

Association Between Gut *Akkermansia* and Metabolic Syndrome is Dose-Dependent and Affected by Microbial Interactions: A Cross-Sectional Study

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Objective: *Akkermansia muciniphila* is among the most abundant bacterial species in the human intestine; however, its relationship to metabolic syndrome (MetS)—which is linked to gut dysbiosis—is not known. In this study, we investigated the association between *Akkermansia* abundance and risk of MetS and its components, as well as dose–response effects and the influence of microbial interactions on the association.

Methods: This cross-sectional study included 6896 Chinese participants aged 18 to 97 years from the Guangdong Gut Microbiome Project. MetS was defined according to Joint Committee for Developing Chinese Guidelines on Prevention and Treatment of Dyslipidemia in Adults criteria. The abundance of *Akkermansia* was assessed by 16S rRNA sequencing. Logistic regression analysis with adjustment for common confounders was performed to evaluate the association between *Akkermansia* and MetS and its components. Models with restricted cubic splines and interaction terms were used to examine the dose–response association and microbial interactions, respectively.

Results: The prevalence of MetS was 20.4%, and the median abundance of *Akkermansia* was 0.08% (interquartile range: 0.04–0.93%). Increased *Akkermansia* abundance was associated with decreased risk of MetS ($P_{\text{nonlinear}} < 0.05$), but this effect was not observed until the *Akkermansia* level was 0.2% of the total gut microbiota abundance (odds ratio=0.96, 95% confidence interval: 0.94–0.98). Of the 5 MetS components, obesity and hypertriglyceridemia showed the strongest association with *Akkermansia*, followed by reduced high-density lipoprotein cholesterol, hypertension, and hyperglycemia. Microbial interaction analyses showed that Ruminococcaceae and Lachnospiraceae were the predominant bacterial families and were not only correlated with *Akkermansia* abundance but also influenced the *Akkermansia*–MetS association.

Conclusion: There is a dose–response association between reduced risk of MetS and increased abundance of *Akkermansia*. The association between *Akkermansia* and 5 MetS components is variable and affected by microbial interactions.

Keywords: metabolic syndrome, gut microbiota, *Akkermansia muciniphila*, dose–response, microbial interaction

Introduction

Metabolic syndrome (MetS)—which is characterized by central obesity, insulin resistance, hypertension, and dyslipidemia—is a major global health problem.¹ Evidence from epidemiologic and physiologic studies indicates that metabolic disorders are closely linked to gut dysbiosis.^{2,3} Specifically, changes in microbial diversity and composition can alter intestinal permeability,⁴ induce low-grade inflammation,⁵ and influence liver gluconeogenesis by stimulating the production of short-chain fatty

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acids.⁶ Gut microbes are potential targets in the treatment of metabolic diseases. The role of some probiotics including *Faecalibacterium prausnitzii*, *Akkermansia muciniphila*, and *Clostridium* spp. in metabolic disorders has been explored in animal models and humans.⁷

A. muciniphila is among the most abundant bacterial species in the human intestine and is a potentially beneficial microbe in the treatment of metabolic disease.^{8–10} Although the protective effects of *A. muciniphila* against MetS components such as obesity and hyperglycemia have been investigated,^{7,11} there have been no studies on the association between *Akkermansia* and the 5 main MetS components. One study reported that administration of *A. muciniphila* for 3 months had variable effects on MetS components, with decreases in fat mass and hip circumference but no changes in fasting glycemia and triglyceride levels compared to a placebo.¹²

Gastrointestinal homeostasis depends on the gut microbiome.⁷ A probiotic can only exert beneficial effects if it can colonize its host under competition and cooperation with native microbiota.¹³ *Akkermansia* is a mucin-degrading bacterium that resides in the mucus layer of the gut;¹⁴ its effect on host physiology depends on complex interactions with other intestinal microorganisms. Indeed, depletion of *Akkermansia* along with enrichment of other mucolytic bacteria such as *Fusobacterium* spp. has been reported in both humans and animal models of inflammatory bowel disease.¹¹ However, it remains unclear which microorganisms affect the association between *Akkermansia* and MetS; moreover, the threshold level of *Akkermansia* required to reduce the risk of MetS is not known. Clarifying these points has important clinical implications for the treatment of MetS through modulation of *Akkermansia* and the gut microbiome.

In this cross-sectional study of 6896 subjects from the Guangdong Gut Microbiome Project (GGMP),^{15,16} we analyzed the association between *Akkermansia* in the gut and 5 components of MetS, and examined the dose–response effect and influence of the gut microbiome on this association.

Materials and Methods

Data Sources

This was a cross-sectional study based on data from the GGMP,^{15,16} which aimed to establish the generalizability of microbiota-based diagnostic models for metabolic disease. Participant data were obtained from stool and blood samples

and a questionnaire. The GGMP was approved by the Ethical Review Committee of the Chinese Centre for Disease Control and Prevention (approval no. 201519-A) with written, informed consent obtained from each participant.

For stool samples, participants were given a stool sampler, ice bag, and ice box to collect and transport their samples within 1 day to a nearby collection point where there were -20°C freezers for sample storage. The Fecal DNA Bead Isolation kit (Bioeasy, Shenzhen, China) was used to extract total bacterial DNA; the 16S rRNA gene V4 region was amplified with barcoded primers (V4F, 5'-GTGYCAGCMGCCGCGGTAA-3' and V4R, 5'-GGACTACNVGGGTWTCTAAT2-3') and used to construct a sequencing library. Paired-end sequencing was performed on the HiSeq2500 system (HiSeq SBS Kit v4; Illumina, San Diego, CA, USA).

Raw sequencing reads (<https://github.com/SMUJYYXB/GGMP>) and metadata (<https://github.com/SMUJYYXB/GGMP>) were downloaded from the European Nucleotide Archive (accession no. PRJEB18535). Metadata consisted of MetS-related risk factors including systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting plasma glucose (FPG), triglyceride (TG), high-density lipoprotein cholesterol (HDL-c), and waist circumference; potential confounders were age, sex, Bristol stool scale, geographic location, annual income, antibiotic consumption, and diet (plant or animal oil, fruits, grains, synbiotics, and vegetables).

Definitions of MetS and Its Components

MetS and its risk components were defined according to the criteria of the Joint Committee for Developing Chinese Guidelines on Prevention and Treatment of Dyslipidemia in Adults¹⁷ as follows: 1) hypertension, SBP ≥ 130 mmHg and/or DBP ≥ 85 mmHg and/or use of antihypertensive drugs; 2) hyperglycemia, FPG ≥ 6.1 mmol/l and/or use of blood glucose-lowering medication and/or previous diagnosis of type 2 diabetes mellitus (T2DM); 3) hypertriglyceridemia, TG ≥ 1.70 mmol/l and/or use of triglyceride-lowering medication; 4) reduced high-density lipoprotein cholesterol (HDL-c) (< 1.04 mmol/l); and 5) central obesity, waist circumference ≥ 90 cm in men and ≥ 85 cm in women. MetS was diagnosed when a subject had at least 3 of the 5 metabolic risk components.

Assessment of *Akkermansia* Abundance

Raw 16S rRNA gene sequencing reads were split according to an online script (paired.extract_sequences.pl,

<https://github.com/SMUJYYXB/GGMP>) provided by the GGMP project. Paired-end reads were merged with FLASH 1.2.11¹⁸ and qualified (with a maximum error rate of 0.01) with VSEARCH 2.1.4.2.¹⁹ Filtered reads were clustered into operational taxonomic units (OTUs) with a similarity of 97%. Representative OTUs were classified into taxonomic groups (syntax_cutoff, 0.8) based on the Silva_16s_v123 dataset.²⁰ OTUs present in <10% of samples were excluded to reduce the computational load, leaving 98.54% (834 OTUs) of the initial abundance. Analyses were performed at the OTU or genus level.

Statistical Analysis

Descriptive statistics and comparisons are reported as mean±standard deviation (SD) or median with interquartile range (IQR) for continuous variables, or as percentages for categorical variables. Trend tests for variables across different quintiles of *Akkermansia* abundance were based on linear regression analysis or the Cochran–Armitage test. The Mann–Whitney *U*-test and chi-squared test were used to evaluate intergroup differences for continuous and categorical variables, respectively.

Logistic or multivariate linear regression was used to examine the associations between *Akkermansia* and MetS and its components. Models were first adjusted for age and sex and then further adjusted for Bristol stool scale, geographic location, and annual income. Trend tests of odds ratio (OR) and 95% confidential intervals (95% CI) across quintiles were conducted by entering the median value of each quintile of *Akkermansia* abundance into the models.

Cubic splines with 4 knots at the 5th, 25th, 75th, and 95th percentiles were used to evaluate the dose–response relationship between *Akkermansia* and the risk of MetS and its components. Nonlinearity of the associations was identified by testing whether the coefficients associated with the nonlinear components were equal to zero.²¹ Fitted possibilities of outcomes for different levels of *Akkermansia* were used to visualize the shape of dose–response curves. In parallel, we separated *Akkermansia* abundance into 20 quantiles, estimated the ORs (compared to the first percentile [Q1]) from the lowest to the highest quantile, and analyzed the change in MetS risk with increasing *Akkermansia* abundance as a line plot. Finally, we assessed the reproducibility of the models by changing parameters such as the position of knots for cubic splines and potential confounders for the later model.

We investigated the effects of interactions between *Akkermansia* and other microbes on MetS in 3 steps. 1)

We identified *Akkermansia*-correlated and MetS-associated microbes by multivariate linear regression with adjustment for age and sex. MetS components were treated as continuous response variables and the abundance of OTUs was arcsine-square root-transformed. A significant association was defined when the coefficient of OTUs in the model had a false discovery rate (FDR) <0.05. 2) We estimated the multiplicative interactions between *Akkermansia* and other OTUs (obtained from step 1 by logistic regression). OTUs with a meaningful interaction coefficient term ($P<0.05$) were considered to significantly influence the *Akkermansia*–MetS association and were identified as interacting (i)OTUs. 3) The iOTUs in step 2 were grouped according to change in OR (Δ OR). Microbial associations with *Akkermansia* OTUs were plotted with Circos 0.69–9. The ‘interactions’ package of R software was used to assess the change in the *Akkermansia*–MetS association when iOTU abundance increased with SD or from the Q1 to Q5.

All data analyses were performed using R×64 v4.0.0. Statistical significance was evaluated with 2-tailed tests using a cutoff value of $P\leq 0.05$. For multiple testing, FDR adjustment was performed with the Benjamini–Hochberg method.

Results

Characteristics of Study Participants

This study included 6896 participants aged 18–97 years. The average age was 52.7±14.6 years and 55.2% of participants were female. The median relative abundance of *Akkermansia* was 0.08% (IQR: 0.04–0.34%) and 97.7% of participants had at least 0.01% *Akkermansia*. The prevalence of MetS was 20.4% and that of its components were as follows: central obesity, 23.8%; hyperglycemia, 18.1%; hypertension, 50.6%; hypertriglyceridemia, 23.7%; and reduced HDL-c, 28.8%.

Table 1 shows the demographic and clinical characteristics of the study participants across quintiles of *Akkermansia*. Young females had a higher abundance of *Akkermansia*, and participants with a higher abundance had a lower prevalence of MetS, central obesity, hyperglycemia, hypertension, hypertriglyceridemia, and reduced HDL-c. Likewise, *Akkermansia* level was inversely related to DBP, SBP, waist circumference, HDL-c, and TG (FDR<0.05) in both crude and sex- and age- adjusted models (Supplementary Table 1 and Supplementary

Table 1 Characteristics of Participants According to Quintile of *Akkermansia* Abundance (n=6896)

Characteristics	Q1	Q2	Q3	Q4	Q5	P for Trend
n	1379	1379	1379	1379	1380	
<i>Akkermansia</i> ^a , (%)	0.02 (0.02–0.03)	0.04 (0.04–0.05)	0.08 (0.06–0.09)	0.20 (0.15–0.33)	7.05 (2.04–18.93)	
Age, years, mean(sd)	53.6 (14.5)	53.7 (14.6)	52.0 (14.5)	51.4 (14.6)	52.9 (15.0)	< 0.001
Sex, male, n (%)	621 (45.0)	623 (45.2)	653 (47.4)	661 (47.9)	535 (38.7)	0.02
MetS, n (%)	263 (19.1)	288 (20.9)	326 (23.6)	303 (22.0)	224 (16.23)**	0.18
Central obesity, n (%)	341 (24.7)	330 (23.9)	370 (26.8)	341 (24.7)	259 (18.8)**	< 0.001
Hyperglycemia, n (%)	225 (16.3)	262 (19.0)	274 (19.9)	252 (18.3)	234 (16.9)**	0.86
Hypertension, n (%)	712 (51.6)	739 (53.5)	703 (51.0)	694 (50.3)	638 (46.2)	< 0.001
Hypertriglyceridemia, n (%)	300 (21.8)	328 (23.8)	366 (26.5)	396 (28.7)	243 (17.3)**	0.35
Low HDL, n (%)	376 (27.3)	401 (29.1)	417 (30.2)	434 (31.5)	356 (25.8)**	0.88

Notes: ^aMedian (interquartile range) relative abundances of *Akkermansia*. **Data of Q5 was significantly different from those of Q1, Q2, Q3 and Q4 ($P < 0.001$).

[Figure 1](#)). However, the association between *Akkermansia* and FBG was nonsignificant.

We also investigated the associations between *Akkermansia* and potential confounders ([Supplementary Figure 2](#)). The abundance of *Akkermansia* differed across geographic regions (FDR=0.001). However, stool type; a diet that included plant or animal oil, fruits, grains, synbiotics and vegetables; annual income; and consumption of antibiotics were not significantly correlated with *Akkermansia*.

Associations Between *Akkermansia* and MetS and Its Components

The relative abundance of *Akkermansia* was inversely associated with the risk of MetS; a 1% increase in *Akkermansia* reduced the risk of MetS by ~0.3% ([Figure 1A](#) and [Supplementary Table 2](#)). Compared to Q1, ORs (95% CIs) for MetS from the lowest to the highest quintiles of *Akkermansia* abundance were as follows: Q2, 1.04 (0.89–1.20); Q3, 1.27 (1.11–1.47); Q4, 1.13 (0.98–1.30); and Q5, 0.71 (0.61–0.83). The P values in this model were <0.001 ([Figure 1B](#) and [Supplementary Table 2](#)). The protective effect remained significant after adjusting for age, sex, and other potential confounders; the P values for trends in models 2 and 3 were <0.001 and <0.05, respectively. Additionally, the abundance of *Akkermansia* was inversely correlated with the number of MetS components ([Figure 1C](#) and [D](#)).

Disparities in the effects of *Akkermansia* were observed among the MetS components. We found that *Akkermansia* was most closely associated with central obesity and hypertriglyceridemia, followed by reduced HDL-c, and hypertension; notably, no significant associations were found with hyperglycemia in any of the models ([Figure 1A](#) and [B](#)). For

instance, a 1% increase in *Akkermansia* abundance significantly reduced the risk of central obesity by ~2% (model 1, OR=0.983, 95% CI: 0.975–0.990; model 2, OR=0.973, 95% CI: 0.972–0.998; and model 3, OR=0.982, 95% CI: 0.989–0.991), while the protective effect against hyperglycemia was not statistically significant (model 1, OR=1.000, 95% CI: 0.993–1.006; model 2, OR=0.998, 95% CI: 0.991–1.004; and model 3, OR=0.996, 95% CI: 0.989–1.003) ([Figure 1A](#)). When the abundance of *Akkermansia* was treated as a categorical variable, we obtained similar results—ie, the protective effect was stronger against central obesity and hypertriglyceridemia than against the other components of MetS. ORs for central obesity and hypertriglyceridemia were lower than those for the other components, and P value trends were <0.001 in all models ([Figure 1B](#)).

Although the protective association observed across quintiles of *Akkermansia* showed a strong linear trend, we found that it was limited by lower levels of *Akkermansia*. For example, the effect on MetS for Q2, Q3, and Q4 was not always statistically significant compared to that for Q5 ([Figure 1B](#)), suggesting a dose–response association.

Dose–Response Effect of *Akkermansia* on MetS

To investigate how the risk of MetS changes with *Akkermansia* abundance, we fitted the dose–response curves of *Akkermansia* on MetS in the models with cubic splines ([Figure 2A](#)) and found that the probability of MetS changed nonlinearly with *Akkermansia* level ($P_{\text{nonlinear}} < 0.05$): the distribution curves were flat before ~0.2% *Akkermansia*, declined sharply until ~10%, and then flattened once again. The distribution of MetS probability was

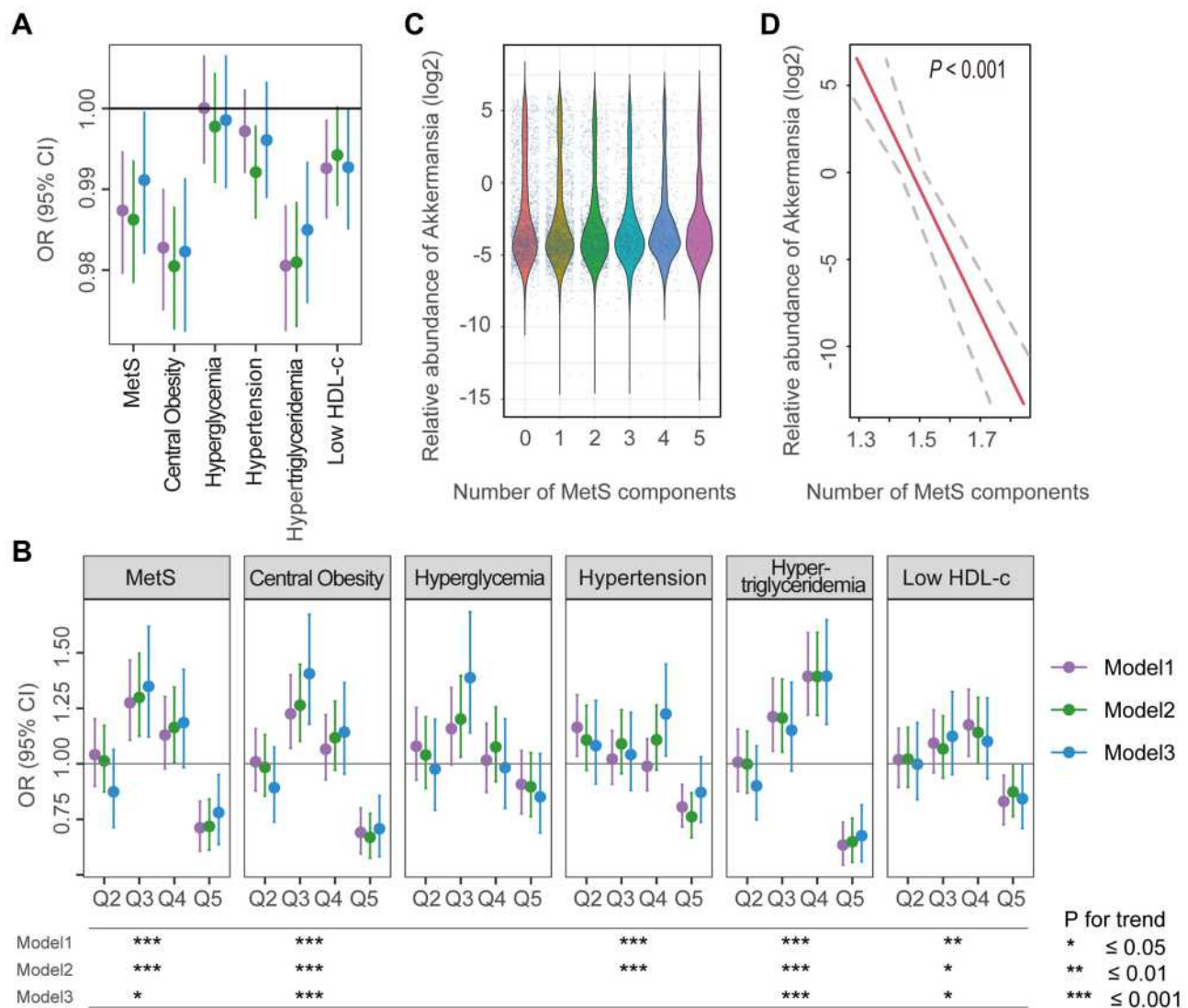


Figure 1 Associations between the relative abundance of *Akkermansia* and MetS and its components. **(A and B)** ORs and 95% CIs for MetS and its components with a 1% increase in the abundance of *Akkermansia* **(A)**, and across quintiles of *Akkermansia* (with Q1 as a reference) **(B)**. **(C)** Relative abundance of *Akkermansia* in participants with different numbers of MetS components; **(D)** Linear association between *Akkermansia* and the number of MetS components; dashed lines represent the 95% CI. Trend test of ORs across quintiles (Q1–Q5) was conducted by entering the median value of each quintile of *Akkermansia* abundance into the models. **P* for trend <0.05; ***P* for trend <0.01; ****P* for trend ≤ 0.001 .

similar in the 3 models, but varied for different MetS components. To ensure the robustness of this result, we also tested different knots in the models that used splines ([Supplementary Figure 3A](#)). Although the inflection point fluctuated according to model parameters, the shape of curves and the nonlinear relationship did not change.

To further evaluate the nonlinear association, we plotted ORs (95% CIs) of 20 quantiles of *Akkermansia* in age- and sex-adjusted logistic regression models and examined how the risk of MetS changed with a 1% increase in *Akkermansia* in each quantile ([Figure 2B](#) and [Supplementary Table 3](#)). In accordance with the fitted

curves, we found that ORs did not decrease markedly until *Akkermansia* abundance was 0.1%–0.2%, and the protective effect was statistically significant until ~3.8% (OR=0.68, 95% CI: 0.463–1.002). The inflection points at which the protective effects of *Akkermansia* became statistically significant varied among MetS components and models ([Supplementary Figure 3B](#) and [Supplementary Table 3](#)).

We next assessed the risk of MetS and its components in subjects with high vs low levels of *Akkermansia* ([Table 2](#); cutoff values were set to 0.1 ([Figure 2A](#)) and 0.2 ([Figure 2B](#))). The results showed that the risk of MetS and its components

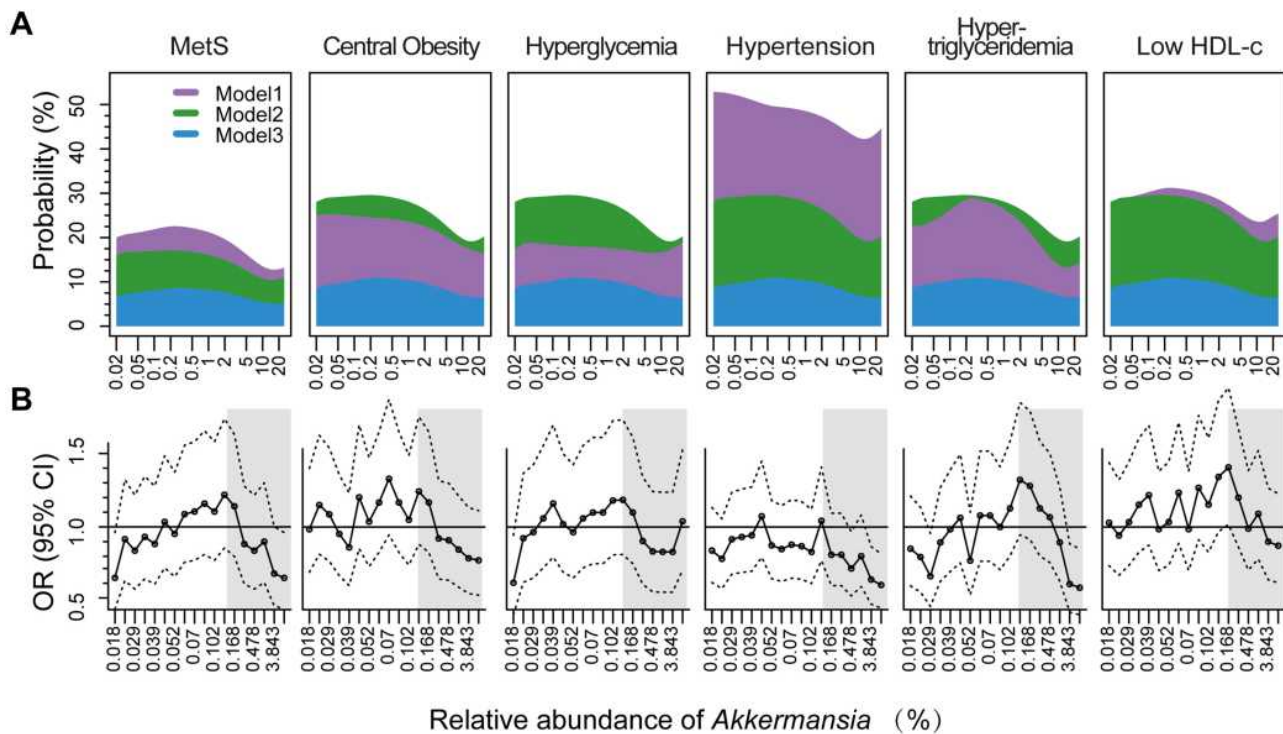


Figure 2 Dose–response effect of *Akkermansia* on MetS and its components. **(A)** Association between risk of MetS and relative abundance of *Akkermansia*. Dose–response curves of *Akkermansia* were fitted to logistic models with cubic splines. Four knots at the 5th, 25th, 75th, and 95th percentiles were used in models. Model 1 was a crude regression without adjusting for confounders; model 2 was adjusted for age and sex; and model 3 was further adjusted for Bristol stool scale, geographic location, and annual income. A trend test of ORs across quintiles (Q1–Q5) was conducted by entering the median value of each quintile of *Akkermansia* abundance into the models. **(B)** ORs and 95% CIs across 20 percentiles of *Akkermansia* (with the first quintile [Q1] as a reference) in crude logistic models.

was reduced to a greater extent when the *Akkermansia* cutoff was set to 0.2% as compared to 0.1%.

Taken together, we found that the association between *Akkermansia* and MetS was nonlinear; dose–response analyses showed that the risk of MetS was not significantly reduced until *Akkermansia* abundance was 0.2% of total gut microbiota.

Effects of Microbial Interactions on the *Akkermansia*–MetS Relationship

Microbial competition and cooperation affect microbe–host interactions in the gut.^{13,22} We therefore investigated whether the *Akkermansia*–MetS relationship changed with the relative abundance of other bacteria. Three

Table 2 Risks of MetS and Its Components in Subjects with Higher *Akkermansia* Compared to Those with Lower *Akkermansia* (or, 95% CI)^a

	MetS	Central Obesity	Hyperglycemia	Hypertension	Hypertriglyceridemia	Low HDL
Cutoff = 0.1 ^b						
Prevalence (%) Akk<0.1/Akk≥0.1 ^c	20.9/19.6	25.2/21.9	18.2/17.9	52.2/48.4	23.5/23.8	28.7/28.9
Model 1	0.99 (0.98–1.02)	0.97 (0.95–0.99)	0.99 (0.98–1.01)	0.96 (0.94–0.99)	1.00 (0.98–1.02)	1.00 (0.98–1.02)
Model 2	0.99 (0.97–1.01)	0.97 (0.95–0.99)	1.00 (0.98–1.02)	0.98 (0.99–1.00)	1.00 (0.99–1.02)	1.00 (0.98–1.03)
Model 3	1.00 (0.98–1.02)	0.98 (0.95–1.00)	1.00 (0.97–1.01)	1.01 (0.99–1.04)	1.00 (0.98–1.04)	1.00 (0.97–1.02)
Cutoff = 0.2						
Prevalence (%) Akk<0.2/Akk≥0.2 ^c	21.7/17.3	25.5/19.9	18.6/17.9	52.1/48.9	24.9/20.7	29.6/26.9
Model 1	0.96 (0.94–0.98)	0.94 (0.92–0.96)	0.98 (0.96–1.00)	0.95 (0.92–0.97)	0.96 (0.93–0.98)	0.97 (0.95–1.00)
Model 2	0.96 (0.94–0.98)	0.94 (0.93–0.97)	0.99 (0.97–1.00)	0.96 (0.94–0.98)	0.96 (0.94–0.98)	0.98 (0.95–1.00)
Model 3	0.98 (0.95–1.00)	0.96 (0.93–0.99)	0.97 (0.95–1.00)	0.98 (0.95–1.00)	0.97 (0.94–0.99)	0.98 (0.94–1.00)

Notes: ^aModel 1 was the crude model; Model 2 was additionally adjusted for age and sex; Model 3 was additionally adjusted for geographic locations, and annual income. OR, odds ratio. 95% CI, 95% confidence interval. ^bThe cutoff values of *Akkermansia* (%) according to the non-linear models. ^cSubjects were divided into 2 groups according to the cutoff abundance of *Akkermansia*.

Akkermansia OTUs were annotated according to the Silva_16s_v123 database; all were distributed in >10% of samples and were named OTU49, OTU115, and OTU236.

The *Akkermansia* OTUs had similar distribution patterns (ie, bimodal). However, their associations with MetS differed. OTU49 and OTU115, the 2 major OTUs (Figure 3A), coexisted in 93.7% of subjects (Figure 3B) but were negatively correlated with each other (Pearson correlation, $r=-0.037$, FDR=0.02) (Figure 3C).

Associations of gut microbes with *Akkermansia* OTUs are shown as Figure 3D and Supplementary Table 4. We detected 717 significant associations (406 positive [+] and 311 negative [-]) with *Akkermansia* OTUs after controlling the FDR (<0.05). These included 548 unique OTUs, 161 genera, and 63 bacterial families. At the family level, Ruminococcaceae had the most associations (n=167), followed by Lachnospiraceae (n=139), Bacteroidaceae (n=35), and Prevotellaceae (n=35). Interestingly, we found that the type of association (+ or -) was largely consistent within the same family. For instance, *Akkermansia* was positively associated with most

Ruminococcaceae (127+ and 40-) and Bacteroidaceae (23+ and 12-) but negatively associated with most Lachnospiraceae (36+ and 103-) and Prevotellaceae (4+ and 22-). At the genus level, *Bacteroides*, *Lachnospiraceae*, and *Alistipes* were the 3 most abundant bacteria, with 35 (23+ and 12-), 24 (7+ and 17-), and 21 (21+ and 0-) associations, respectively.

Of the 3 *Akkermansia* OTUs, only OTU49 was significantly associated with MetS (Figure 4A). We further examined the microbes that potentially influence the association between the OTU49 and MetS. The analysis of interactions showed that 25 iOTUs significantly affected the OTU49–MetS association (P for interactions <0.05) and 17 were also significantly associated with OTU49 (P for association <0.05) (Supplementary Table 5). Moreover, most of the iOTUs showed a positive association with OTU49 (β for associations >0). The iOTUs could be separated into 2 groups according to their effects on the *Akkermansia*–MetS association (Δ OR) (Figure 4B). The high abundance of iOTUs in group 1 weakened the protective effect of OTU49 on MetS, while iOTUs from group 2 strengthened the effect. For example,

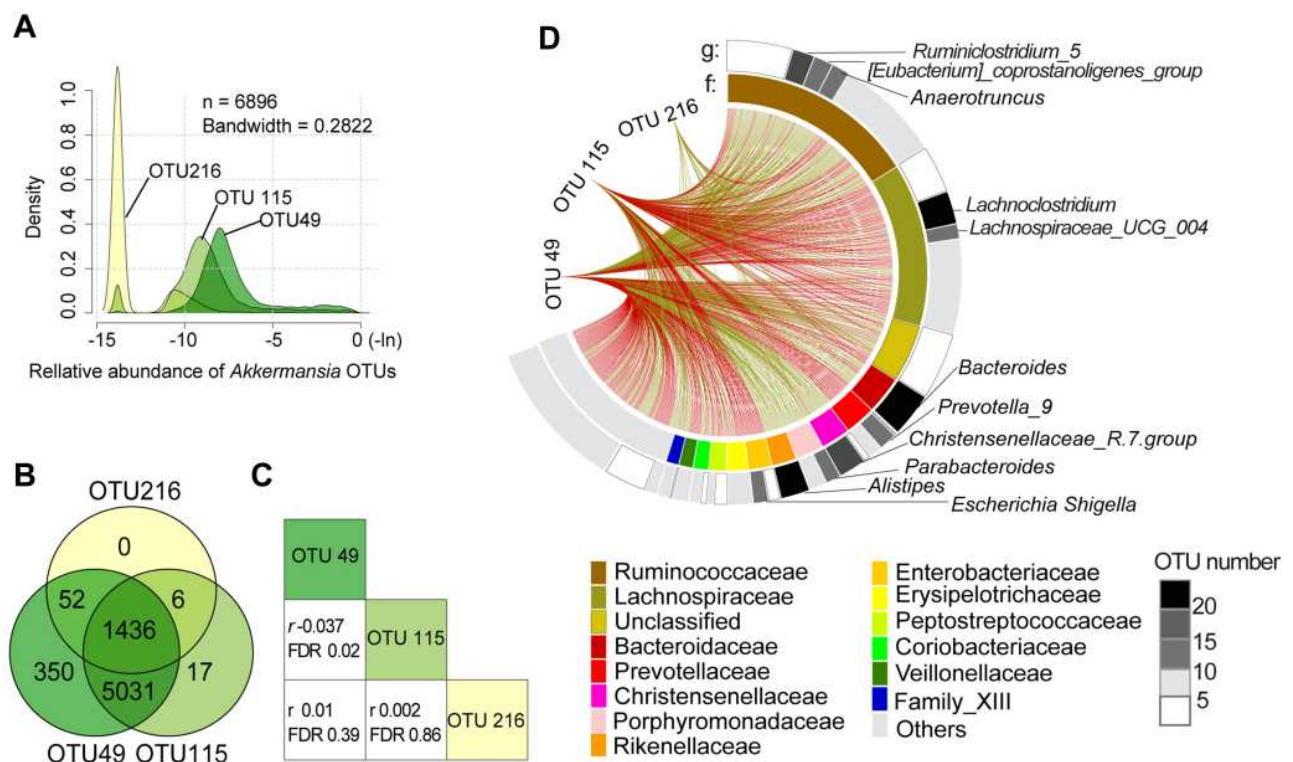


Figure 3 Distribution of *Akkermansia* OTUs and their correlations with other microbiota. (A) Distribution of 3 *Akkermansia* OTUs. (B) Overlap of the distributions of *Akkermansia* OTUs in all participants. (C) Correlations of the 3 *Akkermansia* OTUs. r is the Pearson coefficient. (D) Associations between *Akkermansia* OTUs and other gut microbes. The first and second circles represent OTUs at the genus (g) and family (f) levels, respectively; and red and green lines represent positive and negative correlations, respectively. Only significant correlations are shown.

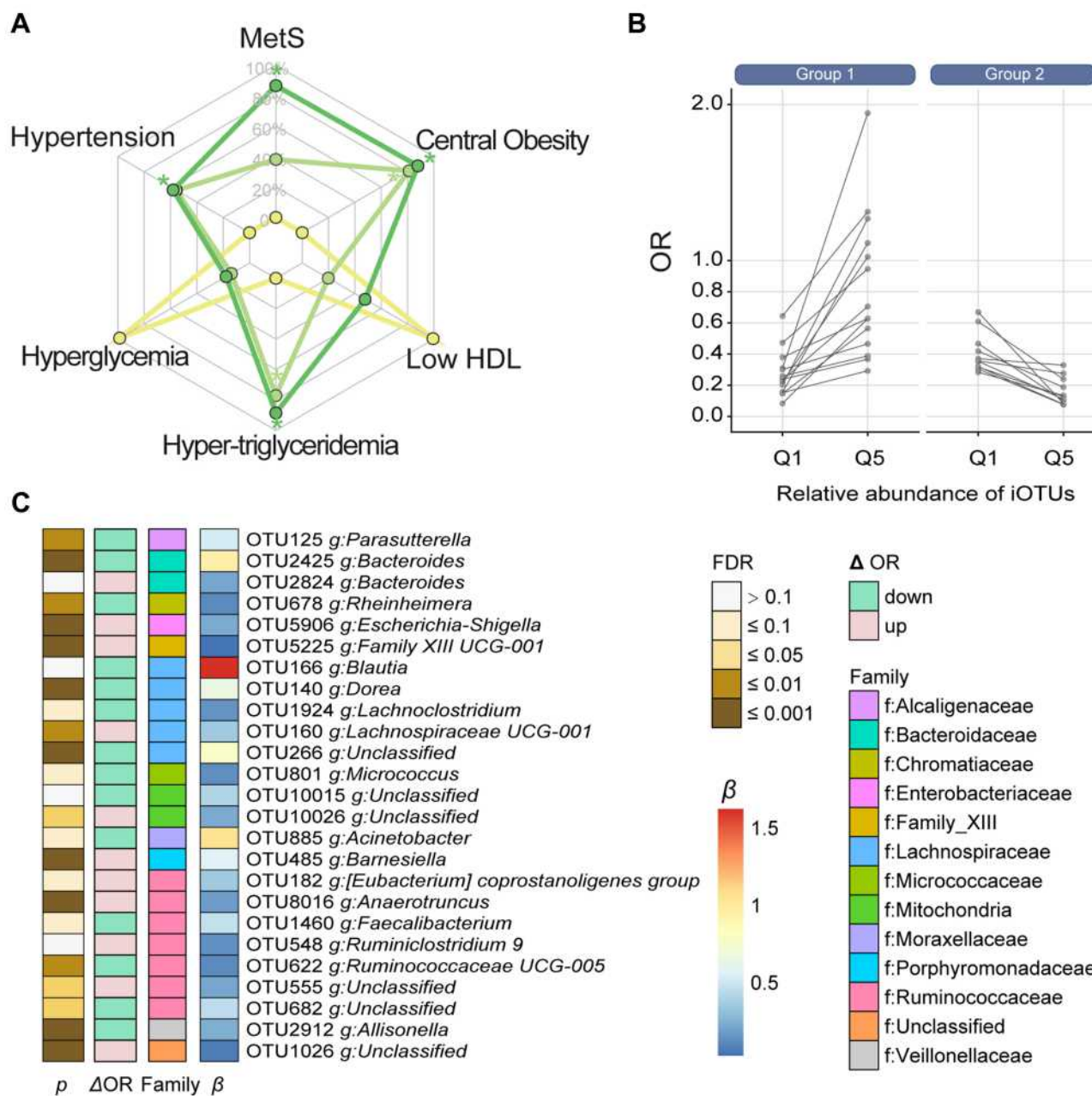


Figure 4 Effects of microbial interactions on the *Akkermansia*–MetS association. **(A)** Associations of MetS and its components with 3 *Akkermansia* OTUs. **(B)** Groups of iOTUs according to their effects on the *Akkermansia*–MetS association. Interactions were visualized by changes in the protective effect of *Akkermansia* against MetS (ORs) when the abundance of iOTUs increased from Q1 to Q5. ORs were estimated by logistic regression in which the abundance of *Akkermansia* was treated as a continuous variable. **(C)** Annotation of iOTUs at the family and genus levels. β and FDR were estimated with the multivariate linear regression. Equation: $\Delta OR = OR_{(iOTU_{atQ5})} - OR_{(iOTU_{atQ1})}$.

Akkermansia had a stronger protective effect against MetS when OTU485 was more abundant (Q5, OR=0.083, 95% CI: 0.011–0.45 and Q1, OR=0.669, 95% CI: 0.155–2.406). This strengthened effect persisted when OTU485 was treated as a continuous variable, and the increment of each SD of OTU485 reduced the ORs for MetS (Supplementary Figure 4). The iOTUs were annotated to 18 genera and 12 bacterial families. In line with the above

results, Ruminococcaceae and Lachnospiraceae were the 2 predominant families enriched in 7 and 5 genera, respectively (Figure 4C). Four genera in Lachnospiraceae (including *Blautia*, *Dorea*, and *Lachnoclostridium*) and 2 in Ruminococcaceae (including *Faecalibacterium* and Ruminococcaceae UCG-005) reduced the ORs of *Akkermansia* for MetS, reflecting a greater protective effect of *Akkermansia*. On the contrary, *Anaerotruncus*,

Lachnospiraceae UCG-001, [*Eubacterium*] *coprostanoligenes* group, and *Ruminiclostridium-9* potentially diminished the protective effect.

Discussion

The present study investigated the associations between gut *Akkermansia* and MetS in a large cohort with adjustment for potential confounders. We found that *Akkermansia* abundance was differentially associated with MetS components. We also demonstrated for the first time a nonlinear relationship between *Akkermansia* and MetS and the influence of microbial interactions on this association.

Some studies have reported an association between increased *A. muciniphila* and reduced prevalence of obesity, untreated T2DM, and hypertension.^{23–25} In this study, we found a significant association between *Akkermansia* and central obesity/waist circumference, hypertriglyceridemia/TG, reduced HDL-c (as a categorical variable)/HDL-c (as a continuous variable), SPB, and DBP, although it was unrelated to hyperglycemia, FBG, and hypertension. The discrepancy between our results and previous findings—especially pertaining to FBG and hyperglycemia—may come from the high degree of heterogeneity of the host and variability of gut microbiota. Our analyses were based on a large population and were adjusted for some confounders such as sex, age, and geography, which could potentially reduce the bias caused by confounders.

Our finding of an *Akkermansia*–obesity association is consistent with another study based on the American Gut Project, which included around 10,000 subjects.²⁶ Both studies adjusted for potential confounders such as age, sex, and geographic region, and we observed a significant relationship between *Akkermansia* and both central and overall obesity. The concordance of these results indicates that the protective effect of *Akkermansia* against obesity is independent of geography, ethnicity, sex, age, and dietary habits in Western and Eastern countries and may be generalizable to a large population.

Previous studies have reported conflicting results on the associations between *Akkermansia* and components of MetS. *A. muciniphila* was shown to control fat storage, adipose tissue metabolism, and glucose homeostasis to reverse diet-induced metabolic disorders in mice and humans.^{27,28} However, another study from the same researchers did not find significant changes in fasting glycemia and HbA1c in human subjects after 3 months of *A. muciniphila* administration, with similar results for

insulin sensitivity.¹² The interaction between lipid or glucose metabolism and *Akkermansia* has been widely studied, but the differential effects of *Akkermansia* on the 2 processes has rarely been addressed.²⁹ Our epidemiologic results showed that hyperglycemia had a weaker association with *Akkermansia* than obesity and hypertriglyceridemia, suggesting differences between lipid–*Akkermansia* and glucose–*Akkermansia* interactions. The protective mechanisms of *Akkermansia* against metabolic disorders include increasing mRNA expression of markers of adipocyte differentiation and lipid oxidation; counteracting colon mucus suppression induced by a high-fat diet; and reducing hepatic glucose-6-phosphatase and increasing acylglycerols in the ileum.^{7,27} However, the interaction of lipid and glucose metabolism under the regulation of gut bacteria requires more detailed investigation. Our results also suggest the need for preventive strategies targeting different components of MetS.

While most gut microbes showed significant correlations with *Akkermansia*, only a few OTUs influenced the *Akkermansia*–MetS association. A previous study found that the gut microbiome of mice was unaltered after *Akkermansia* gavage for 4 weeks.²⁷ On the other hand, 2 studies reported an increase in *Bifidobacterium* abundance following *Akkermansia* administration,^{30,31} which is inconsistent with our results. We found that Ruminococcaceae and Lachnospiraceae were the 2 main families influencing *Akkermansia* abundance and the *Akkermansia*–MetS association. Microbial interactions with *Akkermansia* and their modulation of MetS have rarely been studied, and interactions between different species can vary across environmental conditions, space, and potentially evolutionary time.¹³ Thus, additional studies are needed to clarify the relationships between *Akkermansia*, other gut microbes, and their hosts.

Our study identified the effective dose of *Akkermansia* for protection against MetS. The protective effect of *Akkermansia* was nonlinear and nonsignificant at a low relative abundance, which is in line with previous reports; a dose of 10⁸ CFU/day was found to be required to prevent or treat metabolic disorders,³² whereas one study showed that a daily *A. muciniphila* dose >4×10⁷ CFU was necessary in mice.³³ Although the exact threshold level of *Akkermansia* could not be determined due to the limitations of 16S rRNA sequencing, our results suggest that the risk of MetS is only reduced when *Akkermansia* represents at least of 0.2% of the whole gut microbiota.

We also provide epidemiologic evidence for the safety of *Akkermansia* in the Chinese population. *Akkermansia* is widely distributed in the human gut; >95% of the population harbors *Akkermansia* and ~20% has an abundance >7.05%. A similar distribution has been reported in the American and European populations: *Akkermansia* was detected in 75% of European samples,³⁴ 82% of British samples,²⁶ and 83% of American samples;²⁶ and the median abundance of *Akkermansia* was 0.08% (IQR: 0.04–0.34%) and 0.08% (IQR: 0.006–0.93%) in Chinese and the American samples,²⁷ respectively. The protective effects of *Akkermansia* increased with its abundance and no adverse effects were observed when it was present at high levels. There have been few clinical studies demonstrating the safety of *Akkermansia*, but an interventional study reported a tolerable *A. muciniphila* dose of 10¹⁰ CFU/day in overweight or obese individuals,^{12,35} while another showed no adverse effects even when the proportion of *A. muciniphila* was 60% of total human gut bacteria.³⁶

It is worth noting that we performed analyses at the OTU and genus levels of *Akkermansia* because of the limitations of 16S rRNA sequencing. In fact, there are at least 8 different species reported in this genus,³⁷ although only one *A. muciniphila* strain (MucT) is available and widely used for intervention in metabolic disorders. The associations between MetS and *Akkermansia* at genus and species or strain levels have been previously reviewed;³³ however, it remains unclear how these differ across species. In the present study, we detected 3 *Akkermansia*-like OTUs but only one showed significant associations with MetS and its components.

A strength of our study is that it provides evidence for the relationship between *Akkermansia* and MetS; the large sample size allowed us to estimate the independent protective effect of *Akkermansia* by adjusting for several potential confounders. Moreover, this is the first demonstration of an effective dose of *Akkermansia* for protection against MetS and the first report to highlight the contribution of other gut microbes on this effect.

Our study had certain limitations. Firstly, the results were based on a Chinese population and may not be completely generalizable to Western subjects; that is, the effective dose of *Akkermansia* may differ given the disparities in MetS prevalence and *Akkermansia* abundance across different geographic regions or ethnic groups. Secondly, there may be methodologic bias associated with 16S rRNA sequencing, which is commonly used to detect bacteria at the genus level; shotgun metagenomic sequencing or pure culture-based

analyses at the species or strain level should therefore be performed. Finally, the lack of some medication records may have biased the result. For example, missing 2-h postprandial blood glucose data may have affected the definition of hyperglycemia; and metformin intake can increase *Akkermansia* abundance³⁸ but was not included in this dataset. Accordingly, the present study provides only cross-sectional evidence for the *Akkermansia*–MetS relationship.

Conclusion

The results of this study suggest that a higher abundance of *Akkermansia* was associated with a lower risk of MetS in the Chinese population, whereas the associations with different MetS components varied. We also found that the protective effect of *Akkermansia* is influenced by microbial interactions and may not be significant below a certain abundance threshold. These findings suggest that probiotic intervention with *Akkermansia* can prevent or alleviate MetS and related disorders such as heart disease or T2DM.

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Disclosure

The authors report no conflicts of interest in this work.

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