Journal of Ovarian Research

RESEARCH Open Access

Gut microbiota and gut-derived metabolites are altered and associated with dietary intake in women with polycystic ovary syndrome

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Abstract

Background Disturbances in the gut microbiota may act as mechanisms influencing the interplay between dietary factors and metabolic disorders. Studies have demonstrated that these alterations are associated with the diagnosis of polycystic ovary syndrome (PCOS). Within this context, we aimed to investigate associations between gut microbiota, gut-derived metabolites (short-chain fatty acids [SCFAs] and indole-3-propionic acid [IPA]), and dietary intake in women with PCOS.

Methods We conducted a cross-sectional study of 24 women with PCOS, previously recruited for two studies at our research center, compared with 14 age-matched healthy controls. The mean (SD) age of all 38 participants was 33.3 (7.5) years, and the mean (SD) body mass index was 29.5 (4.8) kg/m^2. Primary outcomes included gut microbiota analysis by sequencing the V4 region of the 16 S rRNA gene, serum IPA levels measured by liquid chromatography/triple-quadrupole mass spectrometry (LC-QqQ-MS), and fecal and plasma SCFA levels measured by LC-MS/MS.

Results Gut microbiota diversity, composition, and metabolic pathways differed between the PCOS and control groups. A higher abundance of two operational taxonomic units specializing in complex carbohydrate metabolism was observed in healthy control women. The PCOS group exhibited a less favorable dietary intake than the control group, and a significant correlation was observed between gut microbiota composition and dietary glycemic load in PCOS ($r=0.314$, $P=0.03$ in Mantel test). Multivariable-adjusted linear regression models indicated that lower levels of IPA and higher circulating levels of two SCFAs (acetic acid and propionic acid) were independently associated with the diagnosis of PCOS.

Conclusions Our data support the differentiation between women with PCOS and healthy controls based on gut microbiota analysis. Furthermore, changes in gut bacteria and their metabolites could be, at least in part, the biological mechanism by which a low glycemic load diet may potentially improve PCOS-related reproductive and cardiometabolic outcomes.

Keywords Polycystic ovary syndrome, Gut microbiome, Short-chain fatty acids, Indole-3-propionic acid, Diet

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Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age and is characterized by hyperandrogenism and oligo-anovulatory infertility [1]. Women with PCOS often develop metabolic disorders such as obesity, insulin resistance, and dyslipidemia, which increase their risk of developing type 2 diabetes, hypertension, and non-alcoholic fatty liver disease [1–4]. The bidirectional association between obesity and insulin resistance can be described as a complex interplay where excessive fat accumulation, particularly visceral fat, contributes to the development of insulin resistance by increasing pro-inflammatory cytokines and androgen production [5–8]. Conversely, insulin resistance can promote weight gain and obesity through mechanisms such as increased appetite [9].

Management of PCOS has increasingly focused on lifestyle modification and quality of life. A healthy diet and exercise should be recommended for all women with PCOS targeting improved metabolic health [10]. However, there is limited evidence to recommend a specific diet composition for the management of PCOS. Low glycemic index (GI)/glycemic load (GL) dietary interventions have been associated with improvements in insulin sensitivity and reproductive hormones compared with high-carbohydrate or control diets, but further research is needed to better understand the biological reasons why a low GI/GL diet may impact PCOS-related reproductive and cardiometabolic outcomes [11].

The mechanisms underlying the interplay between dietary factors and metabolic disorders may be related to gut microbiota disturbances [6, 12, 13]. In recent years, research focus has shifted from gut microbiota composition to functionality, partially mediated by metabolites that could influence host metabolism in health and disease such as short-chain fatty acids (SCFAs) and indole-3-propionic acid (IPA). Dietary fiber and polyphenols, found in fruits, vegetables, and beans, are metabolized by the gut microbiota into SCFAs [14], which have been implicated in glucose and insulin metabolism [15, 16] and immune homeostasis [17]. IPA is a byproduct of tryptophan metabolism by gut microbiota, and lower circulating IPA levels have been associated with metabolic disorders including obesity [18, 19], type 2 diabetes [20], and atherosclerotic cardiovascular disease [21]. Gut microbiota-derived metabolites have the potential to serve as biomarkers for early diagnosis of cardiometabolic diseases and as targets for the development of new therapeutic tools [22].

Changes in the gut microbiota have been associated with PCOS, including a decrease in the diversity of gut bacteria and changes in specific bacterial taxa [23]. Previous studies have suggested that disturbances in the gut microbiota induced by an unhealthy diet can lead to inflammation by increasing the structural components of pathogenic bacteria. This process can trigger a cascade of inflammatory pathways involving interleukins and other cytokines ultimately leading to insulin resistance, hyperandrogenism, and ovarian dysfunction [24, 25]. However, there is limited knowledge regarding the effect of diet on the gut microbiota and how specific dietary factors influence the production of gut-derived metabolites, which may potentially improve the management of PCOS. Within this context, we aimed to investigate associations between gut microbiota, gut-derived metabolites (IPA and SCFAs), and dietary intake in women with PCOS.

Materials and methods

Participants and study design

Women with PCOS who had been recruited for previous studies [26, 27] at our research center were invited to participate in this cross-sectional study conducted from November 2019 to August 2021 at Hospital de Clínicas de Porto Alegre (HCPA), a tertiary hospital in southern Brazil. Women aged 14 to 40 years were eligible for inclusion if they met the following criteria: more than 2 years after menarche and diagnosis of PCOS according to the Rotterdam criteria (presence of at least 2 of the 3 features of hyperandrogenism, oligo-amenorrhea, and polycystic ovarian morphology on ultrasound) [28]. Exclusion criteria were acute illness or evidence of acute inflammation/infection, any systemic disease other than PCOS (e.g., cancer, smoking-related diseases, mental illness), any condition affecting the ability to fully understand the study and give consent, pregnancy or breastfeeding, and antibiotic use in the 6 months preceding enrollment [29].

The control group consisted of age-matched healthy women without PCOS and with regular menses. Controls were recruited from HCPA staff between March and August 2021. The exclusion criteria for controls were the same as for women with PCOS, with the additional requirement of not having used any drugs known to interfere with hormone levels in the 3 months preceding enrollment.

Each study participant provided written informed consent before enrollment. The study was approved by the HCPA Ethics Committee (CAAE 35025414.1.0000.5327) and conducted in accordance with the Helsinki Declaration.

Body composition, dietary intake, and physical activity assessment

Total body fat and lean body mass were measured with a dual-energy X-ray absorptiometry (DXA) scanner, calibrated using a soft-tissue phantom (GE Lunar Prodigy; Radiation Corporation, Madison, WI, USA).

Dietary intake was assessed by a validated food frequency questionnaire (FFQ) [30] consisting of 121 food

items that indicate the usual frequency of consumption over 1 month, as previously reported [26]. Total energy, nutrient, and fiber intakes were estimated using the Brazilian Food Composition Table [31]. The dietary GI and GL were reported as proposed by the Food and Agriculture Organization [32]. The GI and GL of each food were extracted from the International Table of Glycemic Index, with glucose as the standard food, as previously reported [26]. Data from the FFQ were entered in duplicate into EpiData (version 3.1; EpiData Association, Denmark) and subsequently exported to SPSS (version 21.0; IBM Corp., Armonk, NY, USA) for analysis.

Physical activity was assessed with a digital pedometer (BP 148; Techline, SP, Brazil), which was individually configured according to each participant's weight (kg) and step length. Participants were instructed to wear the device for 6 consecutive days, providing the weekly average number of steps [33].

Laboratory measurements

Blood samples were collected after an 8-hour overnight fast for determination of the following data: total testosterone levels, measured by chemiluminescence immunoassay (Centaur XP Siemens); sex-hormone binding globulin levels, measured by chemiluminescence immunoassay (Immulite 2000 S); glucose levels, measured by the hexokinase method (Advia 1800); plasma insulin levels, measured by electrochemiluminescence immunoassay (Centaur XP Siemens); and high-sensitivity C-reactive protein (hsCRP), measured by a validated, high-sensitivity nephelometric method (Dade Behring Marburg, Marburg, Germany). Insulin resistance was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR), calculated as follows: insulin (mU/L) × glucose (mmol/L) / 22.5 [34].

Gut microbiota analysis

All participants collected fecal samples at home into sterile specimen containers. They were instructed to store the container in the household freezer at −6 to −20 °C until delivery to the laboratory within 24 h of collection. The samples were held in a cooler on ice for transport to the laboratory, where they were initially stored at −20 °C and then divided into aliquots for storage at −80 °C until analysis.

Bacterial DNA was isolated from approximately 200 mg of the fecal sample using the QIAamp Fast DNA stool mini kit (Qiagen, USA) following the manufacturer's instructions. Polymerase chain reaction (PCR) amplicons were generated using the following primers that target the V4 region of the 16 S rRNA gene: 515 F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). The amplification was performed using Platinum™ PCR SuperMix High Fidelity (Invitrogen, Carlsbad, CA, USA). We restricted our analysis to the V4 region of the 16 S rRNA gene because this specific region has been widely used in previous studies and is the genetic marker of choice for several reasons, including its presence in almost all bacteria [35, 36].

All samples were sequenced on the Ion Torrent platform (Thermo Fisher Scientific, MA, USA) and processed using a custom pipeline in Mothur v.1.47.0 [37]. Sequences were depleted of barcodes and primers (no mismatch was allowed) and quality filtered by discarding low-quality reads and reads of incorrect length, with ambiguous bases, or with homopolymers longer than 6 bp. All potentially chimeric sequences were identified and removed using VSEARCH [38]. Singletons were also removed to reduce the chance of spurious sequences resulting from PCR or sequencing errors.

Filtered sequences were clustered into operational taxonomic units (OTUs) at a 99% identity level and classified against the SILVA v138 reference database at 97% similarity. Unknown/unclassified sequences and those assigned to eukaryotes, mitochondria, or chloroplasts were removed prior to further analysis. The resulting OTU table consisted of 1,157,389 sequences, with an average of 42,866 sequences per sample, and the dataset was rarefied to the smallest library size (i.e., 25,296 sequences).

Assessment of indole-3-propionic acid and short-chain fatty acids

Blood samples were collected between 8:00 and 10:00 AM after an 8-hour overnight fast. Participants were instructed to abstain from strenuous exercise and alcohol for 24 h prior to the collection.

Serum IPA levels were measured by liquid chromatography/triple-quadrupole mass spectrometry (LC-QqQ-MS) using a reversed-phase separation technique. Commercial IPA was used as a standard, and deuterated IPA (IPA-d2) was used as an internal standard. Details on method validation have been described elsewhere [39].

Fecal and plasma SCFA levels were determined using LC-MS/MS by collaborators in the Department of Life Sciences at the Chalmers University of Technology, Gothenburg, Sweden. Details on method validation have been described elsewhere [40].

Sample size calculation

Sample size calculation was based on the findings of Zhang et al. [41] regarding fecal SCFA levels and conducted using the WinPepi program (PEPI-for-Windows). With a significance level of 5%, a sample size of 22 participants (11 women with PCOS and 11 healthy controls) would provide 80% power to detect a difference of 6.21 μm/g in the concentration of fecal propionic acid between the two groups.

Statistical analysis

Data are presented as mean (SD) or median (IQR) depending on the Shapiro-Wilk test results. Non-normally distributed variables were log-transformed before analysis. The transformed variables were re-checked for normality before conducting further tests. An independent-samples *t*-test was used to assess differences in clinical characteristics, dietary intake, and gut-derived metabolites between women with PCOS and controls.

For the gut-derived metabolites that differed between the PCOS and control groups after controlling for body mass index (BMI), univariate analysis of variance (UNIANOVA) models were applied to explore associations with dietary factors. Subsequently, multivariable-adjusted linear regression models were used to explore relationships between the diagnosis of PCOS and gut-derived metabolites, while adjusting for each significant dietary variable identified in the UNIANOVA. Data were analyzed using SPSS, version 21.0, and a two-tailed *P*-value < 0.05 was considered statistically significant.

Gut microbiota analysis was performed in R, version 4.0.0, using the vegan, phyloseq, ggplot2, and MicrobiomeAnalyst R packages. Alpha diversity was measured using the number of observed taxa and the ACE, Simpson, and Shannon indices. Differences were tested with permutational multivariate analysis of variance (PERMANOVA). For significant differences between bacterial communities (i.e., beta diversity), principal coordinate analysis (PCoA) based on the Bray-Curtis dissimilarity metric was used to determine the clustering of samples and to better understand similarities and differences between the bacterial communities. A multivariate analysis of similarities (ANOSIM) was performed to test for significant differences between women with PCOS and controls. A Venn diagram of the number of differentially expressed genes was generated using InteractiVenn. Linear discriminant analysis (LDA) effect size was used to identify differentially abundant OTUs between women with PCOS and controls [42]—briefly, the algorithm performs a nonparametric factorial Kruskal-Wallis sum-rank test and LDA to determine significantly different features among taxa and then estimates the effect size of each difference, where a logarithmic LDA score threshold of ±1.5 indicated significant differences at a *P*-value < 0.05. A Mantel test based on Bray-Curtis distance with 999 permutations was applied to assess correlations between gut microbiota composition, dietary factors, and gut-derived metabolites in women with PCOS. OTU sequences and abundances were used in PICRUSt2 to predict metabolic pathways [43]. The MetaCyc database was used for the functional annotation of PICRUSt2 predictions. LDA effect size was used to determine differentially abundant pathways, where a logarithmic LDA score threshold of ±3.0 indicated significant differences at a *P*-value < 0.05. We selected these thresholds based on analyses of existing literature and preliminary findings indicating that these values effectively distinguished biologically meaningful differences in our dataset. The ±1.5 threshold for OTUs aligns with previous studies demonstrating its efficacy in identifying significant changes in microbial taxa, while the ±3.0 threshold for pathways was chosen to ensure that we would capture more pronounced effects, which are critical for understanding the underlying biological/metabolic processes.

Results

Characteristics

Of 61 women with PCOS invited to participate in the study, 28 met the inclusion criteria. Four participants dropped out because they could not commit to blood collection or DXA. Of 18 aged-matched women without PCOS enrolled as controls, 4 dropped out because they could not commit to blood collection or DXA. Therefore, the final study sample included 24 women with PCOS and 14 age-matched healthy women without PCOS.

Table 1 shows the characteristics of the participants. Women who self-identified as White accounted for 88% of the PCOS group and 72% of the control group; the remaining participants were of mixed African or European ancestry. Women with PCOS and controls were age-matched and did not differ significantly in their level of education or habitual physical activity. As expected, women with PCOS had higher BMI, waist circumference (WC), body fat, HOMA-IR, hsCRP, and total testosterone levels than controls. In the PCOS group, 1 woman (4%) was using glucose-lowering medication (metformin), and 10 women (42%) were using oral contraceptive pills (OCPs). Women with PCOS who were using OCPs were not different from those who were not using OCPs at the time of data collection in terms of their characteristics and concentrations of gut-derived metabolites (Student's *t*-test, data not shown).

Energy intake was similar in both groups. However, women with PCOS had higher daily dietary GI and saturated fatty acid (SFA) intake than controls. Analysis by food groups showed that women with PCOS had a lower intake of whole-grain cereals and a higher intake of processed meat than controls (Table 1).

Microbiota diversity and composition

Gut microbiota analysis included 31 participants (21 women with PCOS and 10 controls). Two women with PCOS were unable to provide their fecal samples; another participant with PCOS and 4 healthy controls were excluded from gut microbiota analysis due to our failure to amplify and sequence their fecal DNA. The participant using glucose-lowering medication was among those excluded.

Table 1 Clinical characteristics and dietary intake in women with PCOS and controls ($n=38$)

Variable	PCOS ($n=24$)	Controls ($n=14$)	*P*-value [a]
Age, years	32.0 ± 7.8	35.5 ± 6.7	0.170
White, % (n)	88 (21)	72 (10)	0.549
Education			
Elementary, % (n)	8 (2)	0 (0)	0.455
Secondary, % (n)	29 (7)	21 (3)	
Higher, % (n)	63 (15)	79 (11)	
Mean steps per day	5265 ± 2567	5509 ± 2256	0.769
BMI, kg/m^2	**31.1 ± 4.2**	**26.7 ± 4.6**	**0.004**
Waist circumference, cm	**91.9 ± 9.8**	**80.9 ± 9.6**	**0.002**
Body fat, %	**46.3 ± 5.4**	**39.4 ± 9.3**	**0.022**
Trunk fat, %	**19.2 ± 5.0**	**12.9 ± 5.0**	**0.001**
Lean body mass, kg	40.5 ± 7.2	39.5 ± 4.7	0.668
Fasting glucose, mmol/L	4.9 ± 0.5	4.7 ± 0.4	0.110
HOMA-IR [b]	**2.5 (1.6–3.7)**	**1.2 (0.8–1.9)**	**0.008**
hsCRP, mg/L [b]	**3.4 (2.2–8.0)**	**1.0 (0.6–1.8)**	**0.001**
Total testosterone, nmol/L	**1.4 ± 0.9**	**0.9 ± 0.3**	**0.014**
Glucose-lowering medication use, % (n)	4 (1)	0	-
Current oral contraceptive use, % (n)	42 (10)	0	-
Dietary variables			
Kcal	1758.9 ± 806.4	1610.7 ± 327.6	0.541
Carbohydrate, %	47.6 ± 7.8	48.9 ± 7.8	0.432
Protein, %	18.3 ± 4.0	17.6 ± 3.3	0.611
Fat, %	29.1 ± 6.5	27.8 ± 4.9	0.539
SFA, %	**9.1 ± 2.3**	**7.5 ± 1.6**	**0.027**
MUFA, %	10.1 ± 3.4	9.5 ± 2.4	0.564
PUFA, %	3.9 ± 1.3	3.8 ± 0.6	0.743
Fiber, g	21.1 ± 11.5	22.6 ± 5.4	0.663
Fiber, g/kg	0.26 ± 0.14	0.34 ± 0.13	0.086
Glycemic index, %	**57.1 ± 4.7**	**53.8 ± 3.8**	**0.034**
Glycemic load, g	114.4 ± 58.7	98.3 ± 36.6	0.364
Food groups, g			
Fruits and vegetables	305.3 ± 191.0	390.6 ± 233.0	0.229
Legumes	82.6 ± 85.6	93.2 ± 25.6	0.577
Whole-grain cereals [b]	**26.8 (3.5–41.1)**	**59.3 (29.6–105.7)**	**0.008**
Refined-grain cereals [b]	132.1 (44.9–201.0)	61.2 (26.4–142.8)	0.199
Meat and eggs	162.6 ± 119.7	131.3 ± 48.3	0.360
Processed meat	**15.5 ± 14.2**	**9.2 ± 12.2**	**0.049**
Fish [b]	5.3 (0.0–12.0)	16.0 (0.0–20.0)	0.508
Dairy [b]	74.9 (17.1–170.0)	74.0 (37.6–146.1)	0.977
Sweets and desserts	72.5 ± 54.0	55.3 ± 46.2	0.327

PCOS, polycystic ovary syndrome; BMI, body mass index; HOMA-IR, homeostasis model assessment of insulin resistance; hsCRP, high-sensitivity C-reactive protein; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid

Data are shown as mean ± SD, median (IQR), or % as indicated

[a] Independent samples *t*-test

[b] Log-transformed variables

Statistically significant differences are in bold

Alpha diversity (number of observed taxa) was significantly lower ($P=0.04$, ACE $P=0.03$, and Simpson $P=0.04$) in women with PCOS than in controls, except for the Shannon index ($P=0.06$) (Fig. 1A). Beta diversity assessed by PCoA showed cluster differentiation between the PCOS and control groups (ANOSIM $P=0.04$, Fig. 1B). Women with PCOS were subdivided into OCP users ($n=7$) and non-users ($n=14$) for analysis of the potential effect of OCP use on beta diversity, but no significant difference was found between them (ANOSIM, $P>0.05$). PCOS phenotype (classic vs. ovulatory) also did not influence beta diversity (ANOSIM, $P>0.05$).

Gut microbiota composition was characterized in the 31 participants. A total of 1451 OTUs were detected, belonging to 168 genera, 61 families, and 8 phyla. The two most abundant phyla were Firmicutes (62%) and Bacteroidetes (34%), followed by Actinobacteriota and Proteobacteria (1%) (Fig. 2A). Figure 2B and C show the 10 most abundant families and genera, respectively. A total of 146 genus-level taxa were common to the PCOS and control groups. Eight genera were unique to the control group, while 14 genera were unique to the PCOS group (Supplemental list).

Association between PCOS and gut microbiota composition

LDA showed an association between PCOS and altered gut microbiota composition, with a particularly higher abundance of two OTUs, Otu0003 (*Agathobacter*) and Otu0051 (*Lachnoclostridium*). Otu0005 (*Bacteroides*), Otu0009 (*Bacteroides*), Otu0030 (Oscillospiraceae UCG-002), and Otu0082 (Bacteroidales_unclassified) were differentially abundant in the control group (Fig. 3).

Gut-derived metabolites differed between women with PCOS and healthy controls

The IPA concentration was below or above the dynamic quantitation range in 3 samples from women with PCOS, including the only patient using glucose-lowering medication. A total of 35 samples (21 PCOS, 14 controls) were included in the final analysis. The IPA concentration was lower in women with PCOS than in healthy controls, even after controlling for BMI (Table 2).

Regarding SCFAs, as mentioned above, 2 women with PCOS were unable to provide their fecal samples. The final fecal SCFA analysis included 22 participants with PCOS and 14 healthy controls, while the plasma SCFA analysis included the entire sample of 24 women with PCOS and 14 controls. Participants with PCOS had higher propionic and butyric acid levels in fecal samples. Plasma levels of acetic, propionic, isobutyric, and formic acids were significantly higher in women with PCOS. However, women with PCOS had lower plasma levels of valeric acid than controls. After adjusting for BMI, the

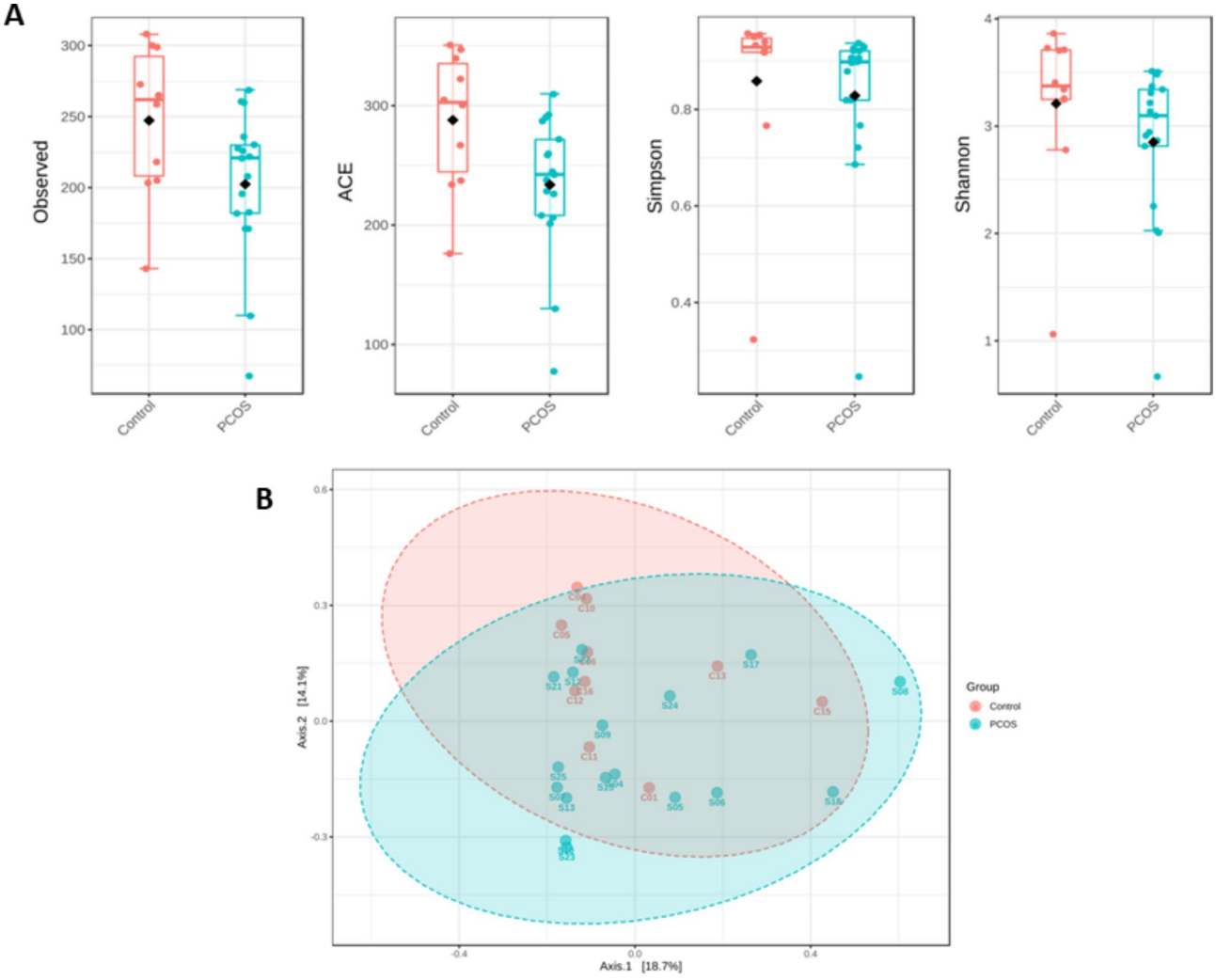

Fig. 1 (**A**) Alpha diversity analysis: women with PCOS ($n=21$) vs. healthy controls ($n=10$); statistical confidence for the sample grouping was assessed using permutational multivariate analysis of variance (PERMANOVA); Observed ($P=0.04$), ACE ($P=0.03$), Simpson ($P=0.04$), and Shannon ($P=0.06$). (**B**) Principal coordinate analysis (PCoA) of bacterial communities based on Bray-Curtis distance, comparison between women with PCOS ($n=21$) and healthy controls ($n=10$) ($P=0.04$ by analysis of similarities, ANOSIM)

levels of acetic, propionic, isobutyric, and valeric acids remained different between the two groups.

Across all participants, dietary intake was associated with gut-derived metabolites. Specifically, dietary GI accounted for 12% of the variation in IPA and 14% of the variation in valeric acid. Percentage SFA intake accounted for 11% and 12% of the variation in acetic and propionic acids, respectively (Supplemental Table 1).

Subsequently, multivariable-adjusted linear regression models were used to test whether the association of the diagnosis of PCOS with circulating levels of IPA, propionic acid, isobutyric acid, and valeric acid was independent of dietary intake. PCOS diagnosis was associated with a lower IPA concentration independently of dietary GI, whereas higher plasma levels of acetic and propionic acids were associated with PCOS diagnosis independently of SFA intake and glucose-lowering medication use (Table 3).

Associations of gut microbiota with gut-derived metabolites and diet

Overall gut microbiota composition was assessed considering only participants with PCOS. Fecal propionic acid levels correlated significantly with gut microbiota composition ($r=0.479$, $P=0.01$ in Mantel test). No correlation was found between gut microbiota composition and the fecal and plasma levels of other SCFAs or serum levels of IPA. Regarding dietary intake, a significant correlation was observed between gut microbiota composition and GL ($r=0.314$, $P=0.03$ in Mantel test). Other clinical and hormonal variables did not correlate with gut microbiota composition in women with PCOS.

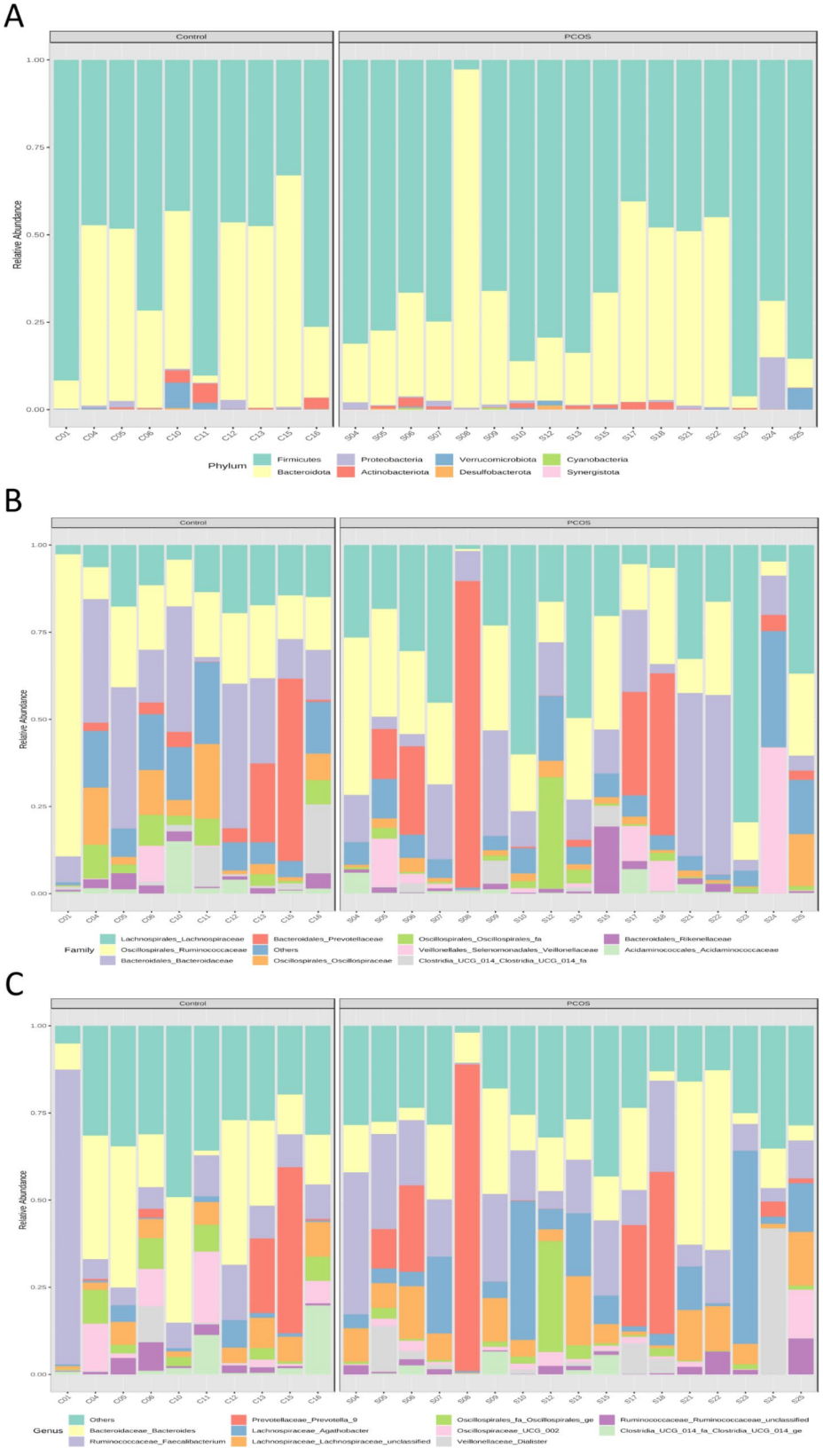

Fig. 2 Relative abundance of the 10 most abundant (**A**) phyla, (**B**) families, and (**C**) genera in women with PCOS ($n=21$) and healthy controls ($n=10$)

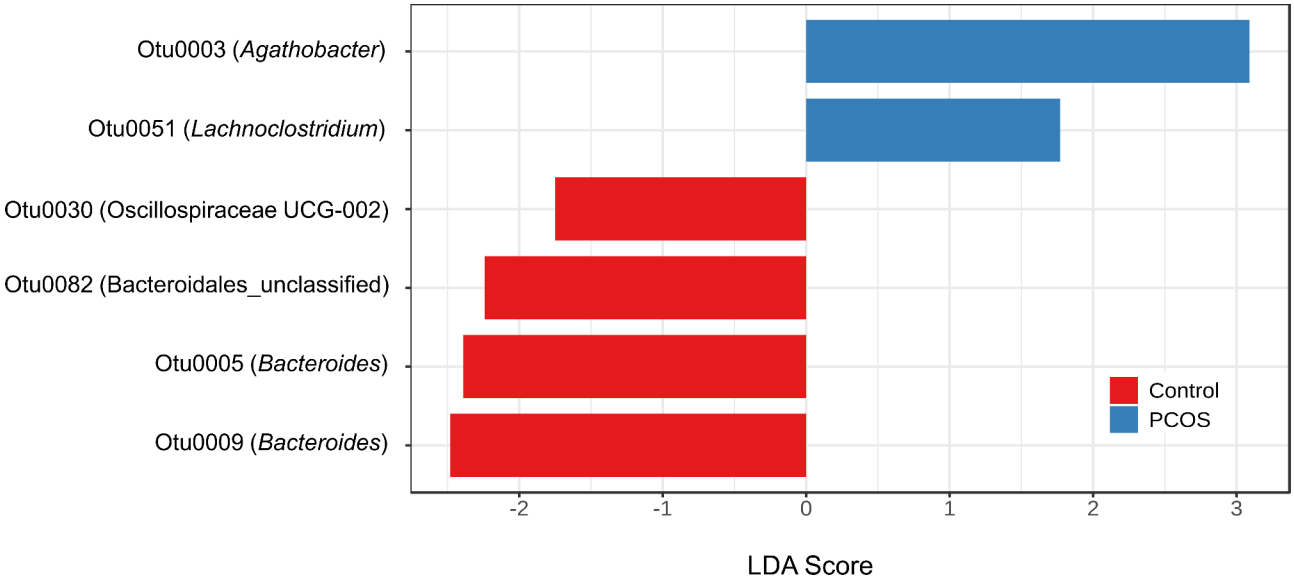

Fig. 3 Linear discriminant analysis (LDA) effect size (LEfSe) at the OTU level between women with PCOS ($n=21$) and healthy controls ($n=10$). Statistical confidence was assessed using the Kruskal-Wallis test. Taxa were ranked by the LDA score, where a logarithmic LDA score threshold of ±1.5 indicated significant differences at $P<0.05$

Table 2 Levels of gut-derived metabolites in women with PCOS and controls

Variable	PCOS ($n=21$)	Controls ($n=14$)	*P*-value [a]	*P*-value [b]
IPA, ng/mL	**77.5 ± 35.3**	**129.5 ± 57.0**	**0.002**	**0.022**
Fecal SCFA	($n=22$)	($n=14$)		
Acetic acid, µm/g	159.8 ± 85.5	114.1 ± 88.8	0.133	
Propionic acid, µm/g	**58.7 ± 37.7**	**36.5 ± 30.7**	**0.021**	0.134
Butyric acid, µm/g	**44.8 ± 25.7**	**26.9 ± 23.8**	**0.044**	0.065
Isobutyric acid, µm/g	5.1 ± 2.1	4.6 ± 1.8	0.490	
Valeric acid, µm/g	10.0 ± 6.4	7.2 ± 4.2	0.167	
Isovaleric acid, µm/g	4.6 ± 2.3	4.1 ± 2.0	0.487	
Caproic acid, µm/g	3.5 ± 3.6	2.3 ± 3.2	0.637	
Formic acid, µm/g	2.4 ± 2.4	1.8 ± 1.7	0.436	
Succinic acid, µm/g	10.3 ± 29.5	8.3 ± 26.4	0.296	
Plasma SCFA	($n=24$)	($n=14$)		
Acetic acid, µM	**76.2 ± 28.2**	**46.2 ± 20.9**	**0.001**	**0.001**
Propionic acid, µM	**3.9 ± 1.3**	**2.5 ± 0.7**	**< 0.001**	**< 0.001**
Butyric acid, µM	0.2 ± 0.1	0.2 ± 0.2	0.470	
Isobutyric acid, µM	**2.6 ± 0.7**	**2.1 ± 0.5**	**0.024**	**0.022**
Valeric acid, µM	**0.1 ± 0.1**	**0.2 ± 0.1**	**0.026**	**0.044**
Isovaleric acid, µM	1.2 ± 0.5	1.1 ± 0.4	0.438	
Caproic acid, µM [c]	0.2 ± 0.3	0.2 ± 0.1	0.879	
Formic acid, µM	**146.4 ± 61.1**	**60.8 ± 17.3**	**< 0.001**	0.842
Succinic acid, µM [c]	2.4 ± 0.7	2.4 ± 0.6	0.847	

PCOS, polycystic ovary syndrome; IPA, indole-3-propionic acid; SCFA, short-chain fatty acid

Data are shown as mean ± SD, median (IQR), or % as indicated

[a] Independent samples *t*-test

[b] UNIANOVA adjusted for body mass index

[c] Log-transformed variables

Statistically significant differences are in bold

Gut microbiota metabolic prediction

A total of 22 differentially abundant metabolic pathways were identified in the PCOS group compared to the control group (Supplemental Fig. 1). These pathways were mainly related to carbohydrate and purine metabolism, lipid biosynthesis, vitamin B12 and S-adenosyl-L-methionine metabolism, survival of *Escherichia coli*, and pathogenicity.

Discussion

In the present study, gut microbiota diversity, composition, and metabolic pathways differed between women with PCOS and healthy controls. LDA revealed a higher abundance of two OTUs specializing in complex carbohydrate metabolism in healthy women. The PCOS group also exhibited a less favorable dietary intake than the control group, and a significant correlation was observed between gut microbiota composition and dietary GL in PCOS. Multivariable-adjusted linear regression models indicated that lower levels of IPA and higher circulating levels of two SCFAs (acetic acid and propionic acid) were independently associated with the diagnosis of PCOS.

The major finding of this study was the presence of gut microbiota alterations in women with PCOS, which could be explained by the differences in dietary intake between them and healthy controls. This is in line with two earlier reports of PCOS-like mice and women with PCOS demonstrating that diet impacts the gut microbiota [44, 45]. However, to the best of our knowledge, this is the first study to observe an association between dietary GL and gut microbiota composition. These results suggest that diet plays a more significant role in shaping the

Table 3 Linear regression analysis of PCOS diagnosis associated with gut-derived metabolites (IPA and SCFA)

Metabolites (dependent variable)	Linear regression Association of the diagnosis of PCOS (yes vs. no)					
	Univariate model			Multivariable-adjusted model		
	Adjusted R^2	β	*P*-value	Adjusted R^2	β	*P*-value
IPA, ng/mL	**0.228**	**-0.478**	**0.004**	**0.247**	**-0.417**	**0.015**[a]
Plasma SCFA						
Acetic acid, µmol/g	**0.228**	**0.499**	**0.001**	**0.285**	**0.434**	**0.010**[b]
Propionic acid, µmol/g	**0.268**	**0.536**	**0.001**	**0.318**	**0.471**	**0.005**[b]
Isobutyric acid, µmol/g	**0.109**	**0.365**	**0.024**	0.161	0.254	0.128 [b]
Valeric acid, µmol/g	**0.130**	**-0.399**	**0.026**	0.187	-0.281	0.127 [a]

PCOS, polycystic ovary syndrome; IPA, indole-3-propionic acid; SCFA, short-chain fatty acid

[a] Adjusted for dietary glycemic index

[b] Adjusted for percentage saturated fat intake and glucose-lowering medication use

Bold indicates statistical significance

gut microbiota in women with PCOS than other clinical and hormonal characteristics associated with the syndrome. Our previous research showed that dietary GI is significantly elevated in the classic PCOS phenotype and correlates with a less favorable anthropometric and metabolic profile [26]. Additionally, compared with high GI/GL or regular diets, dietary interventions with a low GL/GI diet for at least 8 weeks have been shown to significantly reduce WC and BMI and improve insulin sensitivity and reproductive hormones (total testosterone and sex-hormone binding globulin), contributing to improvements in reproductive function and in risk factors for type 2 diabetes and cardiovascular disease in women with PCOS [8]. Accordingly, our results add to a growing body of evidence indicating that alterations in the gut microbiota could be, at least in part, the biological mechanism by which a low GI/GL diet may improve PCOS-related reproductive and cardiometabolic outcomes.

Recent clinical evidence suggests that a decreased alpha diversity and an altered composition of the gut microbiota are associated with PCOS [46–49], an association that might be causal in PCOS development [50, 51]. At the genus level, our results showed that Otu0051 (*Lachnoclostridium)* was an important characteristic OTU in the PCOS group compared with the control group, in line with Zhou et al. [52]. Interestingly, in both studies, obesity was a common feature among women with PCOS. *Lachnoclostridium*, a trimethylamine-producing genus, has been recently reported for the first time to be significantly abundant in individuals with atherosclerosis [53]. In this sense, *Lachnoclostridium* might be a potential link between the diagnosis of PCOS and the increased risk of cardiovascular disease observed in women with PCOS and obesity [54, 55]. Otu0003 (*Agathobacter*) was also differentially abundant in the PCOS group; however, this OTU has not been associated with PCOS previously. In contrast, Otu0005 (*Bacteroides*), Otu0009 (*Bacteroides*), Otu0030 (Oscillospiraceae UCG-002), and Otu0082 (Bacteroidales_unclassified) were more abundant in healthy controls. *Bacteroides* play a key role in breaking down complex carbohydrates, and imbalances in the composition of *Bacteroides* in the gut microbiota have been associated with increased glucose levels [56, 57] and hypertension in a Chinese population [58]. Additionally, there is consistent evidence of reduced abundance of the family Oscillospiraceae in the presence of elevated insulin resistance [15]. The different microbial pathways related to carbohydrate metabolism and lipid biosynthesis are another interesting feature of our participants with PCOS, which may be linked to dietary intake. Taken together, our data support the differentiation between women with PCOS and healthy controls in terms of the gut microbiota. Considering that our specific study sample of women with PCOS had a higher BMI than the control group, this may have influenced our results. Indeed, a previous report showed a lack of significant gut microbiota alterations in healthy-weight women with PCOS compared with controls [59], highlighting the heterogeneity of the condition and the importance of considering weight status when studying the gut microbiota. However, in our participants with PCOS, no correlation was found between BMI and overall gut microbiota composition when applying the Mantel test. Recently, Wang et al. [60] proposed that the gut microbiota is likely not an independent cause of PCOS but may influence the progression of the condition through obesity and related mediators. Thus, interactions among obesity, hormones, and diet could impact gut microbiota composition in women with PCOS and warrant further investigation.

Another important finding of the present study is that not only was gut microbiota composition altered in women with PCOS, but the functionality of the gut microbiota, partially mediated by IPA and SCFAs, was different between the PCOS and control groups, even after adjusting for BMI, which suggests a potential role for these metabolites in the pathogenesis of PCOS. The

lower levels of the antioxidant IPA observed in women with PCOS are consistent with previous reports in other metabolic conditions, such as obesity [18, 19], type 2 diabetes [20], and atherosclerotic cardiovascular disease [21]. The Twins UK cohort demonstrated that microbiota diversity was associated with IPA, and microbiota composition explained approximately 20% of the variation in IPA concentration, with only 4% being explained by nutritional and host genetic parameters [61]. Our study did not detect a significant correlation between IPA and overall gut microbiota composition in women with PCOS, and we were unable to identify the specific gut bacterial strains responsible for IPA production, as this would require shotgun metagenomic analysis. However, since our primary focus was on bacterial diversity and taxonomic classification, 16 S rRNA gene sequencing was the most cost-effective option. Therefore, dietary intake could be affecting IPA production by the gut microbiota in our study participants, especially the type of carbohydrate in the diet [62, 63]. Indeed, dietary GI accounted for 12% of the variation in IPA across all participants. We hypothesized that a high GI diet with a low intake of whole-grain cereals, as observed in women with PCOS, could impact the gut microbiota toward a reduced IPA production. A recent review suggests that IPA levels depend heavily on the diet, particularly on dietary fiber, and can directly affect glucose uptake and metabolism [64]. In our study, we observed a negative correlation between IPA and glucose levels (data not shown). Taken together, our findings indicate that dietary GI influences IPA production, which may subsequently impact glucose metabolism in women with PCOS. To establish causality, however, clinical trials of dietary interventions are needed. In addition, although BMI was adjusted for as a confounder, it cannot entirely rule out the effect of obesity on the lower IPA levels observed in our sample of women with PCOS compared with healthy controls.

Regarding SCFAs, which are proposed to underlie observed relationships between the gut microbiota and glucose metabolism in humans [15], the diagnosis of PCOS accounted for a significant proportion of their variation in our study, especially in plasma levels of acetic and propionic acids. Plasma levels reflect absorption from the gut as well as synthesis by the host. Acetic acid has a more pronounced production from pyruvate in contexts of nutritional excess, such as during hyperactive glucose metabolism, which is often observed in women with PCOS [65]. Increased plasma levels of acetic acid in our sample of women with PCOS may be primarily driven by the interplay between obesity, insulin resistance, and dietary intake rather than by the gut microbiota profile itself, as no significant correlations were found between plasma SCFAs and gut microbiota composition. Supporting this, weight loss induced by caloric restriction has been shown to lead to decreased plasma acetic acid levels [66]. Interestingly, Zhang et al. [67] demonstrated that women with PCOS also had elevated acetic acid levels in follicular fluid. Previous reports have demonstrated that host genetics can influence plasma propionic acid levels [68]. While research has primarily focused on the impact of the intake of whole-grain cereals on SCFA gut production [69], to the best of our knowledge, this is the first study to report an association between increased SFA intake and elevated plasma levels of acetic and propionic acids. This association could have implications for cardiovascular health, as increased SFA intake has been linked to decreased cardiovascular autonomic function in women with PCOS [70]. Nevertheless, this result should be interpreted with caution, as correlation does not imply causation.

Our participants with PCOS also had higher levels of fecal propionic acid than healthy controls, which is consistent with previous reports in Chinese women with PCOS [41, 71], and fecal propionic acid levels were significantly correlated with overall gut microbiota composition in women with PCOS. Recent clinical evidence suggests that the gut microbiota can accurately predict fecal SCFA levels while presenting weaker associations with circulating SCFAs [68]. Previous cross-sectional studies have reported higher fecal SCFA levels in individuals with overweight and obesity than in individuals with normal weight [72, 73], as well as a higher percentage of propionic acid in total SCFA concentration in the fecal samples of participants with obesity than in those of lean participants [74]. Interestingly, experimental evidence shows that genetically obese mice, compared with their lean littermates, have a gut microbiota composition that promotes obesity through excessive SCFA production and increased energy availability [75]. In the context of PCOS, these findings suggest that alterations in gut microbiota composition and propionic acid production may contribute to the weight gain typically observed in this population, which requires further investigation.

Strengths of the present study include a well-characterized sample of women with PCOS, with body composition measurements and dietary recall, and the inclusion of age-matched healthy controls. Furthermore, to our knowledge, this is the first study to assess target concentrations of the circulating gut-derived metabolites SCFAs and IPA in women with PCOS.

Limitations of our study include its observational design and data collected from a relatively limited geographic area, which impacts the generalizability of the results. Different geographic locations often have distinct environmental factors, including diet, climate, sanitation, and pathogen exposure, all of which can influence gut microbiota composition [76]. However, to date, most studies investigating the gut microbiota in women with

PCOS have been conducted in the United States, China, and Europe. To the best of our knowledge, this is the first study investigating women with PCOS from South America, which may contribute to extending the research to more diverse populations. Serum testosterone levels, although significantly different between the PCOS and control groups, were assessed by a low-accuracy method. Employing more accurate hormone assays, such as LC-MS, may be of great interest for future studies, as this can potentially improve data quality and lead to more reliable insights for gut microbiota research. Another limitation is the small sample size calculated for the fecal propionic acid outcome, which limits the ability to draw robust conclusions regarding the other outcomes. Further analyses, such as subgroup analysis stratified by BMI categories or PCOS phenotypes, were not conducted due to this limited sample size. Nevertheless, our sample size is consistent with that of prior studies assessing the gut microbiota in women with PCOS [47, 48, 77]. Additionally, it is important to note that many women with PCOS have underlying mechanisms that contribute to greater longitudinal weight gain and higher BMI, which posed challenges in recruiting women with a normal BMI for our study. As such, generalizing our findings to women with PCOS and normal BMI is not feasible. Therefore, larger-scale studies that specifically target women with PCOS and normal BMI are necessary to replicate and clarify the present results.

Conclusion

Our findings showed that gut microbiota diversity and composition as well as circulating levels of IPA, acetic acid, and propionic acid differed between women with PCOS and healthy controls. Lifestyle management is the first-line treatment for improving complications in women with PCOS. Clinically, it should be recognized that women with PCOS could benefit from adhering to a low GI/GL diet through its effects on gut microbiota composition and the antioxidant metabolite IPA. However, further clinical trials are warranted to investigate this dietary approach comprehensively.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13048-024-01550-w.

Supplementary Material 1

Acknowledgements

We are grateful to the women who participated in our study and gave generously of their time and commitment. We thank Sheila Bunecker Lecke for her support with IPA data analysis.

Author contributions

TRS contributed to the conception and design of the study, analyzed the data, interpreted the results, and drafted the manuscript. LBM collected and analyzed the clinical data and revised the manuscript. PHR and LL analyzed and interpreted the gut microbiome data. TRF analyzed the IPA data. RL analyzed the SCFA data and critically revised the manuscript for important intellectual content. VDM contributed to data analysis and critically revised the manuscript for important intellectual content. PMS conceived and designed the study, interpreted the results, and revised the manuscript. All authors have read, approved the final version, and agreed to the submission of the manuscript.

Funding

TRS is a recipient of a fellowship from FAPERGS - the Fundação de Apoio à Pesquisa do Estado do Rio Grande do Sul (FAPERGS/ Brazilian National Institute of Hormones and Women's Health/Conselho Nacional de Desenvolvimento Científico e Tecnológico (FAPERGS INCT 17/2551-0000519-8). This research was also funded by the Brazilian National Institute of Hormones and Women's Health/Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq INCT 465482/2014-7).

Data availability

No datasets were generated or analysed during the current study.

Declarations

Consent for publication

Consent forms are available from the corresponding author on request.

Competing interests

The authors declare no competing interests.

Human Ethics and Consent to Participate

Each study participant provided written informed consent before enrollment. The study was approved by the HCPA Ethics Committee (CAAE 35025414.1.0000.5327) and conducted in accordance with the Helsinki Declaration.

Clinical trial number

Not applicable.

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Received: 27 June 2024 / Accepted: 31 October 2024
Published online: 22 November 2024

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