ARTICLE

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Epidemiology and Population Health

Gut microbiota accelerates obesity in peri-/post-menopausal women via *Bacteroides fragilis* and acetic acid

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OBJECTIVE: Many animal experiments and epidemiological studies have shown that the gut microbiota (GM) plays an important role in the development of obesity, but the specific biological mechanism involved in the pathogenesis of disease remain unknown. We aimed to examine the relationships and functional mechanisms of GM on obesity in peri- and post-menopausal women.
METHODS: We recruited 499 Chinese peri- and post-menopausal women and performed comprehensive analyses of the gut microbiome, targeted metabolomics for short-chain fatty acids in serum, and host whole-genome sequencing by various association analysis methods.
RESULTS: Through constrained linear regression analysis, we found that an elevated abundance of Bacteroides fragilis (B. fragilis) was associated with obesity. We also found that serum levels of acetic acid were negatively associated with obesity, and that B. fragilis was negatively associated with serum acetic acid levels by partial Spearman correlation analysis. Mendelian randomization analysis indicated that B. fragilis increases the risk of obesity and may causally down-regulate acetic acid levels.
CONCLUSIONS: We found the gut with B. fragilis may accelerate obesity, in part, by suppressing acetic acid levels. Therefore, B. fragilis and acetic acid may represent important therapeutic targets for obesity intervention in peri- and post-menopausal women.

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INTRODUCTION

Obesity has become a global epidemic and is a major risk factor for type 2 diabetes, cardiovascular diseases, and certain cancers [1]. According to the European Association for obesity research (EASO) and the World Health Organization, overweight and obesity cause ~2.8 million deaths each year [2]. Additionally, the global prevalence of obesity in women has more than doubled during the last four decades [2]. As women age, bear children and progress through menopause, their proportions of trunk fat and android fat typically increase substantially [3, 4], they are more likely to become obese [5], and the risk of diabetes and cardiovascular disease is greatly elevated [6, 7]. Obesity is considered as one of the most important public health issues of the twenty-first century, especially in post-menopausal women.

There are many lines of evidence which demonstrate that the gut microbiota (GM) is an important environmental factor leading to obesity by altering the energy acquisition and storage of the host [8, 9]. For instance, *Bacteroides thetaiotaomicron* has been shown to alleviate diet-induced weight gain and adiposity in mice [10]. Previous studies have shown that the GM composition has important implications for nutrient absorption processes by altering the pH of the gut and through interactions with the intestinal mucosal barrier [11, 12]. The microbiota also produces a variety of metabolic byproducts such as short-chain fatty acids (SCFAs, fatty acids produced only by GM in humans) which have a number of important influences on host metabolism [13, 14]. However, the biological mechanisms of the GM contributing to the pathogenesis of obesity are still not fully understood. Investigating the relationships between the GM and its metabolites with obesity traits (including body mass index (BMI), whole-body fat percentage, trunk fat percentage, and android fat percentage) may reveal novel biomarkers for the treatment/prevention of disease.

Therefore, we performed comprehensive analyses of the gut microbiome, targeted metabolomics for SCFAs in serum, and host whole-genome sequencing in a cohort of Chinese peri- and post-menopausal women. We aimed to identify the effects of obesity-associated bacteria and SCFAs, and to uncover the causal effects of specific bacteria on obesity and serum SCFA levels.

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MATERIALS AND METHODS

Subjects recruitment and sample collection

We recruited 499 random independent peri- and post-Chinese women who were self-reported to be unrelated. Menopause is marked by the cessation of menstruation, where peri-menopause is a transition phase beginning at a woman's last menstrual cycle and continuing through the following 12 months without a menstrual cycle; once there are no menstrual cycles for 1 year, we term it post-menopause [15]. All subjects who used antibiotics, estrogens, or anticonvulsant medications in the past 3 months were excluded. Each individual provided written informed consent, and filled out a questionnaire that collected information about demographics, lifestyle (including cigarette smoking, alcohol drinking, and physical activity), medical history, diet habits, etc. This study was approved by the Third Affiliated Hospital of Southern Medical University, Guangzhou City, Guangdong Province, and was performed in accordance with the principle of the Helsinki Declaration II.

We collected blood and stool samples from each subject. Blood samples were collected after an overnight fast for at least 8 h and used for serum analysis and DNA extraction with the SolPure DNA Kit (Magen, Guangzhou, China). Each fecal sample was used for GM DNA extraction with the E.Z.N.A.® Stool DNA Kit (Omega, Norcross, GA, USA). The serum, blood, and GM DNA samples were stored at −80 °C until further analysis. All subjects were weighed in light clothing without shoes. Body height and weight were measured by a height-weight scale, and BMI (kilograms per square meter) was calculated. Whole-body fat percentage, trunk fat percentage, and android fat percentage were measured by dual-energy X-ray absorptiometry (GE Healthcare, Madison, WI, USA, version 13.31.016). Physical activity was measured using a questionnaire and data were summarized in metabolic equivalent-hours/week. The threshold of physical activity was 2.5 h per week based on a published guideline [16]. BMI is widely used as a proxy for obesity in clinical practice and epidemiological research [17]. Whole-body fat percentage, trunk fat percentage and android fat percentage are reported to sometimes be more sensitive than BMI to indicate health risks [18, 19].

Metagenomic shotgun sequencing and annotation

DNA library construction. We performed shotgun metagenomic sequencing at LC-Bio Technologies (Hangzhou) CO., LTD. (Hangzhou City, Zhejiang Province, China, www.lc-bio.com). The fecal DNA library was constructed by the TruSeq Nano DNA LT Library Preparation Kit (FC-121-4001, Illumina, San Diego, CA, USA). And then we used Hiseq 4000 (Illumina, San Diego, CA, USA) and PE150 strategy to conduct metagenomic shotgun sequencing. The raw sequencing reads were processed through multiple cleaning steps.

Bacteria abundance calculation. Quality-filtered reads were de novo assembled to construct metagenomes for each sample by SPAdes v3.10.0. MetaGeneMark v3.26 was used to predict the coding sequences (CDS) of metagenomic contigs. The CD-HIT v4.6.1 was used to cluster the CDS of all samples to obtain unigenes. The relative abundance of unigenes for a sample was estimated by transcripts per kilobase million based on the number of aligned reads and the unigene length by Bowtie2 v2.2.0.

Species biodiversity calculation. The R package "vegan" [20] was used to estimate the α-diversity (Shannon index) of GM in each sample, and the association between the GM biodiversity and obesity traits variation was tested by partial Spearman correlation analysis. The association between β-diversity and obesity traits was assessed by microbiome regression-based kernel association test (MiRKAT) [21]. We constructed various different kernels from commonly used distance metrics for compositional data (including weighted/unweighted UniFrac and Bray–Curtis distances), and then obtained the optimal kernel to estimate the association between GM species profiles and obesity trait variation. These analyses were adjusted for age, years since menopause (YSM), physical activity, and alcohol drinking since these lifestyle factors are known to affect the occurrence of obesity [17]. Results with p value <0.05 were considered statistically significant.

Measurement of SCFAs

We used the gas chromatography-tandem mass spectrometry (GC-MS/MS) to measure SCFAs. We used methyl tert-butyl ether to extract SCFAs from the serum samples by liquid-liquid extraction. Then 2 μl of the supernatants were analyzed with GC-MS/MS (7890B-7000D, Agilent Technologies Inc, Santa Clara, CA, USA) by Wuhan Metware Biotechnology Co., Ltd (Wuhan City, Hubei Province, China, www.metware.cn) based on a fused silica capillary column (DB-FFAP, 30 m × 0.25 mm × 0.25 μm, Agilent Technologies Inc, Santa Clara, CA, USA).

Association analysis among GM, obesity traits and SCFAs

We used various association analysis methods to explore the relationship among GM, SCFAs, and continuous obesity traits. We focused on non-rare species (relative abundance >0.10%), because the contribution of rare species to functional diversity is significantly smaller than that of non-rare species [22].

Spearman correlation analysis was performed to assess the pairwise correlations between the relative abundances of the different bacterial species. For those highly correlated bacterial species (with correlation coefficients [γ's] ≥ 0.80) that might cause a multiple co-linearity issue, we retained the bacterial species with a higher relative abundance and removed others as bacteria with higher abundance may render increased statistical power for association testing. The R package "corrplot" package was used to create a heatmap for visualization of the bacterial species correlation. We also evaluated the Spearman correlations between specific bacteria and probiotic bacteria reported in two previous reviews which summarized the most notable probiotics [14, 23]. The reported probiotic bacteria were pooled for such analyses due to the small relative abundances of each of the individual species (<0.10%).

Constrained linear regression analysis [24] was performed using Stata 14 software with the "cnsreg" function to identify obesity traits-associated bacterial species. Obesity variation was considered as the dependent variable, and the centered log ratio-transformed relative abundances of all bacterial species were set as the independent variables and analyzed simultaneously. The constraint ensures that the regression coefficients of all bacteria species sum to zero, an approach which is frequently used in compositional data analysis. The formula of constrained linear regression analysis is shown below (Formula 1), where p is the phylum level, n is the number of phyla, s is a species indicator, and m is the number of bacterial species in a given phylum. The model was adjusted for age, YSM, physical activity, and alcohol drinking. Bacterial species with a p value of the regression coefficient (β) <0.05 were defined as obesity traits-associated bacterial species.

$$Y(\text{obesity traits}) = \sum_{p=1}^{n}\sum_{s=1}^{m}\beta_{ps}\text{CLR}(X_{ps}) + \beta \times \text{covariates}$$
$$\sum_{s=1}^{m}\beta ps = 0 \quad (1)$$

We then used partial Spearman correlation analysis to identify the association between obesity traits and SCFA levels adjusted for the same set of covariates mentioned above. The SCFA levels were normalized by the maximum likelihood-based Box Cox power transformation. Furthermore, we performed a partial Spearman correlation analysis to identify the relationship between obesity traits-associated SCFAs and obesity traits-associated bacterial species while adjusting for age, YSM, and BMI as covariates.

Mendelian randomization (MR) analysis for potential causality between obesity traits, specific GM, and specific SCFAs

Whole-genome sequencing (WGS). Genomic DNA was isolated from the peripheral blood and used to construct a genomic DNA library for WGS. DNA was extracted and sheared into fragments by Covaris technology followed by amplification, single strand separation, and cyclization by ligation-mediated PCR. High throughput sequencing was performed for each library to ensure that each sample meets the average sequencing coverage requirement. Raw data were stored in FASTQ format and pre-processed by BGISEQ-500 Base-calling software to remove sequences of the adapter, low-quality reads, and unknown reads. The clean data were sequence aligned with the human reference genome (GRCh38/HG38) by Burrows-Wheeler Aligner (BWA) software.

WGS was performed by BGI Genomics Co. Ltd (Shenzhen) on the BGISEQ-500 sequencing system.

Genome-wide association study (GWAS). We used PLINK 1.9 software to identify single nucleotide polymorphisms (SNPs) potentially associated with specific GM, obesity traits, and specific SCFAs. The quality control included eliminating the SNPs with missing rate >0.1, minor allele frequencies <0.01, Hardy–Weinberg equilibrium p value $<1.0 \times 10^{-5}$, and the command of PLINK is -- mind 0.1, -- geno 0.1, and -- hwe 1e−5.

MR analysis. Previous studies have shown that host genome variations affect GM and obesity [25, 26], as well as the interaction between the host and symbiotic bacteria [27]. We conducted bidirectional MR analysis to investigate the causality of specific GM exposure on obesity traits outcome by using the SNPs identified by GWAS above as instrumental variables. We selected independent genetic variants ($r^2 \leq 0.001$) associated with the specific GM (p values $<1 \times 10^{-6}$) and the obesity traits (p values $<1 \times 10^{-6}$) as the instrumental variables [28]. Since SCFAs are exclusively produced by the GM [29] in humans, we conducted single direction MR (specific GM as exposure and specific SCFAs as outcome) to explore the causal relationship between these biological factors. In order to increase the reliability of analysis results, we used three MR analysis methods: (1) simple median method which takes the median estimate assuming all variants carry equal weight, (2) weighted median method which gives more weight to variants with more precise estimates and (3) inverse-variance weighted which uses a weighted linear regression of the ratio of the SNP effects on the outcomes to the SNP effects on the risk factor [30]. Since the MR estimates may be biased by the presence of horizontal pleiotropy, we used the intercept that deviates from the MR-Egger method to examine the presence of horizontal pleiotropy as described in the previous study [28]. If the p value of MR-Egger (intercept) is greater than 0.05, we consider that the instrumental variable affects the outcome only through the exposure of interest and not through any other independent pathways.

RESULTS

Characteristics of study cohort

We recruited 499 peri- and post-menopausal Chinese women and obtained metagenomic profiles for 499 subjects, 497 of which also had targeted metabolomic profiles for SCFAs. Based on YSM [15], 413 women in this cohort (83%) were classified as post-menopausal, while 86 women (17%) were peri-menopausal. According to the BMI guidelines for the Prevention and Control of overweight and Obesity among Chinese Adults, 31 women in this cohort (6.21%) were obese (28 ≤ BMI), 135 women (27.05%) were overweight (24 ≤ BMI < 28), 314 women (62.93%) were normal (18.5 ≤ BMI < 24) and 19 women (3.81%) were underweight (BMI < 18.5). As shown in Table 1, the mean YSM was 1.96 years (standard deviation (SD)) = 0.95), and the average age was 52.84 years (SD = 2.95). The mean BMI was 22.97 kg/m^2 (SD = 2.87), and 60.5% of subjects reported exercising regularly.

Correlation between the biodiversity of GM and obesity traits

On average we obtained ~7.35 Gbp of sequence data for each sample. Through taxonomic annotations, a total of 10,303 taxa were identified at the species level. Among them, 173 species were non-rare species whose relative abundance exceeded 0.10% [31]. The three most common species were *Faecalibacterium prausnitzii* (8.77%), *Bacteroides vulgatus* (7.46%), and *Bacteroides fragilis* (4.16%). We used partial Spearman correlation analysis to evaluate the co-occurrence between the relative abundances of these non-rare species. We found that 26 and 27 of these non-rare species were found to have large positive (γ's > 0.80, p values <0.001) and strong negative (γ's < −0.30, p values < 0.001) correlations with at least one other non-rare species, respectively. Partial Spearman correlation and MiRKAT analyses revealed that there were no significant associations between α-diversity (Shannon index) or β-diversity and obesity traits (Table 2). Although there was no significant global relationship, we hypothesized that there may still be individual species which may influence obesity traits.

Bacterial species associated with obesity traits

The constrained linear regression analysis found that several individual bacterial species were significantly associated with obesity traits (Table 3). For instance, *B. fragilis* ($\beta = 0.734$, p value = 0.023) and *Bacteroides stercoris* (*B. stercoris*, $\beta = 0.260$, p value = 0.025) were significantly associated with BMI. Additionally, *B. fragilis* was also associated with android fat percentage ($\beta = 0.016$, p value = 0.034), trunk fat percentage ($\beta = 0.016$, p value = 0.022), and whole-body fat percentage ($\beta = 0.015$, p value = 0.013). We then investigated the causality between specific GM and obesity traits with bidirectional MR analysis. Finally, we used 13 SNPs associated with BMI, 60 SNPs associated

Table 1. Characteristics of the Chinese study cohort.

Phenotypes	Max	Min	Mean (SD) or percentage
YSM (year)	8.99	0.06	1.96 (0.95)
Age (year)	64.59	41.47	52.84 (2.95)
BMI (kg/m²)	33.73	16.42	22.97 (2.87)
Android fat percentage (%)	58.18	19.28	43.37 (0.07)
Trunk fat percentage (%)	54.07	18.17	37.30 (0.06)
Whole-body fat percentage (%)	52.01	18.74	34.23 (0.05)
Alcohol drinking			No 71.34%
			Yes 28.66%
Physical activity			None 29.86%
			Low 9.62%
			High 60.52%

SD standard deviation, *YSM* years since menopause, *BMI* body mass index.

Table 2. Correlation analysis between obesity traits and gut microbiota (GM) diversity.

Obesity traits	Shannon index		*p (MiRKAT)*
	r	*p* value	
BMI	−0.082	0.087	0.254
Android fat percentage	−0.058	0.142	0.385
Trunk fat percentage	−0.077	0.082	0.234
Whole-body fat percentage	−0.078	0.107	0.389

The test adjusted for years since menopause, age, alcohol drinking, physical activity.
r partial Spearman correlation coefficient, *p* *p*-value of the correlation coefficient.

Table 3. Obesity-related bacterial species identified by constrained linear regression.

Bacteria	β	*p* value	Phenotype
Bacteroides fragilis	0.734	0.023	BMI
Bacteroides fragilis	0.016	0.034	Android fat percentage
Bacteroides fragilis	0.016	0.022	Trunk fat percentage
Bacteroides fragilis	0.015	0.013	Whole-body fat percentage
Bacteroides stercoris	0.260	0.025	BMI
Bacteroides stercoris	0.008	0.004	Android fat percentage
Bacteroides stercoris	0.006	0.013	Trunk fat percentage
Bacteroides stercoris	0.005	0.018	Whole-body fat percentage

The test adjusted for years since menopause, age, alcohol drinking, physical activity.
β constrained linear regression coefficient, *p value* *p*-value of the regression coefficient.

with whole-body fat percentage, 24 SNPs associated with android fat percentage, and 8 SNPs associated with trunk fat percentage. As shown in Table 4, all MR methods indicated that *B. fragilis* (p values < 0.001) and *B. stercoris* (p values <0.02) may causally up-regulate BMI. The non-significance of the MR-Egger (intercept) terms indicate that there were no significant horizontal pleiotropic effects, and there were no major violations of this critical MR assumption. We also detected a significant causal relationship between *B. fragilis* and other obesity traits including whole-body fat percentage, trunk fat percentage, and android fat percentage. However, *B. stercoris* did not have a significant causal relationship with other obesity traits besides BMI.

SCFAs significantly associated with obesity traits

Previous studies have shown that GM can affect host health through the production of SCFAs [14, 29]. We used partial Spearman correlation analysis to test the associations between the individual SCFAs (include caproic acid, isovaleric acid, butyric acid, acetic acid, isobutyric acid, valeric acid) and obesity traits. We found that acetic acid was negatively associated with various obesity traits (Table 5). Furthermore, we found that *B. fragilis* was significantly associated with acetic acid ($\gamma = -0.093$, q value $= 0.022$).

Since SCFAs (e.g., acetic acid) are produced by gut probiotic bacteria [32], we performed partial Spearman correlation analysis to investigate the correlation between *B. fragilis* and known probiotic bacteria [14, 23] (including *Bifidobacterium_longum*, *Bifidobacterium_breve*, *Lactobacillus_rhamnosus*, *Lactobacillus_gasseri*, *Lactobacillus_plantarum*, *Lactobacillus_reuteri*, *Lactobacillus_casei*, *Bifidobacterium_longum_CAG:69.*), all of which are reported to produce acetic acid. We found that the relative abundance of *B. fragilis* was negatively associated with the combined abundance of traditional probiotic bacteria ($\gamma = -0.277$, p value $= 3.75 \times 10^{-10}$). Additionally, MR analysis indicated that *B. fragilis* may causally down-regulate acetic acid (Table 6, p values $<1 \times 10^{-5}$).

DISCUSSION

There is growing evidence that the GM contributes to the development of obesity. Thus, identification of the association between obesity and GM and identification of microbial therapeutic/prevention targets is highly warranted. Although GM biodiversity was not significantly associated with obesity, we found that several individual bacterial species may play an important role in the pathogenesis of disease. *B. fragilis* and *B. stercoris* were positively associated with obesity, while serum levels of acetic acid, a bacterial fermentation product, were negatively associated with both obesity and *B. fragilis*. Furthermore, we found that *B. fragilis* may causally influence both acetic acid levels and obesity.

We were not able to identify a significant association between GM biodiversity and obesity traits variation, perhaps due to the variation of the study participants such as age, lifestyles, and study design-related factors (e.g., sample size). However, the results still suggested a negative direction of effect (e.g., for BMI, $\gamma = -0.082$, p value $= 0.086$), which is in alignment with the previous reports [33]. For example, a previous study [33] found that obesity was associated with a significant decrease in the level of microbiome diversity. Additionally, a growing body of evidence suggests that GM biodiversity has a beneficial effect on human health [34]. We then hypothesized that individual bacterial species may contribute to obesity phenotypes and conducted further analyses to detect potential microbiome-related therapeutic/prevention strategies for obesity.

Among the bacterial species we identified, *B. fragilis* is the most noteworthy. *B. fragilis* is one of the most abundant bacteria in the human gut, and is involved in many activities that influence human health including polysaccharide digestion, gut

Table 4. Potential causality of specific bacteria exposure on BMI outcome with MR approach.

MR methods	*Bacteroides fragilis* to BMI			BMI to *Bacteroides fragilis*			*Bacteroides stercoris* to BMI			BMI to *Bacteroides stercoris*		
	β	Standard error	p value	β	Standard error	p value	β	Standard error	p value	β	Standard error	p value
Simple median	0.09	0.023	7.12E−05	0.11	0.137	0.421	0.057	0.022	0.008	0.093	0.154	0.546
Weighted median	0.086	0.023	0.0002	0.111	0.136	0.415	0.055	0.022	0.012	0.079	0.147	0.59
IVW	0.093	0.016	2.95E−09	0.206	0.098	0.035	0.049	0.015	0.001	0.106	0.139	0.448
MR-Egger (intercept)	0.015	0.029	0.606	0.308	0.679	0.651	−0.015	0.03	0.62	1.485	0.604	0.014

β regression coefficient of the association between *B. fragilis* and BMI with various MR methods, *p-value* *p*-value of the regression coefficient, *MR* Mendelian randomization, *IVW* inverse-variance weighted.

Table 5. The specific bacteria and obesity traits are associated with SCFAs.

SCFAs	β	p value	Q value	Phenotype
Acetic acid	−0.129	0.042	0.042	Body mass index
Acetic acid	−0.188	<0.001	<0.001	Android fat percentage
Acetic acid	−0.175	<0.001	<0.001	Trunk fat percentage
Acetic acid	−0.132	<0.001	<0.001	Whole-body fat percentage
Acetic acid	−0.093[a]	0.011	0.022	*Bacteroides fragilis*

The test adjusted for years since menopause, age, alcohol drinking, physical activity.
SCFAs short-chain fatty acids, *β* constrained linear regression coefficient, *p value* p value of the regression coefficient, *Q value* p value after false discovery rate (FDR) adjustment.
[a]The test adjusted for years since menopause, age, BMI.

Table 6. Potential causality of *B. fragilis* exposure on acetic acid outcome with MR approach.

MR methods	β	Standard error	p value
Simple median	−0.101	0.023	9.64E−06
Weighted median method	−0.101	0.023	9.36E−06
IVW	−0.074	0.016	2.88E−06
MR-Egger (intercept)	−0.008	0.029	0.775

β regression coefficient of the association between *B. fragilis* (as exposure) and acetic acid (as outcome) with various MR methods, *p value* p-value of the regression coefficient, *MR* Mendelian randomization, *IVW* inverse-variance weighted.

development and maturation, and modulation of the immune system [35]. In addition to its role as a commensal microorganism, *B. fragilis* is an opportunistic pathogen associated with anaerobic infections, most commonly intra-abdominal sepsis [36]. Previous studies have found that there is a significant correlation between *B. fragilis* and BMI/weight gain in children, but the specific mechanism is still unclear [37–39]. The results in the present study showed that *B. fragilis* may causally increase BMI. We also identified *B. stercoris* to have a significant causal effect on BMI, which has not previously been identified for association with BMI. Considering these findings, we may conclude that specific bacteria in the GM can accelerate the development of obesity in post-menopausal women.

We then explored the relationship between obesity and serum SCFAs, which are generated exclusively by the GM [29] in humans and function as critical signaling molecules between the GM and host [40]. Previous studies have shown that SCFAs, which are produced by the microbial digestion of dietary fibers, have a largely beneficial effect on energy balance and metabolic homeostasis [41–43] and could have a protective effect against the development of obesity [25]. In fact, most studies link high dietary fiber intake with a lower risk of obesity, which suggests that SCFAs may have a beneficial effect on body-weight regulation [13]. This study found that serum acetic acid was negatively associated with BMI. Previous animal studies revealed that acetate may reduce appetite through interactions with the central nervous system [44]. However, SCFAs also participate in energy harvesting and interfere with host metabolism, so controlled human intervention studies are still needed to draw firm biological mechanism conclusions [13].

We further demonstrated that *B. fragilis* may causally down-regulate acetic acid levels, and that *B. fragilis* was negatively correlated with acetic acid producing probiotic bacteria within the gut. We speculate that *B. fragilis* may competitively inhibit the growth of probiotic bacteria, which in turn affects the production of acetic acid. Previous studies have suggested that the human GM is dominated by negative microbial interactions [45], and that the levels of bacterial fermentation products depend on the relative abundances of different bacterial groups in the gut as well as the competitive and cooperative interactions among different species [46]. The type VI secretion system, a molecular device used by Gram-negative bacteria (such as *Bacteroides*) to transmit toxic substances to other bacteria [45], is a classic mechanism that results in negative interactions/correlations. Through bioinformatics screening, homologous type VI secretion system genes were found in more than half of *Bacteroidales* (a class including the *Bacteroides* genus) species [45], which is consistent with our current results, indicating that *B. fragilis* may competitively inhibit probiotic bacteria. A previous study also reported that when obese children lost weight after probiotic diet interventions, the abundance of *B. fragilis* decreased and the concentration of acetic acid increased [38].

In our study, we also found that android, trunk, and whole-body fat percentages were positively correlated with *B. fragilis* and negatively correlated with acetic acid, consistent with the findings for BMI. Additionally, the correlations between acetic acid and body fat percentages were more significant than BMI. There is some evidence to indicate that these alternative obesity indicators may actually have more utility than BMI for indicating health risks. Among post-menopausal women with normal BMI, trunk fat is associated with increased risk of cardiovascular disease [17]. Post-menopausal women are prone to metabolic alterations resulting in part from a shift from subcutaneous to intra-abdominal visceral fat, which leads to an increase in android and trunk fat [3, 4, 47]. Therefore, we hypothesized that the bacteria also played an important role in the change of body fat distribution in post-menopausal women, thus providing a novel and more precise option for intervention in this physiological process.

The main advantages of our study are as follows. First, to our knowledge, this is the first metagenomic study to directly detect the association between GM and obesity in pre-and post-menopausal women. Secondly, we used an innovative and comprehensive multiomics approach to clarify the relationship between GM and its metabolic byproducts and obesity. Finally, we provide a number of statistical evidences to demonstrate the effects of specific bacterial species and GM derived metabolites on obesity variation and regulation, as well as causality. Nevertheless, the study also has some limitations. Some factors that may affect the results of the study were not considered, such as diet, stress, some medications, family history of obesity and some endocrine diseases. But we have tried our best to control the influence of these factors on the study sample. We recruited volunteers living in the same city for at least 3 months, also consider alcohol drinking. In addition, the sample size of GWAS study used to infer causality may be relatively small. But it can also be used to preliminarily estimate causality, we hope that with the further study of intestinal flora GWAS, there will be data from larger samples to verify our research results.

In summary, our study demonstrated that *B. fragilis* may play a critical role in the increase of body fat, potentially through its effects on acetic acid production. Our findings provide novel insights into the relationship between the GM and obesity, and also provide potential biomarkers for obesity intervention by targeting the GM.

DATA AVAILABILITY

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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AUTHOR CONTRIBUTIONS

H-WD designed the study protocol and directed all parts of the project. JS, XL, R-KL and X-ZZ performed clinical diagnosis and recruited subjects. XL, H-ML and B-YL contributed to the data analysis. W-DS conducted data analysis and drafted the first edition of the document. H-WD revised, rewrote/re-structured some sections and finalized the manuscript. H-MX and JS provided support and constructive criticism in the study. JG, H-ML, XL, W-QL, XQ, and B-YL contributed to text revision and discussion.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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