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Mendelian Randomization Analysis Reveals Causal Effects of the Human Gut Microbiota on Abdominal Obesity

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ABSTRACT

Background: Although recent studies have revealed an association between the composition of the gut microbiota and obesity, whether specific gut microbiota cause obesity has not been determined.

Objectives: The aim of this study is to determine the causal relationship between specific gut microbiota and abdominal obesity. Based on genome-wide association study (GWAS) summary statistics, we performed a 2-sample Mendelian randomization (MR) analysis to evaluate whether the gut microbiota affects abdominal obesity.

Methods: Gut microbiota GWAS in 1126 twin pairs (age range, 18–89 years; 89% were females) from the TwinsUK study were used as exposure data. The primary outcome tested was trunk fat mass (TFM) GWAS in 492,805 participants (age range, 40–69 years; 54% were females) from the UK Biobank. The gut microbiota were classified at family, genus, and species levels. A feature was defined as a distinct family, genus, or species. MR analysis was mainly performed by an inverse variance–weighted test or Wald ratio test, depending on the number of instrumental variables (IVs) involved. A sensitivity analysis was performed on significant results by a weighted median test and a weighted genetic risk score (GRS) analysis.

Results: Results of MR analyses provided evidence of a causal association between 3 microbiota features and TFM, including 1 family [*Lachnosiraceae*; $P = 0.02$; $\beta = 0.001$ (SEE, 4.28×10^{-4})], 1 genus [*Bifidobacterium*; $P = 5.0 \times 10^{-9}$; $\beta = -0.08$ (SEE, 0.14)], and 1 species [*Prausnitzii*; $P = 0.03$; $\beta = -0.007$ (SEE, 0.003)]. Both the weighted median test and GRS analysis successfully validated the association of the genetically predicted family, *Lachnosiraceae* ($P_{\text{weighted median}} = 0.03$; $P_{\text{GRS}} = 0.004$).

Conclusions: Our findings provided evidence of a causal association between gut microbiota and TFM in UK adults and identified specific bacteria taxa that may regulate the fat metabolism, thus offering new direction for the treatment of obesity. *J Nutr* 2021;151:1401–1406.

Keywords: Mendelian randomization, gut microbiota, obesity, trunk fat mass, causal relationship

Introduction

Obesity is a chronic metabolic disease characterized by excessive accumulation of adipose tissue. Obesity is associated with increased risks of cardiovascular disease, type 2 diabetes, and certain cancers (1). It is among the most important risk factors contributing to the overall burden of diseases worldwide. In 2013, the number of overweight and obese individuals globally reached 2.1 billion and the prevalence of obesity had increased substantially (2).

BMI, which is defined as body mass in kilograms divided by the square of height in meters (kg/m^2), is currently the standard measure of obesity due to its simplicity. However, BMI is not the ideal phenotype to measure obesity because it does not give a precise idea about the body composition (3). Human body mass is composed of fat mass, lean mass, bone mass, water, and soft tissues; it is only fat mass that induces obesity and causes a series of adverse clinical manifestations. Therefore, fat mass may provide a more appropriate measure of obesity than BMI. The regional distribution of fat mass also seems to correlate with different levels of cardiovascular risk, even more so than the BMI (4). Fat stored in the abdomen is more harmful than fat stored at other body regions. For example, fat mass stored more

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centrally leads people to be more susceptible to cardiovascular diseases and endocrine disorders (5). Thus, it is believed that an index based on trunk fat mass (TFM) may be more specific to diagnose obesity severity (6). Nonetheless, the research using TFM as a measure of obesity has rarely been studied.

Even though obesity can be attributable to lifestyle, cultural factors, and genetics (7–9), mounting evidence demonstrates that the gut microbiota plays an important role in the development of obesity (10–12). Mice models provided some evidence of obesity linked to gut microbiota, but the findings were far from consistent (13, 14). A cohort study identified 34 bacterial taxa associated with BMI, and the human gut microbiota explained 4.5% of the variance in BMI (15). Several studies have shown an increase of the *Firmicutes*/*Bacteroidetes* ratio in obese individuals. A case-control study found the abundance of *Lactobacillus reuteri* was positively correlated with BMI, while the abundance of *Bifidobacterium animalis*, *Methanobrevibacter smithii*, and *Escherichia coli* were negatively associated with BMI (16). However, the causality between specific gut microbiota and obesity is still ambiguous due to many confounding factors (including lifestyle, diet, and disease status) that occur within the population.

Mendelian randomization (MR) analysis is an efficient approach that utilizes genetic variants as instrumental variables (IVs) to evaluate the potential causal relationship between a specific risk factor and a trait/disease of interest (17). Genetic variants can be used as IVs because they are randomly assigned at birth and are fixed throughout life, and are therefore not affected by confounding factors (e.g., lifestyle, socioeconomic factors). MR analysis relies on 3 essential assumptions: *1*) IVs are strongly associated with the exposure; *2*) IVs are independent of any observed and unobserved confounders of an exposure-outcome association; and *3*) IV-outcome associations are only mediated via exposure rather than by any other pathway. Previous studies have shown that host genetic variations influence the composition of gut microbiota (18–22). In addition, due to the rapidly increasing number of genome-wide association studies (GWASs) for both gut microbiota and complex traits, it is possible to implement an MR analysis for gut microbiota based on GWAS summary statistics. Recent studies using MR have indicated the causal association between gut microbiota and celiac disease (23), bone development (24), ischemic heart disease (25), and neuropsychiatric disorders (26).

Aiming to investigate the causal link from specific gut microbiota to TFM, we conducted a 2-sample MR analysis in which single nucleotide polymorphism (SNP) exposure and SNP outcome associations were identified from independent previous GWASs and subsequently combined into a single causal estimate via a meta-analysis. Specifically, the summary statistics from a gut microbiota GWAS served as the exposure, while the summary statistics from a TFM GWAS served as the outcome.

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Supplemental Table 1 is available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/jn/.
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Abbreviations used: BIA, bioelectrical impedance approach; GRS, genetic risk score; GWAS, genome-wide association study; IV, instrumental variable; LD, linkage disequilibrium; LMM, linear mixed model; MR, Mendelian randomization; PC, principal component; TFM, trunk fat mass; UKB, UK Biobank.

Methods

Ethics statement

Gut microbiota GWAS summary statistics were accessed from a published study (20). No new institutional review board approval was required. The TFM sample came from the United Kingdom Biobank (UKB) project. The ethics approval for the UKB study was obtained from the North West Centre for Research Ethics Committee (11/NW/0382), and informed consent was obtained from all participants. This study (project number 41542) was covered by the general ethical approval for the UKB study. A flowchart briefly describes the whole analysis in **Figure 1**.

GWAS summary statistics for gut microbiota

The gut microbiota sample consisted of 1126 twin pairs from the TwinsUK Registry in the United Kingdom. The average age of the twins was 59 years (range, 18–89 years), and 89% of them were females. In brief, the bacterial taxa relative abundance was normalized by Box-Cox transformation. The genetic associations between 945 bacteria taxa and 1,300,091 host SNPs were tested. A total of 307 host SNPs were identified, and were associated with 61 bacteria taxa (1 kingdom + 6 phyla + 9 classes + 9 orders + 16 families + 16 genera + 4 species) at a false discovery rate < 0.2. The *P* values at these SNPs ranged from 4.94×10^{-9} to 7.33×10^{-5}. Summary statistics of these significant SNPs were assessed through the supplemental table of the study publication (20).

UKB TFM sample

The UKB project is a large, prospective, cohort study with ~500,000 participants aged between 40–69 years from 22 centers across the United Kingdom. The average age at baseline was 56.5 years, and 54% of subjects were females. All the included participants in the UKB sample were those who self-reported as white (data field 21000). Participants who had a self-reported gender inconsistent with the genetic gender, who were genotyped but not imputed, or who withdrew their consent were removed. TFM (data field 23128) was measured by the bioelectrical impedance approach (BIA). The final sample consisted of 492,805 participants and the sample means for BMI and TFM were 27.34 kg/m^2 (SD, 4.77 kg/m^2) and 13.74 kg (SD, 5.17 kg), respectively. Phenotypic outliers were monitored by the Tukey method. Covariates, including age, sex, assessment center, genotyping batch, and the top 10 principal components (PCs) derived from genome-wide genotype data, were used to adjust the raw TFM. The residuals were then normalized into inverse quantiles of standard normal distribution, which were used for a subsequent association analysis.

Genome-wide genotypes were available for all participants at 784,256 genotyped autosome markers, and were imputed into UK10K Haplotype, 1000 Genomes Project Phase 3, and Haplotype Reference Consortium reference panels. A total of ~92 million variants were generated by imputation.

To perform a linear mixed model (LMM) analysis of genetic association (27), we used BOLT-LMM, which adjusts for population structure and cryptic relatedness.

Genetic IVs selection

Based on the GWAS summary data of gut microbiota, a series of quality control criteria were applied to select eligible genetic IVs. Specifically, bacteria taxa were analyzed at the taxonomic levels of family, genus, and species. A feature was defined as a distinct family, genus, or species. In summary, a total of 36 bacteria taxa were grouped into 36 microbiota features, including 4 species, 16 genera, and

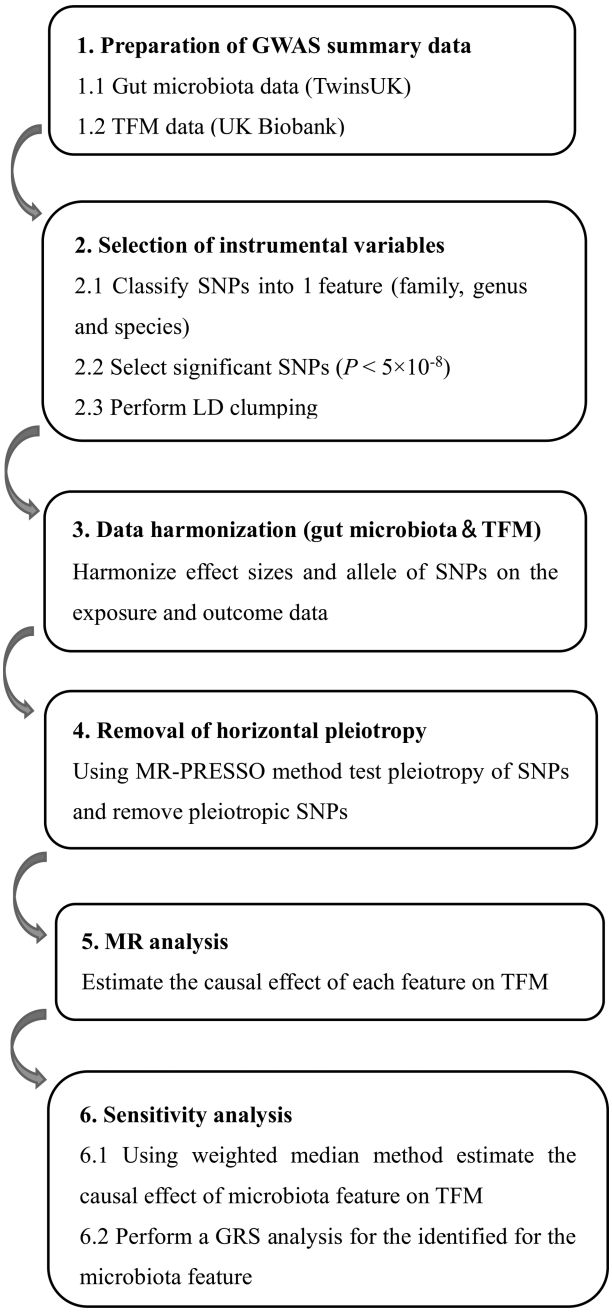

FIGURE 1 Diagrammatic description of MR analysis in this study. Abbreviations: GRS, genetic risk score; GWAS, genome-wide association study; LD, linkage disequilibrium; MR, Mendelian randomization; PRESSO, Pleiotropy RESidual Sum and Outlier; SNP, single nucleotide polymorphism; TFM, trunk fat mass.

16 families. The SNPs associated with gut microbiota at the genome-wide significance level ($P < 5 \times 10^{-8}$) were selected and assigned into distinct microbiota features. For a SNP with multiple signals within 1 feature, the strongest signal was selected for that feature. Then, the SNPs within each feature were clumped to retain independent SNPs only. The linkage disequilibrium (LD) threshold for clumping was set to be $r^2 < 0.001$, and the clumping window size was set to be 10,000 kb, where LD was estimated based on the 1000 Genomes Project sequencing data (Phase 3). Because genus is a subcategory within family while species is a subcategory within genus, certain species, genera, and families may overlap. For features containing identical IVs, only subcategories were reported. For example, the genus *Bifidobacterium* and the family *Bifidobacteriaceae* contain the same IVs. As the genus *Bifidobacterium* is within the family *Bifidobacteriaceae*, we only reported the results for genus *Bifidobacterium*.

Removal of horizontal pleiotropy

We applied the MR-PRESSO (Pleiotropy RESidual Sum and Outlier) global and outlier tests to detect potential horizontal pleiotropy. The global test evaluates a *P* value for overall horizontal pleiotropy among all SNPs, and the outlier test evaluates the presence of specific horizontal pleiotropic outlier variants by calculating the *P* value of pleiotropy significance for each SNP (28). The MR-PRESSO global test was first applied to evaluate overall pleiotropy. In the presence of pleiotropy ($P \leq 0.05$), the MR-PRESSO outlier test was then applied and the SNP with the smallest pleiotropy *P* value was removed. The MR-PRESSO global test was again performed on the remaining SNPs. The process was repeated until the global test became nonsignificant ($P > 0.05$). The final retained SNPs were used as nonpleiotropic IVs to perform the subsequent MR analysis.

MR analysis

We performed a 2-sample MR analysis to examine the causal effect from microbiota features to TFM. Specifically, we tested the association of the identified IVs with TFM. For microbiota features containing multiple IVs, we used the inverse variance–weighted method to evaluate the association between genetically predicted microbiota features and TFM (29). For a microbiota feature containing only 1 SNP, the Wald ratio method was used for the MR analysis. The causal effect was calculated by dividing the SNP outcome effect estimate by the SNP exposure effect estimate. Since the 2-sample MR analysis is based on summary-level data, there are no explicit requirements for the data distributions of exposure and outcome, such as normality. In the present study, the statistical significance of the effects of the gut microbiota on TFM was defined as a 2-sided $P \leq 0.05$.

Sensitivity analysis

To confirm the robustness of our results, we further performed the following sensitivity analyses: *1*) for microbiota features with at least 3 SNPs, the weighted median method (30) was performed to test the causal effects from microbiota feature to TFM; and *2*) in the UKB individual-level genetic data, we derived a weighted genetic risk score (GRS) as a secondary IV for the identified bacterial taxa and then examined the association of GRS with the outcome TFM.

Specifically, the GRS was constructed by the sum of trait-raising alleles, weighted by their regression coefficients, with the following equation:

$$\text{GRS} = \sum_{i=1}^{l} \beta_i g_i \qquad (1)$$

Here, β_i is the regression coefficient of SNP-bacteria association and g_i is the allele dosage of the *i*th SNP, while *l* denotes the total number of IVs. The association of the derived GRS with TFM was examined in a linear regression model in software R (v3.6.1; R Studio). The covariates included age, sex, and the top 10 PCs. By definition, PC1–PC10 were linearly independent of each other. Age or sex were not correlated with each other, nor with the 10 PCs.

The GRS for family *Lachnospiraceae* was calculated based on 5 SNPs (**Supplemental Table 1**). Owing to the fact that species *Prausnitzii* and genus *Faecalibacterium* contained the same 2 SNPs, the GRS was calculated for *Prausnitzii* only (Supplemental Table 1). For genus *Bifidobacterium*, no corresponding GRS was available due to *Bifidobacterium* only containing 1 SNP.

SNP clumping and the MR analysis were performed in R package *TwoSampleMR* (31); a horizontal pleiotropy analysis was performed in R package *MR-PRESSO* (28).

TABLE 1 Significant microbiota features associated with TFM

Level	Feature	MR method	SNP, n[1]	β[2]	SEE[3]	P value
Family	*Lachnospiraceae*	IVW	5	1.03×10^{-3}	4.28×10^{-4}	0.02
		Weighted median	5	9.33×10^{-4}	4.42×10^{-4}	0.03
Genus	*Bifidobacterium*	Wald ratio	1	−0.08	0.140	5.0×10^{-9}
Species	*Prausnitzii*	IVW	2	-6.87×10^{-3}	0.003	0.03

Abbreviations: IVW, inverse variance weighted; MR, Mendelian randomization; SNP, single nucleotide polymorphism; TFM, trunk fat mass.
[1]The number of SNPs used as instrumental variables.
[2]The estimated effect coefficient.
[3]The SEE of the effect coefficient.

Results

To choose appropriate genetic IVs for gut microbiota features, we first selected SNPs that were associated with all features at the genome-wide significance level ($P < 5 \times 10^{-8}$). We then clumped the selected SNPs in each feature to retain independent IVs. After clumping, we identified 16, 12, and 3 SNPs as significant and independent IVs in the family, genus, and species levels, respectively (Supplemental Table 1). We then performed an MR-PRESSO analysis to detect the potential horizontal pleiotropy effect. No evidence of pleiotropy effects was observed (MR-PRESSO global test $P > 0.05$). Finally, a total of 31 SNPs associated with 15 microbiota features at the genome-wide significance level ($P < 5 \times 10^{-8}$), including *Lachnospiraceae*, *Bifidobacterium*, *Lenta*, *Prausnitzii*, and others, were included in the subsequent MR analysis (Supplemental Table 1).

Primary analyses

Nominally significant associations of the primary MR analyses are shown in Table 1. In total, at the family level, the genetically predicated family *Lachnospiraceae* is positively associated with TFM, indicating that a per relative abundance increase in *Lachnospiraceae* causes a per 0.001 SD increase in TFM ($\beta = 0.001$; SEE $= 4.28 \times 10^{-4}$; $P = 0.02$). At the genus level, the genetically predicted genus *Bifidobacterium* is negatively associated with TFM, indicating that a per relative abundance increase in *Bifidobacterium* causes a per 0.08 SD decrease in TFM ($\beta = -0.08$; SEE $= 0.140$; $P = 5.0 \times 10^{-9}$). At last, at the species level, the genetically predicated species *Prausnitzii* is also negatively associated with TFM, indicating that a per relative abundance increase in *Prausnitzii* causes a per 0.007 SD decrease in TFM ($\beta = -0.007$; SEE $= 0.003$; $P = 0.03$).

Sensitivity analysis

The only significant feature that contained more than 2 IVs, *Lachnospiraceae*, was subjected to cross-validation by the weighted median test, which produced a nominally significant result ($P = 0.03$; Table 1). In addition, for the 2 identified features each containing more than 1 IV, *Lachnospiraceae* and *Prausnitzii*, the GRS analysis was applied. The signal of association with the family *Lachnospiraceae* became even stronger ($\beta = 0.002$; SEE $= 0.007$; $P = 0.004$), while *Prausnitzii* became nonsignificant ($\beta = -0.01$; SEE $= 0.007$; $P = 0.129$; Table 2). For the last significant feature, *Bifidobacterium*, there is only 1 IV, so neither the weighted median test nor the GRS analysis is applicable.

Discussion

In the present study, we explored the causal associations between gut microbiota and TFM using a 2-sample MR approach based on summary statistics of GWAS. We found the genetically predicted abundance of species *Prausnitzii* and genus *Bifidobacterium* were negatively associated with TFM, while the genetically predicted abundance of family *Lachnospiraceae* was positively associated with TFM. The causal association between family *Lachnospiraceae* and TFM was further validated by the weighted median test and the GRS analysis.

The gut microbiota in healthy adults is mainly comprised by 4 phyla: *Bacteroidetes*, *Firmicutes*, *Actinobacteria*, and *Proteobacteria* (32). As mentioned above, the ratio of *Firmicutes*/*Bacteroidetes* was closely linked with obesity. The bacteria taxa *Lachnospiraceae* and *Prausnitzii* identified in this study belong to the clostridial cluster of the phylum *Firmicutes* (33). The identified genus *Bifidobacterium* belongs to the phylum *Actinobacteria*, which is abundant in the adult human colon (34). The associations of *Lachnospiraceae* and *Bifidobacterium* with TFM in this study are in line with results from previous observational studies (35–37). A cross-sectional study observed that a higher abundance of family *Lachnospiraceae* was positively associated with obesity (35). A case-control study found the abundance of *Bifidobacterium* was negatively associated with BMI (16), and this negative association was validated in several other studies (36, 37). However, evidence from observational studies remained inconsistent for the association between *Prausnitzii* and obesity. Feng et al. (38) reported that the *Prausnitzii* amount was not significantly different between obese and lean subjects, while another study showed that the relative abundance of *Prausnitzii* was significantly increased in obese subjects compared to normal-weight subjects (37). Our results showed a negative causal association. In addition to the difference of population structure, the

TABLE 2 Validation of the significant features by the GRS analysis in the UKB cohort

Level	Feature	SNP, n[1]	β[2]	SEE[3]	P value
Family	*Lachnospiraceae*	5	0.002	0.007	0.004
Species	*Prausnitzii*	2	−0.010	0.007	0.129

Abbreviations: GRS, genetic risk score; SNP, single nucleotide polymorphism; UKB, UK Biobank.
[1]The number of SNPs used as instrumental variables.
[2]The estimated effect coefficient of the GRS analysis.
[3]The SEE effect coefficient of the GRS analysis.

potential interpretations of the above contradiction include that observational studies mainly rely on self-reported information and are prone to confounding factors and reverse causation bias.

Several mechanisms have been proposed to explain the role of the gut microbiota in obesity development. The gut microbiota–derived metabolites play an important role in the pathways linking specific gut microbiota to the development of obesity (39). The main products of dietary fiber fermented by gut microbiota are SCFAs, which are important source of energy for host intestinal epithelial cells and have a largely beneficial effect on energy homeostasis and appetite control (40). The *Prausnitzii* and members of family *Lachnospiraceae* are the main butyrate producers in the human gut. Butyrate has been shown to increase plasma levels of gastric inhibitory peptide and glucagon-like peptide 1, and therefore increases insulin, inhibits gastric emptying, and increases feelings of satiety (41). However, several studies showed that butyrate amounts are higher in overweight and obese subjects than in lean controls, which suggests its beneficial mechanism may be only valid under a specific limit (42, 43). In addition, Horiuchi et al. (44) observed that *Bifidobacterium animalis* subsp. *lactis* GCL2505 treatment can significantly elevate the plasma acetate levels in mice. The plasma acetate level elevated by GCL2505 activates GPR43, enhances host energy expenditure, and suppresses body fat accumulation. This study also provided compelling evidence for targeting *Bifidobacterium* for the treatment of obesity.

In a previous MR study, Sanna et al. (45) reported that each SD increase in fecal propionate levels was causally associated with a 0.03 SD increase in BMI. However, they did not evaluate the causal associations between specific gut microbiota and BMI or other obesity-related phenotypes (45). In another similar MR analysis, Yang et al. (25) showed that the genetically predicted *Bifidobacterium* was positively associated with a lower BMI ($\beta = -0.011$; $P = 1.6 \times 10^{-4}$), which was somewhat consistent with our findings.

Our study has several advantages. First, the MR approach was based on publicly available, large-scale GWAS summary statistics, thus offering an efficient option to mine reliable genetic information without additional experimental costs. Second, the TFM used in this study was more accurate than BMI in measuring abdominal obesity. The most reliable methods for accurately measuring TFM are DXA and MRI, but they are expensive and therefore difficult to use widely. The TFM used in this study was measured by BIA, which was highly correlated with the measurement from DXA (correlation coefficient = 0.82) (46). In addition, TFM measured by BIA can predict the visceral fat area. Therefore, the TFM measured by BIA is acceptable. Finally, our analyses were performed on gut microbiota at the family, genus, and species levels, which may be helpful to identify bacterial strains that have a true causal association with TFM.

There are certain limitations in our study. Firstly, the gut microbiota GWAS is still in its infancy and the choice of genetic IVs is very limited; thus, our results may be subject to weak instrument bias. Secondly, we did not replicate the identified associations between gut microbiota and TFM in an independent sample. Nevertheless, we performed sensitivity analyses, including a weighted median method and GRS analysis, to test the robustness of our results. Results from sensitivity analyses were slightly consistent with our primary finding: that is, only *Lachnospiraceae* was significantly associated with TFM in sensitivity analyses. Third, the genome-wide summary statistics of gut microbiota GWAS were not publicly available for a reverse MR analysis. Finally, the associations between multiple taxa and TFM were not adjusted by multiple testing. The aim of this study was to evaluate the causal association between specific bacteria and TFM. Considering the limited sample size of the gut microbiota association study, and the redundancy between different microbiota features, we did not correct for multiple testing.

In conclusion, by performing a 2-sample MR analysis based on GWAS summary data, our study generates the hypothesis that *Lachnospiraceae*, *Bifidobacterium*, and *Prausnitzii* may have causal associations with abdominal obesity. Our results may offer a new direction for the treatment of obesity.

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The authors' responsibilities were as follows—LZ and Y-FP: designed the research; S-SZ, R-RW, and QX: collected the data; QX and S-SZ: analyzed data; R-RW, Y-JW, XC, X-TW, QX, and J-JN: performed the literature search; QX: drafted the early version of the manuscript; Y-FP, LZ, and H-GR: jointly supervised the study; and all authors: were involved in writing the paper, had final approval of the submitted and published versions, and read and approved the final manuscript.

References

1. Haslam DW, James WP. Obesity. Lancet 2005;366:1197–209.
2. Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, Mullany EC, Biryukov S, Abbafati C, Abera FS, et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 2014;384:766–81.
3. Engin A. The definition and prevalence of obesity and metabolic syndrome. Adv Exp Med Biol 2017;960:1–17.
4. Coin A, Ruggiero E, Giannini S, Pedrazzoni M, Minisola S, Rossini M, Del Puente A, Inelmen EM, Manzato E, Sergi G. Trunk and lower limb fat mass evaluated by dual-energy X-ray absorptiometry in a 20- to 80-year-old healthy Italian population. Ann Nutr Metab 2012;61:151–9.
5. Pischon T, Boeing H, Hoffmann K, Bergmann M, Schulze MB, Overvad K, Schouw YT, Spencer E, Moons KGM, Tjønneland A, et al. General and abdominal adiposity and risk of death in Europe. N Engl J Med 2008;359:2105–20.
6. Takesian M, Santo MA, Gadducci AV, Santarém GCF, Greve J, Silva PR, Cleva R. Trunk body mass index: a new reference for the assessment of body mass distribution. Arq Bras Cir Dig 2018;31:e1362.
7. Pei YF, Ren HG, Liu L, Li X, Fang C, Huang Y, Hu WZ, Kong WW, Feng AP, You XY, et al. Genomic variants at 20p11 associated with body fat mass in the European population. Obesity 2017;25:757–64.
8. Liu L, Pei YF, Liu TL, Hu WZ, Yang XL, Li SC, Hai R, Ran S, Zhao LJ, Shen H, et al. Identification of a 1p21 independent functional variant for abdominal obesity. Int J Obes 2019;43:2480–90.
9. Chatham RE, Mixer SJ. Cultural influences on childhood obesity in ethnic minorities: a qualitative systematic review. J Transcult Nurs 2020;31:87–99.
10. Festi D, Schiumerini R, Eusebi LH, Marasco G, Taddia M, Colecchia A. Gut microbiota and metabolic syndrome. World J Gastroenterol 2014;20:16079–94.
11. Okeke F, Roland BC, Mullin GE. The role of the gut microbiome in the pathogenesis and treatment of obesity. Glob Adv Health Med 2014;3:44–57.

12. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, Sogin ML, Jones WJ, Roe BA, Affourtit JP, et al. A core gut microbiome in obese and lean twins. Nature 2009;457:480–4.
13. Ley RE, Bäckhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. Obesity alters gut microbial ecology. Proc Natl Acad Sci 2005;102:11070–5.
14. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 2006;444:1027–31.
15. Fu J, Bonder MJ, Cenit MC, Tigchelaar EF, Maatman A, Dekens JA, Brandsma E, Marczynska J, Imhann F, Weersma RK, et al. The gut microbiome contributes to a substantial proportion of the variation in blood lipids. Circ Res 2015;117:817–24.
16. Million M, Angelakis E, Maraninchi M, Henry M, Giorgi R, Valero R, Vialettes B, Raoult D. Correlation between body mass index and gut concentrations of *Lactobacillus reuteri*, *Bifidobacterium animalis*, *Methanobrevibacter smithii* and *Escherichia coli*. Int J Obes 2013;37:1460–6.
17. Sekula P, Del Greco MF, Pattaro C, Köttgen A. Mendelian randomization as an approach to assess causality using observational data. J Am Soc Nephrol 2016;27:3253–65.
18. Davenport ER, Cusanovich DA, Michelini K, Barreiro LB, Ober C, Gilad Y. Genome-wide association studies of the human gut microbiota. PLoS One 2015;10:e0140301.
19. Bonder MJ, Kurilshikov A, Tigchelaar EF, Mujagic Z, Imhann F, Vila AV, Deelen P, Vatanen T, Schirmer M, Weersma RK, et al. The effect of host genetics on the gut microbiome. Nat Genet 2016;48:14073–12.
20. Goodrich JK, Davenport ER, Beaumont M, Jackson MA, Knight R, Ober C, Spector TD, Bell JT, Clark AG, Ley RE. Genetic determinants of the gut microbiome in UK twins. Cell Host Microbe 2016;19:7313–43.
21. Wang J, Thingholm LB, Skiecevičienė J, Rausch P, Kummen M, Hov JR, Degenhardt F, Heinsen FA, Rühlemann MC, Szymczak S, et al. Genome-wide association analysis identifies variation in vitamin D receptor and other host factors influencing the gut microbiota. Nat Genet 2016;48:13963–406.
22. Turpin W, Espin-Garcia O, Xu W, Silverberg MS, Kevans D, Smith MI, Guttman DS, Griffiths A, Panaccione R, Otley A, et al. Association of host genome with intestinal microbial composition in a large healthy cohort. Nat Genet 2016;48:1413–7.
23. García-Santisteban I, Cilleros-Portet A, Moyua-Ormazabal E, Kurilshikov A, Zhernakova A, Garcia-Etxebarria K, Fernandez-Jimenez N, Bilbao JR. A two-sample Mendelian randomization analysis investigates associations between gut microbiota and celiac disease. Nutrients 2020;12;1420.
24. Ni JJ, , Yang XL, Zhang H, Xu Q, Wei XT, Feng GJ, Zhao M, Pei YF, Zhang L. Assessing causal relationship from gut microbiota to heel bone mineral density. Bone 2020;143:115652 ma.
25. Yang Q, Lin SL, Kwok MK, Leung GM, Schooling CM. The roles of 27 genera of human gut microbiota in ischemic heart disease, type 2 diabetes mellitus, and their risk factors: a Mendelian randomization study. Am J Epidemiol 2018;187:1916–22.
26. Zhuang Z, Yang R, Wang W, Qi L, Huang T. Associations between gut microbiota and Alzheimer's disease, major depressive disorder, and schizophrenia. J Neuroinflammation 2020;17:288.
27. Loh PR, Tucker G, Bulik-Sullivan BK, Vilhjálmsson BJ, Finucane HK, Salem RM, Chasman DI, Ridker PM, Neale BM, Berger B, et al. Efficient Bayesian mixed-model analysis increases association power in large cohorts. Nat Genet 2015;47:284–90.
28. Verbanck M, Chen CY, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. Nat Genet 2018;50:693–8.
29. Burgess S, Butterworth A, Thompson SG. Mendelian randomization analysis with multiple genetic variants using summarized data. Genet Epidemiol 2013;37:658–65.
30. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent estimation in Mendelian randomization with some invalid instruments using a weighted median estimator. Genet Epidemiol 2016;40:304–14.
31. Hemani G, Zheng J, Elsworth B, Wade KH, Haberland V, Baird D, Laurin C, Burgess S, Bowden J, Langdon R, et al. The MR-Base platform supports systematic causal inference across the human phenome. eLife 2018;7:e34408.
32. Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R. Diversity, stability and resilience of the human gut microbiota. Nature 2012;489:220–30.
33. Duncan SH, Louis P, Flint HJ. Cultivable bacterial diversity from the human colon. Lett Appl Microbiol 2007;44:343–50.
34. Walker AW, Ince J, Duncan SH, Webster LM, Holtrop G, Ze X, Brown D, Stares MD, Scott P, Bergerat A, et al. Dominant and diet-responsive groups of bacteria within the human colonic microbiota. ISME J 2011;5:220–30.
35. Chávez-Carbajal A, Nirmalkar K, Pérez-Lizaur A, Hernández-Quiroz F, Ramírez-Del-Alto S, García-Mena J, Hernández-Guerrero C. Gut microbiota and predicted metabolic pathways in a sample of Mexican women affected by obesity and obesity plus metabolic syndrome. Int J Mol Sci 2019;20:438.
36. Ignacio A, Fernandes MR, Rodrigues VA, Groppo FC, Cardoso AL, Avila-Campos MJ, Nakano V. Correlation between body mass index and faecal microbiota from children. Clin Microbiolol Infect 2016;22:258.e1–8.
37. Ettehad Marvasti F, Moshiri A, Taghavi MS, Riazi S, Taati M, Sadati SF, Ghaheri A, Masoomi M, Vaziri F, Fateh A, et al. The first report of differences in gut microbiota composition between obese and normal weight Iranian subjects. Iran Biomed J 2020;24:148–54.
38. Feng J, Tang H, Li M, Pang X, Wang L, Zhang M, Zhao Y, Zhang X, Shen J. The abundance of fecal *Faecalibacterium prausnitzii* in relation to obesity and gender in Chinese adults. Arch Microbiol 2014;196:73–7.
39. Ejtahed HS, Angoorani P, Soroush AR, Hasani-Ranjbar S, Siadat SD, Larijani B. Gut microbiota-derived metabolites in obesity: a systematic review. Biosci Microbiota Food Health 2020;39:65–76.
40. Byrne CS, Chambers ES, Morrison DJ, Frost G. The role of short chain fatty acids in appetite regulation and energy homeostasis. Int J Obes 2015;39:1331–8.
41. Wichmann A, Allahyar A, Greiner TU, Plovier H, Lundén G, Larsson T, Drucker DJ, Delzenne NM, Cani PD, Bäckhed F. Microbial modulation of energy availability in the colon regulates intestinal transit. Cell Host Microbe 2013;14:582–90.
42. Schwiertz A, Taras D, Schäfer K, Beijer S, Bos NA, Donus C, Hardt PD. Microbiota and SCFA in lean and overweight healthy subjects. Obesity 2010;18:190–5.
43. Hippe B, Remely M, Aumueller E, Pointner A, Magnet U, Haslberger AG. *Faecalibacterium prausnitzii* phylotypes in type two diabetic, obese, and lean control subjects. Benef Microbes 2016;7:511–7.
44. Horiuchi H, Kamikado K, Aoki R, Suganuma N, Nishijima T, Nakatani A, Kimura I. *Bifidobacterium animalis* subsp. *lactis* GCL2505 modulates host energy metabolism via the short-chain fatty acid recedotor GPR43. Sci Rep 2020;10:4158.
45. Sanna S, van Zuydam NR, Mahajan A, Kurilshikov A, Vich Vila A, Võsa U, Mujagic Z, Masclee AAM, Jonkers D, Oosting M, et al. Causal relationships among the gut microbiome, short-chain fatty acids and metabolic diseases. Nat Genet 2019;51:600–5.
46. Demura S, Sato S. Prediction of visceral fat area at the umbilicus level using fat mass of the trunk: the validity of bioelectrical impedance analysis. J Sports Sci 2007;25:823–33.