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RESEARCH ARTICLE

Association of oral frailty and gut microbiota with hypertension: cross-sectional results in the Shika study

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Abstract **Although recent studies have reported the association between toxins produced by certain gut microbiota and elevated blood pressure, the relationship between oral frailty (OF) and gut microbiota has rarely been investigated. The purpose of this study was to epidemiologically investigate the relationship between the combination of OF and specific gut microbiota on hypertension in the residents of Shika Town, Ishikawa Prefecture, Japan. A total of 322 residents aged ⩾ 50 years in Shika Town agreed to participate and met the criteria. The OF was evaluated difficulty in chewing and swallowing, oral dryness, number of remaining teeth, and frequency of tooth brushing. Blood pressure was measured using an automatic digital blood pressure meter. Next-generation sequencing was used to analyze the gut microbiota. A two-way analysis of covariance revealed a significant interaction between the two OF groups and the two hypertension groups on *Megamonas*. The binomial logistic regression analysis stratified by OF revealed a positive correlation between *Megamonas* and hypertension (OR 1.317; *P* = 0.023). This cross-sectional epidemiological study of the local residents revealed that the abundance of *Megamonas* in the OF group was significantly higher in the hypertension group than in the normotension group; however, no such relationship was observed in the non-OF group.**

Keywords gut microbiota; hypertension; *Megamonas*; oral health; regression analysis

Introduction

The oral frailty (OF) is characterized by overlapping tooth loss and a minor decline in various oral functions, such as eating and speaking, and is associated with an increased risk of oral dysfunction; however, proper intervention and treatment can effectively improve this condition [1]. It is known to roughly double the risk of physical frailty and sarcopenia [2]. Both physical frailty and OF have been reported to increase the risk of mild cognitive impairment 10 years later [3]. Our previous OF studies found that the combination of OF and decreased mineral-containing food intake, bone mineral density, and

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low animal protein intake is associated with decreased bone mineral density [4], renal function [5], and weight-adjusted lower calf circumference, respectively [6]. Conversely, only a few reports investigated the relationship between OF and hypertension. In the relationship between oral health and hypertension, C-reactive protein, interleukin-6, and tumor necrosis factor-α, which are released as a result of periodontitis, have affected vascular endothelial function and may be associated with increased blood pressure [7]. Although OF is related to hypertension through a similar mechanism because OF is initiated by tooth loss and impaired masticatory function due to dental caries and periodontal disease, a direct relationship between OF and hypertension has not yet been reported; thus, it is worth investigating.

One of the factors involved in hypertension has recently attracted focus on changes in the gut microbiota. A cross-sectional study of rural Chinese residents reported that the increased number of *Megasphaera* and *Megamonas* was positively correlated with systolic blood pressure (SBP) [8]. Li *et al*. [9] found the overgrowth of *Prevotella* and *Klebsiella* in patients with hypertension. Because the gut microbiota associated with hypertension varies across different countries and diets, no satisfactory conclusions can be drawn at present.

OF is presumably associated with periodontal disease, which is responsible for the direct increase in blood pressure due to vascular endothelial cell damage [7] and the indirect increase in blood pressure due to periodontopathogenic bacteria, altering the composition of the gut microbiota via the gastrointestinal tract [10]. Because decline in swallowing function due to OF progression is associated with decreased immunity [11,12], the involvement of gut microbiota may enhance the mechanism that increases blood pressure. This study epidemiologically evaluated the relationship between the combination of OF and specific gut microbiota on hypertension in residents of Shika, Ishikawa Prefecture, Japan.

Methods

Study design and participants

This cross-sectional study was conducted among residents of Shika, Ishikawa Prefecture, Japan. Participants were recruited between October 2017 and December 2019. The population of Shika Town was 20 055 (9525 males and 10 530 females), and the number of individuals aged 65 years and older was 8491 (aging rate, 42.3%) [13]. Kanazawa University and Shika signed an agreement on health promotion in 2011 and have been conducting annual super preventive medical checkups since 2013. Over the years, several articles have been reported on the Shika study [14]. Written informed consent was obtained from all 560 participants who agreed to participate in the study. The target population in this study was individuals aged 50 years and older, based on our previous research [6]. Among them, 462 participants were aged 50 years and older. Furthermore, 132 participants with missing blood pressure or blood biochemistry data and 8 participants with unassessed OF and gut microbiota were excluded. Finally, 322 individuals (142 males and 180 females) were analyzed (Fig. 1). Additionally, individuals taking oral antimicrobials and those with infections were not included in this study.

Blood pressure assessment

Well-trained nurses or clinical technologists measured blood pressure during annual super preventive medical checkups using an automatic digital blood pressure meter UM-15P (Parama-tech Co., Ltd., Fukuoka, Japan) or HEM-907 (OMRON Co., Ltd., Kyoto, Japan), with the

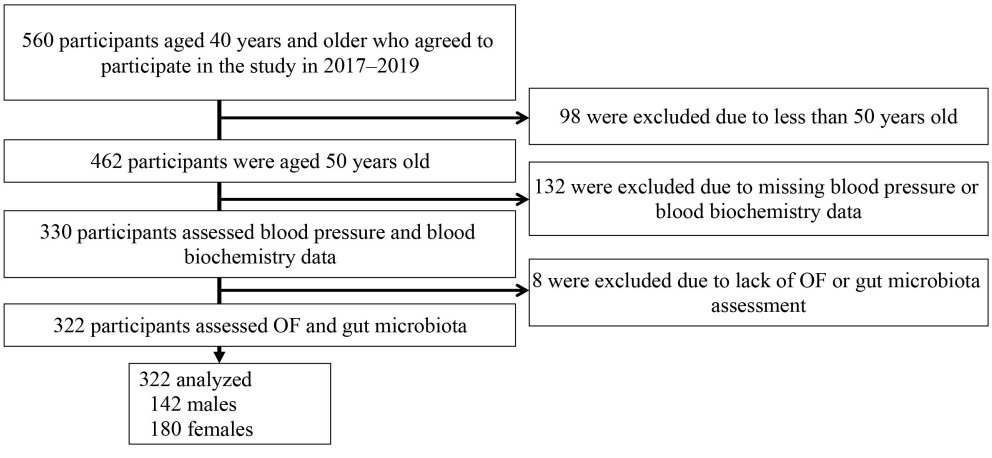

Fig. 1 Participant recruitment chart. Abbreviations: OF, oral frailty.

average of two measurements performed on the right upper arm. Hypertension was defined as SBP of ⩾ 140 mmHg and/or diastolic blood pressure (DBP) ⩾ 90 mmHg [15]. Participants receiving antihypertensive treatment were included in this study.

Blood biochemistry data assessment

Blood samples for biochemistry examinations were collected during annual super medical checkups after fasting for a minimum of 12 h. The study utilized low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), triglyceride (TG), total cholesterol (TC), hemoglobin A1c (HbA1c), and estimated glomerular filtration rate (eGFR). eGFR was calculated using the serum creatinine levels.

OF assessment

OF was evaluated using the same method as in the Shika study [5,6]. This was assessed based on 5 factors: difficulty chewing, difficulty swallowing, oral dryness, number of remaining teeth, and frequency of tooth brushing. Difficulty chewing and swallowing were assessed by rating the difficulty of consuming hard foods compared to 6 months ago (yes: 2 points; no: 0 points) and rating whether they sometimes choked on tea or soup (yes: 2 points; no: 0 points). Oral dryness was assessed by asking if the individual was bothered by it (yes: 1 point; no: 0 points). The number of remaining teeth was evaluated based on the number of their teeth excluding implants, bridge pontics, and removable dentures (19 or fewer: 1 point; 20 or more: 0 points). The frequency of tooth brushing was evaluated by the number of brushing times per day (less than once per day: 1 point; more than twice per day: 0 points). A total of ⩾ 3 points were considered to indicate OF.

Gut microbiota assessment

The gut microbiota assessment followed the procedures used in previous Shika studies [16,17]. Participants collected fecal samples at home using clean paper and a spatula with a plastic tube (AS ONE Corporation, Osaka, Japan). They then froze the samples overnight. All collected samples were stored frozen at −80 °C. DNA was extracted using the NucleoSpin DNA Stool Kit (Machery-Nagel, Düren, Germany).

The DNA extracted from the gut microbiota was processed for 16S rRNA gene sequencing using next-generation sequencing methods. The V3–4 regions of the 16S rRNA gene were amplified with Ex Taq® Hot Start Version polymerase using a TaKaRa polymerase chain reaction (PCR) Thermal Cycler Dice® Gradient (TaKaRa Bio Inc., Shiga, Japan) [18]. The PCR products were purified using Agencourt AMPure XP magnetic beads (Beckman Coulter, Inc., CA, USA) and then indexed using the Nextera XT Index Kit version 2 (Illumina Inc., San Diego, CA, USA). After purifying the indexed products with magnetic beads, the prepared library was sequenced using the MiSeq System (Illumina) with the MiSeq Reagent Kit version 3 and PhiX Control v3 (Illumina).

The QIIME2 program was used to analyze the microbiota [19]. The paired-end sequence data were cleaned up using the DADA2 plugin [20]. Chimeric sequences were removed using USEARCH (version 10.0.240_i86linux32) [21] and SILVA 16S rRNA database (release 132) [22]. From the non-chimeric sequences, the "pick_*de_novo*_otus.py" command in QIIME (version 1.9.1) and the SILVA 16S rRNA gene database (release 132) were used to generate operational taxonomic units (OTUs) (97% similarity threshold) [23]. Finally, global singletons were removed using the "filter_otus_from_otu_table.py" command in QIIME. Samples with fewer than 5000 sequences were excluded from the analysis.

Questionnaire on demographics and body size

Participants completed self-reported questionnaires covering age (years), sex (1: female; 2: male), drinking status (1: non-drinking or drinking fewer than once a month; 2: drinking at least once a month), and smoking status (1: non-smoker or past smoker; 2: current smoker). The body mass index (BMI) was calculated by dividing a person's weight (in kilograms) by the square of their height (in meters). Diabetes, dyslipidemia, and chronic kidney disease (CKD) were based on the results of medical checkups.

Statistical analysis

Participants were categorized into non-OF (NOF) and OF groups, and further divided into normotensive (NT) and hypertensive (HT) groups. IBM SPSS Statistics version 29 for Windows (IBM, Armonk, NY, USA) was used for statistical analyses. The normality of the data distribution was assessed using the Kolmogorov–Smirnov test. Normally distributed data are expressed as mean ± standard deviation (SD), whereas non-normally distributed data are presented as median (25th–75th percentile). Student's *t*-test was used to compare normally distributed data between the two groups, and the Mann–Whitney *U* test for non-normally distributed data. The Mann–Whitney *U* test effect size (r) was obtained from the standardized test statistic and the number of samples. The data for categorical variables are presented as *n* (%) and analyzed using chi-square tests. A two-way analysis of covariance (ANCOVA) adjusted for age, sex,

and BMI was performed to investigate the main effects and interactions between the two OF and two HT groups on gut microbiota. Binomial logistic regression analysis was used to assess the relationship between OF and HT in the gut microbiota to verify the results of the two-way ANCOVA. The analytical method involved stratifying the data by OF. NT and HT were dependent variables evaluated in several models using different variable selections as forced input methods. Alpha diversity was assessed using the Shannon index at a sampling depth of 5000 [24]. The significance level for all tests was set at $P < 0.05$.

Sample size and statistical power

G*power software (free version) was used to calculate the sample size and statistical power. For *F*-tests of ANCOVA, the effect size, alpha error probability, power, number of groups, and number of covariates were set to 0.25, 0.05, 0.95, 4, and 3, respectively. The total sample size and actual power were 210 and 0.950, respectively. For the *Z*-tests for logistic regression, the tails, odds ratio, null hypothesis, alpha error probability, power, X distribution, and X parm π were set to 2, 2, 0.25, 0.05, 0.8, binomial, and 0.5, respectively. The total sample size and actual power were found to be 308 and 0.801, respectively. Therefore, the sample size of this study was verified to be sufficient.

Results

Participant characteristics

Table 1 shows the participant characteristics. Among the 322 participants, the mean age of 65.7 years (SD = 7.0) in 142 males was not significantly different from that of 64.3 years (SD = 7.8) in 180 females. The percentages of males who were drinkers ($P < 0.001$), current smokers ($P < 0.001$), diagnosed with hypertension ($P = 0.027$), diabetes ($P < 0.001$), and CKD ($P = 0.005$) were significantly higher than those of females. BMI ($P < 0.001$), SBP ($P = 0.030$), TG ($P = 0.042$), and HbA1c ($P = 0.005$) were significantly higher in males than in females. Conversely, LDL ($P < 0.001$), HDL ($P < 0.001$), TC ($P < 0.001$), and eGFR ($P = 0.016$) were significantly higher in females than in males. The percentage of participants with OF did not differ significantly by sex.

Characteristics of the two OF groups

Table 2 shows the comparison between the two OF groups. The mean age was significantly older in the 68.0 years (SD = 7.1) of the OF group than in the 63.5 years (SD = 7.2) of the NOF group ($P < 0.001$). HDL ($P = 0.020$), TC ($P = 0.044$), and the number of teeth ($P < 0.001$) were significantly higher in the NOF group than in the OF group.

Characteristics of the two HT groups

Table 3 shows the comparison of the two HT groups. The mean age was significantly older in the 66.6 years (SD = 7.7) of the HT group than in the 63.5 years (SD = 7.0) of the NT group ($P < 0.001$). BMI ($P = 0.002$), SBP ($P < 0.001$), DBP ($P < 0.001$), and TG ($P < 0.036$) were significantly higher in the HT group than in the NT group. The proportion of females was significantly greater in the NT group than in the HT group ($P = 0.027$).

Characteristics of the gut microbiota based on the OF and the HT groups

Table 4 shows the characteristics of the gut microbiota based on the OF and the HT groups. The abundance ratios of *Blautia* ($P = 0.049$) and *Bifidobacterium* ($P = 0.015$) were significantly higher in the NOF group than in the OF group. Namely, *Blautia* was shown to be dominant in the NOF and NT groups, respectively.

Main effects and interactions between the OF and HT groups on the gut microbiota

The NOF group was subdivided into 123 and 96 participants in the NT and HT groups, respectively. The OF group was subdivided into 49 and 54 participants in the NT and HT groups, respectively (Table 5). A two-way ANCOVA was used to assess the main effects and interactions between OF and HT on the gut microbiota after adjusting for age, sex, and BMI. Among the gut microbiota we analyzed, only *Megamonas* showed a significant interaction in the two-way OF and HT groups ($P = 0.002$). Since *Blautia* was significantly more abundant in both NOF and NT groups than in OF or HT groups in the univariate analysis, the *Megamonas*/*Blautia* ratio was calculated, which showed a significant interaction in the two-way OF and HT groups ($P = 0.004$).

Fig. 2A shows the composition of the top 30 genera of intestinal bacteria according to the two-way OF and HT groups. A Shannon index classified in the same way is shown in Fig. 2B. In multiple comparisons using the Bonferroni method in two-way ANCOVA, the HT in the OF group had a significantly higher Shannon index than the NT group ($P = 0.012$); however, no such relationship was found in the NOF group. In similar multiple comparisons, *Megamonas* showed a significantly higher abundance ratio in the HT group than in the NT group in the OF group ($P = 0.013$) but not in the NOF group (Fig. 2C). The *Megamonas*/*Blautia* ratio was also significantly higher in the HT group than in the NT group in the OF group ($P = 0.005$) but not in the NOF group

Table 1 Participant characteristics

Variable	Male (n = 142)		Female (n = 180)		P-value
	Mean/n	SD/%	Mean/n	SD/%	
Age, year	65.7	7.0	64.3	7.8	0.092
BMI, kg/m^2	24.1	3.0	22.5	2.7	< 0.001*
Drinking status					< 0.001*
Non-drinker, n (%)	40	28.2	132	73.3	
Drinker, n (%)	102	71.8	48	26.7	
Smoking status					< 0.001*
Non-or past smoker, n (%)	107	75.4	170	94.4	
Current smoker, n (%)	35	24.6	10	5.6	
Hypertensive diagnosis					0.027*
NT, n (%)	66	46.5	106	58.9	
HT, n (%)	76	53.5	74	41.1	
Diabetes					< 0.001*
Non-diabetes, n (%)	114	80.3	169	93.9	
Diabetes, n (%)	28	19.7	11	6.1	
Dyslipidemia					0.241
Non-dyslipidemia, n (%)	85	59.9	96	53.3	
Dyslipidemia, n (%)	57	40.1	84	46.7	
CKD					0.005*
Non-CKD, n (%)	93	65.5	143	79.4	
CKD, n (%)	49	34.5	37	20.6	
SBP, mm Hg	139.8	16.1	135.6	18.3	0.030*
DBP, mm Hg	79.6	10.5	77.4	10.5	0.062
LDL, mg/dL	120.5	28.8	133.1	32.2	< 0.001*
HDL, mg/dL	60.4	16.1	71.1	16.1	< 0.001*
TG, mg/dL	118.9	71.3	104.5	55.2	0.042*
TC, mg/dL	203.5	35.3	224.7	35.3	< 0.001*
HbA1c, %	6.0	0.5	5.9	0.5	0.005*
eGFR, mL/min/1.73 m^2	65.3	11.3	68.5	12.4	0.016*
OF diagnosis					0.271
NOF, n (%)	92	64.8	127	70.6	
OF, n (%)	50	35.2	53	29.4	
Number of teeth, n	19.5	7.8	19.2	8.7	0.780

Variables presented as means ± SD were analyzed using Student's t-test, and those presented as n and percentage were analyzed using chi-square tests. *, $P < 0.05$. Abbreviations: BMI, body mass index; NT, normotension; HT, hypertension; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; TG, triglycerides; TC, total cholesterol; eGFR, estimated glomerular filtration rate; NOF, nonoral frailty; OF, oral frailty; SD, standard deviation.

(Fig. 2D). Therefore, *Megamonas* was found to be higher only in the OF group with HT.

Relationship between the gut microbiota and HT stratified by OF

Table 6 shows the results of the binomial logistic regression analysis stratified by the NOF and OF groups, with the dependent variable of HT. In model 1, covariates were adjusted for age, sex, and BMI, with *Megamonas* as the independent variable. Significant independent variables for HT were age (OR 1.066, 95% CI 1.002–1.134; P = 0.044) and *Megamonas* (OR 1.274, 95% CI 1.025–1.585; P = 0.029) in the OF group. *Megamonas* was not a significant independent value for HT in the NOF group. Model 2 was the same analysis as model 1, adding drinking and smoking status as covariates. Significant independent variables in the OF group were age (OR 1.081, 95% CI 1.011–1.155; P =

Table 2 Characteristics of the two OF groups.

Variable	NOF (n = 219)		OF (n = 103)		P-value
	Mean/n	SD/%	Mean/n	SD/%	
Age, year	63.5	7.2	68.0	7.1	< 0.001*
Sex					0.271
Male, n (%)	92	42.0	50	48.5	
Female, n (%)	127	58.0	53	51.5	
BMI, kg/m^2	23.1	3.0	23.5	3.0	0.351
Drinking status					0.996
Non-drinker, n (%)	117	53.4	55	53.4	
Drinker, n (%)	102	46.6	48	46.6	
Smoking status					0.835
Non- or past smoker, n (%)	189	86.3	88	85.4	
Current smoker, n (%)	30	13.7	15	14.6	
Hypertensive diagnosis					0.149
NT, n (%)	123	56.2	49	47.6	
HT, n (%)	96	43.8	54	52.4	
Diabetes					0.862
Non-diabetes, n (%)	192	87.7	91	88.3	
Diabetes, n (%)	27	12.3	12	11.7	
Dyslipidemia					0.648
Non-dyslipidemia, n (%)	125	57.1	56	54.4	
Dyslipidemia, n (%)	94	42.9	47	45.6	
CKD					0.079
Non-CKD, n (%)	154	70.3	82	79.6	
CKD, n (%)	65	29.7	21	20.4	
SBP, mm Hg	136.2	17.4	140.2	17.5	0.051
DBP, mm Hg	78.4	10.1	78.2	11.4	0.846
LDL, mg/dL	128.9	30.1	124.6	33.8	0.258
HDL, mg/dL	67.9	16.9	63.2	16.7	0.020*
TG, mg/dL	110.5	65.0	111.6	59.2	0.883
TC, mg/dL	218.2	34.0	209.3	41.7	0.044*
HbA1c, %	5.9	0.5	6.0	0.5	0.519
eGFR, mL/min/1.73 m^2	66.9	12.3	67.4	11.5	0.751
Number of teeth, n	21.0	7.7	15.8	8.3	< 0.001*

Variables presented as means ± SD were analyzed using Student's t-test, and those presented as n and percentage were analyzed using chi-square tests. *, $P < 0.05$. Abbreviations: BMI, body mass index; NT, normotension; HT, hypertension; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; TG, triglycerides; TC, total cholesterol; eGFR, estimated glomerular filtration rate; NOF, nonoral frailty; OF, oral frailty; SD, standard deviation.

0.022), drinking status (OR 3.099, 95% CI 1.041–9.232; P = 0.042), and *Megamonas* (OR 1.317, 95% CI 1.038–1.672; P = 0.023). In the NOF group, *Megamonas* was not a significant independent valuable for HT. Similar results were confirmed for Models 1 and 2 to analyze *Megamonas* as *Megamonas*/*Blautia* ratio (Table 7). Therefore, *Megamonas* was found to be a gut microbe associated with HT in the OF group in various models.

Discussion

Our results revealed that in the OF group, the abundance ratio of *Megamonas* in the HT group was significantly higher than that in the NT group; however, this relationship was not observed in the NOF group. *Megamonas* is a Gram-negative bacterium producing lipopolysaccharide (LPS). Colon-derived LPS enhances systemic inflammation associated with various metabolic

Table 3 Characteristics of the two HT groups

Variable	NT (n = 172)		HT (n = 150)		P-value
	Mean/n	SD/%	Mean/n	SD/%	
Age, year	63.5	7.0	66.6	7.7	< 0.001*
Sex					0.027*
Male, n (%)	66	38.4	76	50.7	
Female, n (%)	106	61.6	74	49.3	
BMI, kg/m^2	22.8	3.1	23.8	2.7	0.002*
Drinking status					0.111
Non-drinker, n (%)	99	57.6	73	48.7	
Drinker, n (%)	73	42.4	77	51.3	
Smoking status					0.328
Non- or past smoker, n (%)	151	87.8	126	84.0	
Current smoker, n (%)	21	12.2	24	16.0	
Diabetes					0.189
Non-diabetes, n (%)	155	90.1	128	85.3	
Diabetes, n (%)	17	9.9	22	14.7	
Dyslipidemia					0.099
Non-dyslipidemia, n (%)	104	60.5	77	51.3	
Dyslipidemia, n (%)	68	39.5	73	48.7	
CKD					0.625
Non-CKD, n (%)	128	74.4	108	72.0	
CKD, n (%)	44	25.6	42	28.0	
SBP, mm Hg	124.7	10.3	152.1	11.7	< 0.001*
DBP, mm Hg	73.2	8.4	84.3	9.6	< 0.001*
LDL, mg/dL	125.4	31.4	130.0	31.1	0.190
HDL, mg/dL	68.0	16.4	64.5	17.4	0.064
TG, mg/dL	104.0	55.1	118.7	70.5	0.036*
TC, mg/dL	213.3	36.6	217.7	37.0	0.285
HbA1c, %	5.9	0.4	6.0	0.6	0.135
eGFR, mL/min/1.73 m^2	67.7	12.3	66.3	11.7	0.307
OF diagnosis					0.149
NOF, n (%)	123	71.5	96	64.0	
OF, n (%)	49	28.5	54	36.0	
Number of teeth, n	20.1	7.6	18.5	8.9	0.084

Variables presented as means and SD were analyzed using Student's t-test, and those presented as n and percentage were analyzed using chi-square tests. *, $P < 0.05$. BMI, body mass index; NT, normotension; HT, hypertension; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; TG, triglycerides; TC, total cholesterol; eGFR, estimated glomerular filtration rate; NOF, nonoral frailty; OF, oral frailty.

diseases, including obesity [25], diabetes, and nonalcoholic liver disease, by increasing plasma LPS levels as it enters the circulatory system [26]. A cross-sectional study by Li *et al*. [8] reported that the presence of *Megamonas* was positively correlated with SBP, demonstrating the relationship between elevated LPS-derived inflammatory substances and increased blood pressure. Although no study has directly evaluated the relationship between OF and *Megamonas*, a study evaluating the correlation between oral and gut bacteria reported a high abundance of *Streptococcus*, *Lactobacillus*, and *Klebsiella* and a low abundance of *Faecalibacterium*, *Blautia*, *Megamonas*, and *Parabacteroides*, indicating aging and plaque accumulation as factors associated with the relationship [27]. As *Megamonas* is unlikely to have a direct effect on the gut–oral axis and was not directly associated with OF in the univariate analysis of this study, OF indirectly

Table 4 Characteristics of the gut microbiota according to the OF and the HT groups

	25%	50%	75%	*P*-value (effect size(r))
Blautia				
Two OF groups				
NOF (*n* = 219)	0.046	0.066	0.101	0.049 (−0.110)
OF (*n* = 103)	0.038	0.058	0.084	
Two HT groups				
NT (*n* = 172)	0.045	0.068	0.111	0.036 (−0.117)
HT (*n* = 150)	0.039	0.059	0.084	
Bifidobacterium				
Two OF groups				
NOF (*n* = 219)	0.011	0.032	0.080	0.015 (−0.135)
OF (*n* = 103)	0.005	0.023	0.061	
Two HT groups				
NT (*n* = 172)	0.011	0.028	0.078	0.349 (−0.052)
HT (*n* = 150)	0.008	0.027	0.069	
Phascolarctobacterium				
Two OF groups				
NOF (*n* = 219)	0.000	0.004	0.014	0.449 (0.042)
OF (*n* = 103)	0.000	0.006	0.014	
Two HT groups				
NT (*n* = 172)	0.000	0.003	0.011	0.035 (0.118)
HT (*n* = 150)	0.001	0.006	0.018	
Ruminococcus.1				
Two OF groups				
NOF (*n* = 219)	0.000	0.001	0.006	0.532 (0.035)
OF (*n* = 103)	0.000	0.001	0.009	
Two HT groups				
NT (*n* = 172)	0.000	0.000	0.006	0.011 (0.142)
HT (*n* = 150)	0.000	0.001	0.008	

The abundance ratio (relative abundance out of 1.0) is shown in quartiles. *P*-values were calculated using the Mann–Whitney *U* test. Abbreviations: NOF, nonoral frailty; OF, oral frailty; NT, normotension; HT, hypertension.

Table 5 Main effects and interactions between the OF and HT groups on the gut microbiota

	NOF (*n* = 219)		OF (*n* = 103)		Main effect		Interaction
	NT (*n* = 123)	HT (*n* = 96)	NT (*n* = 49)	HT (*n* = 54)			
	EMM (95% CI)	EMM (95% CI)	EMM (95% CI)	EMM (95% CI)	OF	HT	OF × HT
Megamonas	0.021 (0.016–0.026)	0.014 (0.009–0.020)	0.011 (0.004–0.019)	0.024 (0.017–0.032)	0.907	0.305	0.002
Megamonas/*Blautia* ratio	0.358 (0.007–0.013)	0.273 (0.145–0.401)	0.199 (0.020–0.379)	0.559 (0.383–0.736)	0.421	0.081	0.004

The *P*-values were calculated using a two-way ANCOVA. Covariates were adjusted for age, sex, and BMI. Abbreviations: NT, normotension; HT, hypertension; NOF, nonoral frailty; OF, oral frailty; EMM, estimated marginal means; CI, confidence interval.

affects *Megamonas*. One possible mechanism is that in periodontal diseases related to OF [28], LPS produced by periodontopathogenic bacteria affects the gut microbiota via the gastrointestinal tract or hematogenous route, and dietary preference and nutritional status changes associated with OF can alter the gut microbiota. The interaction between these changes in the gut microbiota and OF has an inverse relationship: a low count of *Megamonas* is not associated with blood pressure, whereas a high count of *Megamonas* is positively associated with blood pressure. However, further research is warranted to validate this hypothesis as this study did not evaluate periodontal disease, oral microbiome, LPS, or nutrient intake.

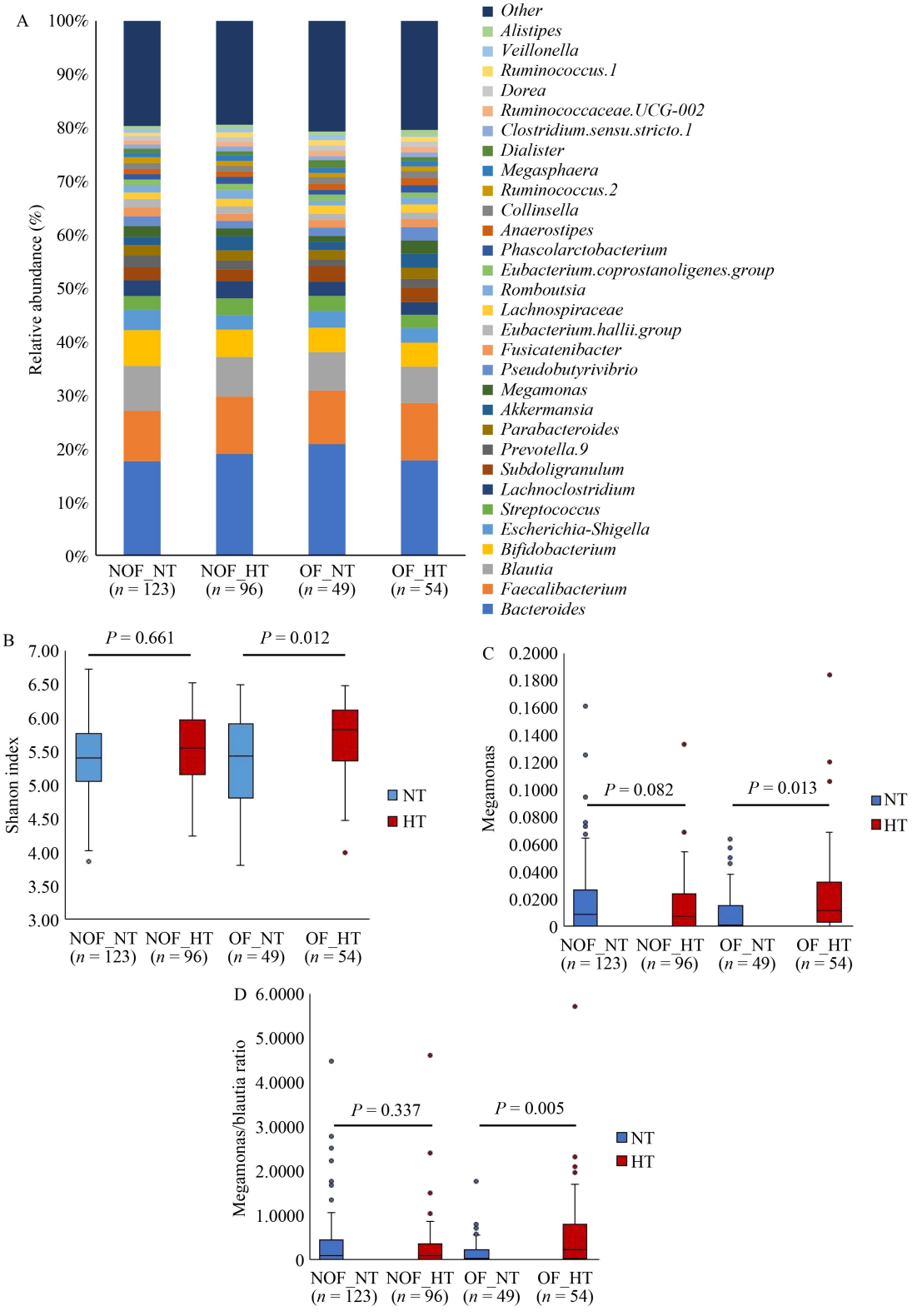

Fig. 2 Differences in the two-way OF and HT groups. (A) Composition of the top 30 genera of the gut bacteria. (B) The α diversity calculated using the Shannon index. (C) Abundance ratio (total = 1.00) of the *Megamonas*. (D) *Megamonas*/*Blautia* ratio. Abbreviations: NT, normotension; HT, hypertension; NOF, nonoral frailty; OF, oral frailty.

Table 6 Results of the binomial logistic regression analysis

	B	*P*-value	OR	95% CI
Model 1				
NOF				
Age	0.048	0.019	1.050	1.008–1.093
Sex	0.326	0.281	1.385	0.766–2.506
BMI	0.105	0.043	1.111	1.004–1.229
Megamonas	−0.091	0.11	0.913	0.817–1.021
OF				
Age	0.064	0.044	1.066	1.002–1.134
Sex	0.313	0.474	1.367	0.581–3.218
BMI	0.102	0.171	1.107	0.957–1.281
Megamonas	0.242	0.029	1.274	1.025–1.585
Model 2				
NOF				
Age	0.052	0.013	1.053	1.011–1.097
Sex	0.134	0.701	1.143	0.577–2.266
BMI	0.113	0.031	1.120	1.010–1.241
Drinking status	0.151	0.634	1.163	0.624–2.168
Smoking status	0.635	0.144	1.886	0.806–4.416
Megamonas	−0.095	0.091	0.909	0.815–1.015
OF				
Age	0.078	0.022	1.081	1.011–1.155
Sex	−0.276	0.613	0.759	0.260–2.212
BMI	0.114	0.132	1.120	0.966–1.299
Drinking status	1.131	0.042	3.099	1.041–9.232
Smoking status	−0.043	0.949	0.958	0.256–3.578
Megamonas	0.276	0.023	1.317	1.038–1.672

The dependent variable is HT. Abbreviations: BMI, body mass index; B, partial regression coefficient; CI, confidence interval; NOF, nonoral frailty; OF, oral frailty; OR, odds ratio.

Table 7 Binomial logistic regression analysis with *Megamonas* as *Megamonas*/*Blautia* ratio in the model used in Table 6

	B	*P*-value	OR	95% CI
Model 1				
NOF				
Age	0.050	0.015	1.051	1.010–1.094
Sex	0.318	0.291	1.374	0.761–2.480
BMI	0.102	0.047	1.108	1.001–1.226
Megamonas/*Blautia* ratio	−0.226	0.357	0.797	0.493–1.291
OF				
Age	0.060	0.057	1.062	0.998–1.130
Sex	0.270	0.540	1.310	0.553–3.102
BMI	0.101	0.173	1.106	0.957–1.280
Megamonas/*Blautia* ratio	1.275	0.018	3.578	1.247–10.262
Model 2				
NOF				
Age	0.054	0.010	1.055	1.013–1.099
Sex	0.125	0.718	1.133	0.574–2.239
BMI	0.111	0.034	1.117	1.008–1.237
Drinking status	0.151	0.632	1.163	0.626–2.160
Smoking status	0.639	0.140	1.895	0.811–4.427
Megamonas/*Blautia* ratio	−0.267	0.282	0.766	0.471–1.245
OF				
Age	0.077	0.024	1.080	1.010–1.154
Sex	−0.381	0.496	0.683	0.228–2.045
BMI	0.110	0.144	1.117	0.963–1.295
Drinking status	1.175	0.038	3.237	1.070–9.790
Smoking status	0.103	0.880	1.108	0.292–4.201
Megamonas/*Blautia* ratio	1.402	0.013	4.063	1.350–12.224

The dependent variable is HT. Abbreviations: BMI, body mass index; B, partial regression coefficient; CI, confidence interval; NOF, nonoral frailty; OF, oral frailty; OR, odds ratio.

Regarding hypertension and gut microbiota, studies have shown increased LPS-producing Gram-negative bacteria, including *Megamonas*, and decreased short-chain fatty acid (SCFA)-producing bacteria, including *Blautia*, in patients with hypertension [29] and the primary aldosteronism that causes it [30]. Our univariate analysis also revealed a decrease in the proportion of SCFA-producing bacteria, *Blautia*, in the HT group. In a study analyzing the relationship between the renin-angiotensin system and gut microbiota in the same Shika residents, *Blautia* was negatively correlated with SBP [17], revealing that SCFA-producing bacteria may have a protective effect against elevated blood pressure. *Blautia* was significantly reduced in OF than in NOF in our results, indicating that it is related to oral health. In addition, the finding that the *Megamonas*/*Blautia* ratio—i.e., the LPS-producing bacteria/SCFA-producing bacteria ratio—was related to the combination of OF and HT in the two-way ANCOVA and binomial logistic regression analysis is a novelty of this study.

Our Shannon index evaluation showed a high diversity in the OF and HT groups. Regarding the relationship between gut microbiota diversity and HT, studies have reported that various taxa of the gut microbiota are associated with HT and may influence each other in a complex metabolic network, not due to a single factor or a limited number of species alone [8] and that reduced bacterial richness or diversity may have an impact on HT [9], without a unanimous view. A systematic review by Palmu *et al*. [31] indicated that key gut microbial characteristics such as diversity index, microbial abundance, and variation rate vary from study to study due to technical factors or biological variability, making the comparison and replication of results difficult across studies. Therefore, although the relationship between gut

microbiota diversity and HT has not yet been concluded in previous studies, the fact that the Shannon index was higher in the OF and HT groups in our results may be hypothesized to be related to hypertension if OF-mediated changes in the composition and diversity of the gut microbiota may have been favorable for an increased expression of *Megamonas*.

It is well known that obesity is a risk factor for hypertension [32]. In our logistic regression analysis stratified by OF, BMI was a significant independent variable only in the NOF group. In contrast, a possible reason why BMI was not a significant variable in the OF group may be that the LPS production by *Megamonas* raises blood pressure independently of obesity. Although excessive drinking is a risk factor for hypertension [33], our results showed that drinking status was a significant independent variable only in the OF group. A study targeting alcohol use disorder reported an abundance of *Megamonas* [34]. However, it is unclear whether the combination of OF and heavy drinking increases *Megamonas*; hence, further investigation is necessary.

Several methods are used to evaluate the OF. The number of evaluation criteria ranges from 5 to 8, with varied points assigned to each criterion [6,35–39]. Consequently, previous studies evaluating the relationship between OF and systemic factors reported different conclusions may occur if one article's evaluation methods are used to other articles that use different evaluation methods. The Japan Geriatrics Society, the Japanese Society of Gerodontology, and the Japanese Association on Sarcopenia and Frailty jointly issued a consensus statement on OF in 2024 [1]. The assessment method adopted was the oral frailty five-item checklist (OF-5) [40]. When comparing the evaluation methods of the OF-5 and Shika study, 4 of 5 items are commonly used. The difference is that the OF-5 assesses low articulatory oral motor skills, whereas the Shika study evaluates whether tooth brushing is less than twice a day. Our evaluation of OF will yield results comparable to the standard method for OF evaluations. However, future systematic reviews on using the OF-5 for OF studies would be preferable to evaluate OF.

One strength of this study is the assessment of the direct relationship between OF and gut microbiota on hypertension, with no similar studies previously reported. Conversely, the limitations of this study are that causal relationships cannot be elucidated in a cross-sectional study, the methods for evaluating OF are not unified, analyses excluding the effects of antihypertensive drugs were not performed, the oral microbiota was not investigated, and periodontal disease indicators were not evaluated. Furthermore, the use of probiotics was not confirmed, suggesting that their influence should be investigated in the future.

Conclusions

This cross-sectional epidemiological study of the local residents revealed that only the OF group had a significantly higher abundance of *Megamonas* in the HT group than in the NT group, but not in the NOF group. In addition to the high abundance of *Megamonas*, the low ratio of *Blautia* was found to be a modifier to the HT with OF. Further studies should elucidate the causal relationship through longitudinal studies and the effects of food intake associated with OF on the gut microbiota.

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Compliance with ethics guidelines

Conflicts of interest Fumihiko Suzuki, Ren Mizoguchi, Shigehiro Karashima, Yasuo Ikagawa, Hiromasa Tsujiguchi, Akinori Hara, Sakae Miyagi, Thao Thi Thu Nguyen, Atsushi Asai, Koji Katano, Tomoko Kasahara, Kuniko Sato, Masaharu Nakamura, Yukari Shimizu, Aki Shibata, Keita Suzuki, Takayuki Kannon, Noriyoshi Ogino, Hirohito Tsuboi, Atsushi Tajima, Shigefumi Okamoto, and Hiroyuki Nakamura declare no competing interests.

The present study was conducted with the approval of the Ethics Committee of Kanazawa University (protocol code 1491 and date of approval: 18 December 2013) and the Ethics Committee of Ohu University (protocol code 365 and date of approval: 12 September 2022). The study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Informed consent was obtained from all patients for being included in the study.

Data availability and compliance statement

The authors declare that the acquisition and subsequent use of all data presented in this manuscript comply fully with all relevant local, national, and international laws, regulations, ethical guidelines, and the terms of use associated with the original data sources.

The authors bear full legal responsibility for ensuring the legality of data acquisition and all subsequent uses.

Data in the present study are available upon request from the corresponding author. Data are not publicly available due to privacy and ethical policies.

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