipeline. The tag sequence with the highest abundance was selected as a representative sequence within each cluster. The representative sequences were classified into organisms by a naive Bayesian model using RDP classifier[22] (V.2.2) based on the SILVA database[23] (https://www.arb-silva.de/), with the confidence threshold values ranging from 0.8 to 1.

Statistical analysis

This was a self-control experiment. For α diversity analysis, Chao1, Simpson and all other α diversity indices were calculated with QIIME. The OTU rarefaction curve and rank abundance curves were plotted in QIIME. The α index comparison between groups was calculated with Welch's t-test and the Wilcoxon rank test. Differences in α index among the three groups were assessed with the Kruskal-Wallis H test.

For β diversity analysis, the weighted UniFrac distance matrix was generated by QIIME, and the Bray-Curtis distance matrix was generated by function vedist in vegan R package. PCoA (principal coordinates analysis) of these two distances was calculated and plotted in Omicsmart, an online bioinformatics analysis platform of Gene Denovo Co. Ltd. (www.omicsmart.com). For statistical comparisons of β diversity among groups, the non-parametric multivariate analysis of variance (MANOVA) methods using the Adonis function in vegan R package were performed.

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Table 2 Illumina sequencing data summary

	Shanghai			Shenzhen		
	T1	T2	T3	T1	T2	T3
Effective tags	83 197±19 139	75 577±18 403	77 089±19 943	85 050±36 948	79 237±35 683	84 965±32 850
Unique tags	40 744±6024	37 566±5290	39 244±7605	36 079±12 643	32 514±12 117	36 502±12 151
Taxon tags	79 849±19 758	72 404±18 759	73 599±20 207	83 177±36 659	77 851±35 285	82 950±31 808
OTUs	574±70	554±57	566±71	447±93	424±90	471±94

Effective tags: high quality tags used for further analysis. Unique tags: the representative sequences for the unequal number of same tags. Taxon tags: the tags were classified into an organism based on the SILVA database.
OTUs, operational taxonomic units; T1, before diving; T2, during diving; T3, after diving.

The bacteria with different abundances among three groups were assessed with the Kruskal-Wallis rank test, and Metastats was used for comparison between groups. The Short Time-series Expression Miner (STEM)[24] was used to analyse the bacteria abundance pattern based on the abundance differential features screened by Metastats.[25] Briefly, in the order of sequential time points, the relative abundance data of each sample were normalised to 0, log2 (T2/T1) and log2 (T3/T1), and then clustered by the STEM, and the clustered profiles with p value<0.05 were considered as significant profiles.

RESULTS

Statistical characteristics of Illumina sequencing data

A total of 47 sets and 141 samples were sequenced. A total of 13 207 604 raw tags (ranging from 33 910 to 167 331 tags/sample) and 13 145 370 clean tags (ranging from 33 580 to 166 639 tags/sample) were obtained. The sequencing data are summarised in table 2. For most samples, the Chao1 value increased slightly with the increase in rarefaction sequences, and the Shannon diversity reached a plateau, indicating that most diversity had been well-captured with the sequencing effort (figure 1A,B).

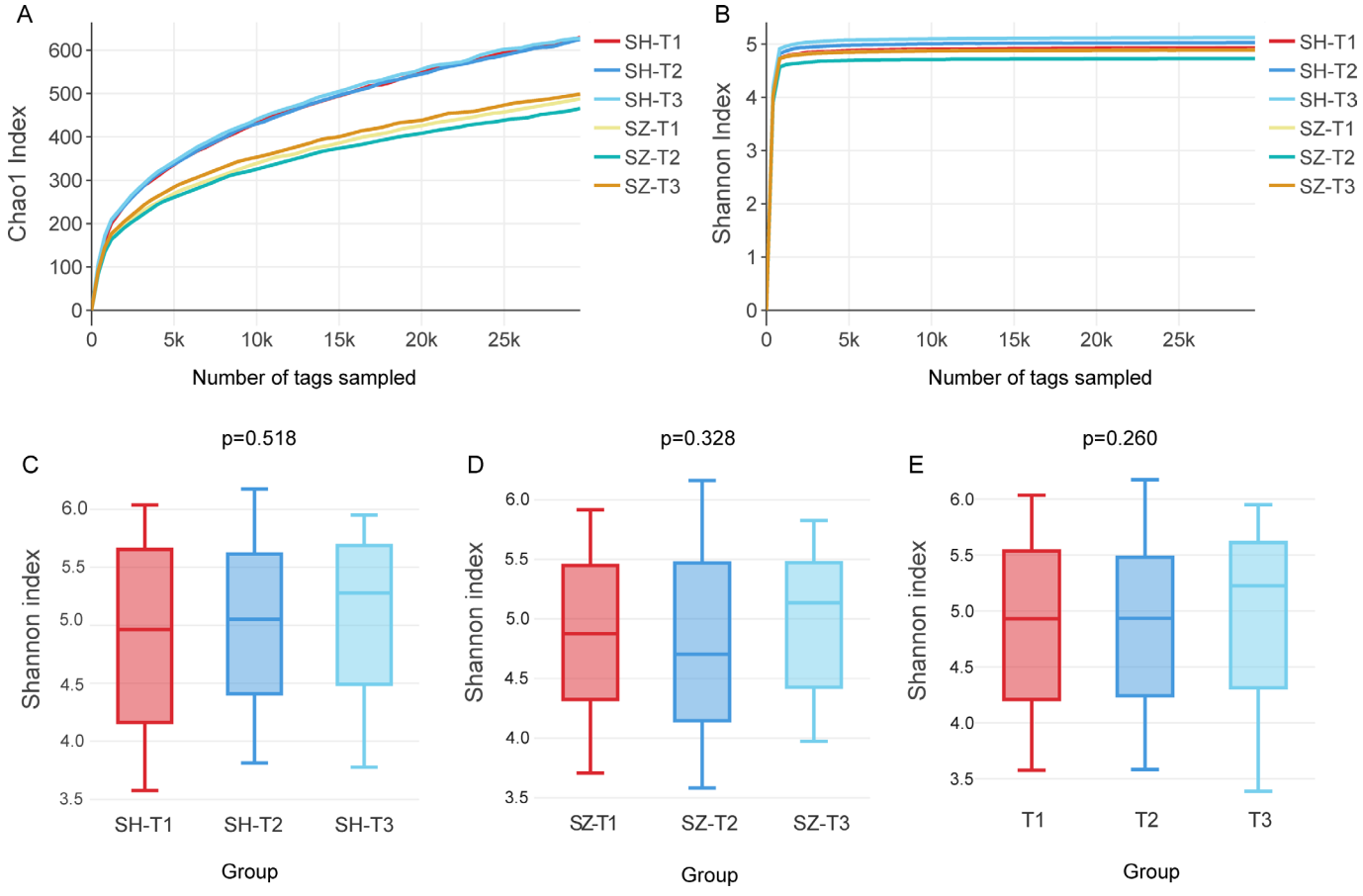

Figure 1 Alpha diversity analysis of gut microbiota of divers. (A and B) Chao1 and Shannon rarefaction curves. The Chao1 value increased marginally with an increase in rarefaction sequences, and the Shannon diversity plateaued. (C–E) Box-plot of Shannon index. Little difference in α diversity was evident at the different time points in Shanghai (C), Shenzhen (D) and pooled data (E). The top and bottom boundaries of each box represent the 75th and 25th quartile values, respectively, and lines within each box indicate the 50th quartile (median) values. Ends of whiskers mark the lowest and highest diversity values in each group. The p value was calculated using the Kruskal-Wallis H test and is indicated in the figure. SH, Shanghai; SZ, Shenzhen; T1, before saturation diving; T2, during saturation diving; T3, after saturation driving.

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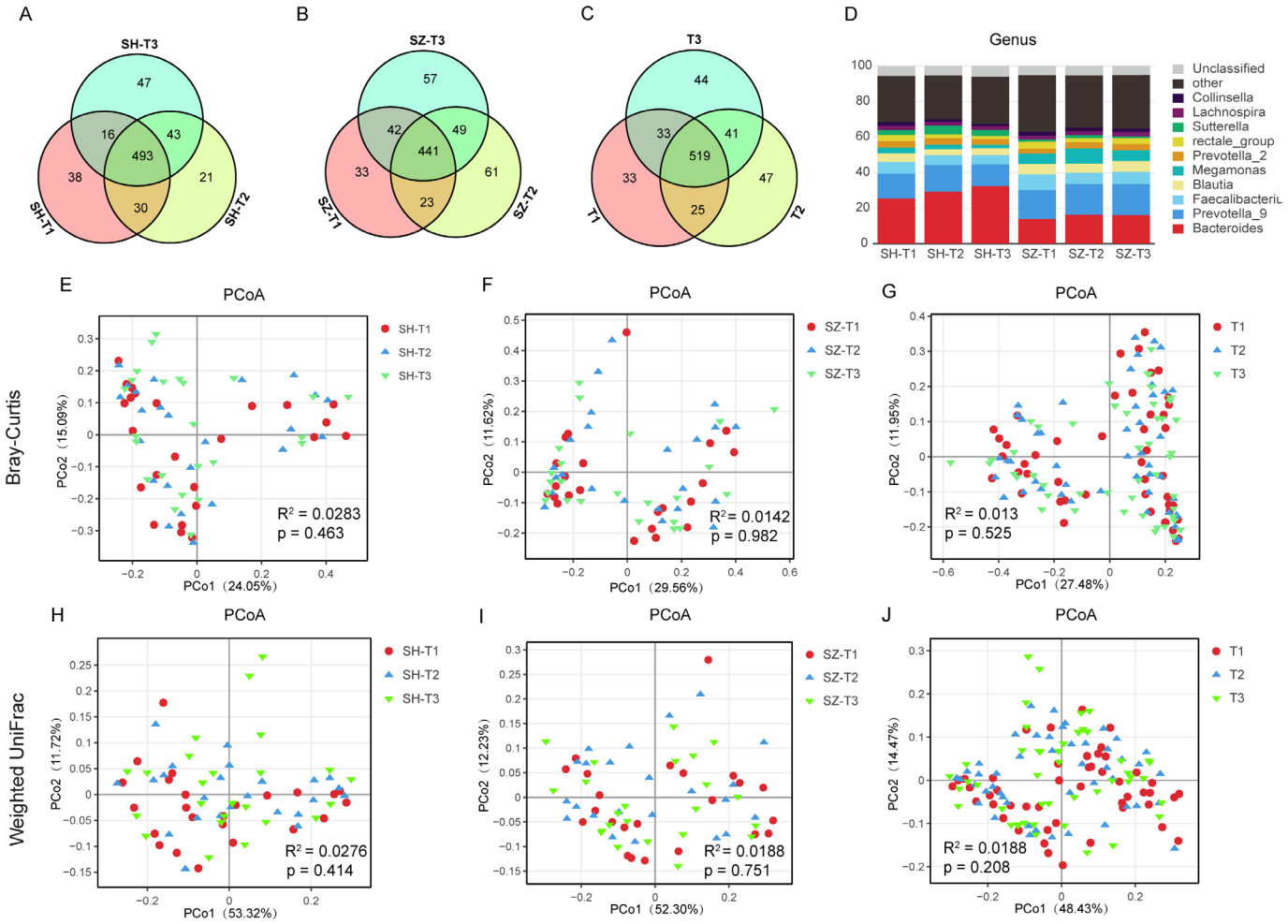

Figure 2 Beta diversity analysis of gut microbiota of divers. (A–C) Venn plots showing the shared and unique bacteria at different time points in Shanghai (A), Shenzhen (B) and pooled samples (C). (D) Gut microbiota composition of each sample group. Relative abundances of the top 10 bacteria at the genus level at different time points and locations are shown. Bacteria in other genera were grouped into the 'other' category, and those that could not be classified into a known genus were grouped into the 'unclassified' category. (E–J) Principal coordinate analysis (PCoA) plots for groups at different time points in Shanghai (E and H), Shenzhen (F and I) and pooled samples (G and J), based on the Bray-Curtis distances (E–G) and weighted UniFrac distances (H–J). Adonis analysis revealed no statistically significant differences. The R^2 and p values are marked on the figure. PCo, principal coordinate; SH, Shanghai; SZ, Shenzhen; T1, before saturation diving; T2, during saturation diving; T3, after saturation driving.

Similarity of the faecal microbial communities before, during and after saturation diving

Reduced biodiversity of the gut microbiota has been reported to be associated with gut microbiota dysbiosis and disease status, such as inflammatory bowel disease and so on.[26] To evaluate the diversity of the gut microbiota within individuals, the α diversity was calculated using the Chao1 index, which indicates the richness, and the Shannon index, which reveals the richness as well as the evenness of the microbial community. No significant differences were found in either of them among the three time points in either Shanghai or Shenzhen group (figure 1).

To evaluate the microbiota diversity among individuals, the β diversity was calculated using PCoA, which indicates a shift in the gut bacterial composition profile along with the time. Since most bacteria were shared among the three time points in both Shanghai and Shenzhen and pooled samples (figure 2A–C), and the gut microbiota structure did not show much change among different time points at both locations either (figure 2D), PCoA was applied based on the Bray-Curtis distance (figure 2E–G) and weighted UniFrac distance (figure 2H–J), which took relative abundance into consideration when calculating the distance. The Adonis results showed no difference in either Shanghai, Shenzhen or pooled samples regardless of Bray-Curtis distance or weighted UniFrac distance.

Decrease in the abundance of the genus *Bifidobacterium* during and after saturation diving

The Kruskal-Wallis rank test was used to investigate the differential abundant bacteria among the three time points. The abundances of 13, 4 and 12 genera were altered in Shanghai, Shenzhen and pooled groups, respectively ($p<0.05$, figure 3A–C). However, in all the three analyses, *Bifidobacterium* was the only one that showed a significant difference. Moreover, when we raised the threshold to $q<0.05$, *Bifidobacterium* still remained in the results in Shanghai group, and it was the only selected one in the pooled samples. The abundance of *Bifidobacterium* was decreased from 2.43%±3.83% (mean±SD) at T1 to 0.79%±1.23% at T2 ($p=0.003$ compared with T1) and further to 0.59%±0.79% at T3 ($p=0.001$ compared with T1) in pooled data. At the species level, *Bifidobacterium longum subsp. longum* was one of the two abundant differential species in Shanghai

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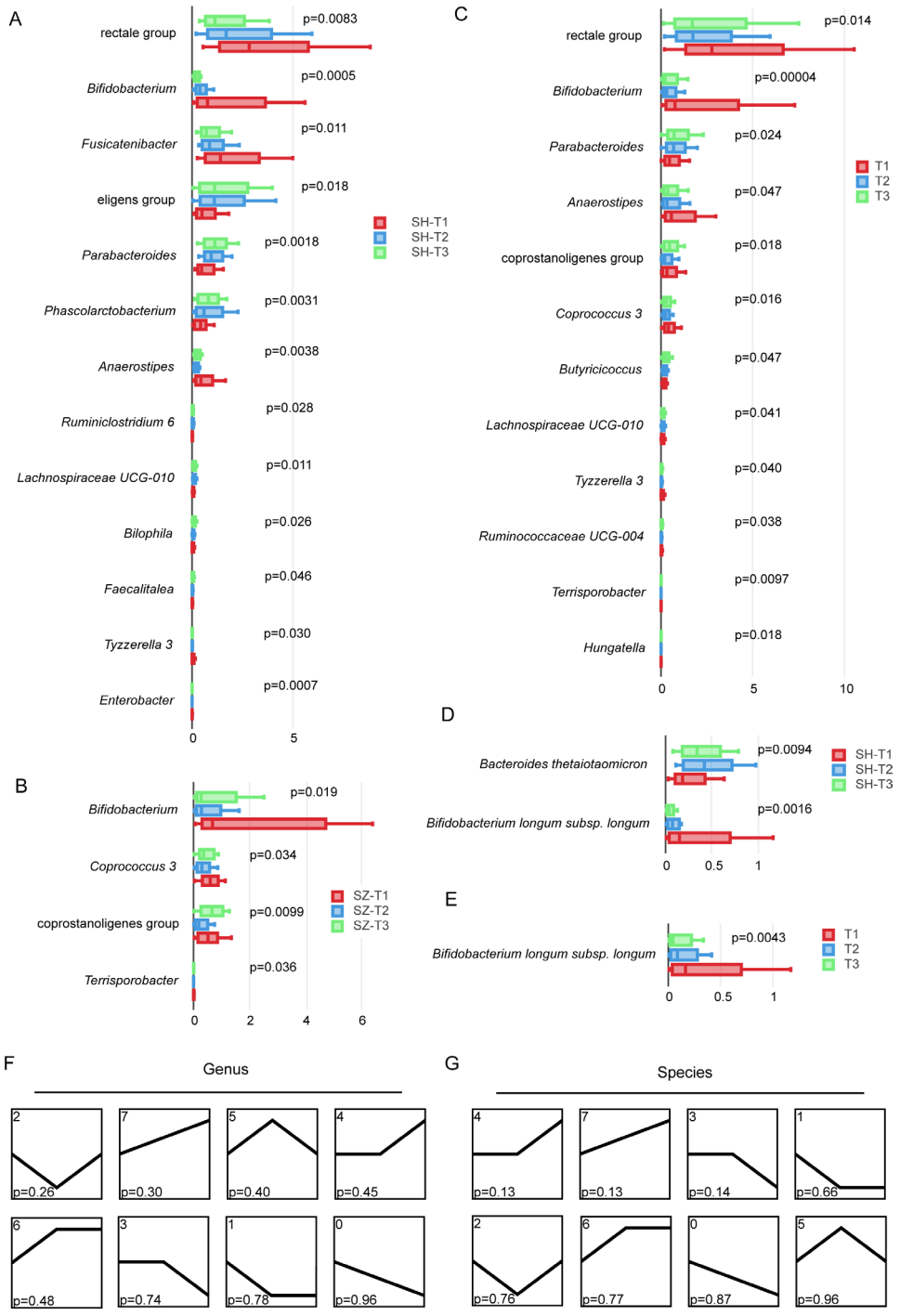

Figure 3 Analysis of differential abundance of bacteria. (A–E) Differential abundance of bacteria assessed using the Kruskal-Wallis rank test. (A–C) Box plots of differentially abundant bacteria at three time points at the genus level in Shanghai (A), Shenzhen (B) and pooled samples (C). (D and E) Box plots of differentially abundant bacteria at three time points at the species level in Shanghai (D) and pooled samples (E) in the order of decreasing relative abundance. The horizontal axis represents the relative abundance and the vertical axis represents differential abundance bacteria. The left and right boundaries of each box represent the 25th and 75th quartile values, respectively, and lines within each box indicate the 50th quartile (median) values. Ends of whiskers mark the lowest and highest relative abundance values in each group. The p values are indicated. (F and G) Eight trend profiles clustered through STEM analysis at the genus (F) and species (G) levels in the order of increasing p value. The profile ID is marked on the top left of each panel. The p value of each profile is shown on the bottom of each panel. P<0.05 was considered as significant. SH, Shanghai; STEM, Short Time-series Expression Miner; SZ, Shenzhen; T1, before saturation diving; T2, during saturation diving; T3, after saturation driving.

groups, and the only species in pooled groups (p=0.0094 and p=0.0043, respectively, figure 3D,E), with the average abundance of 0.43%±0.62%, 0.17%±0.22% and 0.16%±0.27% at T1, T2 and T3, respectively. These changes were not found in Shenzhen samples (detailed data are presented in online supplementary table 2).

Since great individual differences existed, we checked the ratio of divers whose *Bifidobacterium* levels changed (we considered more than 50% as significant). In Shanghai T2 and T3 groups, *Bifidobacterium* abundance decreased in 45.83% (11/24) and 66.67% (16/24) of divers, respectively, and the average fold change in these divers was 0.18 (±0.13) and 0.16 (±0.17) of T1,

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respectively. At the same time, the abundance of *Bifidobacterium* increased only in 12.50% (3/24) and 4.17% (1/24) of divers, respectively, and the fold change was 4.87 (±4.45) and 4.78 of T1, on average, in these individuals, respectively. The trend was similar in Shenzhen groups: In T2 and T3, *Bifidobacterium* richness decreased in 60.87% (14/23) and 56.62% (13/23) of divers, respectively, and the fold change in these divers was 0.21 (±0.13) and 0.24 (±0.15) of T1, on average, respectively. Meanwhile, the abundance of *Bifidobacterium* increased only in 4.35% (1/23) and 8.70% (2/24) of divers, respectively, and the fold change was 5.26 and 2.86 of T1, on average, in these individuals, respectively.

Decrease in the abundance of short-chain fatty acid-producing bacteria and increase in the abundance of pathogenic bacteria during and after saturation diving

With STEM analysis of the bacteria abundance changing trend on the genus and species levels following the sequential time points, eight alteration trend profiles were clustered, but no profile was significantly enriched (figure 3F,G). We hypothesised that the influence of saturation diving on the bacteria abundance would be maintained in the same direction or reached a plateau after a period of time. Based on that, we focused on the bacteria whose abundance kept decreasing (profile 0), decreased first and then was maintained at a similar level (profile 1), increased first and then was maintained at a similar level (profile 6), or kept increasing (profile 7). The data are presented in online supplementary table 3 (genus level) and online supplementary table 4 (species level). On the genus level, consistent with the previous result, *Bifidobacterium* was shown in profile 1. On the species level, *B. longum subsp. longum* and *B. adolescentis* were also shown in profile 1. In addition, on the genus level, 3 and 12 genera were clustered in profiles 0 and 1, respectively. Among them, attention was fixed on the genus with more than 1% relative abundance before diving, *that is, Fusicatenibacter, Faecalibacterium*, rectale group and *Anaerostipes*. Interestingly, they all belong to the short-chain fatty acid (SCFA)-producing group, especially butyrate-producing bacteria. Their average relative abundances at the three time points were 2.08%±1.75% vs 1.59%±1.76% vs 1.30%±1.23%, 7.65%±4.24% vs 5.97%±3.10% vs 6.09%±3.35%, 3.72%±2.83% vs 2.22%±1.65% vs 2.52%±2.16%, and 1.13%±1.37% vs 0.55%±0.59% vs 0.60%±0.67%, respectively. Gut commensals in *Bifidobacterium* and SCFA-producing bacteria are linked to many beneficial host effects.[27 28] Therefore, most of the decreased bacteria were beneficial for host health.

On the contrary, on the species level, four and six species were clustered in profiles 6 and 7, respectively. Among them, all the species in profile 7, *that is, Lactococcus garvieae*, *Actinomyces odontolyticus*, *Peptoclostridium difficile*, *Butyricimonas virosa*, *Streptococcus mutans* and *Porphyromonas asaccharolytica*, were reported to be associated with human diseases, including bacteraemia, colitis, endocarditis and dental caries.[29–34] Since the relative abundance of these species was quite low, we assessed the ratio of divers who were detected (tags number ≥2 was set to avoid false positive) in at least one of the species in profile 7. As a result, the ratio increased from 23.4% at T1 to 38.30% at T2, and further to 65.96% at T3.

DISCUSSION

In our study of gut microbiota composition during saturation diving, the abundance of *Bifidobacterium* and some SCFA-producing bacteria was found to be decreased during and after diving, despite no significant difference in either α or β diversity in samples from Shanghai or Shenzhen or pooled samples. Meanwhile, the abundance of some pathogenic bacteria increased during and after diving. These results suggested that commercial saturation diving may affect the gut microbiota homoeostasis and increased the risk of pathogen infection.

What caused the reduction in *Bifidobacterium* and some SCFA-producing bacteria during and after saturation diving? Hyperoxia is one possible contributing factor. Oxygen has been reported to induce gut dysbiosis, driving an uncontrolled luminal expansion of facultative anaerobic bacteria, such as the family *Enterobacteriaceae*[35] (although this did not change in our study). When living in the saturation chambers and performing excursions, divers were exposed to about 40–60 kPa PaO_2, respectively, which is double to triple of that in normal conditions, for up to about a month. Since gut commensals in *Bifidobacterium* and SCFA-producing bacteria are known as obligated anaerobes,[27] their growth may be affected by the higher PaO2.

Besides extra oxygen, diet is another possible contributing factor. Diet affects the gut microbiota rapidly and reproducibly.[16] We performed our investigation at two different companies with two locations to rule out the location-specific factors. The diets of the divers were recorded during saturation diving. Except for the high-protein content in the food required for the body mass maintenance needs, nothing special was observed in their diets. It was reported that an animal-based diet increases the abundance of bile-tolerant microorganisms, such as *Alistipes*, *Bilophila* and *Bacteroides*, and decreases the levels of Firmicutes that metabolise dietary plant polysaccharides, such as *Roseburia*, *Eubacterium rectale* and *Ruminococcus bromii*.[16] Actually, in our data, *Bilophila* showed a mild increasing trend, and the average relative abundance was 0.075%, 0.092% and 0.12% before, during and after diving, respectively. A high-protein diet might partially explain the reduction of previously mentioned SCFA-producing bacteria in our study.

On the contrary, cross-feeding interactions exist between bifidobacteria and butyrate-producing colon bacteria,[27] and the decrease in *Bifidobacterium* might cause a reduction in SCFA-producing bacteria. Indeed, in our data, when comparing the relative abundance between T3 and T1, in 21/29 of divers whose *Bifidobacterium* declined more than 50%, at least one of the four SCFA-producing bacteria mentioned before decreased more than 50%; in 3/3 of divers whose *Bifidobacterium* increased more than onefold, at least one of the four SCFA-producing bacteria also increased more than onefold, supporting the relationship between *Bifidobacterium* and butyrate-producing bacteria.

Our data revealed that pathogenic bacteria were detected in more divers during and after diving compared with the baseline. The confined hyperbaric chambers with relatively high humidity (60%–80%) and temperature (28±2°C) is an appropriate environment for bacteria growth,[36] and the thriving of bacteria in this environment will increase the possibility of pathogen infection. In addition, *Bifidobacterium* and SCFA-producing bacteria were reported to play an important role in protection against pathogens and maintenance of the gut barrier functions.[26] Correspondingly, we found that 19 out of 35 divers whose *Bifidobacterium* abundance in faeces at T2 and/or T3 decreased at least 50% compared with T1, and carried at least one kind of previously mentioned pathogenic bacterium at T2 and/or T3.

Although no obvious symptoms were reported in these divers, the susceptibility to pathogens might increase the risk of illness, and therefore, prevention has become our major concern. *B. adolescentis* and *B. longum* are reported to dominate the adult

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gut microbiota and were both reduced during and after saturation diving in our study. The consumption of probiotics can stimulate favourable bacterial communities in the human gastrointestinal tract,[27] and both *B. adolescentis* and *B. longum* are often used as food supplements. Selected species in *E. rectale* have also generated interest for their use as probiotics. In addition to probiotics, prebiotics, such as inulin-type fructans and arabinoxylan oligosaccharides, can be also added to food to increase the number of *Bifidobacterium* in the gut.[27] Therefore, the addition of these probiotics and prebiotics to divers' diets may prevent the decrease in 'good' bacteria during saturation diving, and their role in the improvement of the health and performance of divers needs further assessment.

In addition to bacteria, other microorganisms, such as archaea, viruses, phages, yeast and fungi, are present in the gut, and their role may be as important as bacteria. However, the present study only focused on bacteria. Except for the composition of microbiota, their activity is equally important.[9] In addition, we only investigated Chinese divers. Whether the influence of saturation diving is similar in divers of other countries is still unknown. Moreover, the saturation diving depth in our study was from 55 to 134 metres, and we do not know the influence of deeper diving depth on the gut microbiota. We collected stool samples from four divers before and after saturation diving in a 200-metre simulation trial and found an obvious trend of α diversity reduction as well as a decrease in *Roseburia*, a butyrate-producing bacterium (data not shown), suggesting that increased diving depth may cause more severe consequences. Much more work is required to obtain a bigger picture of how the diver gut microbiota is affected by the extreme environment during saturation diving.

To summarise, our data provided evidence that saturation diving reduced some 'good' gut commensals and increased some pathogenic bacteria, which may harm diver health. Therefore, we recommend that divers increase their consumption of probiotics and prebiotics for health promotion and disease prevention.

Contributors Conceptualisation and design: YY and ZJ; Methodology: YY and BP; Investigation and data collection: GZ, WJ, HJ, ZH and XC; Data analysis: YY, HZ and GT; Writing and editing: YY and ZJ; Funding acquisition: ZJ and HG; Implementation assistance: HZ and GT; Supervision: ZJ.

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