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Association of body mass index and intestinal (faecal) *Streptococcus* in adults in Xining city, China P.R.

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RESEARCH ARTICLE

Abstract

Body mass index (BMI) and gut microbiota show significant interaction, but most studies on the relationship between BMI and gut microbiota have been done in Western countries. Relationships that are also identified in other cultural backgrounds are likely to have functional importance. Hence here we explore gut microbiota in adults living in Xining city (China P.R.) and relate results to subject BMI. Analysis of bacterial 16s rRNA gene was performed on faecal samples from participants with normal-weight (n=24), overweight (n=24), obesity (n=11) and type 2 diabetes (T2D) (n=8). The results show that unweighted but not weighted Unifrac distance was significantly different when gut microbiota composition was compared between the groups. Importantly, the genus *Streptococcus* was remarkably decreased in both obese subjects and subjects suffering from T2D, as compared to normal-weight subjects. Accordingly, strong association was identified between the genus *Streptococcus* and BMI and especially *Streptococcus salivarius* subsp. *thermophiles* was a major contributor in this respect. As previous studies have shown that *Streptococcus salivarius* subsp. *thermophiles* is also negatively associated with obesity in Western cohorts, our results suggest that this species is a potential probiotic for the prevention of obesity and related disorders.

Keywords: gut microbiota, *Streptococcus*, *Streptococcus salivarius* subsp. *thermophiles*, obesity, type 2 diabetes

1. Introduction

Body mass index (BMI) is a widely used indicator for establishing whether a person is overweight or obese and consequently at risk for medical conditions as diabetes, high blood pressure, heart disease and certain cancers. As a major global health threat, obesity is a key risk factor for insulin resistance and type 2 diabetes, and also contributes to increased risk for other chronic diseases, including but not limited to hypertension, fatty liver, heart diseases and cancer (Kopelman, 2000). Over the last decades, gut microbiota have been demonstrated to be closely associated with obesity and type 2 diabetes (T2D) (Qin *et al.*, 2012; Stephens *et al.*, 2018), and are also associated with aberrant adiposity and metabolism (Boulangé *et al.*, 2016). Most data, however, have been obtained in Western populations and thus the exact gut bacterial species that mediate the interaction between obesity and the bacterial microbiota remain obscure at best. Studies performed in alternative cultural backgrounds may help identifying the relevant species in this respect, but such studies are still relatively scarce. Thus prompted, here we aim to investigate the relationship between gut microbiota and increasing BMI in adults in Xining city of Qinghai province, China P.R.

2. Materials and methods

Participants

All participants were recruited at The Fifth People's Hospital of Qinghai province. Based on BMI calculation, participants with no known significant health problems were categorised as normal (BMI <24.9 kg/m^2), overweight (BMI 25-29.9 kg/m^2) or obese (BMI ≥30 kg/m^2). For participants with T2D, the inclusion criteria had to meet the condition of fasting blood glucose ≥7.0 mmol/l or that the subjects received antidiabetic treatment. Exclusion criteria included inability to provide consent or the use of antibiotics one month prior to the study. The body weight and height of all participants was measured by a body composition analyser SH-900G (Zhenzhou Shanghe Electronic Technology Co., Zhengzhou, China P.R.). Fasting blood samples were collected and sent to Zhejiang DIAN Diagnostics Co., Ltd. (Xining sub-company, Hangzhou, China P.R.) for the measurement of blood parameters, such as glucose and Hemoglobin A1c (HbA1C). Yoghurt consumption was estimated based on frequency categories ranging from 'never consumed', to 'consume daily', 'consume weekly', 'consume monthly' and 'consume yearly'. Participants reported their yoghurt intake through a recall interview. In addition, a recall interview with respect to medication use at the time of the study was performed. This study was approved by the medical ethical committee of Northwest Minzu University (XBMZ-YX-2.016.001) and all subjects provided written informed consent before enrolment.

Microbiota characterisation and statistical analysis

Individual stool samples for each subject were following production and collection transported on dry ice to the NoveGene Company, Beijing, China P.R., where faecal DNA extraction and next-generation sequencing of 16S ribosomal RNA gene amplicons were performed as previously described (Su *et al.*, 2020).

In short, the V3-4 region of the bacterial 16S rRNA gene was amplified and sequenced. The raw reads were deposited in the NCBI Sequencing Read Archive (SRA) database under BioProject accession number PRJNA789136 (https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA789136). Paired-end reads (250 bp) were assigned to each sample by a unique barcode. After removing the barcode, merged reads were quality-checked by using split-libraries-fastq.py in QIIME (Bokulich *et al.*, 2013; Caporaso *et al.*, 2010). Applying the UCHIME algorithm, chimeric sequences were removed by comparing the reads with the reference database (SILVA database). The resulting sequences were analysed with the Uparse software and those with ≥97% similarity assigned to the same OTU (operational taxonomic unit) (Haas *et al.*, 2011). Representative sequences were classified against the SILVA reference taxonomy using a negative Bayesian classifier implemented within mother (Edgar, 2013; Wang *et al.*, 2007). Finally, a rarefied OTU table was generated at one depth of sequence per sample, with which all of the downstream analyses were performed. All OTUs for each sample are shown in Supplementary Table S1 with total tags number and in Supplementary Table S2 with relative abundance.

The QIIME software was used to evaluate alpha and beta diversity in the gut microbial community (Bokulich *et al.*, 2013). Alpha diversity was estimated by calculating the observed species, Chao1 and Shannon indices. Beta diversity was analysed by using UniFrac, which is a distance measure for comparing microbial communities (Lozupone and Knight, 2005). Briefly, weighted UniFrac (a semi-quantitative measure) accounts for the relative abundance of each taxon within a microbial community, while unweighted UniFrac (a qualitative measure) emphasizes the presence/absence of taxa (Lozupone *et al.*, 2007). Principal Co-ordinates Analysis (PCoA) of the Bray-Curtis distance was performed using the 'vegan' package available in the R programming language (Dixon, 2003). Correlation analysis of BMI with gut microbiota was performed using Spearman method employing the function cor.test() in R.

For analysis of the relative abundance of the genera detected, a comparison between groups was performed using the Mann-Whitney test. The data were shown as mean ± standard deviation (SD). A two-sided *P*-value less than 0.05 was considered to be a statistically significant difference.

3. Results and discussion

All participants were from Xining city of Qinghai province, China. Following preselection, finally a total of 67 adults were included in the study. Of those included, 24 were overweight, 11 obese, 8 diabetic and all were compared to 24 healthy controls, who were age- and gender-matched to the overweight/obese/diabetic cohort (Table 1 and Supplementary Table S3). Of note, the non-obese samples collected were also used in a previous study (Su *et al.*, 2021), but the obese sample group and the group of subjects both obese and suffering from metabolic disease, were specifically collected for the present study. We first determined the faecal microbiota community of all participants using 16S rRNA gene sequencing analysis. Rarefaction curves were plotted to determine the sequence number per group at the same depth. As shown in Figure 1A, the number of OTUs became saturated at 35,000 sequences for each of the groups, indicating that this sequencing depth was sufficient to identify the majority of bacterial species present in each group. We observed no overall significant differences in species richness in faecal microbiota when assessed by OTU number, Chao1 index or Shannon index in comparing the overweight, obese or T2D participants and normal weight

Table 1. Baseline demographics of the volunteers.[1,2]

Characteristics	Healthy (n=20)	Overweight (n=21)	Obesity (n=18)	T2D (n=8)	*P*-value
Age year (mean)	43.60 (39.83-47.37)	40.29 (37.06-43.51)	42.89 (38.68-47.09)	46.63 (42.70-48.55)	0.3067
Sex male n (%)	7 (35.00)	12 (57.14)	11 (61.11)	4 (57.14)	0.3344
Body weight (kg)	56.81 (54.09-59.53)	70.20 (66.80-73.59)	88.78 (83.60-93.96)	83.09 (78.02-88.16)	<0.0001
BMI	22.08 (21.34-22.82)	26.17 (25.66-26.68)	31.93 (30.44-33.42)	30.20 (28.89-31.51)	<0.0001
Disease for years	-	-	-	3.56 (2.29-4.84)	
GLU (mmol/l)	4.00 (3.82-4.17)	4.24 (4.06-4.43)	4.20 (3.95-4.45)	7.959 (5.25-10.67)	<0.0001
HbA1C (%)	5.12 (4.92-5.31))	5.24 (5.04-5.44)	5.35 (5.10-5.61)	7.00 (5.82-8.18)	<0.0001
Triglycerides (mmol/l)	1.25 (0.98-1.51)	1.70 (1.29-2.12)	1.86 (1.39-2.33)	2.70 (1.38-4.01)	0.0047
TCHOL (mmol/l)	4.02 (3.68-4.35)	4.01 (3.66-4.36)	4.17 (3.82-4.52)	4.28 (3.70-4.86)	0.7492
HDL-CH (mmol/l)	1.25 (1.11-1.38)	1.09 (0.95-1.23)	1.05 (0.92-1.18)	0.86 (0.68-1.04)	0.0113
LDL-CH (mmol/l)	2.41 (2.14-2.67)	2.42 (2.08-2.75)	2.40 (2.07-2.74)	2.45 (1.61-3.29)	0.9990
TBIL	13.82 (10.96-16.68)	12.39 (10.47-14.30)	14.72 (11.39-18.04)	12.49 (8.29-16.68)	0.5805

[1] Each value was expressed as mean (95%CI). Statistical analysis was performed by one-way ANOVA. *P*=value less than 0.05 was considered significantly.
[2] GLU = glucose; HbA1C = haemoglobin A1c; TCHOL = total cholesterol; HDL-CH = high density lipoprotein cholesterol; LDL-CH = low density lipoprotein cholesterol; TBIL = total bilirubin; T2D = type 2 diabetes.

subjects (Figure 1). However, the unweighted (Figure 2B) but not weighted (Figure 2A) UniFrac distance showed a significant shift in the gut microbiota composition of overweight, obese or T2D participants away from healthy subjects. The PCoA reveals a significant separation between the overall microbiota composition of the groups (Figure 2C,D). Given the lack of differences in gut microbiota composition using weighted UniFrac, the shift in the gut microbial community between groups was probably driven by rare lineages. Nevertheless, these results indicate that gut microbiota composition in adults was affected by changes in BMI. This is in line with the general idea in the field that BMI and gut microbiota are related, a notion supported for instance by previous studies in adult monozygotic twins (Le Chatelier *et al.*, 2013) and in patients with coronary heart disease (Haro *et al.*, 2016).

The exact species functionally related to obesity remains largely obscure (Schwiertz *et al.*, 2010). We reasoned that species associated in our Chinese cohort with obesity that

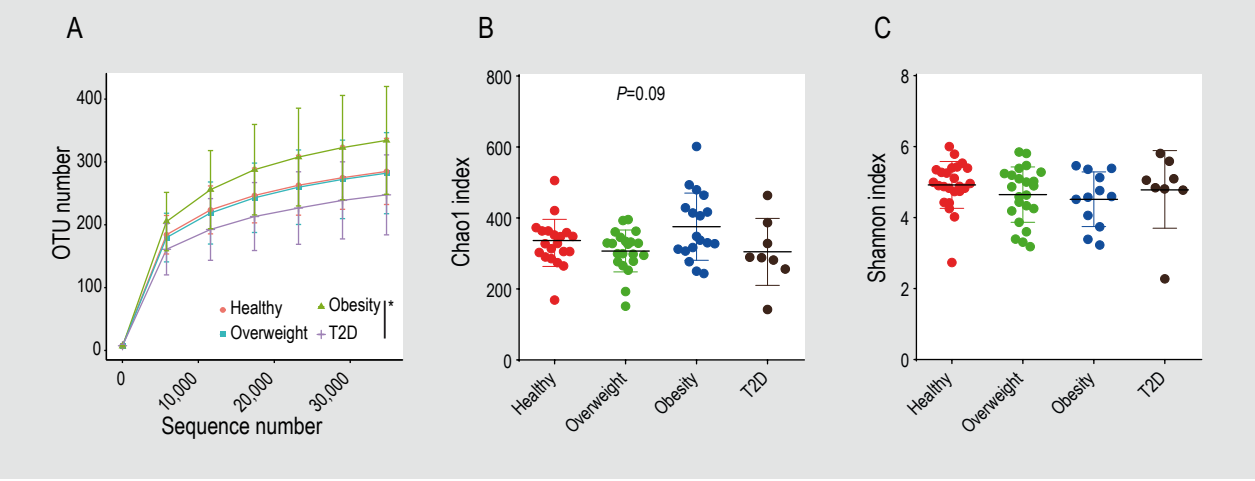

Figure 1. Analysis of gut bacterial communities by 16S rRNA sequencing. (A) Cumulative species curves. Sequences with ≥97% similarity were assigned to the same operational taxonomic units (OTUs). Sample-based rarefaction curves were constructed with sequence counts at 35,000 cut-off value to determine the sequence number of each group at the same depth. The cumulative species numbered was plotted to establish the increasing number of species in relation to the increase in sampling effort for each group. The number of OTUs is presented as mean ± standard deviation. * Adjusted *P*-value <0.05; Kruskal-Wallis test with Bonferroni correction. (B,C) Gut microbial diversity evaluation, as indicated by (B) Chao1 and (C) Shannon indices (Mann-Whitney test).

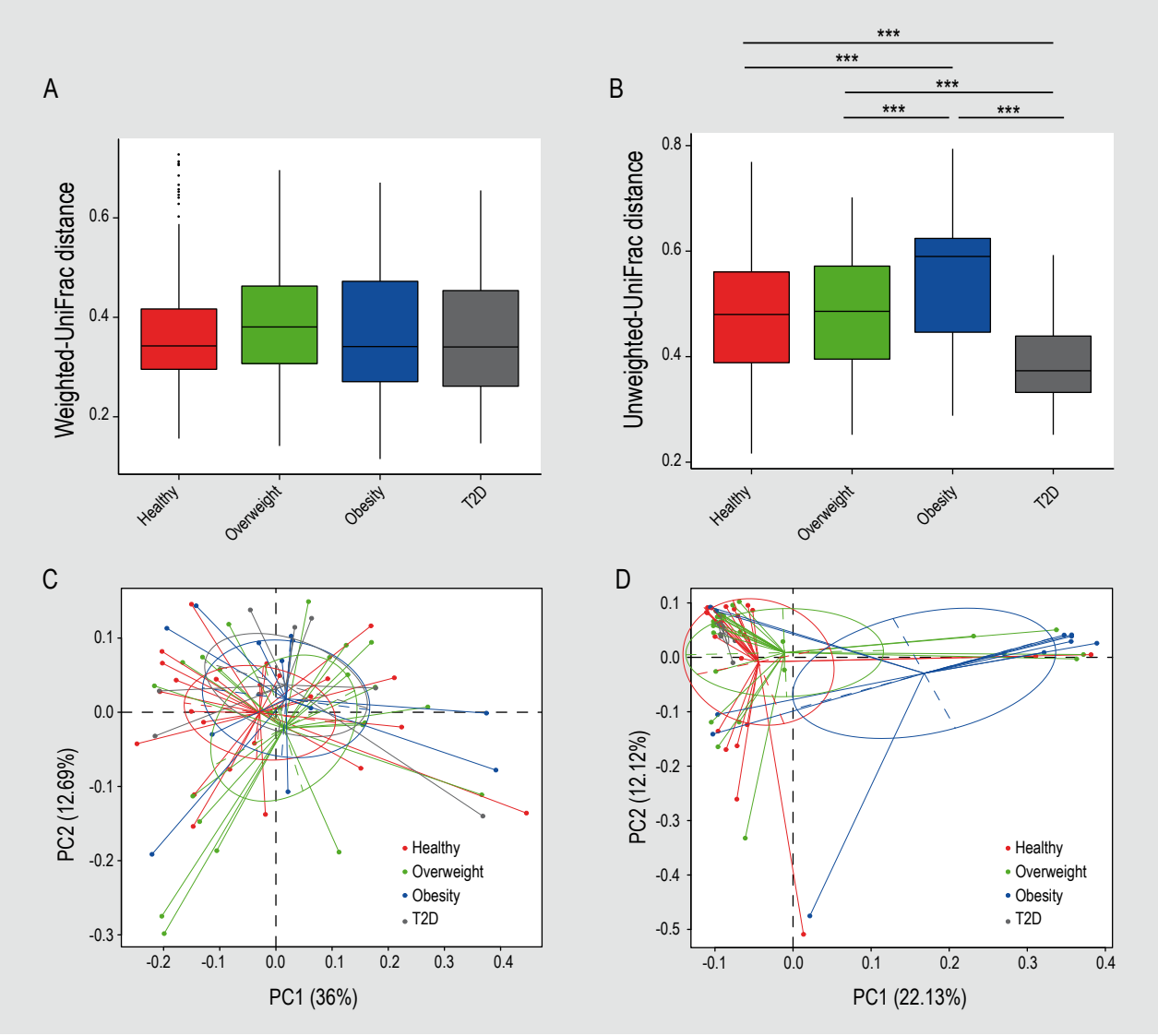

Figure 2. Analysis of gut bacterial communities by 16S rRNA sequencing. (A) Weighted and (B) unweighted UniFrac-based box-plots and (C) weighted and (D) unweighted Principal Coordinate Analysis (PCoA) indicate the shift in gut microbiota between groups. ** *P*<0.01; * *P*<0.001; Wilcoxon signed-rank test. Each point in the PCoA corresponds to a community from an individual. Colours indicate community identity. Ellipses show the 95% confidence intervals.**

were previously linked in Western cohorts with obesity would be strong candidates for the species that functionally link BMI and microbiome. Thus, we first analysed gut microbiota at the genus level. The mean relative abundance of the top 30 genera representing 72.8% of overall relative abundance of all samples is provided for each group in the figures (Supplementary Figure S1). In particular, we observed that the abundance of *Streptococcus* was significantly decreased in obesity and T2D when compared to the healthy group (Figure 3A).

Next, we performed a Spearman association analysis to test whether there is a relationship between *Streptococcus* and BMI. As expected, a moderate negative association was identified between the two variables (Figure 3B). Interestingly, this association was mainly driven by *Streptococcus salivarius* subsp. *thermophiles*, also known as *Streptococcus thermophilus* (Figure 3C and Supplementary Table S4), a notable member of the genus *Streptococcus* that has often detected in fermented milk products and is generally used for the production of yoghurt. Intriguingly, a recent study reported that oral supplementation of *Streptococcus salivarius* subsp. *thermophiles* in conjunction with other probiotic species, confers beneficial effects in metabolic disease (Hajifaraji *et al*., 2018). However, we did not find any significant associations between blood glucose and *Streptococcus* or *Streptococcus salivarius* subsp. *thermophiles* (Supplementary Figure S2).

To further examine if yoghurt consumption contributes to the negative association established between *Streptococcus salivarius* subsp. *thermophiles* and BMI, we performed a

questionnaire for the evaluation of yoghurt consumption in the present cohort. As shown in Figure 4A, no significant difference in this parameter was found between any groups. However, yoghurt consumption was slightly and negatively associated with BMI, although it was not statistically significant (Figure 4B). Also, a small but significant positive association was identified between yoghurt consumption and *Streptococcus salivarius* subsp. *thermophiles* (Figure 4C). These data suggest that yoghurt consumption may contribute to the established negative association between *Streptococcus salivarius* subsp. *thermophiles* and BMI.

Notably, there were three participants in the present cohort with significantly greater abundance of streptococcus (>9%) compared to the other samples (<5%) (Figure 3A). To confirm whether these samples skew the results, we reanalysed the data without including these samples. We found that *Streptococcus* still showed a significant decrease in other three groups compared to the healthy group (Figure 5A). The negative association was also still seen between BMI and this genus (Figure 5B) as well as with *Streptococcus salivarius* subsp. *thermophiles* (Figure 5C). Therefore, inclusion of these three samples did not significantly skew the results.

Overall, our study supports the notion that *Streptococcus salivarius* subsp. *thermophiles* is the principal determinant in the gut microbiota with respect to metabolic regulation and adipose tissue low-grade inflammation. The notion that biological processes in general and the reaction of the body to bacterial microbiome components in particular does not show a linear dependency on the challenge involved has often been observed. BMI in general (which is a quadratic measure by itself) is controlled by many factors that might show an exponential relationship with the concentration of *Streptococcus* in the intestine. These factors include

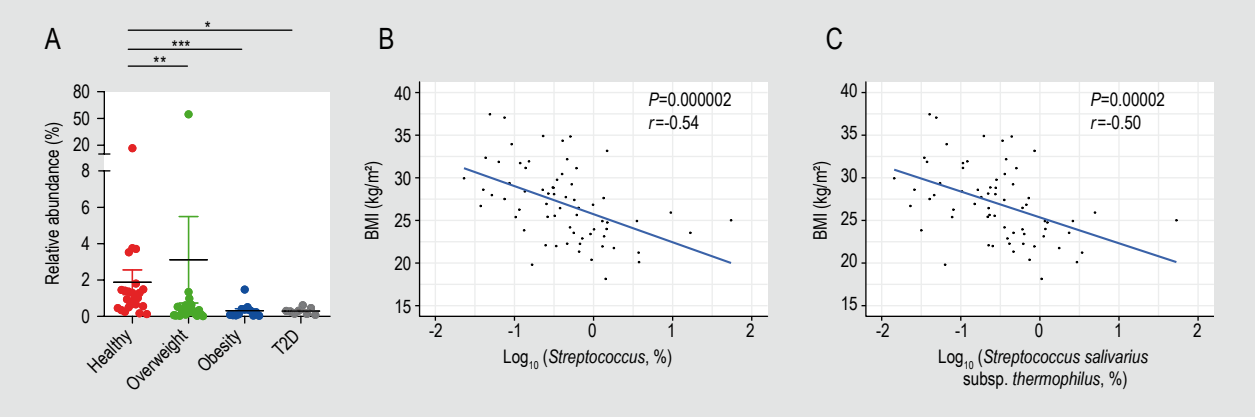

Figure 3. (A) Comparison of the relative abundance of the genus *Streptococcus* between different groups. * *P*<0.05; ** *P*<0.01; * *P*<0.001; Mann-Whitney test. (B) Spearman correlation between the genus *Streptococcus* and body mass index (BMI). (C) Spearman correlation between the species *Streptococcus salivarius* subsp. *thermophilus* and BMI.**

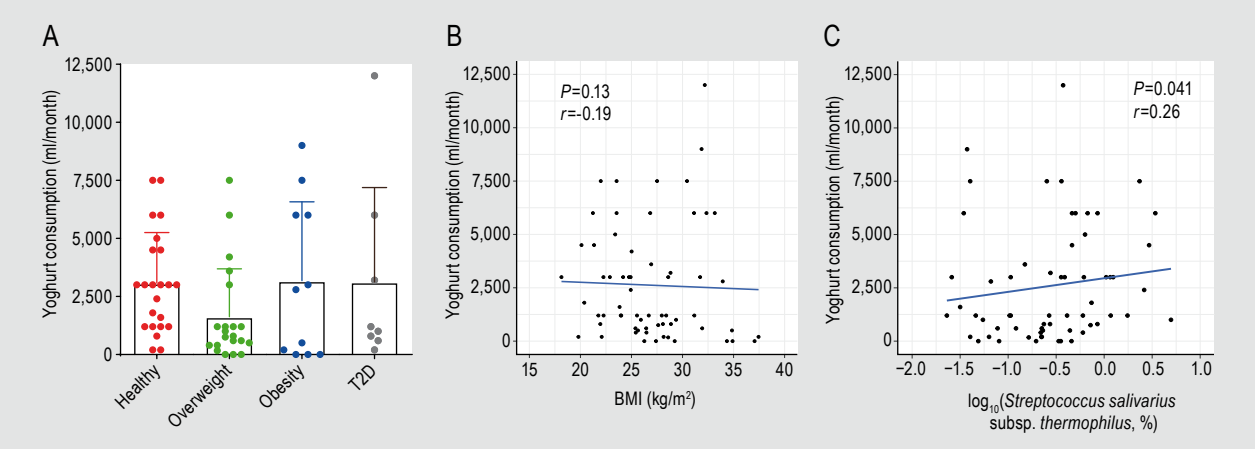

Figure 4. Association between yoghurt consumption and *Streptococcus salivarius* subsp. *thermophiles*. (A) Comparison of the yoghurt consumption per month between different groups. The significance was calculated using the Mann-Whitney test. (B) Spearman correlation between the yoghurt consumption and body mass index (BMI). (C) Spearman correlation between the yoghurt consumption and the species *Streptococcus salivarius* subsp. *thermophilus*.

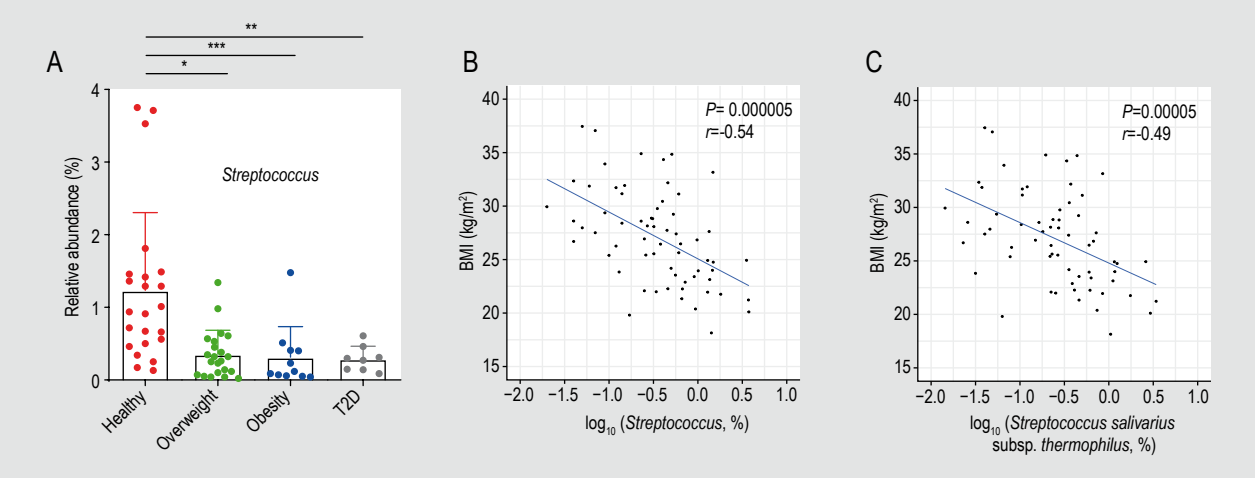

Figure 5. Confirmation of the association between the genus *Streptococcus* and body mass index (BMI). (A) Three samples with the abundance of *Streptococcus* above 5% were thus excluded from the study to avoid the possible skewness. Comparison of the relative abundance of *Streptococcus* between different groups. * *P*<0.05; ** *P*<0.01; * *P*<0.001; Mann-Whitney test. (B) Spearman correlation between the genus *Streptococcus* and BMI. (C) Spearman correlation between the species *Streptococcus salivarius* subsp. *thermophilus* and BMI.**

absorption of *Streptococcus*-produced metabolites (which involves mucus penetration (Wu *et al.*, 2017), size of the intestinal area and transport through the body) and possible influence of altered pH (a logarithmic measure by itself; (Dunn *et al.*, 2019)), the binding of those products to their cognate receptors and subsequent induction of negative feedback mechanisms (Wang *et al.*, 2018) and the intrinsic properties of adipose tissue compartment (Warne *et al.*, 2006) amongst others. Limitations of our study include the modest sample size and the fact that this is a single centre study, which could cause bias and underestimate the association between yoghurt consumption with BMI and the gut microbiome or between the latter two variables. Thus, our results warrant replication in larger cohorts and also outside the Xining city.

In conclusion, BMI influenced the composition of the gut microbiota in adult population in Xining City and negatively associated with *Streptococcus salivarius* subsp. *thermophiles* belonging to the genus *Streptococcus*. Our results might help to extend the understanding of the potential role of *Streptococcus salivarius* subsp. *thermophiles* strains in the prevention and treatment of metabolic disorders.

Supplementary material

Supplementary material can be found online at https://doi.org/10.3920/BM2021.0046.

Table S1. OTU_table_absolute.

Table S2. OTU_table_relative.

Table S3. Antidiabetic drugs.

Table S4. *Streptococcus* OTUs identified in the present cohort.

Figure S1. Stacked bar chart representing the mean relative abundance of top 30 genera in healthy, overweight, obese and T2D group.

Figure S2. Spearman correlation between blood glucose with *Streptococcus* and *Streptococcus salivarius* subsp. *thermophiles*.

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Conflict of interest

The authors declare no conflict of interest.

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