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Legume Consumption and Gut Microbiome in Elderly Chinese Men and Women

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ABSTRACT

Background: Legumes, important components of a healthy diet, may exert their health benefits through the influence of the gut microbiome. However, this hypothesis has not been well investigated.

Objective: This study aimed to examine the associations between long-term legume consumption and the gut microbiome among elderly Chinese.

Methods: The gut microbiome was profiled by 16S ribosomal RNA sequencing in 2302 Chinese adults enrolled in 2 large cohort studies, the Shanghai Women's Health Study and Shanghai Men's Health Study. Legume consumption, including peanuts, soy foods, and other beans, was assessed by food-frequency questionnaires prior to the stool collection. The associations of legume consumption with microbiome diversity and taxa abundance were evaluated by linear or negative binomial hurdle models, adjusting for sociodemographics, lifestyle factors, and BMI. False discovery rate (FDR)–corrected *P* values (P_{FDR}) < 0.1 were considered significant.

Results: Respectively, 52% and 48% of study participants were male and female. The mean age at stool collection was 68.03 y for females and 70.28 y for males. Total legume consumption was not associated with gut microbiome α-diversity; however, male peanut consumers had a higher Chao1 index ($\beta = 22.52$, $P = 0.01$), whereas peanut consumption was associated with decreased Shannon ($\beta = -0.03$, $P = 0.02$) and Simpson ($\beta = -0.002$, $P = 0.04$) indexes among females. In female and male combined analyses, total legume consumption was associated with increased *Enterobacteriales* ($\beta = 0.30$, $P_{FDR} = 0.06$). Within this order, an unclassified genus in the family *Enterobacteriaceae* was positively associated with total legume ($\beta = 0.46$, $P_{FDR} = 0.03$) and peanut ($\beta = 0.59$, $P_{FDR} = 0.01$) consumption. Stratified analyses showed significant associations were primarily confined to females and participants without metabolic conditions.

Conclusions: Legume consumption was associated with gut microbiome diversity and abundance of some bacteria in elderly Chinese. Associations were significant only among 1 sex group. Further research, including large-scale prospective studies and feeding trials, is needed to fully understand the role of the gut microbiome in legume–health associations. *J Nutr* 2021;151:2399–2408.

Keywords: gut microbiome, legume consumption, peanut, soy food, beans, elderly Chinese

Introduction

Legumes are an important part of the human diet. Commonly consumed legumes include green beans and peas, peanuts, soybeans, broad beans, chickpeas, and lentils (1). They are rich in nutrients such as protein, dietary fiber, minerals, and phytochemicals that are necessary to maintain normal gut function and support a healthy gut microbiota (2). Legume consumption is recommended for Chinese in the 2016 Dietary Guidelines (3) and for Americans in both the 2015–2020 Dietary Guidelines (4) and Dietary Approaches to Stop Hypertension Eating Plan of the National Heart, Lung, and Blood Institute (5).

Legume consumption has been associated with several beneficial health effects, such as reducing metabolic risk factors and excess adiposity, decreasing absorption of cholesterol from the gut, and regulating energy intake (1). For example, soybean protein and soluble fiber have been shown to reduce serum total and LDL-cholesterol concentrations and improve glycemic control, which have been linked to a lower risk of coronary artery disease (6). In addition, legumes are a major source of dietary folate and plant polyphenols, important for the metabolism of homocysteine and plasma homocysteine concentrations (7), which, respectively, protect against environmental stresses and pathogenic microbial infections (1).

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The human gastrointestinal tract is colonized by $>10^{14}$microbes, collectively known as the gut microbiota (8). The gut microbiota is the most complex microbial community across the human body, composed of microorganisms that interact with each other and host cells (8). It has been increasingly recognized that the gut microbiota plays an important role in human health, by involvement in the host's metabolic functions and energy balance (9) and interactions with the immune system to promote normal development of immune functions (10). Microbiota can modulate pathogenesis, progression and treatment of various diseases, such as autoimmune diseases and metabolic disorders (11). Several population-based studies have revealed diet as one of the major factors in shaping the overall composition of the human gut microbiota and a key component impacting the abundance of specific species, and such associations have been found in both short-term (12) and long-term (13) dietary interventions. Reshaping host–microbiota interactions through dietary interventions has emerged as a new therapeutic method for disease control and prevention (14).

Legume consumption has been suggested to improve gut health (15). However, few population-based studies have specifically investigated this topic. Utilizing resources from 2 large cohorts, this study aims to fill this gap.

Methods

Study population

Participants of our study were drawn from 2 cohort studies, the Shanghai Women's Health Study (SWHS) and the Shanghai Men's Health Study (SMHS). Details of these studies have been described elsewhere (16, 17). Briefly, a total of 74,742 women (40–70 y old) and 61,480 men (40–74 y old) in 8 communities of urban Shanghai, China, were recruited to the SWHS (1996–2000) and SMHS (2002–2006), respectively. In-person interviews were conducted at baseline to collect information on sociodemographics, disease history, diet/lifestyles, and anthropometrics. Participants were followed up through in-person surveys every 2–4 y (response rates >92%), with supplemental annual linkages to the Shanghai Vital Statistics and Shanghai Cancer Registry (completion rates >99%) to collect information on occurrence of cancer and other chronic disease and vital status, as well as to update information on diet, lifestyle, and anthropometrics. A participant flowchart is shown in **Supplemental Figure 1**.

Dietary assessment

Dietary information was collected using validated food-frequency questionnaires (18, 19) at baseline and follow-up surveys of the SWHS (first and fourth) and SMHS (first). A total of 77 (SWHS) and 81 (SMHS) food items and food groups were included in the questionnaires, covering ~90% of common foods in urban Shanghai. For each food item or food group, participants were asked about the frequency (daily, weekly, monthly, yearly, or never) and the amount of consumption of foods or food groups. Total legume consumption included in the current study was calculated as the sum of peanuts, soy foods (in dry weight), and other beans, all of which were measured by grams per day, from the most recent survey prior to stool sample collection (time interval from survey to stool sample collection: 5.21–19.99 y). Soy foods included soy milk, tofu and tofu products, dried and fresh soybeans, and bean sprouts. Other beans included fresh beans, mung beans, red beans and other dried beans, mung bean sprouts, fresh peas, fresh broad beans, yard-long beans, green beans, and hyacinth beans. Consumption of total legumes, soy foods, and other beans was dichotomized into low and high groups using the median consumption level as the cutoff.

Stool sample collection

Stool samples from volunteer cohort participants were collected during the fifth follow-up of the SWHS and third follow-up of the SMHS during 2015–2018. All participants from the SWHS and SMHS provided informed consent at baseline. During 2015 and 2018, half of the living cohort members who remained in 5 of 8 of our study districts were invited to donate a stool sample for research. Stool samples were collected from all willing participants without any exclusion criteria. Fifteen RMB (Chinese yuan, ~$2.3) was provided to study participants as a study incentive. Ethical approval was obtained from participating institutions prior to stool sample collection.

Participants were provided with stool sample collection kits, a step-by-step instruction sheet, and a sample collection form. A peanut-size (1 full scoop) stool sample was collected and placed into a sample collection tube containing 5 mL 95% ethanol with glass beads. The tube was shaken until the stool sample was well mixed with ethanol. Participants filled out sample collection forms to record the date and time of sample collection, use of antibiotics and medications in the last 7 d and 6 mo, usual bowel movement frequency, and diarrhea in the last 7 d. Stool samples were shipped to the laboratory within 3 d after collection and were then placed into aliquots and stored in −80°C freezers until DNA extraction. Stool samples from 1804 participants from the SWHS and SMHS, who had biomarker data or developed cancer after stool sample collection, were selected for the microbiome study, and 1554 additional stool samples from the SWHS and SMHS were randomly selected, bringing a total of 3358 samples for microbiome profiling. After excluding 171 samples that failed the 16S ribosomal RNA (rRNA) gene-sequencing process due to low DNA amount or failure of quality control, stool samples for 3187 participants were profiled for data analysis.

Due to potential changes in diet after diagnosis of a major disease, we excluded participants who had cancer, stroke, acute myocardial infarction, or diabetes prior to stool sample collection from the current study. We further excluded participants who used antibiotics within 6 mo or had diarrhea within 7 d before stool sample collection. Finally, 16S rRNA gene-sequencing data from 2302 individuals, including 1100 women from the SWHS and 1202 men from the SMHS, were included in the present study.

Microbiome data processing

DNA was extracted from stool samples using QIAGEN's DNeasy PowerSoil kit, following the manufacturer's instructions. DNA sequencing libraries were prepared using Bioo Scientific NEXTflex 16S V4 Amplicon-Seq kit. Sequencing was performed on Illumina HiSeq at 2 × 250-bp paired-end reads. Sequence reads were trimmed to remove adapters and filtered to remove low-quality reads using Sickle (20). BayesHammer was used to correct sequencing errors (21). PANDAseq was used to assemble paired-end reads (22). Clean assembled reads were clustered into operational taxonomic units (OTUs) at 97% identity against the Green Genes database (23) using a closed-reference OTU picking strategy via Quantitative Insights Into Microbial Ecology (QIIME, version 1.9) (24). The OTU table was rarefied to the minimum sequencing depth among the 2302 samples (17,013 reads) for further α- and β-diversity analyses. The unrarefied OTU table was used to derive count and relative abundance of taxa from phylum to species levels for downstream association analyses.

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Supplemental Figure 1 and Supplemental Tables 1 and 2 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/jn/.
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Abbreviations used: BC, Bray-Curtis; clr, centered log-ratio; FDR, false discovery rate; OTU, operational taxonomic unit; rRNA, ribosomal RNA; SMHS, Shanghai Men's Health Study; SWHS, Shanghai Women's Health Study.

Statistical analysis

α-Diversity was assessed by the Chao1, Shannon, and Simpson indexes. Their associations with legume consumption were evaluated by linear regression analyses. β-Diversity was measured by Bray-Curtis (BC) dissimilarity (25) matrix, and its association with legume consumption was assessed using PERMANOVA in R (R Foundation for Statistical Computing) package "vegan" (26).

For common taxa (i.e., those observed among ≥50% of samples), centered log-ratio (clr) transformation was used to normalize raw count data after substituting zero count with a pseudo count "1", and linear regression was conducted to estimate the associations of legume consumption with clr-transformed taxa abundance. For rare taxa, observed among <50% of samples, we limited our analyses to those shown in >10% of samples, and negative binomial hurdle regression [R package "pscl" (27)] was applied to evaluate the associations of legume consumption with the abundance of taxa without transformation. β-Coefficients and *P* values were based on the zero hurdle part of the model. Analyses were conducted for all 2302 participants and stratified by sex, because females and males differ substantially in sociodemographic and lifestyle factors, especially smoking and alcohol drinking, which may modify gut microbiome–legume associations. The following covariates were adjusted in the analyses: time interval between dietary assessment and stool sample collection, age at stool sample collection, sex (only in the combined analyses), education, income (in yuan per capita per year; low: <500, middle: 500–1999, high: ≥2000), smoking status (for females and combined analyses) or pack-years (for males), alcohol drinking status (for females and combined analyses) or amount (for males), physical activity, BMI, and total energy intake. We further conducted stratified analyses by metabolic conditions, defined as having hypertension, hyperlipidemia, or obesity [BMI (kg/m^2) ≥30]. All statistical analyses were performed in R 3.6.3. False discovery rate (FDR) correction was performed within each taxonomic level separately for combined or stratified analyses, and associations with FDR-corrected *P* values (P_{FDR}) of < 0.1 were considered significant.

Results

A total of 1110 women and 1202 men were included in the study (**Table 1**). The median consumption of legumes was 27.9 g/d among women and 31.9 g/d among men. The time interval from the most recent dietary assessment to the stool sample collection ranged from 5.21 to 19.99 y. Female participants were, on average, 2.4 y older than men (women: 70.0 y; men: 67.6 y) at stool sample collection. Men had higher educational levels: 60% completed high school or had a college education compared with women (37%). Notably, only a small portion of women, 1% and 6%, were ever-smokers or regular drinkers compared with 73% and 33% men, respectively. The prevalence of metabolic conditions was higher in women (52%) than in men (33%).

Among both women and men, participants who had a legume consumption greater than the median tended to drink alcohol ($P = 0.02$ for women and 0.01 for men) and have a higher energy intake ($P < 0.01$ for both) (**Table 2**). Women who had a higher legume consumption had a shorter time interval between dietary assessment and stool sample collection ($P = 0.03$) and a higher mean BMI ($P = 0.02$). Men with a higher legume consumption had a higher income ($P = 0.03$) and were more likely to have hyperlipidemia ($P = 0.03$).

After quality control and rarefaction, a total of 1099 OTUs were identified among all participants, based on which α-diversity indexes (Chao1, Shannon, and Simpson indexes) and β-diversity matrices (BC dissimilarity matrix) were calculated. In combined data of men and women, total legume consumption was not associated with any α-diversity index or β-diversity (**Supplemental Table 1**). Analyses stratified by sex showed that the amount of total legume consumption (in grams/day) explained 0.1% of variations in β-diversity among men ($P = 0.003$) and 0.2% among men without metabolic conditions ($P = 0.02$). Peanut consumption (consumption vs. nonconsumption) explained 0.3% of variations in β-diversity among participants with metabolic conditions ($P = 0.02$). Among men, peanut consumers had a higher Chao1 index than nonconsumers ($\beta = 22.52$, $P = 0.01$), with a stronger association among men with metabolic conditions ($\beta = 41.45$, $P = 0.009$). Among women, the amount of peanut consumption (in grams/day) was associated with lower Shannon ($\beta = -0.03$, $P = 0.02$) and Simpson ($\beta = -0.002$, $P = 0.04$) indexes. Consumption of soy foods above the median amount (19.9 g/d) was associated with a decreased Shannon index among men with metabolic conditions ($\beta = -0.18$, $P = 0.04$), and the amount of soy food intake explained 0.2% of the variations in β-diversity among male participants without metabolic conditions ($P = 0.03$).

After excluding taxa with a prevalence <10%, a total of 13 phyla, 21 classes, 37 orders, 70 families, 142 genera, and 205 species were investigated. Taxa that showed significant associations ($P_{FDR} < 0.1$) in any of the analyses of combined female and male participants are presented in **Tables 3** and **4** and **Supplemental Table 2**. Most significant associations with legume consumption were observed among common taxa (Table 3). Meanwhile, although most significant associations showed consistent directions between women and men, the significant associations were primarily confined to women. In the combined analyses of women and men, high legume intake (>29.9 g/day, median) was associated with a higher abundance of the order *Enterobacteriales* ($\beta = 0.30$, $P_{FDR} = 0.06$) and a higher proportion of carriers of an unclassified order ($\beta = 0.43$, $P_{FDR} = 0.01$), both in the class *Gammaproteobacteria*. High legume intake was also positively associated with 3 taxa under *Enterobacteriales*, including 2 genera of *Enterobacteriaceae*—an unclassified genus ($\beta = 0.46$, $P_{FDR} = 0.03$) and *Citrobacter* ($\beta = 0.34$, $P_{FDR} = 0.04$)—and an unclassified species in *Citrobacter* ($\beta = 0.33$, $P_{FDR} = 0.03$). When stratified by sex, we found the same direction of associations, but they were mostly only significant in women.

Among legume subtypes, any consumption of peanuts (i.e., consumption vs. nonconsumption) was associated with an increased abundance of the order *Actinomycetales* ($\beta = 0.19$, $P_{FDR} = 0.08$) and an unclassified genus of the family *Enterobacteriaceae* ($\beta = 0.59$, $P_{FDR} = 0.009$) in the combined analyses of women and men. We found the associations of peanut consumption with the proportion of carriers of family *Peptococcaceae* to be negative in women. Among women, soy food consumption >17.5 g/d (median in women) was associated with a higher proportion of carriers of an unclassified order in *Gammaproteobacteria* ($\beta = 0.54$, $P_{FDR} = 0.07$). In addition, consumption of other beans of >7.8 g/d (median in women) was associated with an increased abundance of *Bacteroides plebeius* in women ($\beta = 0.81$, $P_{FDR} = 0.08$).

When consumption of legumes was treated as a continuous variable (Supplemental Table 2), total legume consumption was also associated with an increased abundance of an unclassified genus in the family *Enterobacteriaceae* ($\beta = 0.01$, $P_{FDR} = 0.06$). Similar to the binary legume consumption results, significant associations were only evident in women. Among women, in addition to *Enterobacteriaceae*, total legume consumption was associated with a higher proportion of carriers of the phylum *Lentisphaerae* ($\beta = 0.02$, $P_{FDR} = 0.0004$), which was driven

TABLE 1 Characteristics of the study population in the Shanghai Women's and Men's Health Studies[1]

	Overall ($n = 2302$)	Women ($n = 1100$)	Men ($n = 1202$)
Consumption, g/d			
Legumes	29.9 (0, 174)	27.9 (0, 120)	31.9 (0, 174)
Peanuts	1.1 (0, 94.6)	0.76 (0, 26.1)	1.5 (0, 94.6)
Soy foods	19.9 (0, 156)	17.5 (0, 106)	22.0 (0, 156)
Other beans	7.2 (0, 72.7)	7.8 (0, 67.4)	6.7 (0, 72.7)
Time interval, y	9.5 ± 2.5	8.7 ± 3.2	10.3 ± 1.3
5 to ≤7.4 y	564 [25]	564 [51]	0 [0]
7.5 to ≤10 y	781 [34]	340 [31]	441 [37]
>10 y	957 [42]	196 [18]	761 [63]
Age at stool sample collection, y	68.7 ± 8.99	70.0 ± 8.62	67.6 ± 9.17
50–59 y	392 [17]	118 [11]	274 [23]
60–69 y	986 [43]	500 [45]	486 [40]
70–79 y	554 [24]	279 [25]	275 [23]
≥80 y	370 [16]	203 [18]	167 [14]
Highest level of education			
Below high school	1173 [51]	691 [63]	482 [40]
High school	949 [41]	371 [34]	578 [48]
College	180 [8]	38 [3]	142 [12]
Annual income[2]			
Low	365 [16]	181 [16]	184 [15]
Middle	1762 [77]	846 [77]	916 [76]
High	175 [8]	73 [7]	102 [8]
BMI, kg/m^2	24.3 ± 3.54	24.3 ± 3.84	24.3 ± 3.24
Smoking			
Never smoker	1407 [61]	1087 [99]	320 [27]
Ever smoker	895 [39]	13 [1]	882 [73]
Pack-years among smokers	22.5 ± 17.4	3.13 ± 5.53	22.8 ± 17.3
Alcohol drinking			
Nondrinker	1837 [80]	1035 [94]	802 [67]
Drinker	465 [20]	65 [6]	400 [33]
Drinks/day among drinkers	2.11 ± 2.01	0.126 ± 0.365	2.43 ± 1.98
Physical activity, MET/wk	13.0 ± 16.0	13.7 ± 16.2	12.5 ± 15.8
Total energy intake, kcal/d	1760 ± 466	1530 ± 327	1960 ± 478
Hypertension[3]			
No	1572 [68]	618 [56]	954 [79]
Yes	730 [32]	482 [44]	248 [21]
Hyperlipidemia[4]			
No	1885 [82]	890 [81]	995 [83]
Yes	417 [18]	210 [19]	207 [17]
Metabolic condition[5]			
No	1339 [58]	528 [48]	811 [67]
Yes	963 [42]	572 [52]	391 [33]

[1]Values are means ± SDs for continuous variables or *n* [%] for categorical variables; consumption amount is shown in median (range). MET, metabolic equivalent task.
[2]Low: <500 yuan per capita per year; middle: 500–1999 yuan per capita per year; high: ≥2000 yuan per capita per year.
[3]Ever reported hypertension at baseline or during follow-up.
[4]Ever reported hyperlipidemia at baseline or during follow-up.
[5]Having hypertension, hyperlipidemia, or obesity [BMI (kg/m^2) ≥30].

by an unclassified genus in the family *Victivallaceae*. Subtypes of legumes were also found to be associated with several taxa among women, including peanut consumption with an increased proportion of carriers of the order *SHA-98* under class *Clostridia* ($\beta = 0.15$, $P_{FDR} = 0.001$), soy foods with increased *Lentisphaerae* ($\beta = 0.02$, $P_{FDR} = 0.001$), and other beans with decreased *Actinobacteria* ($\beta = -0.02$, $P_{FDR} = 0.06$) and an increased abundance of an unclassified family under *Enterobacteriales* ($\beta = 0.05$, $P_{FDR} = 0.06$).

We further conducted stratified analyses by metabolic disease status (Table 4). Among participants without metabolic conditions ($n = 1339$), we found that legume consumption was significantly and inversely associated with an abundance of *Bacteroidetes* ($\beta = -0.10$, $P_{FDR} = 0.02$) and positively associated with an abundance of *Gammaproteobacteria* ($\beta = 0.41$, $P_{FDR} = 0.006$). Peanut consumption was associated with a decreased proportion of carriers of an unclassified genus in the family *Coriobacteriaceae* ($\beta = -0.55$, $P_{FDR} = 0.04$) and an increase in an unclassified genus in the family *Enterobacteriaceae* ($\beta = 0.70$, $P_{FDR} = 0.07$). Soy food consumption was associated with a decreased abundance of *Firmicutes* ($\beta = -0.08$, $P_{FDR} = 0.09$) and *Bacteroidetes* ($\beta = -0.10$,

TABLE 2 Characteristics of the study population in the Shanghai Women's and Men's Health Studies[1]

	Women			Men		
	≤27.9 g Legumes/d ($n = 550$)	>27.9 g Legumes/d ($n = 550$)	P^2	≤31.9 g Legumes/d ($n = 601$)	>31.9 g Legumes/d ($n = 601$)	P^2
Dietary assessment to stool collection interval, y	8.86 ± 3.52	8.44 ± 2.76	0.03	10.2 ± 1.22	10.3 ± 1.29	0.08
5 to ≤7.4 y	284 [52]	280 [51]		0 [0]	0 [0]	
7.5 to ≤10 y	162 [29]	178 [32]		230 [38]	211 [35]	
>10 y	104 [19]	92 [17]		371 [62]	390 [65]	
Age at stool sample collection y	70.1 ± 8.83	69.8 ± 8.42	0.54	67.6 ± 9.32	67.5 ± 9.03	0.88
50–59 y	65 [12]	53 [10]		142 [24]	132 [22]	
60–69 y	238 [43]	262 [48]		240 [40]	246 [41]	
70–79 y	137 [25]	142 [26]		131 [22]	144 [24]	
≥80 y	110 [20]	93 [17]		88 [15]	79 [13]	
Highest level of education						
Below high school	340 [62]	351 [64]	0.39	256 [43]	226 [38]	0.20
High school	194 [35]	177 [32]		275 [46]	303 [50]	
College	16 [3]	22 [4]		70 [12]	72 [12]	
Annual income[3]						
Low	100 [18]	81 [15]	0.18	106 [18]	78 [13]	0.03
Middle	410 [75]	436 [79]		452 [75]	464 [77]	
High	40 [7]	33 [6]		43 [7]	59 [10]	
BMI, kg/m^2	24.0 ± 3.88	24.5 ± 3.79	0.02	24.1 ± 3.20	24.4 ± 3.27	0.09
Smoking						
Never smoker	543 [99]	544 [99]	1.00	152 [25]	168 [28]	0.33
Ever smoker	7 [1]	6 [1]		449 [75]	433 [72]	
Pack-years among smokers	5.37 ± 6.89	0.505 1.01	0.11	22.0 ± 17.1	23.6 ± 17.6	0.16
Alcohol drinking						
Nondrinker	527 [96]	508 [92]	0.02	424 [71]	378 [63]	0.01
Drinker	23 [4]	42 [8]		177 [29]	223 [37]	
Drinks/day among drinkers	0.11 ± 0.23	0.13 ± 0.43	0.77	2.28 ± 1.86	2.55 ± 2.06	0.18
Physical activity, MET/wk	12.9 ± 15.6	14.4 ± 16.8	0.13	12.0 ± 14.3	12.9 ± 17.2	0.32
Total energy intake, kcal/d	1410 ± 276	1650 ± 328		1800 ± 399	2130 ± 494	
			<0.001			<0.001
Hypertension[4]						
No	300 [55]	318 [58]	0.30	473 [79]	481 [80]	0.62
Yes	250 [45]	232 [42]		128 [21]	120 [20]	
Hyperlipidemia[5]						
No	454 [83]	436 [79]	0.19	512 [85]	483 [80]	0.03
Yes	96 [17]	114 [21]		89 [15]	118 [20]	
Metabolic condition[6]						
No	260 [47]	268 [49]	0.67	414 [69]	397 [66]	0.33
Yes	290 [53]	282 [51]		187 [31]	204 [34]	

[1] Values are means ± SDs for continuous variables or *n* [%] for categorical variables. MET, metabolic equivalent task.
[2] *P* values are based on Student's *t* test for continuous variables and chi-square test for categorical variables. $P < 0.05$ is considered statistically significant.
[3] Low: <500 yuan per capita per year; middle: 500–1999 yuan per capita per year; high: ≥2000 yuan per capita per year.
[4] Ever reported hypertension during follow-up.
[5] Ever reported hyperlipidemia during follow-up.
[6] Having hypertension, hyperlipidemia, or obesity [BMI (kg/m^2) ≥30].

$P_{FDR} = 0.009$), as well as an increased proportion of carriers of the order *YS2* in class *4C0d-2* ($\beta = 0.66$, $P_{FDR} = 0.03$). Results among people with metabolic conditions differed by sex. For example, peanut consumption was associated with decreased *Peptococcaceae* in women ($\beta = -0.69$, $P_{FDR} = 0.10$) and with increased abundances of 2 species in *Faecalibacterium* and an unclassified species in *Corynebacterium* in men.

Discussion

In this study of mostly older men and women living in urban China, we found that total legume consumption was associated with β-diversity of gut microbiota among males. The amount of peanut consumption was associated with a lower α-diversity among women, but a higher α-diversity in males. In the combined analyses of women and men, total legume consumption was associated with an increase in the family *Enterobacteriaceae* and peanut consumption with an unclassified genus within this family. Meanwhile, peanut consumption was also associated with an increase in the order *Actinomycetales*. Stratified analyses showed that the significant associations were primarily confined to women and people without metabolic conditions. Among legume subtypes, consumption of peanuts and soy foods had a greater effect than other legumes on the gut microbiome. The consumption of peanuts and soy foods was associated with α- and β-diversity among certain subsets of participants, whereas no association

TABLE 3 Association between legume consumption and microbiome on taxa levels in the Shanghai Women's and Men's Health Studies[1]

	Overall ($n = 2302$)				Women ($n = 1110$)				Men ($n = 1202$)			
Taxa	Relative abundance,[2] median, %	Prevalence, %	β	P_{FDR}[3]	Relative abundance,[2] median, %	Prevalence, %	β	P_{FDR}[3]	Relative abundance,[2] median, %	Prevalence, %	β	P_{FDR}[3]
Legumes		> vs. ≤29.9 g/d				> vs. ≤27.9 g/d				> vs. ≤31.9 g/d		
Phylum *Proteobacteria*												
Class *Betaproteobacteria*	2.88	99.70	−0.18	0.44	2.87	99.45	−0.11	1.00	2.89	99.92	−0.28	0.10*
Class *Gammaproteobacteria*	1.28	100	0.30	0.005*	1.31	100	0.34	0.09*	1.24	100	0.30	0.20
Order unclassified[4]	0.001	17.07	0.43	0.01*	0.001	16.09	0.63	0.01*	0.001	17.97	0.37	0.78
Order *Enterobacteriales*	0.72	99.91	0.30	0.06*	0.76	100	0.33	0.56	0.70	99.83	0.31	0.78
Family *Enterobacteriaceae*												
Genus unclassified	0.04	96.09	0.46	0.03*	0.04	96.09	0.78	0.002*	0.05	96.09	0.18	1.00
Genus *Citrobacter*	0.004	71.11	0.34	0.04*	0.003	70.64	0.53	0.01*	0.004	71.55	0.18	1.00
Species unclassified	0.001	61.51	0.33	0.03*	0.001	62.18	0.44	0.10*	0.001	60.90	0.27	1.00
Peanuts		Yes vs. no				Yes vs. no				Yes vs. no		
Phylum *Actinobacteria*												
Order *Actinomycetales*	0.01	97.87	0.19	0.08*	0.01	97.82	0.24	0.23	0.01	97.92	0.10	1.00
Phylum *Proteobacteria*												
Class *Gammaproteobacteria*	1.28	100	0.25	0.20	1.31	100	0.41	0.07*	1.24	100	0.10	1.00
Order *Enterobacteriales*	0.72	99.91	0.33	0.15	0.76	100	0.50	0.07*	0.70	99.83	0.19	1.00
Family *Enterobacteriaceae*												
Genus unclassified	0.04	96.09	0.59	0.009*	0.04	96.09	0.58	0.78	0.05	96.09	0.62	0.47
Phylum *Firmicutes*												
Family *Peptococcaceae*[4]	0.01	34.36	−0.15	1.00	0.01	34.36	−0.54	0.05*	0.01	34.36	0.21	1.00
Genus unclassified[4]	0.01	27.54	−0.20	1.00	0.01	27.09	−0.62	0.03*	0.01	27.95	0.20	1.00
Soy foods		> vs. ≤19.9 g/d				> vs. ≤17.5 g/d				> vs. ≤22.0 g/d		
Phylum *Proteobacteria*												
Class *Gammaproteobacteria*												
Order unclassified[4]	0.001	17.07	0.28	0.58	0.001	16.09	0.54	0.07*	0.001	17.97	0.14	1.00
Other beans		> vs. ≤7.2 g/d				> vs. ≤7.8 g/d				> vs. ≤6.7 g/d		
Phylum *Bacteroidetes*												
Species *Bacteroides plebeius*	0.01	91.05	0.14	1.00	0.01	89.18	0.81	0.08*	0.02	92.76	-0.49	1.00

[1] Models were adjusted for time interval between dietary assessment to stool sample collection, age at stool sample collection, sex (only in the combined analyses), education, income, smoking status (for women and combined analyses) or pack-years (for men), alcohol drinking status (for women and combined analyses) or amount (for men), physical activity, BMI, and total energy intake. P_{FDR}, false discovery rate–corrected *P* value.

[2] Median relative abundance for rare taxa was calculated among carriers.

[3] $P_{FDR} < 0.1$(*) is considered statistically significant.

[4] Rare taxa: 10% ≤prevalence <50% in the population.

TABLE 4 Association between legume consumption and microbiome on taxa levels by metabolic conditions in the Shanghai Women's and Men's Health Studies[1]

	Overall				Women				Men			
Taxa	Relative abundance,[2] median, %	Prevalence, %	β	P_{FDR}[3]	Relative abundance,[2] median, %	Prevalence, %	β	P_{FDR}[3]	Relative abundance,[2] median, %	Prevalence, %	β	P_{FDR}[3]
Without metabolic conditions, *n*	1339				528				811			
Legumes		> vs. ≤29.9 g/d				> vs. ≤27.9 g/d				> vs. ≤31.9 g/d		
Phylum *Bacteroidetes*	57.99	100	−0.10	0.02*	58.84	100	−0.07	1.00	57.54	100	−0.10	0.08*
Phylum *Proteobacteria*												
Class *Gammaproteobacteria*	1.19	100	0.41	0.006*	1.19	100	0.34	1.00	1.19	100	0.46	0.04*
Peanuts		Yes vs. no				Yes vs. no				Yes vs. no		
Phylum *Actinobacteria*												
Family *Coriobacteriaceae*												
Genus unclassified[4]	0.002	30.92	−0.55	0.04*	0.001	30.16	−0.43	1.00	0.002	31.42	−0.61	0.28
Phylum *Proteobacteria*												
Family *Enterobacteriaceae*												
Genus unclassified	0.04	95.88	0.70	0.07*	0.03	96.03	0.89	0.55	0.04	95.79	0.58	1.00
Soy foods		> vs. ≤19.9 g/d				> vs. ≤17.5 g/d				> vs. ≤22.0 g/d		
Phylum *Firmicutes*	28.81	100	−0.08	0.09*	29.03	100	−0.10	0.41	28.60	100	−0.05	1.00
Phylum *Bacteroidetes*	57.99	100	−0.10	0.009*	58.84	100	−0.07	1.00	57.54	100	−0.10	0.05*
Phylum *Cyanobacteria*												
Class *4C0d-2*[4]	0.005	10.57	0.57	0.06*	NA	NA	NA	NA	0.01	11.37	0.60	0.28
Order *YS2*[4]	0.01	10.18	0.66	0.03*	NA	NA	NA	NA	0.01	10.86	0.72	0.14
Phylum *Actinobacteria*												
Genus *Corynebacterium*[4]	0.001	18.41	0.43	0.96	0.001	15.48	−0.09	1.00	0.001	20.31	0.67	0.08*
With metabolic conditions, *n*	963				572				391			
Legumes		> vs. ≤29.9 g/d				> vs. ≤27.9 g/d				> vs. ≤31.9 g/d		
Phylum *Proteobacteria*												
Class *Gammaproteobacteria*												
Order unclassified[4]	0.001	16.06	0.51	0.20	0.002	15.44	0.83	0.04*	0.001	16.95	0.21	1.00
Peanuts		Yes vs. no				Yes vs. no				Yes vs. no		
Phylum *Firmicutes*												
Family *Peptococcaceae*[4]	0.01	32.91	−0.31	1.00	0.01	33.05	−0.69	0.10*	0.01	32.70	0.31	1.00
Genus unclassified[4]	0.01	25.71	−0.40	1.00	0.01	26.51	−0.80	0.06*	0.01	24.58	0.36	1.00

(*Continued*)

TABLE 4 (*Continued*)

Taxa	Overall				Women				Men			
	Relative abundance,[2] median, %	Prevalence, %	β	P_{FDR}[3]	Relative abundance,[2] median, %	Prevalence, %	β	P_{FDR}[3]	Relative abundance,[2] median, %	Prevalence, %	β	P_{FDR}[3]
Family *Ruminococcaceae*												
Genus *Faecalibacterium*	5.14	99.90	0.33	1.00	4.81	99.83	−0.03	1.00	5.79	100	0.86	0.03*
Species *prausnitzii*	4.94	99.90	0.33	1.00	4.69	99.83	−0.04	1.00	5.59	100	0.85	0.05*
Species other	0.17	96.85	0.39	0.73	0.17	96.31	0.08	1.00	0.18	97.61	0.83	0.02*
Phylum *Actinobacteria*												
Species *Corynebacterium* unclassified[4]	0.001	17.64	−0.40	1.00	0.001	18.46	0.18	1.00	0.001	16.47	−1.23	0.03*

[1]Models were adjusted for time interval between dietary assessment to stool sample collection, age at stool sample collection, sex (only in the combined analyses), education, income, smoking status (for women and combined analyses) or pack-years (for men), alcohol drinking status (for women and combined analyses) or amount (for men), physical activity, BMI, and total energy intake. P_{FDR}, false discovery rate–corrected *P* value. NA, not applicable.
[2]Median relative abundance for rare taxa was calculated among carriers.
[3]$P_{FDR} < 0.1$(*) is considered statistically significant.
[4]Rare taxa: 10% ≤prevalence <50% in the population.

with diversity was identified with the consumption of other beans. In the taxa analyses, more taxa showed significant associations with the consumption of peanuts and soy foods than with other beans.

Our findings on the associations between legume intake and the gut microbiome are supported by previous reports (28–30). A study of mice fed a high-fat diet found that consumption of black legumes was associated with increased α-diversity (Chao 1, Shannon, and Simpson indexes) and an increased abundance of *Bacteroidetes* and *Proteobacteria* (28). Few population-based studies have been conducted to investigate the association of legume consumption with the gut microbiome. A study in older people of European descent reported that a Mediterranean diet rich in legumes was associated with an increased abundance of *Faecalibacterium prausnitzii* (29), which has been associated with delayed frailty onset in the elderly (31). Similarly, intakes of dietary fiber and polysaccharides were found to be associated with an increased abundance of *F. prausnitzii* (30). This is in line with our findings of an increase in *F. prausnitzii* among male peanut consumers with metabolic conditions.

The legume–taxa associations identified in our study have complex functional potentials. *Enterobacteriaceae*, which showed an increase among participants with higher consumption of total legumes, peanuts, and soy foods, includes harmless bacteria as well as pathogens. For example, *Citrobacter*, whose abundance was higher in participants with higher legume consumption, is generally considered a commensal resident in the healthy human gut but also includes species that cause opportunistic infections (32). *Peptococcaceae*, which had a lower abundance in women who consumed peanuts, has been related to prenatal anxiety in females (33) and a high-fat diet and stress in female mice (34), whereas a weight-loss probiotic supplement was shown to have a positive association (35). *Aggregatibacter*, which had a higher abundance in men who consumed peanuts, is part of the human oral microbiota and could cause periodontal infection (36). *Bacteroides plebeius*, associated with a higher consumption of other beans, is a prevalent bacteria found in fecal samples of Japanese and is responsible for breaking down the marine algae containing polysaccharides by expressing β-porphyranases (37).

Several significant associations that we found in the study differed by sex. In addition to the biological differences between males and females and measurement errors, multiple other factors could contribute to the differential associations, including age at stool sample collection of study participants (mean age: 67.6 y for men and 70.0 y for women), time interval between dietary assessment and stool sample collection (mean: 10.3 y for men and 8.7 y for women), smoking/drinking habits, and comorbidities. Although we have attempted to address the confounders in multivariate analyses, residual confounding cannot be ruled out. Our study also does not have sufficient statistical power to evaluate the effect modification from these factors.

One of the major strengths of this study is the population-based study design and a large sample size. Validated food-frequency questionnaires were applied in the parent cohort studies, which captured information on long-term intake of legumes and other foods/nutrients (20, 21). In our parent studies, we have implemented repeated dietary surveys and found that legume intake assessed at these surveys correlated reasonably well (Spearman's correlation was 0.36). However, we cannot exclude the influence of dietary habit change, particularly the recent diet, on the legume and microbiome association. Such influence is likely to lead to an underestimation of the true

association. In addition, extensive information was available on sociodemographic factors, health conditions, and other lifestyle factors, such as smoking and alcohol drinking status, which allowed a comprehensive adjustment for confounders and evaluation of potential effect modifications by stratified analyses.

There are several limitations to this study. First, the 16S rRNA sequencing used in our study offers limited taxonomical and functional resolutions, especially at the species level, prohibiting a more in-depth evaluation (38). Second, stool samples were collected from 2015 to 2018, which was 5 to 20 y after the assessment of dietary intake. Although the human gut microbiome is considered stable over time (39), it is possible that the legume–gut microbiome associations could be altered by major changes in diet or health status shortly preceding the stool collection. The influence of recent intake on the gut microbiome is an important research question and can be best investigated in clinical trials. Although repeated stool samples were not available in our study, we tried to minimize the influence of health conditions and medication use on the legume–gut microbiome associations by excluding participants with cancer, stroke, acute myocardial infarction, or diabetes and those who used antibiotics within 6 mo or had diarrhea within 7 d before stool sample collection. In addition, the survey-based exposure assessment may introduce potential information bias. Such misclassification is likely to bias the study results towards the null. Selection bias is also likely, given that only ~30% of invited SWHS/SMHS participants provided a stool sample to the study. We noticed that participants in our study differed significantly from the entire cohort of participants on demographic and lifestyle factors, as well as legume intake, although almost all differences were small. We would also like to emphasize that participants in the study were elderly Chinese living in urban Shanghai. The generalizability of our study findings thus will need to be evaluated.

In summary, this large study found that peanut consumption was associated with a decrease in α-diversity among women and an increase in α-diversity among men; legume consumption was associated with a higher relative abundance of *Gammaproteobacteria*, including *Enterobacteriaceae*, especially among women. Although our study provides important information about legume intake in association with human gut microbiota, our findings need confirmation, particularly regarding the differences between sexes. Large-scale prospective studies and feeding trials are highly desirable to fully understand the role of the gut microbiome in the legume–health connection.

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