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Smoking-related gut microbiota alteration is associated with obesity and obesity-related diseases: results from two cohorts with sibling comparison analyses

Yiting Duan[1,2†], Chengquan Xu[1,2†], Wenjie Wang[1,2], Xiaoyan Wang[1,2], Nuo Xu[1,2], Jieming Zhong[3], Weiwei Gong[3], Weifang Zheng[4], Yi-Hsuan Wu[5], April Myers[5], Lisa Chu[5], Ying Lu[6,7], Elizabeth Delzell[5], Ann W. Hsing[5,7,8], Min Yu[3*], Wei He[1,2*] and Shankuan Zhu[1,2*]

Abstract

Background Individuals who smoke tend to have a lower body mass index (BMI) but face an increased risk of obesity-related diseases. This study investigates this paradox from the perspective of gut microbiota.

Methods We conducted microbiome analyses to identify smoking-related microbial genera and created a smoking-related microbiota index (SMI) using 16S rRNA sequencing data from 4000 male participants in WELL-China cohort and Lanxi cohort. We employed logistic regression to explore the association between SMI and obesity indices derived from dual-energy X-ray absorptiometry. Cox regression analyses were conducted to explore the association of SMI with incident of obesity-related diseases. To further control for unmeasured familial confounders, sibling comparison analyses were conducted using between-within (BW) model.

Results The smoking-related microbiota index (SMI) showed a positive association with BMI and other obesity indices. Further analyses revealed that SMI is linked to obesity-related diseases, with hazard ratios (95% confidence intervals) of 1.97 (1.41–2.75) for incident diabetes, 1.31 (1.01–1.71) for major adverse cardiovascular events, and 1.70 (1.05–2.75) for obesity-related cancers. Results from sibling comparison analyses reinforced these findings.

Conclusions While smoking may reduce weight through various mechanisms, alterations in gut microbiota related to smoking are associated with weight gain. Further research is required to determine if changes in the smoking-related microbiome contribute to weight gain following smoking cessation.

Keywords Gut microbiota, Smoking, Obesity, Obesity-related disease

†Yiting Duan and Chengquan Xu contributed equally to this work.

*Correspondence:
Min Yu
myu@cdc.zj.cn
Wei He
zjuhewei@zju.edu.cn
Shankuan Zhu
zsk@zju.edu.cn
Full list of author information is available at the end of the article

Background

Tobacco use remains the first-leading modifiable risk factor for death among men in 2019 [1]. While certain studies suggest that smoking may reduce the risk of obesity [2, 3], others indicate that it heightens the risk of obesity-related illnesses such as diabetes and cardiovascular disease (CVD) [4–7]. The longstanding paradox—that smokers exhibit lower body weight but suffer from higher risks of obesity-related diseases—suggests a need for a novel approach to understand these dynamics better and to enhance tobacco control efforts.

The human gut microbiota, comprising trillions of microbial cells and thousands of bacteria species, engages in a complex mutualistic symbiosis with the host [8, 9]. Several population-based studies, often limited by small sample sizes and low reproducibility, have demonstrated that cigarette smoking may alter gut microbiota composition [10, 11]. In animal studies, exposure to smoke components has been shown to elevate gut pH levels or decrease the production of organic acids, thereby reducing the abundance of specific microbial taxa and leading to dysregulation of the gut microbiome [12]. Additionally, the gut microbiota also has been implicated in the pathogenesis of obesity [13] and obesity-related diseases including diabetes [14, 15], CVD [16], and obesity-related cancers [17, 18]. For example, *Ruminococcaceae* and *Lachnospiraceae* are involved in a variety of metabolic pathways by producing a variety of short-chain fatty acids (SCFAs) such as butyric acid. Butyrate not only enhances the integrity of the epithelial barrier and inhibits inflammatory responses but also modulates energy homeostasis by stimulating intestinal endocrine cells to secrete leptin, a hormone primarily produced by adipocytes, which plays a significant role in obesity and multiple chronic diseases [19, 20].

However, the implications of these smoking-induced changes in gut microbiota on obesity remain largely unexplored. To date, only one animal study has investigated this phenomenon, finding that mice who received fecal bacteria from counterparts exposed to cigarette smoke experienced weight gain [21]. Further research is necessary to determine whether these findings are applicable to humans, particularly in light of epidemiological studies indicating that smoking may reduce body weight.

To address these gaps, we analyzed data from two cohorts—the WELL-China cohort (the discovery cohort) [22] and the Lanxi cohort (the replication cohort) [23]—to investigate the role of gut microbiota in the paradox posed by the observation that individuals who smoke may have lower body weight but a higher risk of obesity-related diseases. Our study used both cross-sectional and longitudinal analyses to determine (1) whether smoking alters gut microbiota; (2) whether smoking-related microbiota alteration is associated with obesity and central fat distribution; (3) whether smoking-related microbiota alteration is linked to obesity-related outcomes; (4) whether smoking-related microbiota alteration mediates the association of smoking with obesity, central fat distribution, and obesity-related diseases.

Smoking, gut microbiota, obesity, and obesity-related diseases all exhibit familiar clustering [24, 25], implying that the association of gut microbiota with obesity and related diseases may be confounded by unmeasured familial factors like genetic similarity and shared environment early in life. To this end, we also conducted sibling comparison analyses to account for these confounding factors and to provide more robust findings.

Methods

Data sources

We used data from the WELL-China (the Wellness Living Laboratory China) cohort (the discovery cohort) and the Lanxi cohort (the replication cohort) for all the following gut microbiota analyses. Briefly, the WELL-China cohort enrolled a total of 10,268 residents from three districts of Hangzhou, China, during 2016 to 2019 [22]. The Lanxi cohort included a total of 4503 participants recruited from Lanxi, China, during 2017 to 2019 [23].

Study population

This study included only male participants from the WELL-China cohort and the Lanxi cohort, as female participants had very low smoking prevalence (WELL-China cohort 1.33%, Lanxi cohort 0.37%). Additional file 1: Fig. S1 describes the participant selection process. After excluding individuals with baseline cancers, inflammatory bowel diseases and BMI < 18.5 kg/m^2 (due to underlying health conditions [26]), or missing information on smoking or gut microbiome, final analyses included a total of 2709 male participants from the WELL-China cohort (the discovery cohort) and 1291 male participants from the Lanxi cohort (the replication cohort).

Sibling comparison analyses included 220 participants in the Lanxi cohort. Eligibility for this analysis required each sibling set to include at least two brothers. Sibling information was collected through a questionnaire survey.

Smoking status

Smoking information was collected through face-to-face interviews. According to definition previously published [11], current smokers were individuals who had smoked at least 100 cigarettes in their lifetime and were smoking at baseline either daily or occasionally. Former smokers were those who had quit smoking after surpassing the

100 cigarettes mark. Participants who did not fall into either category were classified as never smokers.

Smoking-related microbiota index

A smoking-related microbiota index (SMI) served as a comprehensive representation of the gut bacterial pattern associated with smoking, calculated as described in a previous publication [15]. Specifically, we calculated SMI based on the relative abundance of identified microbial genera using the following formula:

$$I_i^P = \sum_{j=1}^{n} A_{ij}$$

$$I_i^N = \sum_{j=1}^{m} A_{ij}$$

$$SMI = \left(\frac{I_i^P}{n} - \frac{I_i^N}{m} \right)$$

where A_{ij} represents the relative abundance of smoking-related genus j for individual i. P is a subset of all gut microbial genera positively correlated with smoking. N is a subset of all gut microbial genera negatively correlated with smoking. Details regarding the gut microbial DNA extraction process, amplicon sequencing, and the analysis of gut microbiota can be found in Additional file 1: Method S1 [27, 28]. Since SMI is not normally distributed, we divided it into tertiles in our analyses, further allowing for the examination of a potential dose–response relationship.

Obesity data at baseline

Body weight, height, and waist and hip circumferences were measured by trained staff at baseline. BMI was calculated as weight in kilograms divided by the square of height in meters. Waist-to-hip ratio (WHR) was calculated as waist circumference (cm) divided by hip circumference (cm). The body fat mass and regional fat mass were measured using dual-energy X-ray absorptiometry (DXA) scans (Software version 11.40.004, GE Lunar Prodigy; GE Healthcare, Milwaukee, WI, USA). Body fat percentage was calculated as total fat mass divided by body weight. For the android region, the measurement starts at the pelvic line and goes upward, covering 20% of the distance to the femoral neck. The gynoid region begins slightly below the pelvic line, extending down 1.5 times the android region's height. The edges of both regions are determined by the legs' outermost lines [29]. The percentage of android fat mass and gynoid fat mass were calculated as android fat mass and gynoid fat mass divided by total body fat mass, respectively. Android-gynoid fat ratio (AOI) was calculated as android fat mass divided by gynoid fat mass.

Obesity-related disorders at baseline

Fasting glucose, HbA1c, and lipid profiles were assessed at baseline. Diabetes was defined as fasting blood glucose ≥ 7.0 mmol/L, HbA1c ≥ 6.5%, or self-reported medication history [30]. Dyslipidemia was defined as elevated total cholesterol (TC ≥ 6.2 mmol/L), elevated triglycerides (TG ≥ 2.3 mmol/L), elevated low-density lipoprotein cholesterol (LDL ≥ 4.1 mmol/L), decreased high-density lipoprotein cholesterol (HDL < 1.0 mmol/L), or use of lipid-lowering medications [31]. Metabolic syndrome (MetS) was defined using revised criteria from the International Diabetes Federation (IDF) [32]. Metabolic dysfunction-associated steatotic liver disease (MASLD) was diagnosed by a consensus review through ultrasound images described previously [33, 34].

Obesity-related diseases during the follow-up

Participants were followed for death and hospitalization events through electronic linkage via unique personal identification number. Patients were followed from baseline period of 2016 to 2019 until death, the onset of the obesity-related diseases, or the end of the study period (June 24, 2024), whichever came first. The median follow-up time was 6.0 years in the WELL-China cohort and 4.9 years in the Lanxi cohort. ICD-10 codes were used to define obesity-related diseases and all cancers, including incident diabetes (E11-E14), major adverse cardiovascular events (MACE) (including death, myocardial infarction (I20–I25), heart failure (I50), or stroke (I60–I64, I69) [35, 36]), obesity-related cancers (esophageal cancer (C15), gastric cancer (C16), colorectal cancer (C18-C20), liver cancer (C22), gallbladder cancer (C23-C24), pancreatic cancer (C25), laryngeal cancer (C32), prostate cancer (C61), and kidney cancer (C64-C65) [37, 38]), and all cancers (C00-D48).

Covariates

Information on demographic and lifestyle factors, including age (years), marital status (married, others), education level (≤ 6 years, 6–12 years, or ≥ 12 years), annual income (≤ 50,000 CNY, 50,000–109,999 CNY, or ≥ 110,000 CNY), district of residence (urban or rural), alcohol consumption (non-drinker, occasional, regular or former drinker), and nutritional supplement (yes or no) and antibiotics use (yes or no), were obtained through standard structured questionnaires. Diet energy intakes (Kcal / day) in the WELL-China cohort was measured using a validated 26-item Food Frequency Questionnaire (FFQ) [39], while the Lanxi cohort utilized a validated 46-item FFQ [40].

Physical activity levels were categorized as low, moderate, or high based on response to the International Physical Activity Questionnaire (IPAQ) [41].

Statistical analyses

Continuous variables were presented as means (SDs) and categorical variables were presented as numbers (proportions). The one-way analysis of variance (ANOVA) and the chi-square test were used to test whether baseline characteristics differed by smoking status.

Microbiome analyses

We used multivariable linear regression to assess associations between smoking and gut microbiota α-diversity. The relationship between smoking and gut microbiota β-diversity was investigated through permutational multivariate analysis of variance (PERMANOVA) with 999 permutations [42]. The Microbiome Multivariable Associations with Linear Models (MaAsLin) [43] was used to identify gut microbial genera potentially associated with smoking. To account for the false discovery rate (FDR) due to multiple testing, we applied the Benjamini–Hochberg method with a Q value (FDR-adjusted *p*-value) of < 0.25, which is the default threshold in the MaAsLin method, considered statistically significant.

Cross-sectional analyses

The MaAsLin was applied to assess the association between individually identified genus and obesity indices derived from anthropometric and DXA measurements. Additionally, multivariable logistic regression was employed to investigate the association among the collective effects of individual genera (SMI), obesity and obesity-related disorders detected at baseline including diabetes, dyslipidemia, metabolic syndrome, and MASLD. To explore the role of adiposity in the association between SMI and baseline obesity-related disorders, we further adjusted BMI and AOI based on the full adjusted model.

Longitudinal analyses

Cox regression for proportional hazards model was conducted to investigate the association between SMI and time to obesity-related diseases (including diabetes, MACE, and obesity-related cancers) and all cancers. The proportional hazard assumption of the Cox regression was verified by using the Schoenfeld residual test, with no observed model violation.

Meta-analyses

To obtain combined effect estimates from the WELL-China cohort and the Lanxi cohort, we performed a meta-analysis using the Mantel–Haenszel method for both cross-sectional and longitudinal analyses. Heterogeneity within the meta-analysis was assessed using Cochran's Q test, with no observed heterogeneity, so the fixed-effects model [44] was applied across two cohorts.

Mediation analyses

Prior to the mediation analyses, we assessed the relationships between smoking status, obesity, and obesity-related disorders using multivariable logistic regression model. We explored the potential mediating role of SMI in the links between smoking status, obesity, and obesity-related disorders, which was achieved through a mediation analysis employing the '*mediation*' package in R [45] (version 4.5.0). In the mediation analyses, smoking was considered as exposure, while SMI, obesity, and obesity-related disorders (diabetes, dyslipidemia, metabolic syndrome, and MASLD) were treated as mediator and outcomes, respectively.

Sibling comparison analyses

To control for unmeasured confounders shared within families, we repeated our analyses of SMI with obesity and related disorders using sibling comparison analyses with the between-within (BW) model [46].

Sensitivity analyses

To justify the validity and reliability of SMI, additional analyses were conducted to examine the association of SMI with smoking and nicotine dependence assessed by Fagerström test for nicotine dependence (FTND) in both cohorts. To investigate the robustness of our findings, we repeated our analyses restricted to individual genera instead of SMI.

Statistical analyses were performed using R version 4.1.2. A two-sided *P*-value < 0.05 was considered statistically significant unless otherwise specified.

Results

Population characteristics

Baseline characteristics, categorized by smoking status, of the WELL-China cohort (the discovery cohort) and the Lanxi cohort (the replication cohort) are presented in Additional file 1: Table S1. Across both cohorts, there were no statistically significant differences in age, marital status, or levels of physical activity observed between current smokers and never smokers. However, current smokers in both cohorts had higher alcohol consumption and lower levels of education compared to never smokers. This table also includes detailed baseline characteristics of the Lanxi sibling subcohort.

Smoking and gut microbiota

No statistically significant differences in gut microbial richness and evenness (α-diversity) were observed among current smokers, former smokers, and never smokers (Additional file 1: Fig. S2). However, a significant shift in gut microbial composition (β-diversity) was noted when comparing current smokers to never smokers in both the WELL-China cohort (the discovery cohort) ($P<0.001$) and the Lanxi cohort (the replication cohort) ($P=0.004$) (Fig. 1 A_1 & B_1). This shift persisted across both cohorts when assessing β-diversity using weighted Unifrac distance, which accounts for phylogenetic relationships and shared lineages among microbial taxa (Fig. 1 A_2 & B_2).

In the WELL-China cohort (the discovery cohort), 50 microbial genera displayed an association with current smoking compared to never smoking (Additional file 1: Table S2). Similarly, in the Lanxi cohort (the replication cohort), 19 microbial genera showed an association with current smoking. Nine smoking-related gut microbial genera in the WELL-China cohort were successfully replicated in the Lanxi cohort (FDR < 0.25).

Smoking-related microbial genera and obesity

Among the nine microbial genera, four (*Atopobium*, *Actinomyces*, *Solobacterium*, and *Tyzzerella_4*) were enriched in current smokers (Fig. 1 C_1 & D_1). These genera demonstrated a positive association with various obesity metrics, including BMI, body fat percentage, and central fat distribution parameters such as waist circumference, WHR, android fat percentage, and AOI, while exhibited a negative association with gynoid fat percentage (Fig. 1 C_2 & D_2). In contrast, the remaining five microbial genera (*Ruminococcaceae_UCG-013*, *Gemella*, *Incertae_Sedis*, *Lachnospiraceae_NK4A136_group*, and *Haemophilus*), which were depleted in current smokers, showed a contrary association with overall adiposity and central fat distribution. These results suggest that smoking-associated genera are linked to an increased—rather than decreased—risk of obesity, along with increased central fat distribution.

Smoking-related microbiota index (SMI) and obesity

Table 1 presents the association between the SMI and obesity. No statistically significant heterogeneity was observed between the WELL-China cohort and the Lanxi cohort. Meta-analyses of the two cohorts revealed a robust positive association of SMI with various obesity indicators including BMI, body fat percentage, and central fat distribution parameters (waist circumference, WHR, android fat percentage, and AOI), while a negative association with gynoid fat percentage.

In the sibling comparison analyses, findings were consistent regarding the association between SMI and obesity, with statistically significant correlations observed for BMI and waist circumference (Table 1).

Smoking-related microbiota index (SMI) and prevalent obesity-related disorders

Table 1 presents the association between the SMI and prevalent obesity-related disorders at baseline. In analyses of the total cohorts, no statistically significant heterogeneity was detected between the WELL-China cohort and the Lanxi cohort. Meta-analyses of the two cohorts revealed a robust positive correlation of SMI with obesity-related disorders. Compared to participants in the lowest tertile of SMI, those in the highest tertile exhibited elevated odds ratios (ORs) of 2.30 (95% CI 1.82–2.90) for diabetes, 1.59 (95% CI 1.35–1.87) for dyslipidemia, 1.65 (95% CI 1.38–1.97) for metabolic syndrome, and 1.92 (95% CI 1.54–2.39) for MASLD. Particularly, the effects of SMI on obesity-related disorders were attenuated with further adjustments for BMI and AOI (Additional file 1: Fig. S3).

In sibling comparison analyses, consistent findings emerged regarding the association between SMI and obesity-related disorders, with statistically significant correlations observed for MASLD (Table 1).

Smoking-related microbiota index (SMI) and incident obesity-related diseases

Table 2 presents the association between baseline SMI and subsequently risk of developing obesity-related diseases. No statistically significant heterogeneity was observed between the WELL-China cohort and the Lanxi cohort. The meta-analysis of the two cohorts demonstrated that a higher baseline SMI was linked to an increased risk of incident obesity-related diseases and all cancers. In comparison to individuals in the lowest tertile of SMI, those in the middle and highest tertile had adjusted HRs (95% CIs) of 1.47 (1.05–2.06) and 1.97 (1.41–2.75) for diabetes, 1.01 (0.75–1.36) and 1.31 (1.01–1.71) for MACE, 1.50 (0.94–2.40) and 1.70 (1.05–2.75) for obesity-related cancers, and 1.36 (1.03–1.78) and 1.31 (1.00–1.73) for all cancers.

Smoking-related microbiota index (SMI) mediated the effect of smoking on obesity and obesity-related disorders

Figure 2 presents the association of smoking with obesity, and obesity-related disorders, both before and after adjustment for SMI. After adjusting SMI, the associations between smoking and overall adiposity (BMI, body fat percentage) became more pronounced. In contrast, the

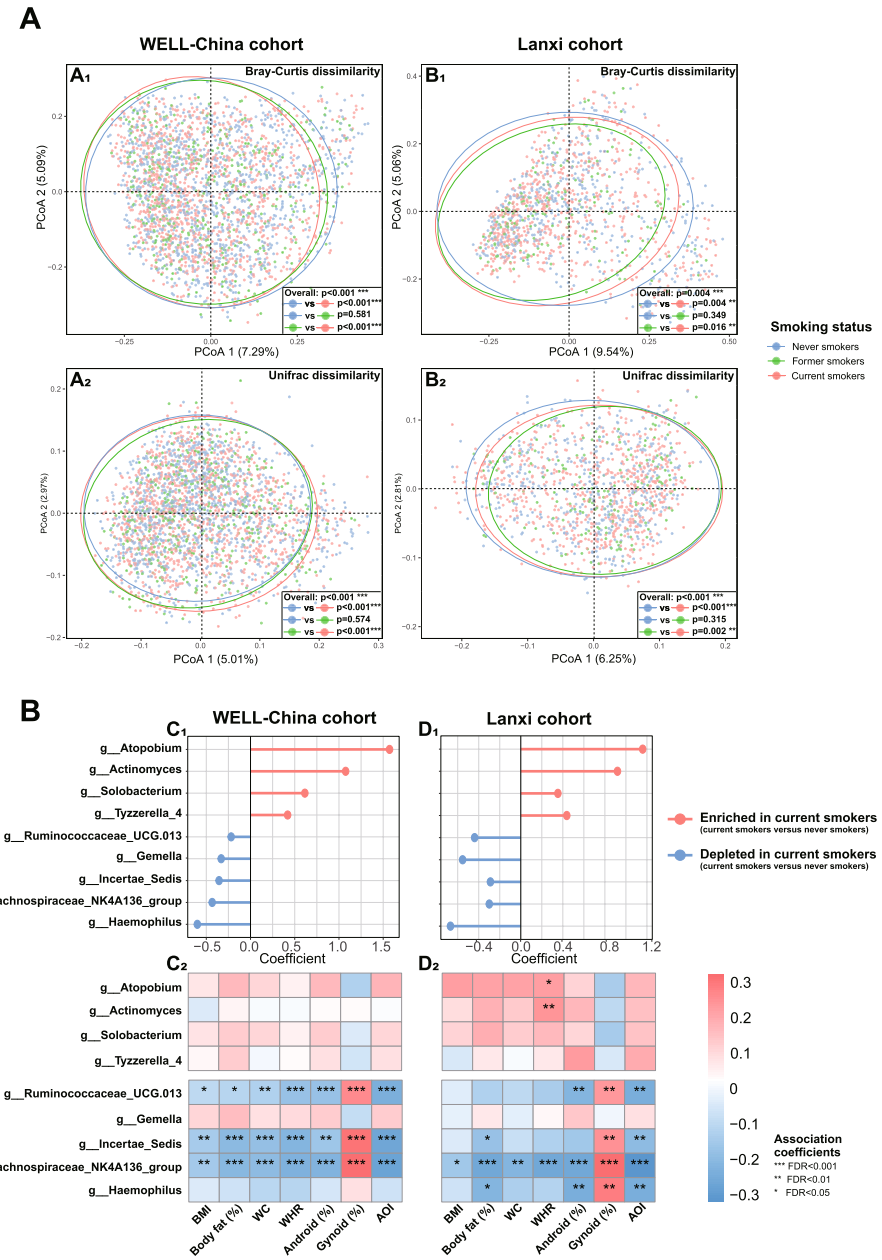

Fig. 1 Smoking status and gut microbiota composition in the male participants of the WELL-China cohort (the discovery cohort) and the Lanxi cohort (the replication cohort). **A** β-diversity analyses. A_1& B_1: Principal coordinates analysis (PCoA) based on Bray-Curtis dissimilarity was performed in the WELL-China and the Lanxi cohorts: A PERMANOVA test (999 permutations) was used to evaluate the variation of β-diversity in gut microbiota composition structure comparing different smoking status, adjusted for age, marital status, education, annual income, alcohol consumption, physical activity, diet energy intakes, nutritional supplements, district of residence, antibiotics use (except for Lanxi cohort due to the lack of corresponding information), sequencing batch and sequencing depth. A_2& B_2: Principal coordinates analysis (PCoA) based on weighted Unifrac dissimilarity was performed in the WELL-China and the Lanxi cohorts, adjusted for the same covariates as mentioned above. **B** Distinct bacterial genera analyses. C_1& D_1: Multivariate Analysis by Linear Models (MaAsLin) were used to identify microbial genera between never smokers and current smokers, adjusted for the same covariates as mentioned above. The Q values (false discovery rate adjusted *p* value) were calculated using the Benjamini-Hochberg method (Q value < 0.25). C_2& D_2: Smoking-related microbial genera and obesity in the WELL-China cohort and Lanxi cohort. Multivariate Analysis by Linear Models (MaAsLin) were adjusted for the same covariates as mentioned above. WC, Waist-circumference; WHR, Waist-hip ratio; AOI, Android-gynoid ratio

Table 1 Smoking-related microbiota index (SMI), obesity, and prevalent obesity-related disorders in the male participants of the WELL-China cohort, Lanxi cohort, and Lanxi sibling subcohort

	Total cohort analyses[a]				Sibling comparison analyses[b]
	WELL-China cohort	Lanxi cohort	Meta-analyses		Lanxi sibling subcohort
	OR (95% CI)	OR (95% CI)	OR (95% CI)	$P_{heterogeneity}$	OR (95% CI)
Overall fatness					
BMI ≥ 24 kg/m^2					
SMI tertile 1	1.00 (reference)	1.00 (reference)	1.00 (reference)		1.00 (reference)
SMI tertile 2	0.92 (0.76,1.11)	1.28 (0.97,1.68)	1.02 (0.88,1.20)	0.052	**1.82 (1.81,1.83)** ***
SMI tertile 3	**1.41 (1.16,1.72)** ***	**1.41 (1.07,1.86)** *	**1.41 (1.20,1.66)** ***	1.000	**2.68 (2.66,2.69)** ***
Body fat percentage ≥ 25%					
SMI tertile 1	1.00 (reference)	1.00 (reference)	1.00 (reference)		1.00 (reference)
SMI tertile 2	0.98 (0.81,1.19)	1.43 (1.07,1.91)	1.10 (0.94,1.29)	0.033	1.26 (0.45,3.53)
SMI tertile 3	**1.32 (1.09,1.61)** **	**2.12 (1.58,2.85)** ***	**1.52 (1.30,1.79)** ***	0.009	2.19 (0.65,7.34)
Central fat distribution					
Waist-circumference ≥ 90 cm					
SMI tertile 1	1.00 (reference)	1.00 (reference)	1.00 (reference)		1.00 (reference)
SMI tertile 2	0.99 (0.82,1.20)	**1.36 (1.02,1.81)** *	1.09 (0.93,1.28)	0.071	1.72 (0.60,4.97)
SMI tertile 3	**1.64 (1.35,1.99)** ***	**1.61 (1.21,2.14)** ***	**1.63 (1.39,1.91)** ***	0.916	**4.20 (1.22,14.41)** *
Waist-hip ratio ≥ 0.9					
SMI tertile 1	1.00 (reference)	1.00 (reference)	1.00 (reference)		1.00 (reference)
SMI tertile 2	1.18 (0.95,1.47)	1.21 (0.87,1.70)	1.19 (0.99,1.43)	0.902	0.58 (0.19,1.77)
SMI tertile 3	**1.84 (1.45,2.35)** ***	**1.55 (1.10,2.21)** *	**1.74 (1.43,2.12)** ***	0.428	1.67 (0.43,6.51)
Android fat percentage ≥ median					
SMI tertile 1	1.00 (reference)	1.00 (reference)	1.00 (reference)		1.00 (reference)
SMI tertile 2	1.19 (0.98,1.44)	1.20 (0.91,1.58)	1.19 (1.02,1.40)	0.961	1.77 (0.75,4.20)
SMI tertile 3	**1.66 (1.36,2.02)** ***	**1.63 (1.23,2.15)** ***	**1.65 (1.40,1.94)** ***	0.917	1.58 (0.56,4.44)
Gynoid fat percentage ≥ median					
SMI tertile 1	1.00 (reference)	1.00 (reference)	1.00 (reference)		1.00 (reference)
SMI tertile 2	0.96 (0.79,1.17)	**0.70 (0.53,0.93)** *	0.87 (0.74,1.02)	0.071	0.70 (0.29,1.71)
SMI tertile 3	**0.66 (0.54,0.81)** ***	**0.47 (0.35,0.62)** ***	**0.59 (0.50,0.70)** ***	0.058	0.35 (0.12,1.04)
Android-gynoid fat ratio ≥ median					
SMI tertile 1	1.00 (reference)	1.00 (reference)	1.00 (reference)		1.00 (reference)
SMI tertile 2	1.12 (0.92,1.35)	**1.42 (1.07,1.87)** *	**1.21 (1.03,1.42)** *	0.170	1.64 (0.66,4.03)
SMI tertile 3	**1.73 (1.42,2.12)** ***	**1.98 (1.49,2.62)** ***	**1.81 (1.54,2.13)** ***	0.445	1.94 (0.66,5.65)
Prevalent obesity-related disorders[c]					
Diabetes					
SMI tertile 1	1.00 (reference)	1.00 (reference)	1.00 (reference)		1.00 (reference)
SMI tertile 2	**1.50 (1.12,2.01)** **	1.48 (0.97,2.27)	**1.49 (1.17,1.90)** ***	0.959	0.67 (0.18,2.56)
SMI tertile 3	**2.49 (1.88,3.32)** ***	**1.93 (1.28,2.94)** **	**2.30 (1.82,2.90)** ***	0.322	2.12 (0.44,10.16)
Dyslipidemia					
SMI tertile 1	1.00 (reference)	1.00 (reference)	1.00 (reference)		1.00 (reference)
SMI tertile 2	1.19 (0.98,1.44)	1.29 (0.97,1.72)	**1.22 (1.04,1.43)** *	0.647	1.12 (0.45,2.81)
SMI tertile 3	**1.51 (1.24,1.83)** ***	**1.78 (1.34,2.38)** ***	**1.59 (1.35,1.87)** ***	0.353	1.65 (0.56,4.84)
Metabolic syndrome					
SMI tertile 1	1.00 (reference)	1.00 (reference)	1.00 (reference)		1.00 (reference)
SMI tertile 2	1.01 (0.81,1.27)	1.18 (0.86,1.62)	1.06 (0.89,1.28)	0.432	0.87 (0.28,2.74)
SMI tertile 3	**1.75 (1.42,2.17)** ***	**1.45 (1.06,1.99)** *	**1.65 (1.38,1.97)** ***	0.332	1.77 (0.47,6.70)
MASLD[d]					
SMI tertile 1	1.00 (reference)	1.00 (reference)	1.00 (reference)		1.00 (reference)
SMI tertile 2	0.99 (0.73,1.36)	**1.46 (1.09,1.97)** *	1.21 (0.98,1.50)	0.076	**2.55 (2.53,2.57)** ***
SMI tertile 3	**1.73 (1.25,2.40)** ***	**2.09 (1.56,2.82)** ***	**1.92 (1.54,2.39)** ***	0.400	**4.84 (4.81,4.88)** ***

Table 1 (continued)

[a] Multivariable logistic regression models were used to estimate the association among SMI, obesity and obesity-related disorders in total cohort analyses, adjusted for the same covariates as mentioned in Fig. 1. Estimates were meta-analyzed using fixed effect model. Bold values denote statistical significance (***$p < 0.001$, **$p < 0.01$, *$p < 0.05$)

[b] Between-within (BW) models were used to estimate the association between SMI and obesity and obesity-related disorders in sibling comparison analyses, adjusted for the same covariates as mentioned in Fig. 1

[c] Obesity-related disorders were defined using baseline data

[d] The diagnosis of MASLD in the WELL-China cohort was performed only in Gongshu district, including 995 participants

Table 2 Smoking-related microbiota index (SMI) and incident of obesity-related diseases in the male participants of the WELL-China cohort and the Lanxi cohort[a]

Incident	WELL-China cohort	Lanxi cohort	Meta-analyses	
	HR (95% CI)	HR (95% CI)	HR (95% CI)[b]	$P_{heterogeneity}$
Diabetes[c e]				
SMI tertile 1	1.00 (reference)	1.00 (reference)	1.00 (reference)	
SMI tertile 2	1.44 (0.98,2.11)	1.57 (0.78,3.18)	**1.47 (1.05,2.06)***	0.832
SMI tertile 3	**1.96 (1.34,2.88)*****	**2.00 (1.02,3.93)***	**1.97 (1.41,2.75)*****	0.959
Major adverse cardiovascular events[e]				
SMI tertile 1	1.00 (reference)	1.00 (reference)	1.00 (reference)	
SMI tertile 2	0.98 (0.69,1.38)	1.09 (0.62,1.90)	1.01 (0.75,1.36)	0.752
SMI tertile 3	**1.40 (1.01,1.96)***	1.17 (0.76,1.81)	**1.31 (1.01,1.71)***	0.519
Obesity-related cancers[d e]				
SMI tertile 1	1.00 (reference)	1.00 (reference)	1.00 (reference)	
SMI tertile 2	1.72 (0.99,3.02)	1.08 (0.45,2.58)	1.50 (0.94,2.40)	0.379
SMI tertile 3	**2.04 (1.15,3.62)** *	1.11 (0.46,2.67)	**1.70 (1.05,2.75)** *	0.256
All cancers[d e]				
SMI tertile 1	1.00 (reference)	1.00 (reference)	1.00 (reference)	
SMI tertile 2	**1.49 (1.08,2.04) ***	1.05 (0.62,1.76)	**1.36 (1.03,1.78) ***	0.262
SMI tertile 3	1.32 (0.95,1.85)	1.30 (0.79,2.15)	**1.31 (1.00,1.73) ***	0.960

[a] Incident obesity-related diseases were defined using data from the disease registration system, including inpatient system and outpatient system

[b] Cox regression models were used to estimate the association between SMI and diabetes, major adverse cardiovascular events (MACE), obesity-related cancers, and all cancers, adjusted for the same covariates as mentioned in Fig. 1. Obesity-related diseases (diabetes, MACE and obesity-related cancers) and all cancers events were defined as the occurrence of an obesity-related diseases or cancers, or death from obesity-related diseases or cancers. Patients were followed until obesity-related diseases or cancers events, death from causes other than obesity-related diseases or cancers, or end of the study period (June 24, 2024), whichever came first. Estimates were meta-analyzed using fixed effect model. Bold values denote statistical significance (***$p < 0.001$, **$p < 0.01$, *$p < 0.05$)

[c] Diabetes: Individuals diagnosed with diabetes at baseline based on self-reported medication history or who had a fasting blood glucose level greater than 7.0 mmol/L at baseline, or diabetes recorded in the disease registration system were excluded from the analyses

[d] Obesity-related cancers: Individuals with obesity-related cancers at baseline recorded from disease registration system were excluded. All cancers: Individuals with cancers at baseline recorded from disease registration system were excluded

[e] Events in the WELL-China cohort and the Lanxi cohort: 179 (events)/2291 for diabetes, 212 (events)/2709 for MACE, 83 (events)/2695 for obesity-related cancers and 235 (events)/2671 for all cancers in WELL-China cohort, while 60 (events)/1117 for diabetes, 107 (events)/1291 for MACE, 32 (events)/1283 for obesity-related cancers and 94 (events)/1279 for all cancers in Lanxi cohort

association between smoking and central fat distribution, along with obesity-related disorders, were attenuated after accounting for SMI. Mediation analyses indicated that most of shifts in associations before and after SMI adjustment were statistically significant.

Sensitivity analyses

Compared to never smokers and former smokers, current smokers exhibited a higher SMI (Additional file 1: Fig. S4) in both the WELL-China cohort (the discovery cohort) ($P < 0.001$) and the Lanxi cohort (the replication cohort) ($P < 0.05$), further emphasizing the validity and reliability of our SMI. No statistically significant differences in SMI were observed between former smokers and never smokers (Additional file 1: Fig. S4) in both the WELL-China cohort (the discovery cohort) and the Lanxi cohort (the replication cohort). Moreover, SMI was positively associated with the FTND in both the WELL-China cohort (the discovery cohort)

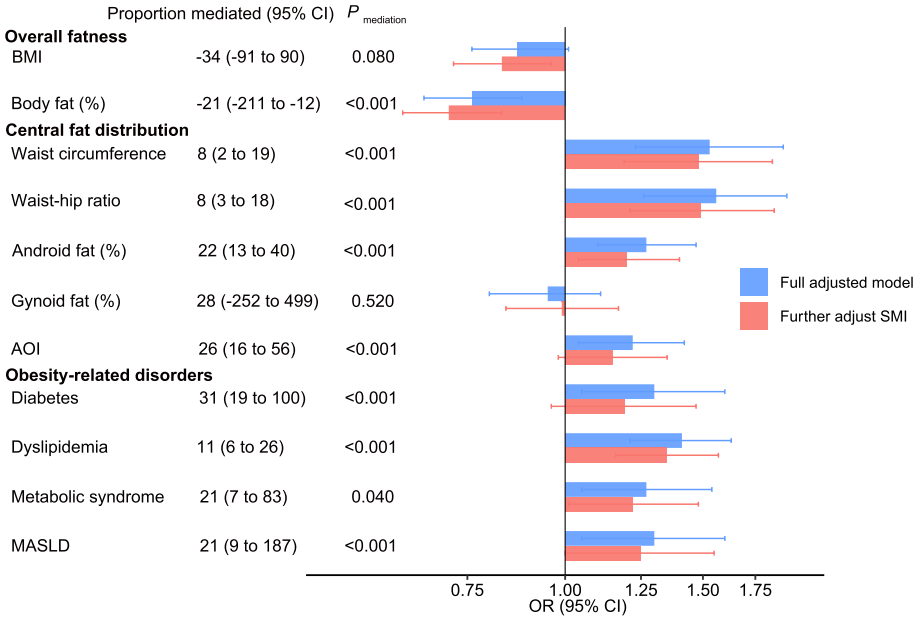

Fig. 2 Smoking-related microbiota index (SMI) mediated the effect of smoking on obesity and obesity-related disorders in the male participants. To increase power, this analyses combined data from the WELL-China cohort and the Lanxi cohort. Multivariate logistic regression model was used to estimate the associations of smoking with overall fatness, central fat distribution, and obesity-related disorders. The full adjusted model for overall fatness adjusted for the covariates mentioned in Fig. 1. The full adjusted model for central fat distribution and obesity-related disorders adjusted for BMI and the covariates mentioned in Fig. 1. Mediation analyses were conducted to estimate the proportion of the effect of smoking on obesity and obesity-related disorders that is mediated by SMI

($P_{trend} < 0.001$) and the Lanxi cohort (the replication cohort) ($P_{trend} < 0.001$), suggesting that SMI may serve as a quantitative indicator for the effect of smoking on the gut microbiota (Additional file 1: Table S3) [47]. Restricting our analyses to single genera (Additional file 1: Table S4-S5) provided results similar to those of the main analyses, further validating SMI's reliability.

Discussion

In this population-based study comprising two large-scale cohorts, we found a notable difference in microbiota composition between current smokers and never smokers, as evidenced by variation in β-diversity. Further analyses identified nine distinct genera that differed between these two groups, observed in both the WELL-China cohort (the discovery cohort) and Lanxi cohort (the replication cohort). Smoking-related microbiota index (SMI), based on these nine distinct genera, exhibited a positive association with obesity, central fat distribution, and various obesity-related disorders in both cross-sectional and longitudinal analyses. Sibling comparison analyses consistently supported these findings, confirming that smoking-related microbiota alterations are linked to obesity and its related disorders. Mediation analyses demonstrated that SMI masked the association between smoking and overall adiposity, yet mediated the association between smoking and central fat distribution, as well as obesity-related disorders.

Our findings suggest that the relationship between smoking and obesity is quite complex. Various mechanisms might underpin this underlying observation. On the one hand, previous studies have shown that nicotine in cigarette not only accelerates the basal metabolic rate [48], but also decreases appetite by influencing appetite regulation mechanisms in the brain [49, 50]. Furthermore, smoking can modify an individual's perception of food [51] and decelerate the rate of gastrointestinal emptying [52], thereby reducing food intake.

On the other hand, we found that smoking-related gut microbiota alterations are positively, rather than negatively, associated with body fat mass and body fat percentage. This finding aligns with a recent animal study showing that alteration in the smoking-related gut microbiome could underlie weight gain after smoking cessation, with the main mechanism involved upregulation of Dimethylglycine (DMG) (a glycine derivative leading to obesity-related pro-inflammatory processes [53]) expression and downregulation of N-acetylglycine (ACG) (a glycine derivative inhibiting weight gain [54]) expression [21]. Moreover, the inverse association between smoking and BMI become more pronounced upon

further adjustment for changes in the smoking-related gut microbiome.

In line with previous studies [10], our findings reveal a significant disparity in β-diversity between current smokers and never smokers. Additionally, we detected a total of nine smoking-related gut bacteria genera in both cohorts. Previous studies typically had sample sizes less than 1000 individuals, with most constrained to about 100 participants [10, 55]. To our knowledge, our study represents the most extensive investigation of smoking-related gut microbiota to date. While cohort-specific differences may influence the microbial profiles observed, the smoking-related genera that we identified were validated in both of our cohorts, thereby enhancing the credibility of the SMI as a reliable indicator of smoking status.

Our study revealed correlation in smoking-related genera and central fat distribution indices including WHR and android-gynoid fat ratio, in addition to overall adiposity. These correlations were maintained when genera were analyzed both individually and in aggregate. These findings validate prior observations of a connection between smoking and central fat accumulation for a given BMI [29, 56], and reveal that alteration in gut microbiota may contribute to central fat accumulation among smokers.

Both BMI and smoking are essential indicators of Life's Essential 8 proposed by the American Heart Association [57], and both are closely linked to vascular inflammation [58, 59]. We observed a positive correlation between smoking-related microbiota index and obesity-related diseases (e.g., diabetes, major adverse cardiovascular events, and obesity-related cancers) in both cross-sectional and longitudinal analyses. Notably, the associations appeared to be partially mediated by body mass index (BMI) and central fat distribution. The strength of the correlation between SMI and obesity-related disorders decreased upon adjusting for BMI and the android-gynoid fat ratio, as shown in Additional file 1: Fig. S3. Moreover, existing studies have supported the role of the gut microbiota as a key mediator of vascular inflammation [60, 61]. Inflammatory pathways involving *Actinomyces* [62, 63], and *Lachnospiraceae_NK4A136_group* [64, 65], as well as metabolic pathways involving *Lachnospiraceae_NK4A136_group* [64, 65] and *Ruminococcaceae _UCG.013* [66] may play roles in these associations. Further studies are necessary to determine whether interventions specifically targeting these smoking-related genera or the entire microbiota ecosystem could attenuate the adverse effects of smoking, emphasizing the need for synergistic approaches to reduce cardiovascular and metabolic disease burdens.

The World Health Organization (WHO) acknowledges smoking as a preventable risk factor for diabetes and cardiovascular disease [67, 68]. Despite this, tobacco control efforts remain largely ineffective in many countries [69, 70], including China. The ineffectiveness may be attributed, in part, to the perception that smoking aids in weight loss, potentially fostering smoking initiation among youth [71]. Multiple studies indicate that heavy smokers, smokers who perceived themselves as overweight, and those who express concerns about their weight are more likely to use smoking as a way to manage their weight [72]. However, our study reveals that smoking-related alterations in gut microbiota are linked to an increased—rather than decreased—risk of obesity. These findings suggest a complex relationship between smoking and obesity. Furthermore, our study demonstrated that alterations in smoking-related microbiome are linked with obesity-related disorders. This suggests that while smoking may reduce body weight, it concurrently increases the risk of obesity-related diseases, thereby providing new evidence to support tobacco control interventions.

We observed no statistically significant differences in α-diversity, β-diversity, and SMI between former smokers and never smokers. These findings contrast with a mouse experiment demonstrating that gut microbiota mediate weight gain in mice following discontinued smoke exposure [21]. These contradictory results may be due to difference in time since smoking cessation. The mouse study measured gut microbiota within 2 months after discontinuing smoking exposure, while our human study involved a cohort with a longer cessation duration (mean of 12.7 years in the WELL-China cohort and 9.6 years in the Lanxi cohort). This discrepancy suggests that while gut microbiota alterations may lead to weight gain shortly after smoking cessation among former smokers [73], after a prolonged period of smoking cessation, the gut microbiota of former smokers gradually normalizes and becomes more similar to that of non-smokers. Further research is warranted to investigate the long-term dynamics of gut microbiota following smoking cessation, including how the timing of cessation affects microbial recovery and its potential role in obesity-related diseases.

Our study benefits from employing a sibling comparison design. Shared genetic and environmental factors significantly impact smoking, gut microbiota, obesity, and obesity-related diseases [24, 25]. Traditional cohort studies face challenges in accurately assessing and accounting for latent shared genetic and early-life environmental influences. The sibling comparison design presents a unique methodology to address these confounding factors, enhancing the credibility of our findings [74].

Several limitations warrant consideration when interpreting the findings of this study. First, despite adjusting

for various potential confounders, the possibility of residual confounding remains. Second, while our study included two cohorts comprising over 4000 participants with available gut microbiota data, our statistical power was insufficient for analyzing obesity-related outcomes with low occurrence rates, such as specific obesity-related cancers. Third, due to the observational nature of our study, establishing a causal relationship between smoking and gut microbiota is challenging. However, randomized controlled trials of smoking are not feasible in human studies. Fourth, the smoking status was determined based on self-reported classifications, further research is needed to incorporate objective measurements of blood nicotine levels, which would provide a more accurate assessment of smoking exposure. Last, since our study included only Chinese men, the findings should be interpreted with caution in other populations and women.

Conclusions

In conclusion, our study revealed that smoking-related alteration in gut microbiota are associated with an increased risk of obesity, contrary to the anticipated decreased. Furthermore, the smoking-related gut microbiota alterations are also linked to an increase in central fat distribution and obesity-related diseases. These discoveries introduce a novel mechanism through which smokers, traditionally perceived as relatively lean, face an increased risk of obesity-related diseases. Disseminating these findings could support public health interventions targeting smoking prevention or cessation, especially among young adults who might initiate smoking under the misconception.

Abbreviations

ACG	N-acetylglycine
AOI	Android-gynoid fat ratio
ASV	Amplicon Sequence Variant
BMI	Body mass index
CVD	Cardiovascular disease
DMG	Dimethylglycine
DXA	Dual-energy X-ray absorptiometry
FDR	False discovery rate
FFQ	Food Frequency Questionnaire
FTND	Fagerström Test for Nicotine Dependence
IPAQ	International Physical Activity Questionnaire
MaAsLin	Microbiome multivariable associations with linear models
MACE	Major adverse cardiovascular events
MASLD	Metabolic dysfunction-associated steatotic liver disease
PERMANOVA	Permutational multivariate analysis of variance
SCFAs	Short-chain fatty acids
SMI	Smoking-related microbiota index
USEARCH	Ultra-fast sequence analysis
WELL-China	Wellness Living Laboratory China
WHR	Waist-to-hip ratio

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12916-025-03969-4.

Additional file 1: Method S1: Fecal sample collection and 16S rRNA gene sequencing. Table S1: Baseline characteristics of the male participants according to smoking status in the WELL-China cohort, Lanxi cohort and Lanxi sibling subcohort. Table S2: Smoking-related microbial genera identified in male participants of the WELL-China cohort and the Lanxi cohort. Table S3: Smoking-related microbiota index (SMI) and Fagerström test for nicotine dependence (FTND) score in the male participants of the WELL-China cohort and the Lanxi cohort. Table S4: Smoking-related microbial genera and prevalent obesity-related disorders in the male participants of the WELL-China cohort and the Lanxi cohort. Table S5: Smoking-related microbial genera and incident obesity-related diseases in the male participants of the WELL-China cohort and the Lanxi cohort. Fig. S1: Flow diagram in the WELL-China cohort and the Lanxi cohort. Fig. S2: Smoking status and α-diversity in the male participants of the WELL-China cohort and the Lanxi cohort. Fig. S3: Smoking-Related Microbiota Index (SMI) and prevalent obesity-related disorders in the male participants of the WELL-China cohort and the Lanxi cohort. Fig. S4: Smoking-Related Microbiota Index (SMI) and smoking status in the male participants of the WELL-China cohort and the Lanxi cohort.

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Authors' contributions

Y.D., C.X., W.H. and S.Z. designed the study and drafted the manuscript. Y.D. and C.X. performed the data analyses and prepared the figures. W.W., X.W. and N.X. provided constructive analytical suggestions. All the authors provided critical revision of the article for important intellectual content. M.Y., W.H. and S.Z. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity and accuracy of the data analyses.

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Data availability

The raw 16S rRNA sequencing data used in this study have been deposited at the Genome Sequence Archive (GSA) (http://ngdc.cncb.ac.cn/gsa/) at accession number CRA015037 (https://ngdc.cncb.ac.cn/gsa/s/WpyWelQY) for the WELL-China cohort and CRA015036 (https://ngdc.cncb.ac.cn/gsa/s/g7Z54740) for the Lanxi cohort. The R programming codes used for this study are publicly available at: https://github.com/Kruskal–Wallis/Smoking-microbiome-and-obesity.

Declarations

Ethics approval and consent to participate

The protocol of WELL-China cohort study was approved by the Institutional Review Boards of Zhejiang University (No. ZGL201507-3) and Stanford University (IRB-35020). The protocol of Lanxi cohort study received approval from the Ethics Committee of the School of Public Health, Zhejiang University (No. ZGL201905-1). Written informed consents were obtained from all participants in both cohorts.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Nutrition and Food Hygiene, Children'S Hospital, Zhejiang University School of Medicine, National Clinical Research Center for Child Health, Hangzhou, Zhejiang, China. [2]School of Public Health, School of Medicine, Chronic Disease Research Institute, the Children'S Hospital, National Clinical Research Center for Child Health, Zhejiang University, 866 Yu-Hang-Tang Road, Hangzhou, Zhejiang 310058, China. [3]Department of NCDs Control and Prevention, Zhejiang Provincial Center for Disease Control and Prevention, 3399 Bin-Sheng Road, Hangzhou, Zhejiang 310051, China. [4]Lanxi Red Cross Hospital, Lanxi, Zhejiang 321102, China. [5]Department of Medicine, Stanford Prevention Research Center, Stanford School of Medicine, Stanford University, Palo Alto, CA 94304, USA. [6]Department of Biomedical Data Sciences, Stanford School of Medicine, Stanford University, Palo Alto, CA 94305, USA. [7]Department of Epidemiology and Population Health, Stanford School of Medicine, Stanford University, Palo Alto, CA 94305, USA. [8]Stanford Cancer Institute, Stanford School of Medicine, Stanford University, Palo Alto, CA 94305, USA.

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