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Indole derivatives and their associated microbial genera are associated with the 1-year changes in cardiometabolic risk markers in Chinese adults

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Abstract

Background Although emerging evidence suggests that indole derivatives, microbial metabolites of tryptophan, may improve cardiometabolic health, the effective metabolites remain unclear. Also, the gut microbiota that involved in producing indole derivatives are less studied. We identified microbial taxa that can predict serum concentrations of the key indole metabolite indole-3-propionic acid (IPA) at population level and investigated the associations of indole derivatives and IPA-predicting microbial genera with cardiometabolic risk markers.

Methods In a cohort of 318 community-dwelling adults, serum indole metabolites and fecal microbiota (16S ribosomal RNA) were measured at baseline. Total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and fasting blood glucose were repeatedly measured at baseline and again after 1 year. Brachial-ankle pulse wave velocity (baPWV) and ankle-brachial index (ABI) were measured after 1 year. The association between indole derivatives and the 1-year changes in blood lipids and glucose, and association of indole derivatives with baPWV and ABI were investigated using linear regression models.

Results Each 1 μmol/L increase in indole-3-acetic acid (IAA) levels was associated with 5.08% ($P=0.046$) decrease in LDL-C. IPA levels were inversely associated with baPWV (percentage difference = -1.32%, $P=0.036$). Per 1 μmol/L increase in Indole-3-aldehyde (IAld) levels was associated with 1.91% ($P=0.004$) decrease in TC and 0.58% ($P=0.019$) increase in ABI, but 1.79% decrease in HDL-C with borderline significance ($P=0.050$). We identified 18 bacterial genera whose relative abundance was positively associated with serum IPA concentrations ($P_{FDR} < 0.05$) and constructed a microbial score to reflect the overall IPA-producing potential. This score was inversely associated with baPWV (percentage difference = -0.48%, $P=0.007$).

Conclusions Our results suggest that IAA, IPA, IAld, and IPA-predicting microbial score are favorably associated with several cardiometabolic risk markers, although IAld may decrease HDL-C levels.

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Keywords Indole metabolites, Indole-3-propionic acid, Cardiometabolic risk, Gut microbiome, Brachial-ankle pulse wave velocity

Introduction

Cardiovascular diseases (CVDs) have become a public health-threatening problem [1]. Accumulating evidence has shown a link between CVDs and altered gut microbiota composition [2–4]. The gut microbiota metabolizes or ferments food components into a variety of metabolites, which may mediate the impact of gut microbiota on cardiovascular health [5].

Tryptophan (Trp) is an essential amino acid that can only be obtained from protein-rich foods such as meat, fish, eggs, dairy, beans, nuts, and soy [6]. Trp in the intestine is mainly catabolized through the kynurenine pathway (KP), which represents 95% of ingested Trp [7]. A small portion of Trp (1–2%) can lead to the production of serotonin [8]. While the unabsorbed Trp (4–6%) can be directly transformed by the gut microbiota into indole and its derivatives (Supplementary Fig. 1) [9]. Among indole metabolites, indole pyruvic acid can give rise to indole-3-propionic acid (IPA), which is the final product of reductive Trp metabolism [10]. Indole pyruvic acid can also be further converted into indole-3-acetic acid (IAA), and subsequently into indole-3-aldehyde (IAld) [11]. The host and microbial Trp catabolite have been identified to manifest divergent effects on the progression of metabolic disorders and CVDs. Some indole derivatives, such as IPA, IAA, and IAld can activate the aryl hydrocarbon receptor (AHR), which may favorably regulate inflammation and immune responses [12–14]. According to recent studies, IPA can improve intestinal barrier function through the activation of the pregnane X receptor (PXR) [15]. While higher levels of KP catabolites, such as kynurenine, have been linked to inflammation and oxidative stress, both of which are known to contribute to the onset of cardiometabolic diseases [16].

The gut microbiota profiles are indeed crucial in modulating the production of indole metabolites from dietary Trp. Prior culture-based studies have identified multiple bacterial genera capable of metabolizing Trp into indole and its derivatives [10, 17–19]. These studies may have not considered all microbes that contribute to the production of indole metabolites, given the known difficulties in culturing many of the microbes comprising the human gastrointestinal microbiome. Additionally, the evidence may not be applied to general populations, because human diet is complex, and the capacity of indole metabolites depends not only on host-microbial compositions, but also on the habitual diet consumed. Thus, identifying the indole metabolite-producing microbial taxa at population levels may better reflect the real-world settings.

Therefore, we leveraged an integrated microbiome-metabolome data from a representative sample of 754 community-dwelling adults in Huoshan, China. We aimed to identify gut microbial genera that could be involved in the production of IPA in a Chinese population and investigate the association between serum indole metabolites as well as IPA-predicting microbial genera and 1-year changes in cardiometabolic risk markers.

Methods

Study population

The Anhui Liver Diseases Study (ALDS) is an ongoing community-based cohort that started in 2020 in Lu'an and Ma'anshan, China. To ensure representativeness of the study population, we used a multistage cluster sampling design. For example, among 3 districts and 4 counties in Lu'an, we selected 4 study sites including Huoshan County, Shucheng County, Jin'an District, and Yu'an District. We then randomly selected 4 towns or streets (an administrative unit of the county or district) in each study site, and 4 villages or communities in each town or street. Third, we selected 1 residential group in each village or community, and 50 households in each residential group. Last, we selected 1 adult aged 18 years or older per household using the Kish selection grid technique.

In this study, we used data from 754 participants from the ALDS in Huoshan. Among them, 482 completed the baseline survey and the first round of follow-up interview after 1 year. We excluded participants who did not provide fecal samples ($n=90$) or had no data on cardiometabolic risk markers at baseline or after 1 year ($n=74$). Therefore, 318 participants were included in the final analysis (Supplementary Fig. 2). All participants were unaware of the specific hypotheses being tested. The study was approved by the ethics committee of Anhui Medical University (Protocol Number: 20210730), and all subjects provided informed written consent.

Indole metabolites analysis

Baseline fasting blood samples were thawed and assayed for gut microbiota–derived tryptophan metabolites, including IPA, IAld, IAA, tryptamine (TAM), indole-3-acetamide (IAM), and indole-3-acrylic acid (IA). Serum concentrations of indole metabolites were measured using high-throughput liquid chromatography-tandem

mass spectrometry techniques. Details of sample extraction, separation, and MS analysis have been described elsewhere [20].

Assessment of covariates

A structured questionnaire was used to collect age, sex, family income, education, smoking, alcohol consumption, physical activity, and history of chronic diseases. Body weight and height were measured by trained investigators at baseline, and the body mass index (BMI) was calculated as weight in kilograms (kg) divided by height in meters squared (m^2). Physical activity was quantified as the metabolic equivalent task (METS) hours per week. Hypertension and diabetes were identified if participants had been told by a healthcare professional that they had such diseases or took prescribed medications due to the diseases. Diabetes (fasting plasma glucose ≥ 126 mg/dL) and hypertension (systolic blood pressure ≥ 140 or diastolic blood pressure ≥ 90 mmHg) were also identified through laboratory tests or physical examinations at baseline. Diet was assessed at baseline using a validated 141-item food frequency questionnaire. The dietary approaches to stop hypertension (DASH) were calculated to assess overall diet quality [21].

Cardiometabolic risk markers

Serum glucose, triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were repeatedly measured at baseline and after 1 year of follow-up. To quantify the degree of arterial stiffness, we measured brachial-ankle pulse wave velocity (baPWV) and ankle-brachial index (ABI) using an oscillometric device (BP-203RPEIII; Omron) after a 1-year follow-up. Blood lipids and glucose were quantified by electrochemiluminescence. The method for measuring baPWV and ABI has been detailed elsewhere [22]. High baPWV and low ABI are independent predictors of cardiovascular events and mortality [23, 24].

Fecal sample collection and microbiome profiling

Participants provided fecal samples at the same time with blood sample collection at baseline. Fecal samples were collected at home using a commode specimen collection system and a stool collection container (Fisher Scientific) by the participants, and were delivered to the nearest Community Health Center within 4 h. Upon arrival, each sample was immediately stored in −80 °C freezers until nucleic acid extraction. In the current study, we only included participants without probiotic or antibiotic use at least 1.5 months before fecal sample collection to reduce the impact of probiotic or antibiotic use [25].

We profiled the fecal microbiome using 16S ribosomal RNA (rRNA) gene sequencing. The microbial community DNA was extracted using MagPure Stool DNA KF kit B (Magen, China). DNA was quantified with a Qubit fluorometer by using the Qubit dsDNA BR Assay Kit (Invitrogen, USA), and the quality was checked by running an aliquot on 1% agarose gel. Variable region V4 of the bacterial 16 S rRNA gene was amplified with degenerate PCR primers. Both forward and reverse primers were tagged with Illumina adapter, pad, and linker sequences. PCR enrichment was performed in a 50-μL reaction containing 30 ng of template, fusion PCR primer, and PCR master mix. PCR cycling conditions were as follows: 95 °C for 3 min, 30 cycles of 95 °C for 45 s, 56 °C for 45 s, 72 °C for 45 s, and final extension for 10 min at 72 °C for 10 min. The PCR products were purified using Agencourt AMPure XP beads and eluted in elution buffer. Libraries were qualified by the Agilent Technologies 2100 bioanalyzer. The validated libraries were used for sequencing on Illumina HiSeq 2500 platform (BGI, Shenzhen, China), and generating 2 × 250 bp paired-end reads. Genera with an average relative abundance over 0.001% were selected for our downstream analysis. In total, 75 genera were identified.

Statistical analysis

Baseline characteristics of the participants were presented as mean with standard deviation (SD) for continuous normally distributed variables, median [inter-quarter range (IQR)] for continuous non-normally distributed variables, and percentages for categorical variables. Statistically significant differences in general characteristics among tertiles of IPA levels were compared using one-way ANOVA, Kruskal-Wallis test, or chi-square test. The 1-year changes in blood glucose and lipids were calculated as the differences between the levels after 1 year and the levels at baseline, and all variables were log-transformed prior to calculation. Linear regression models were used to evaluate the association between serum concentrations of gut microbiota-derived tryptophan metabolites in indole pathway and cardiometabolic risk markers, adjusting for age, sex, education, family income, BMI, physical activity, smoking, alcohol drinking, total energy intake, and DASH score.

After applying Arc-sin square root transformation to relative abundances of taxonomic features, the feature-wise associations between bacterial genera and serum indole metabolites were analyzed using linear regression. The models were adjusted for age, sex, BMI, total energy intake, physical activity, DASH score, education, family income, smoking, and alcohol drinking. The Benjamini-Hochberg false discovery rate (FDR) method was used for multiple testing corrections. Bacterial genera associated

with serum indole metabolites at an FDR < 0.05 were considered statistically significant.

Considering that IPA belongs to the end product of the indole pathway produced by gut microbiota [10], which is what most of the previous studies focused on [26, 27], we constructed an IPA-predicting microbial score (IPAMS) based on identified IPA-associated genera. This score summarizes the relative abundance of microbial genera that were positively associated with IPA levels in the analysis at an FDR < 0.05. Microbial genera detected in ≥ 50% of the samples were categorized as "high" (median levels or high) or "low" (less than the median level) according to the median relative abundance, whereas microbial genera detected in < 50% of the samples were dichotomously categorized according to the presence or absence of the genus. We assigned 1 point for higher abundance or presence of genera or 0 otherwise. The scores of all genera were then summed to calculate a total score. We also investigated the association between the IPAMS and cardiometabolic risk markers. We did not exclude participants who had missing data on covariates (generally < 1%) in the analysis and assigned a separate 'missing' indicator variable in the models. To facilitate the interpretation, the effect sizes were back transformed if the variables were log transformed in the models [28]. We reported percentage differences and 95% confidence intervals (CIs) in cardiometabolic risk markers for each 1 μmol/L increase in serum indole metabolites or 1 point increase in IPAMS. All analyses were performed using R version 4.2.0.

Results

Participant characteristics

The analysis encompassed 318 participants, including 127 men and 191 women (mean [SD] age, 49.8 [14.7] years). The median serum concentrations of IPA in the population were 2.25 μmol/L (IQR 1.69 to 3.21 μmol/L). Compared to participants with the lowest tertile of serum IPA, those in the highest tertile were more likely to be women, and were less likely to be current smokers and current drinkers (Table 1).

Serum indole metabolites and cardiometabolic health

As shown in Table 2, per 1 μmol/L increase in baseline serum concentrations of IAA was associated with a decrease in LDL-C, with the percentage differences of −5.08% (95%CI: −9.83, −0.09, $P = 0.046$). Higher serum IPA levels were associated with lower baPWV, with the percentage differences of −1.32% (95%CI: −2.53, −0.09, $P = 0.036$). Each 1 μmol/L increase in IAld was associated

Table 1 Characteristics of 318 participants by tertiles of serum IPA levels in Huoshan, China[a]

Characteristics	IPA			*P*
	Tertile 1	Tertile 2	Tertile 3	
No. of participants	106	106	106	
Age, years	48 (36-56)	53 (38-64)	53 (43-62)	0.07
Female, %	47.7	63.7	76.3	<0.01
Annual Household per capita income, %				0.70
<10 000 Yuan	38.3	35.8	31.9	
10 000-20 000 Yuan	32.2	21.1	26.6	
>20 000 Yuan	29.5	43.1	41.5	
Education, %				0.55
Uneducated	19.2	16.4	20.7	
Primary school or below	31.3	31.2	22.0	
Junior high school or above	49.5	52.3	57.3	
Current smokers, %	29.0	19.6	6.5	<0.01
Current drinkers, %	21.8	17.1	4.7	<0.01
BMI, kg/m^2	25.2 (4.0)	24.0 (3.5)	24.3 (3.0)	0.08
Total energy intake, kcal/d	2193 (1685-2962)	2170 (1671-2681)	1928 (1665-2359)	0.07
Physical activities, METS-h/week	150 (93-189)	139 (95-198)	138 (100-201)	0.81
Hypertension, %	53.9	47.6	52.5	0.25
Diabetes, %	15.9	11.5	10.0	0.97
DASH scores	24 (22-27)	24 (21-27)	25 (22-27)	0.24

Abbreviations: *BMI* Body mass index, *DASH* Dietary approaches to stop hypertension, *IPA* Indole-3-propionic acid, *METS* Metabolic equivalent tasks

[a] Continuous variables are expressed as the mean (SD) or median (interquartile range) according to the distribution of the variables, while categorical variables are presented as %. *P* values were calculated from the one-way ANOVA or Kruskal-Wallis test for continuous variables and chi-squared test for categorical variables

Table 2 Associations between serum indole metabolites and cardiometabolic risk markers in Huoshan, China ($N=318$)[a]

	Percentage difference (%) and 95% confidence interval					
	IAA	**TAM**	**IA**	**IPA**	**IAld**	**IAM**
TC[b]	−2.94 (−6.15, 0.37)	22.12 (−2.55, 53.05)	−0.09 (−2.40, 2.28)	−0.43 (−1.81, 0.96)	−1.91 (−3.17,−0.63)*	3.27 (−2.98, 9.92)
TG[b]	−6.78 (−15.2, 2.54)	8.52 (−42.9, 106.3)	−1.89 (−8.18, 4.83)	−2.50 (−6.25, 1.41)	1.76 (−1.95, 5.62)	−9.75 (−24.4, 7.69)
HDL-C[b]	−2.90 (−7.33, 1.74)	−0.96 (−27.7, 35.65)	0.98 (−2.25, 4.31)	−0.51 (−2.41, 1.42)	−1.79 (−3.56, −0.00)*	2.45 (−6.06, 11.72)
LDL-C[b]	−5.08 (−9.83, −0.09)*	−10.0 (−36.4, 27.34)	−2.40 (−5.83, 1.15)	0.01 (−2.10, 2.16)	−0.79 (−2.76, 1.23)	3.81 (−5.66, 14.22)
Glucose[b]	−2.76 (−5.98, 0.58)	−0.89 (−21.1, 24.46)	−1.12 (−3.42, 1.23)	−0.63 (−2.01, 0.76)	−0.36 (−1.66, 0.96)	−2.30 (−8.24, 4.02)
baPWV	1.74 (−1.27, 4.86)	11.20 (−9.15, 36.11)	0.05 (−2.02, 2.17)	−1.32 (−2.53, −0.09)*	−0.46 (−1.61, 0.72)	−3.71 (−8.93, 1.80)
ABI	0.85 (−0.41, 2.13)	−5.79 (−13.4, 2.52)	−0.07 (−0.94, 0.81)	−0.31 (−0.83, 0.21)	0.58 (0.09, 1.07)*	−0.11 (−2.41, 2.26)

Abbreviations: *ABI* Ankle-brachial index, *baPWV* Brachial ankle pulse wave velocity, *HDL-C* High-density lipoprotein cholesterol, *IA* Indole-3-acrylic acid, *IAA* Indole-3-acetic acid, *IAld* Indole-3-aldehyde, *IAM* Indole-3-acetamide, *IPA* Indole-3-propionic acid, *LDL-C* Low-density lipoprotein cholesterol, *TAM* Tryptamine, *TC* Total cholesterol, *TG* Triglycerides

* *P* value ≤ 0.05

[a] Linear regression model was adjusted for age (18-39, 40-49, 50-59, and ≥60 years), sex (women, men), education (no formal education, primary school or below, junior high school or above), annual household per capita income (<10,000 yuan, 10,000-20,000 yuan, >20,000 yuan), body mass index (<28.0 and ≥28.0 kg/m^2), drinking status (never, past, current drinking), smoking status (never, past, current smoking), physical activity (metabolic equivalent tasks-h/week, tertile), total energy intake (kcal/day, tertile), and Dietary Approaches to Stop Hypertension index (continuous). Within the endings of baPWV and ABI, additional adjustments were made for hypertension and diabetes

[b] The 1-year changes were derived from the difference from baseline data after one year, and all variables were log-transformed to approximate a normal distribution of the residuals

with 1.91% (95% CI: −3.17, −0.63, $P=0.004$) and 1.79% (95% CI: −3.56, −0.00, $P=0.050$) decrease in TC and HDL-C from baseline to 1 year later, respectively. In addition, serum IAld concentrations were positively associated with ABI determined at 1 year, with the percentage differences of 0.58% (95%CI: 0.09%, 1.07%, $P=0.019$). We did not find any statistically significant associations for TAM, IAM, and IA. We excluded each covariate one by one in the full-adjusted models and found that BMI most significantly contributed to the associations as a confounder (data not shown).

IPA, fecal microbiome and cardiometabolic health

Of the 75 identified bacterial genera, the relative abundance of 18 genera was positively associated with serum IPA concentrations at FDR Q < 0.05 (Table 3). These genera belong to the following 4 phyla families: Firmicutes (*Roseburia*, *Dorea*, *Butyricicoccus*, *Ruminococcus*, *Eubacterium*, *Faecalibacterium*, *Gemmiger*, *Intestinimonas*, *Sporobacter*, *Oscillibacter*, *Clostridium_XlVb*, *Coprococcus*), Actinobacteria (*Adlercreutzia*, *Collinsella*), Bacteroidetes (*Prevotella*, *Alistipes*, *Odoribacter*), and Verrucomicrobia (*Akkermansia*). Additionally, we observed a null association between gut microbial genera and other serum indole derivatives (Supplementary Table 1).

We found an inverse association between the IPAMS and baPWV, with the percentage differences of −0.48% (95%CI: −0.82, −0.13, $P=0.007$). We did not find any statistically significant association between the IPAMS and 1-year changes in blood lipids and glucose (Table 4).

Discussion

In this 1-year longitudinal study, we found that indole metabolites, including IAA, IPA, and IAld, were favorably associated with several cardiometabolic risk markers in Chinese community-dwelling adults, although IAld was inversely associated with the levels of HDL-C. We identified 18 bacterial genera whose relative abundance can predict higher serum levels of IPA, and constructed a microbial score to reflect the overall IPA-producing potential. This score showed an inverse association with the risk of arterial stiffness. Our findings may contribute to the development of novel approaches to improve cardiometabolic health by enhancing IPA-producing capacity by modulating the host gut microbiome.

To date, several studies have investigated the associations between cardiometabolic health and indole metabolites, including IAA, IPA, and IAld. For example, administration of IAA by intraperitoneal injection was found to decrease fasting blood glucose, plasma TC, and LDL-C levels in high-fat diet (HFD)-fed mice [29]. Another study revealed that fecal samples from individuals with metabolic syndrome showed lower concentrations of IAA, compared to those from healthy individuals [30]. These findings are consistent with our results that serum IAA concentrations were associated with a decrease in LDL-C levels. Experimental studies found that IPA markedly decreased markers for metabolic impairments such as fasting blood glucose, fasting insulin, TC, LDL-C, and TG levels [31, 32]. Human observational studies suggested a favorable association between IPA and risk of several cardiometabolic

Table 3 Abundance of bacterial genera associated with serum IPA among 318 participants in Huoshan, China*

Phylum; class; order; family; genus	Prevalence (%)	Average abundance (%)	β	SE	*P* value	Q value
Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; *Roseburia*	98.43	4.68	2.05	0.61	<0.01	<0.01
Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; *Dorea*	87.74	0.20	14.13	3.35	<0.01	<0.01
Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; *Butyricicoccus*	96.54	0.24	8.96	3.40	0.01	0.03
Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; *Ruminococcus*	83.33	1.15	4.72	1.12	<0.01	<0.01
Firmicutes; Clostridia; Clostridiales; Eubacteriaceae; *Eubacterium*	60.69	0.23	6.18	2.16	<0.01	0.02
Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; *Faecalibacterium*	98.74	7.60	2.53	0.62	<0.01	<0.01
Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; *Gemmiger*	81.45	0.76	5.48	1.27	<0.001	<0.01
Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; *Intestinimonas*	41.51	0.02	19.51	6.92	0.01	0.02
Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; *Sporobacter*	60.06	0.14	7.48	2.63	<0.01	0.02
Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; *Oscillibacter*	89.62	0.34	8.81	2.12	<0.01	<0.01
Actinobacteria; Actinobacteria; Coriobacteriales; Coriobacteriaceae; *Adlercreutzia*	53.46	0.02	29.61	8.22	<0.01	<0.01
Actinobacteria; Actinobacteria; Coriobacteriales; Coriobacteriaceae; *Collinsella*	62.89	0.20	6.35	2.31	0.01	0.02
Verrucomicrobia; Verrucomicrobiae; Verrucomicrobiales; Verrucomicrobiaceae; *Akkermansia*	40.88	0.65	3.26	1.12	0.01	0.02
Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; *Prevotella*	86.48	13.80	0.75	0.24	<0.01	0.01
Bacteroidetes; Bacteroidia; Bacteroidales; Rikenellaceae; *Alistipes*	86.16	1.00	3.06	1.15	0.01	0.03
Bacteroidetes; Bacteroidia; Bacteroidales; Porphyromonadaceae; *Odoribacter*	69.81	0.08	13.90	4.07	<0.01	0.01
Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; *Clostridium_XlVb*	83.96	0.15	9.00	2.33	0.01	0.02
Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; *Coprococcus*	74.84	0.22	9.00	2.33	<0.01	<0.01

**P* values were estimated from linear regression after adjustment for age (18-39, 40-49, 50-59, and ≥60 years), sex (women, men), education (no formal education, primary school or below, junior high school or above), annual household per capita income (<10,000 yuan, 10,000-20,000 yuan, >20,000 yuan), body mass index (<28.0 and ≥28.0 kg/m^2), total energy intake (kcal/day, tertile), physical activity (metabolic equivalent tasks-h/week, tertile), smoking status (never, past, current smoking), drinking status (never, past, current drinking), and Dietary Approaches to Stop Hypertension index (continuous). All taxa with Q < 0.05 are included in the table. Q value, false discovery rate-corrected $P < 0.05$. Prevalence indicates the proportion of participants in each genus

Abbreviations: *IPA* Indole-3-propionic acid

disorders, including type 2 diabetes, dyslipidemia, and hepatic steatosis [27, 33–36]. We did not find any statistically significant association between IPA and blood lipids as well as blood glucose. The reasons for these discrepant findings are unclear, although another animal experiment found that water supplemented with IPA did not protect Western diet-fed mice from the cardiometabolic consequences [37]. In our study, we found that individuals with higher serum concentrations of IAld tended to have lower TC levels, which indicates a lower risk of CVDs [38]. Interestingly, we also observed a correlation between baseline IAld levels and a subsequent decrease in HDL-C levels, which are inversely associated with the risk of CVDs [39]. However, the health benefits of high HDL-C remain unclear. The randomized clinical trials on HDL-C raising pharmaceuticals generally showed no or harmful effects on cardiovascular health [40]. Due to the intricate composition and inter-changeability of the serum lipid profile, clarifying the role of HDL-C in cardiovascular health is much more challenging. Thus, to completely comprehend the complex association between IAld and cholesterol metabolism, further study is necessary.

Atherosclerosis is the underlying cause of most CVDs. One observational study showed that plasma concentrations of IPA and IAld were lower in patients with severe atherosclerosis compared to an age- and gender-matched control group. It also showed a positive association between IPA and ABI (low ABI is an indicator of peripheral atherosclerosis) [41]. Another study offered experimental evidence that supplementation with IPA could facilitate macrophage reverse cholesterol transport to inhibit atherosclerosis [42]. Moreover, emerging evidence has revealed that IPA can activate the PXR, which inhibits inflammation, and can induce vasodilation [15, 43].

IPA, as the final product of the Trp reduction metabolism, was found to be entirely dependent upon gut microflora [44]. One animal study demonstrated that the abundances of the two genera, *Oscillibacter* and *Odoribacter*, which were implied to be important for intestinal homeostasis, were decreased in the HFD-fed group and recovered in the IPA group [45]. Consistently, in our investigation, *Oscillibacter* and *Odoribacter* genera displayed a significant positive correlation with IPA. We found that the richness of *Akkermansia*, *Coprococcus*, *Eubacterium*, *Faecalibacterium*, and

Table 4 Associations between IPA-predicting microbial score and cardiometabolic risk markers ($N = 318$)[a]

	Linear regression model coefficients	
	Percentage difference (95% CI)	***P***
TC[b]	-0.28 (-0.67, 0.11)	0.161
TG[b]	-0.24 (-1.35, 0.87)	0.667
HDL-C[b]	-0.31 (-0.85, 0.23)	0.262
LDL-C[b]	-0.25 (-0.85, 0.35)	0.413
Glucose[b]	-0.30 (-0.69, 0.09)	0.130
baPWV	-0.48 (-0.82, -0.13)	0.007
ABI	-0.05 (-0.19, 0.10)	0.544

Abbreviations: *ABI* Ankle-brachial index, *baPWV* Brachial ankle pulse wave velocity, *HDL-C* High-density lipoprotein cholesterol, *IPA* Indole-3-propionic acid, *LDL-C* Low-density lipoprotein cholesterol, *TC* Total cholesterol, *TG* Triglycerides

[a] Linear regression model was adjusted for age (18-39, 40-49, 50-59, and ≥60 years), sex (women, men), education (no formal education, primary school or below, junior high school or above), annual household per capita income (<10,000 yuan, 10,000-20,000 yuan, >20,000 yuan), body mass index (<28.0 and ≥28.0 kg/m^2), drinking status (never, past, current drinking), smoking status (never, past, current smoking), physical activity (metabolic equivalent tasks-h/week, tertile), total energy intake (kcal/day, tertile), and Dietary Approaches to Stop Hypertension index (continuous). Within the endings of baPWV and ABI, additional adjustments were made for hypertension and diabetes

[b] The 1-year changes were derived from the difference from baseline data after one year, and all variables were log-transformed to approximate a normal distribution of the residuals

Ruminococcus genera was positively associated with serum IPA concentrations, which have been reported in other studies [17, 26, 46]. Due to the limitation of 16S rRNA sequencing, we cannot identify the specific species that may produce IPA. However, the pathway producing IPA has been mostly investigated in *Clostridium sporogenes* [47]. Moreover, several gut bacterial genera, such as *Adlercreutzia*, *Collinsella*, and *Alistipes*, can predict serum IPA levels in our population, which have not yet been reported in other populations. This could be due to the distinct microbial compositions among different ethnic groups. It is possible that these bacteria together constitute a complex environment that is favorable for the IPA-producing genera. In summary, our study validates previous findings at the genus level and introduces novel bacterial genera that could potentially contribute to IPA production in the Chinese population.

Given the intricate and interdependent nature of the human gut microbiota, our study systematically identified the specific microbial taxa that may potentially be involved in IPA production, and developed a microbial score to reflect the overall IPA-producing potential. This score showed an inverse association with baPWV. Our results may help develop potential strategies to improve cardiometabolic health by shaping the host gut microbiome through enhancing the production of IPA.

The strengths of the present study include the prospective design, the use of repeated measures for cardiometabolic risk markers, and the representative sample of community-dwelling adults in Huoshan, China. However, our study has several limitations. First, despite the prospective design, we were unable to assess the associations of serum indole metabolites and gut microbiota with long-term cardiometabolic outcomes due to the short (i.e., 1 year) follow-up period. Second, despite the adjustments for a wide range of dietary and lifestyle variables, there was still the possibility of residual confounding. Third, due to the use of 16S rRNA gene sequencing, the taxonomic resolution was limited to the genus level in most cases. Thus, the evaluation of fecal microbial gene expression using deeper sequencing (e.g., metagenomics) may enhance our study. Last, our study was conducted in a Chinese population, which might limit its generalizability to other populations.

In conclusion, we discovered multiple new bacterial genera that may be involved in the production of IPA in a Chinese population, and confirmed earlier studies on IPA-producing microbial taxa at the genus level. We also developed a microbial score to reflect the overall IPA-producing potential. We showed that this microbial score and several indole metabolites are favorably associated with cardiometabolic risk markers. These findings, if confirmed, will aid in the development of personalized nutrition to improve cardiometabolic health by targeting gut flora.

Abbreviations

ABI	Ankle-brachial index
AHR	Aryl hydrocarbon receptor
ALDS	Anhui Liver Diseases Study
baPWV	Brachial-ankle pulse wave velocity
CI	Confidence interval
CVD	Cardiovascular disease
DASH	Dietary approaches to stop hypertension
FDR	False discovery rate
IA	Indole-3-acrylic acid
IAA	Indole-3-acetic acid
IAld	Indole-3-aldehyde
IAM	Indole-3-acetamide
IPA	Indole-3-propionic acid
KP	Kynurenine pathway
METS	Metabolic equivalent tasks
PXR	Pregnane X receptor
TAM	Tryptamine
Trp	Tryptophan

Supplementary Information

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Supplementary Material 1.

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Authors' contributions
Yutong Pan and Yamin Li had full access to all the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Wanshui Yang. Acquisition, analysis, or interpretation of data: All authors. Drafting of the manuscript: Yutong Pan, Wanshui Yang. Critical revision of the manuscript for important intellectual content: All authors. Statistical analysis: Yutong Pan, Yamin Li. Obtained funding: Wanshui Yang. Administrative, technical, or material support: Wanshui Yang. Study supervision: Wanshui Yang.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The study was approved by the Ethics Committee of Anhui Medical University (Protocol Number: 20210730), and the written informed consent was obtained from all participants. All procedures performed in this study were in accordance with the 1964 Helsinki declaration.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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