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Original Article

Gastric microbiome composition in obese patients and normal weight subjects with functional dyspepsia

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Abstract

Introduction: Despite the numerous studies demonstrating gut microbiota dysbiosis in obese subjects, there is no data on the association between obesity and gastric microbiota. The aim of this study was to address this gap in literature by comparing the composition of gastric microbiota in obese patients and a control group which included normal weight volunteers diagnosed with functional dyspepsia (FD).

Methodology: A total of 19 obese patients, and 18 normal weight subjects with FD and normal endoscopy results were included in the study. The gastric tissue samples were collected from participants in both groups by bariatric surgery and endoscopy, respectively, and profiled using 16S ribosomal RNA gene sequencing.

Results: There was no significant difference in the α-diversity scores, while distinct gastric microbial compositions were detected in both groups. Significantly lower levels of Bacteroidetes and Fusobacteria, and higher Firmicutes/Bacteroidetes ratio were recorded in the obese patients. A total of 15 bacterial genera exhibited significant difference in gastric abundance with *Prevotella*_7, *Veillonella*, *Cupriavidus*, and *Acinetobacter*, present in frequencies higher than 3% in at least one subject group.

Conclusions: Our study suggests a significant association between obesity and gastric microbiome composition. Future studies with larger sample size and gastric samples from subjects without any gastrointestinal complications are required to confirm our conclusions.

Key words: obese; normal; gastric; microbiota; 16S rRNA.

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Introduction

Obesity is the second most important (after smoking) preventable disease with complex and multifactorial etiology. According to data provided by the World Health Organization (WHO), more than 1.9 billion adults aged ≥ 18 years were overweight, and more than 650 million were classified as obese in 2016 [1]. These numbers will continue to rise as the prevalence of obesity has been rising globally in recent years, and is predicted to reach 20% by 2030 [2]. This poses a serious threat to public health since obesity is an established risk factor for chronic health problems such as type-2 diabetes, cardiovascular diseases, cancer, and mortality [3].

The mammalian gastrointestinal tract (GIT) is home to a wide range of commensal microorganisms including bacteria, archaea, viruses and protozoa, which are collectively known as “the microbiome”. With trillions of microorganisms in the GIT, the microbiome plays an important role in shaping human health by contributing to digestion, metabolism, protection against pathogens and immune function [4]. Its structure goes through a dynamic state in the first years of life, and adopts a more stable adult-like composition after the age of ~3 years, which is mainly shaped by environmental factors including diet [5]. Accordingly, subjects with obesity displayed altered GIT microbiome composition which, apart from increased energy harvesting capacity by the host, was

also associated with obesity-related diseases including type-2 diabetes, cardiovascular diseases, and non-alcoholic fatty liver disease [6].

While stomach, the most acidic part of digestive tract, was initially thought to be a sterile environment because of its hostile nature, recent studies with more sensitive DNA sequencing technologies reported the presence of microorganisms including bacteria and fungi in gastric tissue specimens [7,8]. Gastric tissue health and the microbial community are in correlation with each other as reported by studies showing altered microbiome composition in subjects with gastric health problems including chronic gastritis and gastric cancer [9]. The gastric microbiome composition also exhibited fluctuations in cases of extra-gastrointestinal diseases including hematological, cardiovascular, neurological, endocrine and dermatological diseases [9]. Moreover, the gastric microbial content was shown to interact with the microbial communities from the other locations of the GIT (i.e. mouth, duodenum, and small intestine) [8] which are also in correlation with diverse range of diseases on local, systemic and remote organs, including inflammatory bowel disease, celiac disease, and colorectal cancer [10].

Nevertheless, despite these data and the fact that obesity is associated with microbiome dysbiosis, there has yet to be a study evaluating gastric microbiota composition in subjects with obesity. In an attempt to fill this gap in literature, the present study performed molecular analysis to obtain relative abundances of gastric microbial phyla and genera in the gastric tissue samples collected from obese patients. The results were then compared with those obtained from age-matched normal weight subjects with functional dyspepsia (FD) who were used as the control group since it was not possible to recruit healthy subjects without any gastrointestinal complaints.

Methodology

Subjects and settings

The volunteers were categorized according to body mass index (BMI) cut off-points specified by WHO (normal weight: 18.5-24.9 kg/m^2; obese > 30.0 kg/m^2). The normal weight (NW) group included 18 subjects (mean age: 37.2 ± 15.1 years) admitted to the gastroenterology department with dyspeptic complaints who had normal endoscopy results and were diagnosed with FD (without any organic or physiological disease that could explain the complaints). The obese weight (OW) group included 19 obese patients (mean age: 36.4 ± 15.0 years) without any dyspeptic complaints. None of the volunteers had received any antibiotics or gastric medication three months prior to sample collection. All gastric tissue samples were collected from OW and NW group patients by bariatric surgery and endoscopy, respectively. All samples were stored at -80 °C until used.

Genomic DNA extraction and storage

Genomic DNA was extracted from the gastric tissue samples by using EurX GeneMATRIX Tissue & Bacterial DNA Purification kit (EURx, Gdansk, Poland). The DNA concentration was determined using a NanoDrop spectrophotometer (2000c) (Thermo Fisher Scientific, Waltham, USA), while the integrity and purity were detected by 1% agarose gel electrophoresis. All DNA samples were stored at -20 °C until further processing.

16S rRNA gene amplification and sequencing

Bacterial 16S rRNA amplification and library preparation were performed by following the 16S sequencing library preparation protocol (Illumina, San Diego, USA). The variable V3–V4 region of the bacterial 16S rRNA was amplified using 16S 341F (TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG CCT ACG GGN GGC WGC AG) and 785R (GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GGA CTA CHV GGG TAT CTA ATC C) primers. The polymerase chain reaction (PCR) was performed in a Thermal Cycler (Kyratec; Model SC300, Wembley, Australia) using 25 µL reaction volume containing 2.5 µL of microbial DNA (15 ng/µL), 5 µL of amplicon PCR forward primer (1 µM), 5 µL of amplicon PCR reverse primer (1 µM), and 12.5 µL of 2X KAPA HotStart PCR mix. The cycling parameters were as follows: 95 °C for 3 min; followed by 25 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s; and a final extension at 72 °C for 5 min. The PCR products were quantified and then purified by using AMPpure XP beads (Beckman Coulter, Pasadena, USA). Barcoded V3 and V4 amplicons were sequenced by using NovaSeq Reagent Kit (Illumina, San Diego, USA).

Data processing and bioinformatics analysis

Raw sequencing data were converted to FastQ format using bcl2fastq v1.8.4 software and trimmed to remove adapter sequences by Scythe (v0.994 BETA) (https://github.com/vsbuffalo/scythe) and Sickle programs (https://github.com/najoshi/sickle). The FastQC program (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) was used for quality control, and data which had

low sequencing quality (Phred Score < Q20, 30 bp sequences) were removed from the entire data set. Low quality base pairing in the ends, possible adaptor contaminations, and chimerics were removed by using Trimmomatic 4 algorithm based on Genomes Online Database (GOLD). The sequences were merged using DADA2 tool, which was also used for denoising and chimera removal. Sequences for taxonomic identification were aligned to target microorganisms by using SILVA database using QIIME2 tool [11]. After the alignment process, operational taxonomic unit (OTU) groups were determined in each sample. R scripts were used in data reporting, statistical analysis and data visualization processes.

Statistical analysis

The phylum and genus levels were represented as mean abundance (%) ± standard deviation (SD). The non-parametric Mann-Whitney test was used to compare the relative abundances of taxonomic units and alpha diversity indices between the groups. Alpha diversity of the samples was estimated by the Chao 1, Simpson, Shannon indices.

Chao1 index is used to measure the richness of the microbial community, and can detect rare taxa. Shannon and Simpson indexes are used to determine the community heterogeneity; while the former provides more weight to evenness, the latter measures both the richness and evenness of the microbial community.

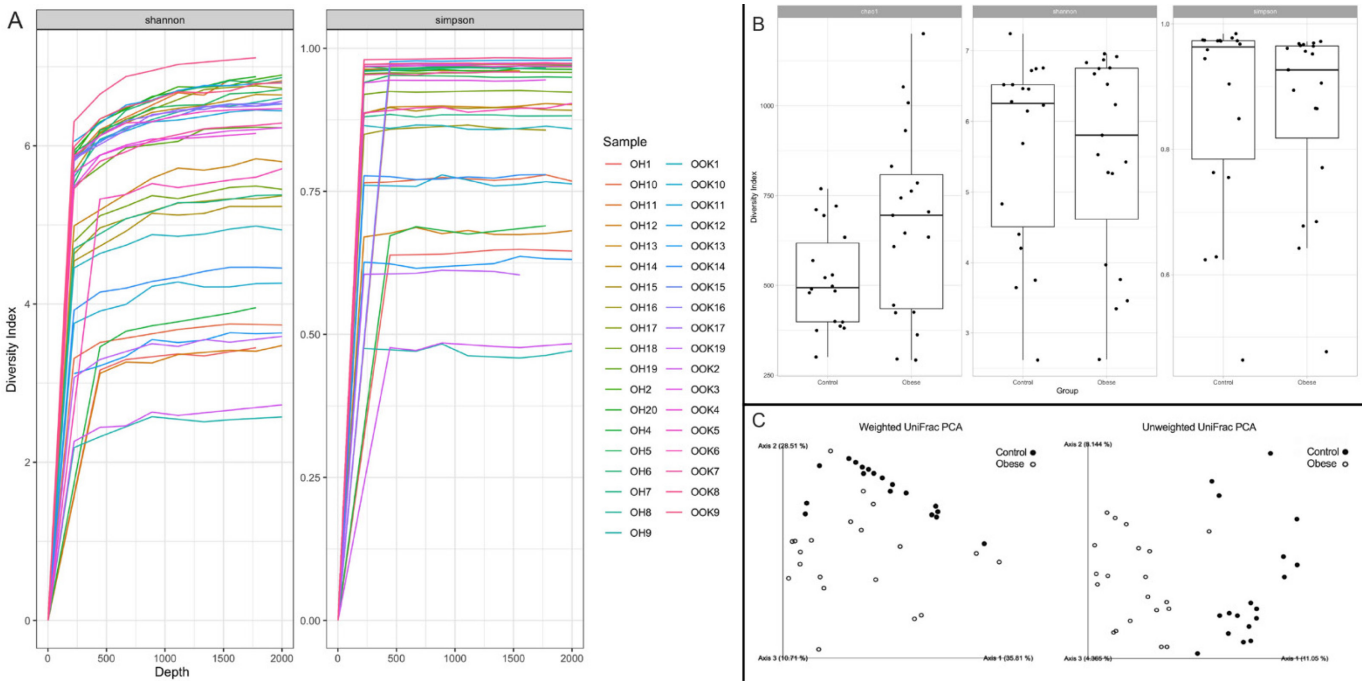
The rarefaction curves were generated by using Simpson and Shannon index values. The relationship of gastric microbiome composition with obesity was assessed by principal coordinate analysis (PCoA) based on unweighted and weighted UniFrac distances. Permutational multivariate analysis of variance (PERMANOVA) was performed to test for statistically significant difference in β-diversity between OW and NW subjects. False discovery rate (FDR) was applied for multiple testing, and significant differences were only considered for *p* values below 0.05.

Results

Differences in the gastric microbiota diversity

In total, 7,212,824 paired end reads (2 × 150 bp) with an average of 194,941 ± 14,124 analyzed sequences (min-max: 114,675–512,614) and a mean length of 298 base-pairs per sample (min-max: 297-300) were acquired from 19 OW and 18 NW volunteers. Moreover, a total of 694 OTUs were obtained at a sequence-similarity level of 97%, with 224 ± 92 (min-max: 112-518) as the mean number of OTUs per sample.

Figure 1. Rarefaction curves and α- and β-diversity analysis of gastric microbial samples.



A. Shannon and Simpson rarefaction curves of control and obese group subjects. **B.** Box plots of α-diversity calculated by Chao1, Shannon, and Simpson indices. The Mann-Whitney U test did not show any significant difference in the indices between the two groups (p = 0.066, 0.843 and 0.207, respectively). **C.** PCoA plots based on weighted and unweighted UniFrac distance, where each dot represents a single subject from the control and obese groups. The PERMANOVA confirmed that the community composition significantly differed between the two groups of subjects (p = 0.001).

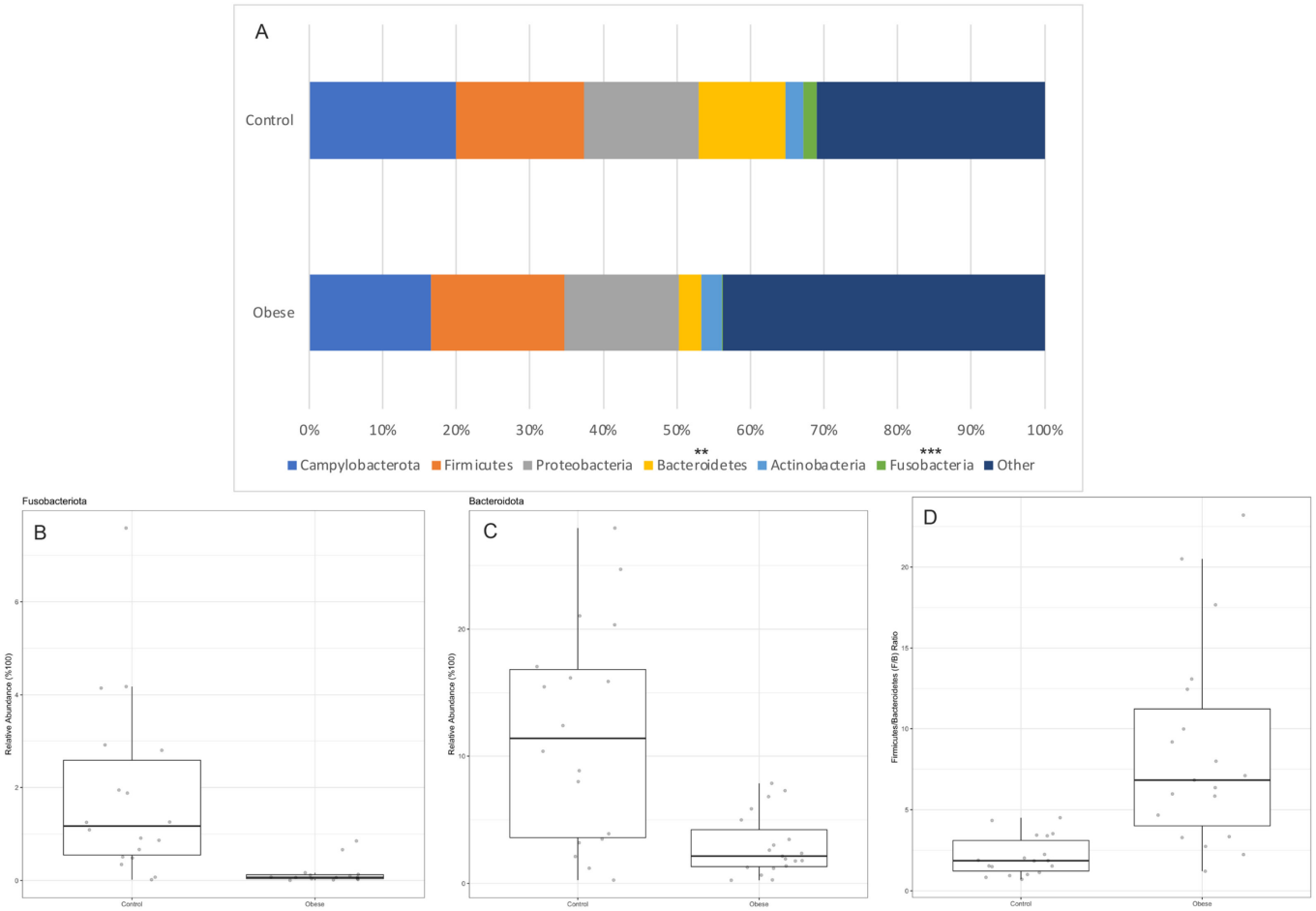
Table 1. Average abundances of the major gastric microbial phyla in obese and control subjects.

Phylum	Abundances (%) of major microbial phyla (mean ± standard deviation)		*p* value
	Control	Obese	
Campylobacterota	19.98 ± 26.78	16.57 ± 27.42	0.7819
Firmicutes	17.41 ± 10.49	18.10 ± 12.92	1.0000
Proteobacteria	15.51 ± 10.62	15.60 ± 10.31	1.0000
Bacteroidetes	11.80 ± 8.52	3.00 ± 2.40	0.0045*
Actinobacteria	2.48 ± 1.57	2.82 ± 2.54	1.000
Fusobacteria	1.83 ± 1.92	0.16 ± 0.25	0.0005**

* and ** indicate statistical significance, where $p \leq 0.005$ and $p \leq 0.0005$, respectively.

In our analysis, the α-diversities of gastric microbiome samples were estimated by using Chao 1 (richness), Simpson (diversity), and Shannon (diversity) indices. The rarefaction curves of Shannon and Simpson indexes approached the plateau phase, suggesting that sequencing depth was enough and sufficient for microbial community structure analysis (Figure 1A). Nevertheless, neither of the three α-diversity indices (Chao 1, Simpson, and Shannon) showed statistically significant difference between OW and NW patient samples (Figure 1B). On contrary, PCoA based on weighted and unweighted UniFrac distances demonstrated distinct clustering patterns between the two study group subjects, which was found to be statistically significant by PERMANOVA (p = 0.001) (Figure 1C).

Figure 2. Phylum-based comparisons of gastric microbiome between control and obese subject groups.



A. Horizontal bar chart displaying the frequencies of the five major microbial phyla with more than 1% of abundance in control and obese gastric samples. (**B-C**) Box plots showing the difference in the gastric Fusobacteria **B.** and Bacteroidetes **C.** relative abundances between obese weight (OW) and normal weight (NW) group subjects. Data are represented as mean frequency ± SD. **D.** The F/B box plot showing the ratio of bacterial phyla Firmicutes to Bacteroidetes in control and obese gastric samples. The Mann-Whitney U test confirmed statistically significant alterations in Fusobacteria (p = 0.0004) and Bacteroidetes (p = 0.0082) levels and F/B ratio (p = 0.0000336) between the two groups. Note: ** and *** represent p < 0.01 and, p < 0.001 respectively.

Comparison of the gastric microbiota at phylum level

The major microbial phyla with more than 1% of abundance in the OW and NW patient samples were Campylobacterota (19.98 ± 26.78% vs. 16.57 ± 27.42%), Firmicutes (17.41 ± 10.49% vs. 18.10 ± 12.92%), Proteobacteria (15.51 ± 10.62% vs. 15.60 ± 10.31%), Bacteroidetes (11.80 ± 8.52% vs. 3.00 ± 2.40%), Actinobacteria (2.48 ± 1.57% vs. 2.82 ± 2.54%), and Fusobacteria (1.83 ± 1.92% vs. 0.16 ± 0.25%) (Figure 2A) (Table 1). Statistical analysis revealed significantly lower gastric levels of Bacteroidetes (Figure 2B) and Fusobacteria (Figure 2C) in OW than that in NW group patients ($p < 0.01$ and $p < 0.001$, respectively). Samples from obese volunteers also displayed elevated rates of Firmicutes, and reduced levels of Campylobacterota than those obtained from NW patients; however, these differences were not statistically significant (Table 1). Additionally, comparison of the gastric Firmicutes/Bacteroidetes (F/B) ratios between the two study groups showed statistically significantly elevated F/B ratio in the obese gastric microbiome ($p < 0.0001$) (Figure 2D).

Comparison of gastric microbiota at the genus level

The most dominant OTUs (mean relative abundance values > 1% in both OW and NW group subjects) at genus level in each sample are shown in the heatmap (Figure 3A). *Helicobacter* was the most abundant bacterial genus, with a mean abundance of 18.19 ± 27.15%, which displayed a reduced frequency in the OW patients compared to NW group (16.56 ± 27.42% vs. 19.83 ± 26.87%); however, the difference was not statistically significant. On the other hand, the subjects with obesity displayed significantly lower gastric levels of *Prevotella*_7¬ (3.75 ± 2.93% vs. 0.19 ± 0.23%), *Veillonella* (3.38 ± 2.58% vs. 0.28 ± 0.62%), *Cupriavidus* (3.03 ± 2.58% vs. 0.03 ± 0.06%), *Alloprevotella* (2.13 ± 2.40% vs. 0.12 ± 0.17%), *Haemophilus* (2.13 ± 2.42% vs. 0.22 ± 0.29%), *Porphyromonas* (2.06 ± 2.29% vs. 0.09 ± 0.08%), *Massilia* (1.70 ± 3.58% vs. 0.94 ± 2.41%), *Neisseria* (1.42 ± 1.69% vs. 0.31 ± 0.84%), *Gemella* (1.38 ± 2.45% vs. 0.25 ± 0.35%), *Prevotella* (1.35 ± 1.13% vs. 0.14 ± 0.15), *Actinomyces* (1.33 ± 0.96% vs. 0.24 ± 0.55%), and *Fusobacterium* (1.27 ± 1.36% vs. 0.13 ± 0.21%); and higher levels of *Acinetobacter* (0.10 ± 0.16% vs. 2.94 ± 3.64%), *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* (0.20 ± 0.36% vs. 2.04 ± 4.63%), and *Bacillus* (0.04 ± 0.03% vs. 1.09 ± 2.91%) relative to the NW subjects (Table 2). Among these, only *Prevotella*_7, *Veillonella*, *Cupriavidus*, and *Acinetobacter* exhibited frequencies ≥ 3% in either of the two subject groups (Figure 3B-E).

Discussion

Gastric microbiota has become the focus of recent studies following the detection of microbial communities in the stomach using sensitive and culture-independent approaches. Nevertheless, there is no data on the association between gastric microbiome composition and obesity, which is long known to affect gut microbiota.

Our study is the first to identify major gastric microbial phyla in obese subjects. These phyla included Campylobacterota, Firmicutes, Proteobacteria, Bacteroidetes, Actinobacteria, and Fusobacteria. The same was also observed for NW volunteers with FD, which was in alignment with data previously suggested for healthy subjects [7,8]. Among those bacterial phyla, Campylobacterota and Firmicutes had the highest abundancy in the gastric microenvironment of both OW and NW group patients in our study. Campylobacterota is a new phylum that was formerly known as the Epsilon proteobacteria class of Proteobacteria [12]. The

Table 2. Average abundances of the most dominant gastric microbial genera that displayed significantly altered frequencies between obese and control gastric samples.

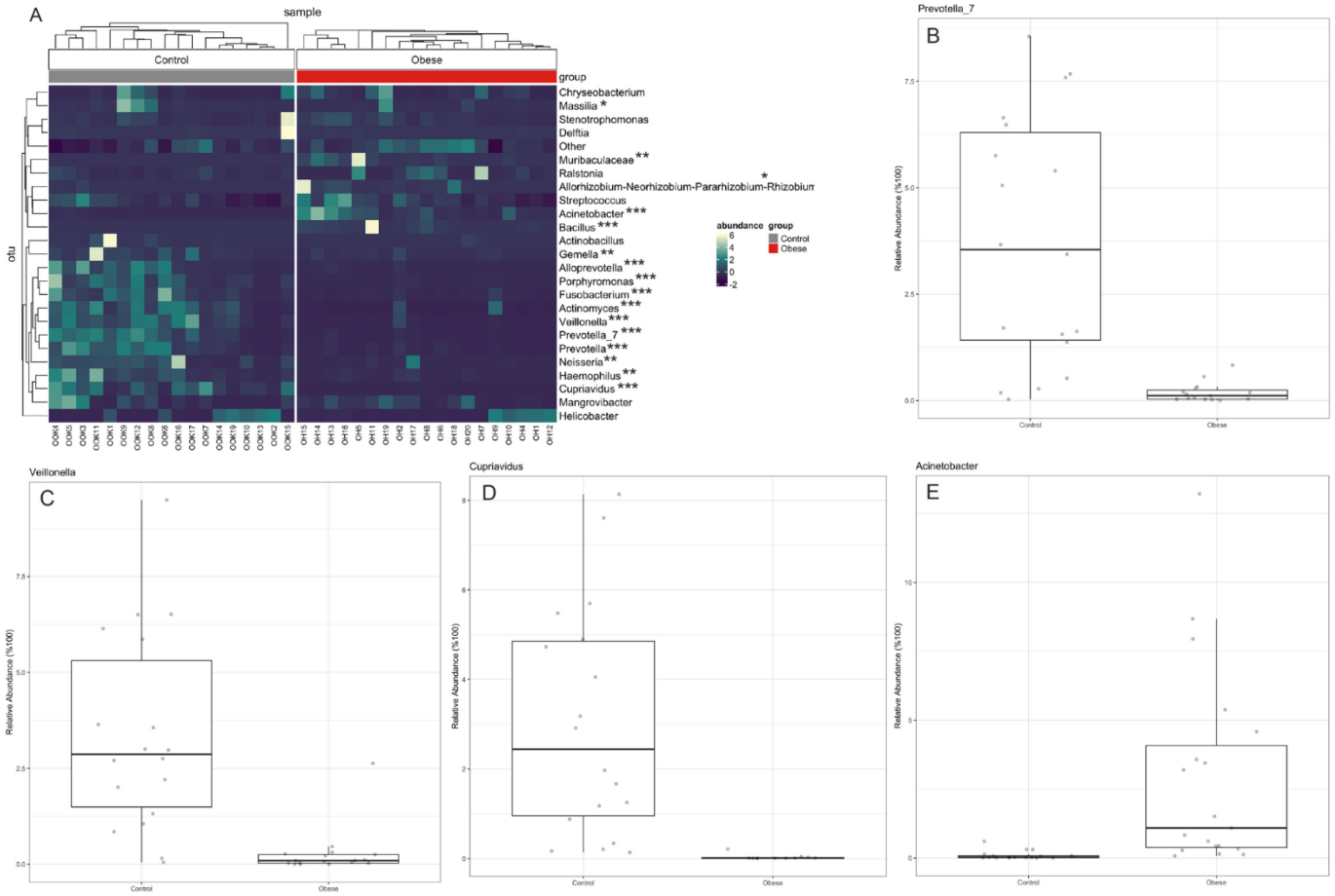
Genus	Average abundances of bacterial genera (%)		*p* value
	Control	Obese	
Prevotella_7	3.75 ± 2.93	0.19 ± 0.23	**0.00038**
Veillonella	3.38 ± 2.58	0.28 ± 0.62	**0.00031**
Cupriavidus	3.03 ± 2.58	0.03 ± 0.06	**0.00009**
Alloprevotella	2.13 ± 2.40	0.12 ± 0.17	**0.00038**
Haemophilus	2.13 ± 2.42	0.22 ± 0.29	**0.00197**
Porphyromonas	2.06 ± 2.29	0.09 ± 0.08	**0.00055**
Massilia	1.70 ± 3.58	0.94 ± 2.41	**0.02747**
Neisseria	1.42 ± 1.69	0.31 ± 0.84	**0.00299**
Gemella	1.38 ± 2.45	0.25 ± 0.35	**0.00675**
Prevotella	1.35 ± 1.13	0.14 ± 0.15	**0.00078**
Actinomyces	1.33 ± 0.96	0.24 ± 0.55	**0.00055**
Fusobacterium	1.27 ± 1.36	0.13 ± 0.21	**0.00046**
Acinetobacter	0.10 ± 0.16	2.94 ± 3.64	**0.00024**
Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	0.20 ± 0.36	2.04 ± 4.63	**0.02568**
Bacillus	0.04 ± 0.03	1.09 ± 2.91	**0.00020**

data presented in this study is in agreement with literature demonstrating Proteobacteria and Firmicutes as the most abundant gastric phyla in subjects with dyspeptic complaints [13,14]. On the other hand, the relative frequencies of the two bacterial phyla are lower than what was previously reported by Delgado *et al.* [13]. This can be due to factors including differences in the dietary traditions, geographical origin, and ethnicity [15].

Both OW and NW groups displayed similar dominant bacterial phyla. The comparison analysis reported significantly lower levels of Fusobacteria and Bacteroidetes, and increased F/B ratio in obese subjects. Moreover, while the two study populations did not display any difference in microbial diversity as assessed by α-diversity indices, the PERMANOVA test confirmed statistically significant differences in community compositions. This supports the previous study by Lin *et al.* demonstrating the correlation of BMI with β-diversity, but not with α-diversity of microbial species in the upper digestive tract that included the saliva, esophagus, and gastric contents [16].

On the other hand, in contrast to our data and data from Lin *et al*. [16], significant alteration was previously reported in both α- and β-diversities of the obese gut microbial communities [17–20]. This implies possible differences in the effect of obesity on α-diversity indices between upper and lower GIT regions which could be due to distinct microenvironmental features such as local immune response, interphylum network, gastric pH, and nutrients. Similar differences in the niche factors can also explain the conflict between our and previous data which demonstrated elevated Fusobacteria, Actinobacteria, and Proteobacteria levels in gut microbiota of subjects with obesity [21–25].

Figure 3. Genus-based comparisons of gastric microbiome between control and obese subject groups.



A. Heatmap showing operational taxonomic units (OTUs) at the genus level with relative abundance > 1% in control and obese gastric microbiome. The colour code indicates the range of relative abundance for a given genus. (B-E) Box plots showing the difference in the gastric *Prevotella*_7 **B.**, *Veillonella* **C.**, *Cupriavidus* **D.**, and *Acinetobacter* **E.** relative abundances between NW and OW group volunteers. Data are represented as mean frequency ± SD. The Mann-Whitney U test confirmed statistically significant changes in the gastric levels between control and obese subjects ($p = 0.000384$, 0.00031, 0.00009, 0.00024, respectively). Note: *, ** and *** represent $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

One important process that is crucial for the development of healthy microbiome and distinct microenvironment along the GIT is regulation of oxygen concentration. Epithelial cells are essential in this regulation process as they reduce oxygen levels by beta-oxidation, which is disrupted during inflammation leading to enhanced oxygen concentration in the microenvironment [26]. During chronic infections, creation of aerobic environment is further facilitated by the increased levels of hemoglobin carrying oxygen and production of reactive oxygen species [27,28]. This supports the growth of facultative anaerobic Proteobacteria which was recently proposed as a microbial signature of gut microbiota dysbiosis [29].

However, having a more aerobic environment in the stomach than the distal parts of GIT [30–33] may result in the former to suffer from less dramatic effects from the elevated oxygen levels during obesity-associated chronic inflammation. This could at least partially explain our data showing lack of change in the gastric Proteobacteria and Campylobacterota levels between OW and NW group patients, despite the literature showing increased contribution of Proteobacteria to the obese gut microbiome [21,29,34]. Nevertheless, our observation of reduced but not significantly changed Campylobacterota levels in the obese gastric samples also indicate the possible effects of factors other than oxygen concentration, including differences in the control groups used by our and other studies.

Firmicutes were suggested to have greater contribution to energy absorption than Bacteroidetes through the expression of a wider range of carbohydrate metabolizing enzymes [35]. Accordingly, decrease in Bacteroidetes and increase in Firmicutes abundances resulted in elevated energy harvest [36]. Moreover, increased gut F/B ratio was proposed as a hallmark for obesity by various reports [21,37]. Similar increase was also detected in our study, which was suggested to be mainly caused by significantly reduced gastric Bacteroidetes levels. Our results also showed elevated but not significantly altered Firmicutes abundance in obese samples, which can be due to the low sample size. Therefore, future studies with larger sample sizes are recommended for validation of our findings and more reliable conclusions.

This is the first report in literature of comparison of the gastric microbiome structure between NW and OW subjects at the genus level. The gastric core bacterial microbiota with highly prevalent taxa is yet to be clarified due to heterogeneous and diverse data in the literature. Therefore, a recent systemic review by Rajilic-Stojanovic *et al.* proposed a definition for the 'typical' gastric bacteria that depended on the bacterial groups reported in at least 20% of previous gastric microbiota studies [8]. While most of the bacterial genera with > 1% abundance in our study were among the suggested 'typical' gastric bacteria population, they also included *Alloprevotella* (phylum: Bacteroidetes), *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* (phylum: Proteobacteria), *Cupriavidus* (phylum: Proteobacteria), *Chryseobacterium* (phylum: Bacteroidetes), *Mangrovibacter* (phylum: Proteobacteria), and *Massilia* (phylum: Proteobacteria). Our data showing *Helicobacter* as the most abundant bacterial genus is in agreement with the previous results showing *Helicobacter pylori* as the most dominant species in the stomach [8,38]. Accordingly, *H. pylori* was suggested as a core gastric microbiota member due to its high prevalence. In fact, its colonization, rather than its presence, was associated with pathogenic outcomes, reduced microbial diversity, and decreased abundance of other bacterial groups [8,38].

Our analysis identified 15 bacterial genera that displayed significant difference in gastric abundance between NW and OW group subjects, among which only *Prevotella_7* (phylum: Bacteroidetes), *Veillonella* (phylum: Firmicutes), *Cupriavidus* (phylum: Proteobacteria), and *Acinetobacter* (phylum: Proteobacteria) exhibited frequencies higher than 3% in at least one subject group. To the best of our knowledge, there is no published data correlating obesity with *Cupriavidus*, and *Prevotella_7* levels, which were all lower in the obese gastric microbiome in our study. Obese subjects also displayed lower gastric levels of *Veillonella* which was in alignment with previous studies showing inverse relation between oral *Veillonella* frequency and BMI [39,40]. As *Veillonella* is regarded as one of the most abundant bacterial genera in the oral cavity [41], the reduced gastric levels could be due to lower transport of oral microbial community to stomach, as well as altered gastric microenvironment in the obese subjects. Moreover, in agreement with the literature demonstrating positive association of obesity with gut *Acinetobacter* levels [42] and *Acinetobacter* infections [43], higher levels of *Acinetobacter* were detected in the obese gastric tissue samples. While *Acinetobacter* species is recognized as an important opportunistic nosocomial pathogen [44], and regarded as a critical group of antibiotic-resistant bacteria by WHO [45], they were also associated with gastric carcinoma [46,47]. Therefore, whether the observed differences in gastric microbiome structure precedes any gastric and extra-gastric diseases, including

carcinogenesis needs further attention in future follow-up studies.

The major limitation of our study is the low sample size. In addition, since both OW and NW groups in our study contained volunteers with hospital admission, our results are also prone to a hospital-based bias. Further bias may be introduced by the lack of gastric biopsy samples from healthy subjects without any gastrointestinal complaints and the use of FD patients as the control group. It was not possible to obtain detailed data at species level due to limited resolution of 16S rRNA gene sequencing. Moreover, since it was not a prospective study with multiple measurements mainly because of ethical issues in having repeated gastric biopsy samples from volunteers, the association detected in our study may have occurred without a causal relation to obesity. Therefore, future animal studies with multiple time measurements would not only contribute to our understanding in this area, but also help to enlighten the relationship between the observed changes in gastric microbiota and obesity-associated complications, including cancer.

Conclusions

In summary, our study detected significant difference in gastric microbiome composition between OW patients and NW subjects with FD, with lower levels of Bacteroidetes and Fusobacteria and higher Firmicutes/Bacteroidetes ratio in the former. The results also showed significant differences in gastric abundance of *Prevotella*_7, *Veillonella*, *Cupriavidus*, and *Acinetobacter* between the two subject groups. Future studies are required to confirm our findings and investigate whether the reported changes precede obesity-associated complications.

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Authors' contributions

Study conception and design: GK, UG; data collection: GK, UG, MC, ACD, OB; analysis and interpretation of results: GK, UG, ER, CH, OT; draft manuscript preparation: GK, UG. All authors reviewed the results and approved the final version of the manuscript.

Ethics approval and consent to participate

The study was approved by the Health Sciences Ethics Committee of the Near East University Ethics Review Board (YDU/2017/51-472) and performed in accordance with the Declaration of Helsinki. Written informed consents were obtained from each person prior to enrolment.

Availability of data and materials

The datasets generated and/or analysed during the current study are available in the BioProject database under accession number PRJNA835353 (http://www.ncbi.nlm.nih.gov/bioproject/835353).

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