Gut Microbiota and the Polycystic Ovary Syndrome: Influence of Sex, Sex Hormones, and Obesity

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Context: Gut microbiota play a major role in health and disease by influencing physiology, metabolism, nutrition, and immune function.

Objective: To evaluate the composition of gut microbiota in women with polycystic ovary syndrome (PCOS), focusing on the influence of sex, sex hormones and obesity on the associations found.

Design: Cross-sectional study.

Setting: Academic hospital.

Participants: We recruited 15 women with PCOS, 16 nonhyperandrogenic control women, and 15 control men. Participants were classified as nonobese (<30 kg/m^2) or obese (≥ 30 kg/m^2) according to their body mass index.

Interventions: Standardization of diet for 3 consecutive days (at least 300 g of carbohydrates per day) followed by fecal sampling and a standard 75-g oral glucose tolerance test.

Main Outcome Measures: Analysis of bacterial abundance and composition of gut microbiota by massive sequencing of 16S ribosomal DNA amplicons in a MiSeq platform (Illumina).

Results: α Bacterial diversity was reduced in women compared with men, and β diversity was reduced particularly in obese patients with PCOS. Women with PCOS presented with specific abnormalities in gut microbiota consisting of an increased abundance of the *Catenibacterium* and *Kandleria* genera. When all participants as a whole were considered, indexes of bacterial diversity and the abundance of several bacterial genera correlated positively with serum androgen concentrations and negatively with estradiol levels.

Conclusions: The diversity and composition of the gut microbiota of young adults are influenced by the combined effects of sex, sex hormone concentrations, and obesity, presenting with specific abnormalities in women with PCOS. ***(J Clin Endocrinol Metab*** **103: 2552–2562, 2018)**

Polycystic ovary syndrome (PCOS) is now considered a complex multigenic disorder with strong environmental influences (1). Several metabolic disorders are associated with PCOS, including insulin resistance, diabetes, and obesity (2). The latter exerts a major effect on the metabolic associations and complications of the syndrome (3). Among other factors, obesity is clearly related to the infertility of PCOS and increases the risk for

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Abbreviations: BMI, body mass index; DHEAS, dehydroepiandrosterone-sulfate; HOMA-IR, homeostasis model assessment of insulin resistance; PCOS, polycystic ovary syndrome; rDNA, ribosomal DNA.

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metabolic syndrome and the clustering of cardiovascular risk factors in these women (4, 5).

During the last two decades, several studies revealed the major influence of gut microbiota on physiology, metabolism, nutrition, and immune function (6). Germ-free mice—which therefore lack gut microbiota—have reduced adiposity and improved tolerance to glucose and insulin when compared with colonized counterparts (7). Moreover, fecal transplantation of the obese gut microbiome into germ-free mice promotes increase in adiposity, resulting in obese phenotype (8). Moreover, several studies have reported the gut microbiota particularities related to not just obesity but also diabetes and the metabolic syndrome (9).

The possible influence of host sex and/or sex steroids on the composition of gut microbiota remains unclear at present (10–13). The current study aims to evaluate the composition of gut microbiota in women with PCOS, focusing on the possible influence of sex, sex hormones, obesity, and their interactions, on any putative association.

Materials and Methods

Participants

A total of 46 young adult volunteers (15 patients with PCOS, 16 nonhyperandrogenic women, and 15 healthy men) were recruited. Participants had similar mean body mass index (BMI) and age. They were classified as nonobese (<30 kg/m^2) or obese (≥ 30 kg/m^2) according to their BMI. The diagnosis of PCOS was based on the presence of clinical and/or biochemical hyperandrogenism, oligo-ovulation, and exclusion of secondary causes (14, 15). Before inclusion, the participants had no history of obesity-associated comorbidities, such as impaired glucose tolerance, hypertension, cardiovascular events, or sleep apnea. Smokers were excluded. None of the individuals had received treatment with oral contraceptives, antiandrogens, lipid-lowering or blood pressure–lowering drugs, or insulin sensitizers within the previous 3 months. None of the women in the control group had signs or symptoms of hyperandrogenism, history of menstrual dysfunction, or infertility. Men with androgen deficiency, as defined by a serum total testosterone concentration <8 nmol/L, were excluded.

The Ethics Committee of Hospital Universitario Ramón y Cajal approved the study protocol, and all participants gave written informed consent.

Study design

All individuals underwent a comprehensive clinical, anthropometric, and physical evaluation. Participants were advised to follow a diet with 300 g of carbohydrate per day for 3 days before sampling to avoid false-positive results on the 75-g oral glucose load performed thereafter; the latter test was used not only for research purposes but also to check patients for disorders of glucose tolerance. In women, the study was performed during days 3 to 10 of the follicular phase of the menstrual cycle or in amenorrhea after exclusion of pregnancy. Occurrence of infectious disease, including vaginal candidiasis and periodontal disease, during the previous 3 months and recent use of antibiotics were recorded.

Laboratory measurements

Baseline blood samples were collected for hormonal and metabolic measurements. Plasma and serum aliquots were frozen at -80°C until use. Technical characteristics of the assays used for laboratory measurements have been described previously (16). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated from fasting glucose and insulin concentrations (17), and the composite insulin sensitivity index was estimated from the glucose and insulin concentrations measured during the oral glucose tolerance test (18).

DNA extraction from fecal sample and next-generation sequencing

Patients were instructed to collect fecal samples into sterile plastic bottle topped with a screw cap. The samples were immediately divided into aliquots and stored at -80°C after collection until analysis was performed. Samples were defrosted slowly at 4°C to avoid DNA degradation and further thawed at room temperature. An aliquot of 0.5 g of feces was solubilized in 5 mL of distilled water and centrifuged at 157*g* and 4°C for 10 minutes to eliminate undigested fiber. Total DNA from 1 mL of the supernatant was obtained with the QIAamp DNA Mini KitPellet commercial kit (Qiagen, Hilden, Germany).

After DNA samples were checked for quality and concentration, they were sent to Fundacio per al Foment de la Investigacio i Biomedica (FISABIO, Valencia, Spain) for massive sequencing of 16S ribosomal DNA (rDNA) amplicons in a MiSeq platform (Illumina). Bioinformatics analysis of the readings was conducted at FISABIO, and only sequences longer than 200 nucleotides were considered. The bacterial diversity was estimated by using Shannon, Chao 1, Jaccard, and Sorensen indexes with their SDs. Taxonomic affiliations were assigned by using the RDP_classifier from the Ribosomal Database Project (RDP), and readings with RDP score value <0.8 were assigned to the upper taxonomic rank, leaving the last rank as unidentified.

Statistical analysis

Data are expressed as mean $\pm$ SD unless otherwise stated. We tested the normality of distribution by using the Kolmogorov-Smirnov test. Two-step transformations were applied as needed to ensure normal distribution of data (19). General linear models were used to determine the influence of group and obesity on hormonal, metabolic, and inflammatory variables and indexes of α and β diversity. The differences between the groups of participants in bacterial composition were evaluated by the Kruskal-Wallis test followed by Dunn nonparametric *post hoc* test (20, 21). The comparisons between nonobese and obese subgroups on bacterial composition and abundance were evaluated by Mann-Whitney U test. The interaction between groups and obesity were evaluated, in each subgroup of participants separately, by a Mann-Whitney U test. We applied a Bonferroni correction to the level of statistical significance to take into account multiple comparisons ($P <$ 0.0125 was considered to indicate a statistically significant difference). Categorical variables were analyzed by a χ^2 or Fisher exact test as appropriate. Relationships between described genera and clinical and biochemical variables were

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analyzed by Spearman correlation analysis. We used SPSS statistical software, version 18.0 (IBM, Chicago, IL) and PAST for statistical analyses of biodiversity. A P value <0.05 was considered to indicate statistical significance with the exception mentioned previously.

Results

Clinical, hormonal, and metabolic variables in patients with PCOS and controls

The clinical, hormonal, and metabolic data are summarized in Table 1. Age and BMI did not differ between groups. The hirsutism score was higher in patients with PCOS than in control women (hirsutism score was not assessed in men). Besides their higher total testosterone, free testosterone and dehydroepiandrosterone-sulfate (DHEAS) levels, men also had higher waist-to-hip ratio and ratio of free testosterone to free estradiol than did control women, who, in contrast, had higher SHBG and free estradiol than did men. Women with PCOS had increased concentrations of free testosterone, ratio of free testosterone to free estradiol, and androstenedione compared with control women. In addition, women with PCOS presented lower levels of total and free testosterone and ratio of free testosterone to free estradiol, but higher SHBG and androstenedione levels, than did men.

Regarding metabolic parameters, high-density lipoprotein cholesterol levels were higher in female controls and women with PCOS than in men. Fasting glucose levels were reduced in patients with PCOS compared with controls. Total cholesterol, low-density lipoprotein cholesterol, triglycerides, insulin sensitivity index,

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Table 1. Clinical, Hormonal, and Metabolic Characteristics of Participants

	Control Women		Women With PCOS		Men				
Characteristic	Nonobese (n = 8)	Obese (n = 8)	Nonobese (n = 7)	Obese (n = 8)	Nonobese (n = 8)	Obese (n = 7)	Group *P* Value	Obesity *P* Value	Interaction *P* Value
Age, y	27.3 ± 4.0	27.3 ± 6.5	23.0 ± 7.9	29.9 ± 5.1	23.3 ± 3.3	23.6 ± 3.2	0.116	0.129	0.138
BMI, kg/m^2	23.4 ± 1.9	35.9 ± 4.2	24.4 ± 2.8	37.0 ± 4.8	22.9 ± 2.0	34.6 ± 3.4	0.309	<0.001	0.888
Waist-to-hip ratio[a,b]	0.74 ± 0.1	0.83 ± 0.12	0.74 ± 0.04	0.85 ± 0.1	0.84 ± 0.04	0.9 ± 0.1	0.005	<0.001	0.542
Hirsutism score	1.3 ± 1.3	1.8 ± 1.2	10.1 ± 5.1	9.3 ± 4.5	NA		<0.001	0.887	0.589
Total testosterone, nmol/L[a,b]	1.6 ± 0.3	2.0 ± 0.5	2.5 0.7	2.4 1.0	19.2 ± 3.4	16.6 ± 2.8	<0.001	0.993	0.134
FT, pmol/L[a,b]	20 ± 5	31 ± 8	38 ± 10	45 ± 24	462 ± 106	464 ± 81	<0.001	0.012	0.022
Total estradiol, pmol/L[a,b]	153 ± 66	276 ± 200	188 ± 230	149 ± 49	64 ± 13	95.8 ± 25.3	<0.001	0.024	0.598
FE, pmol/L[a]	2.7 ± 1.2	5.3 ± 3	3.9 ± 4.9	3.5 ± 1.4	1.7 ± 0.5	2.7 ± 0.7	0.027	0.003	0.549
Ratio FT/FE[a,b,c]	8.3 ± 2.6	7.5 ± 4.6	19 ± 12.7	14 ± 5.4	286.2 ± 60.4	179.0 ± 39.3	<0.001	0.087	0.272
SHBG, nmol/L[a,b,c]	60 ± 24	43 ± 14	44 ± 18	32 ± 13	28 ± 10	18 ± 5	<0.001	0.007	0.788
DHEAS, mmol/L[a]	5.1 ± 0.9	5.3 ± 1.5	8.5 ± 3.9	5.7 ± 2.1	6.5 ± 1.8	8.5 ± 3.2	0.025	0.798	0.027
Androstenedione, nmol/L[b,c]	9.1 ± 3.1	9.4 ± 2.8	16.1 ± 3.5	13.3 ± 5.9	7.3 ± 1.8	9.8 ± 4.2	<0.001	0.992	0.175
Total cholesterol, mmol/L	4.5 ± 0.9	4.7 ± 1.2	4.4 ± 1.2	4.4 ± 1.0	3.9 ± 0.6	4.61 ± 0.75	0.635	0.263	0.607
LDL-cholesterol, mmol/L	2.8 ± 0.7	2.8 ± 0.4	2.6 ± 1.0	2.6 ± 1.	2.4 ± 0.5	3.1 ± 0.6	0.811	0.263	0.291
HDL-cholesterol, mmol/L[a,b]	1.3 ± 0.3	1.2 ± 0.2	1.4 ± 0.2	1.2 ± 0.2	1.2 ± 0.2	1.0 ± 0.1	0.011	0.020	0.619
Triglycerides, mmol/L	0.8 ± 0.2	0.9 ± 0.4	0.8 ± 0.2	1.2 ± 0.6	0.9 ± 0.3	1.2 ± 0.6	0.423	0.055	0.755
Fasting glucose, mmol/L[b,c]	4.7 ± 0.3	5.6 ± 0.4	4.8 ± 0.4	4.9 ± 0.4	5.3 ± 0.5	5.3 ± 0.5	0.014	0.014	0.039
Fasting insulin, pmol/L	53 ± 28	92 ± 45	47 ± 35	83 ± 28	37 ± 14	76 ± 28	0.383	<0.001	0.969
HOMA-IR	1.6 ± 0.9	3.3 ± 1.6	1.5 ± 1.1	2.6 ± 0.9	1.3 ± 0.5	2.6 ± 1.0	0.348	<0.001	0.780
Insulin sensitivity index	7.0 ± 2.7	3.3 ± 1.2	8.2 ± 5.2	3.5 ± 1.4	7.6 ± 2.9	3.6 ± 1.7	0.899	<0.001	0.997
Antibiotics, n (%)	0 (0)	0 (0)	0 (0)	2 (25)	1 (12.5)	0 (0)	0.217	1.000	NA
Periodontal or dental disease, n (%)	0 (0)	1 (12.5)	0 (0)	0 (0)	0 (0)	0 (0)	0.434	1.000	NA
Urinary tract infection, n (%)	0 (0)	0 (0)	0 (0)	1 (12.5)	0 (0)	0 (0)	0.434	1.000	NA
Vaginal candidiasis or infection, n (%)	0 (0)	0 (0)	0 (0)	1 (12.5)	NA		0.396	1.000	NA

Data are means ± SD or counts (percentage). For categorical variables, differences between groups were analyzed by χ^2 tests. The effects of group and obesity on continuous variables were analyzed by a two-way general linear model after application of two-step transformation as needed to ensure a normal distribution of the variables.

Abbreviations: FE, free estradiol; FT, free testosterone; NA, not applicable.

[a] $P < 0.05$ for the differences between men and control women, independently of obesity.

[b] $P < 0.05$ for the differences between men and women with PCOS, independently of obesity.

[c] $P < 0.05$ for the differences between women with PCOS and control women, independently of obesity.

and fasting HOMA-IR scores did not differ among groups.

Regardless of the study group, obesity was characterized by increased levels of free testosterone, fasting insulin, and glucose and HOMA-IR score and decreased SHBG, high-density lipoprotein cholesterol levels, and insulin sensitivity index. In addition, we observed interactions between group and obesity. Obese control women had higher levels of free testosterone and fasting glucose than did their nonobese counterparts. Obese women with PCOS had decreased levels of DHEAS compared with nonobese patients with PCOS, whereas obese men had increased levels of DHEAS compared with their nonobese counterparts.

Infectious events and use of antibiotics were rare and did not differ in frequency among groups (Table 1). However, to avoid the possible effect of prior use of antibiotics on gut microbiota, the results from the three participants who had used these drugs during the previous 3 months were eliminated from further analyses of gut microbiota.

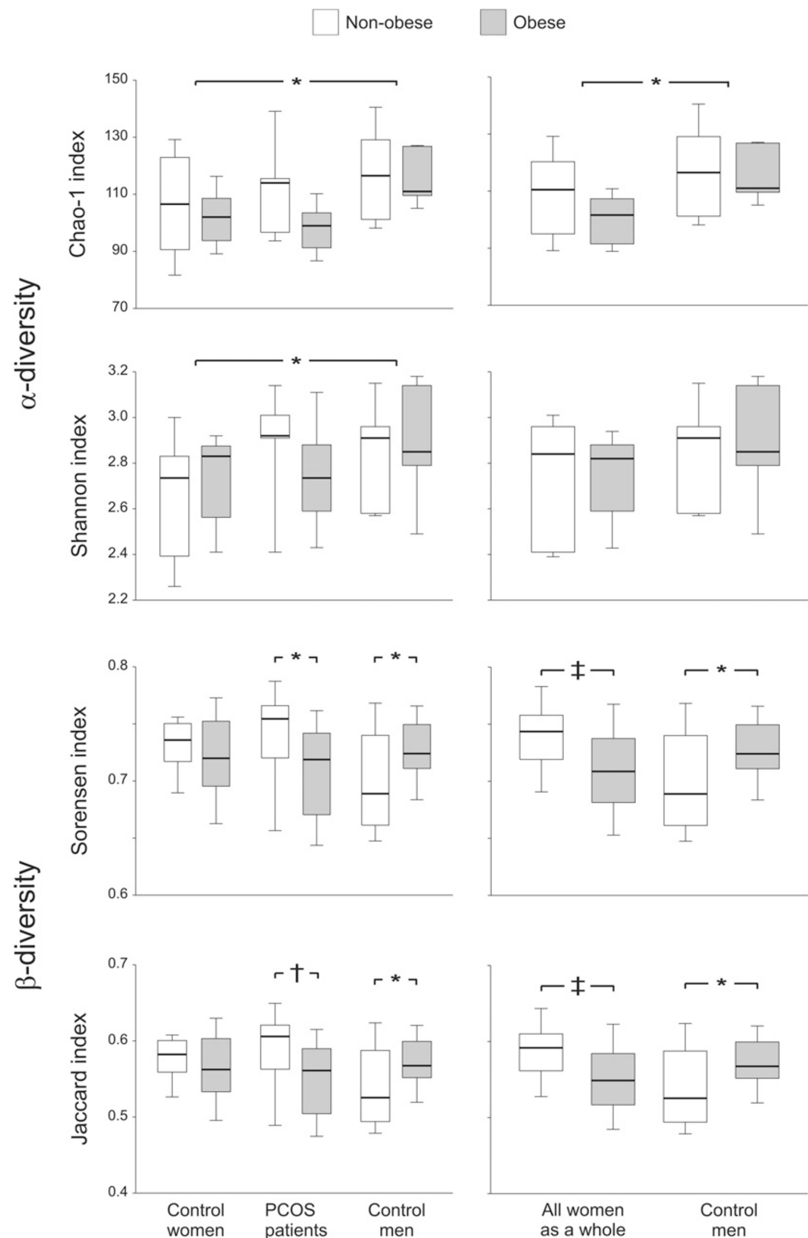

Figure 1. Box-and-whisker plots of the interactions of study group and obesity on the bacterial α and β diversity of gut microbiota. White boxes are nonobese participants and gray boxes correspond to obese individuals. The box represents the interquartile range, which contains the median, and the whiskers indicate the 90th and 10th percentiles. $^{*}P < 0.05$; $^{†}P < 0.01$; $^{‡}P < 0.001$.

Differences in bacterial diversity of gut microbiota according to study group, obesity, and circulating sex hormone concentrations

Sequencing the V4 16S microbial rDNA generated a dataset consisting of 6,507,940 filtered high-quality 16S rDNA gene sequences with 1,659,832 reads for control women, 2,462,150 reads for women with PCOS, and 2,385,958 reads for men, with an average of 151,437 sequences per sample (minimum, 44,795; maximum, 281,779).

We assessed gut microbiota α and β diversity by Chao 1, Shannon, Jaccard, and Sorensen estimators in the three groups. Regardless of obesity, the analysis of α diversity revealed statistically significantly larger values in men than in control women (Fig. 1). We also observed a trend toward higher Chao 1 values in men when compared with women with PCOS that was close to reaching statistical significance ($P = 0.053$) (Fig. 1). When women were considered as a whole, irrespective of the presence or absence of PCOS, sex also influenced gut microbiota by lowering the Chao1 index in women (Fig. 1), and a similar near-significant trend ($P = 0.087$) was observed in the Shannon index (Fig. 1). With all participants considered as a whole, the Chao 1 and Shannon indexes showed positive correlations with total testosterone (Chao 1: $r = 0.345$, $P = 0.023$; Shannon: $r = 0.337$, $P = 0.027$) concentrations and the ratio of free testosterone to free estradiol (Chao 1: $r = 0.362$, $P = 0.017$; Shannon: $r = 0.408$ $P = 0.007$) and showed a negative correlation with total estradiol (Chao 1: $r = -0.313$, $P = 0.041$; Shannon: $r = -0.377$, $P = 0.013$).

Even though obesity did not show independent effects on bacterial diversity in our series of young adults, we

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observed an interaction of obesity with the effects of sex and of the study group on the indexes of bacterial β diversity. When control women and patients with PCOS were considered as a whole, the Sorensen and Jaccard indexes decreased in obese women compared with lean women, whereas these indexes increased in obese men compared with lean men (Fig. 1). The analysis considering control women and patients with PCOS as separate groups revealed that the aforementioned interaction was actually related to the findings in patients with PCOS because obese patients had decreased β diversity indexes compared with their lean counterparts, whereas such differences were not present in control women (Fig. 1).

Differences in bacterial phyla of gut microbiota according to study group and obesity

We then investigated bacterial abundance at the phylum level but found no statistically significant differences among the three groups of participants (data not shown). On the contrary, we found an increased relative abundance of the candidatus *Saccharibacteria* phylum in obese patients compared with nonobese participants regardless of group and sex (obese, 0.06; nonobese, 0.02; $P = 0.018$).

Differences in bacterial genera of gut microbiota according to study group, obesity, and circulating sex hormone concentrations

At the genus level, we observed differences in the relative abundance of three genera among the study groups. The relative abundance of *Catenibacterium* and *Kandleria* genera was higher in women with PCOS and in men compared with control women; in fact, *Kandleria* was absent in the latter. Moreover, the abundance of the *Raoultella* genus was higher in men than in women with PCOS (Fig. 2).

In addition, we also analyzed the presence or absence of bacterial genera in the study groups and observed that men more frequently had *Butyricimonas* (86% vs 44%; OR, 7.0; 95% CI, 1.2 to 42.8), *Megasphaera* (71% vs 25%; OR, 7.5; 95% CI, 1.5 to 37.9), and *Paraprevotella* (86% vs 31%; OR, 13.2; 95% CI, 2.1 to 85.5) genera compared with control women and that women with PCOS more frequently had *Catenibacterium* (77% vs 19%; OR, 14.4; 95% CI, 2.4 to 87.4) and *Oribacterium* (69% vs 19%; OR, 9.7; 95% CI, 1.7 to 54.5) genera compared with control women.

When all participants were considered as a whole, total and free testosterone and the free testosterone to free estradiol ratio showed positive correlations with the relative abundance of *Raoultella* genus (Table 2) and serum androstenedione concentrations showed positive correlations with the abundance of the *Kandleria* genus (Table 2).

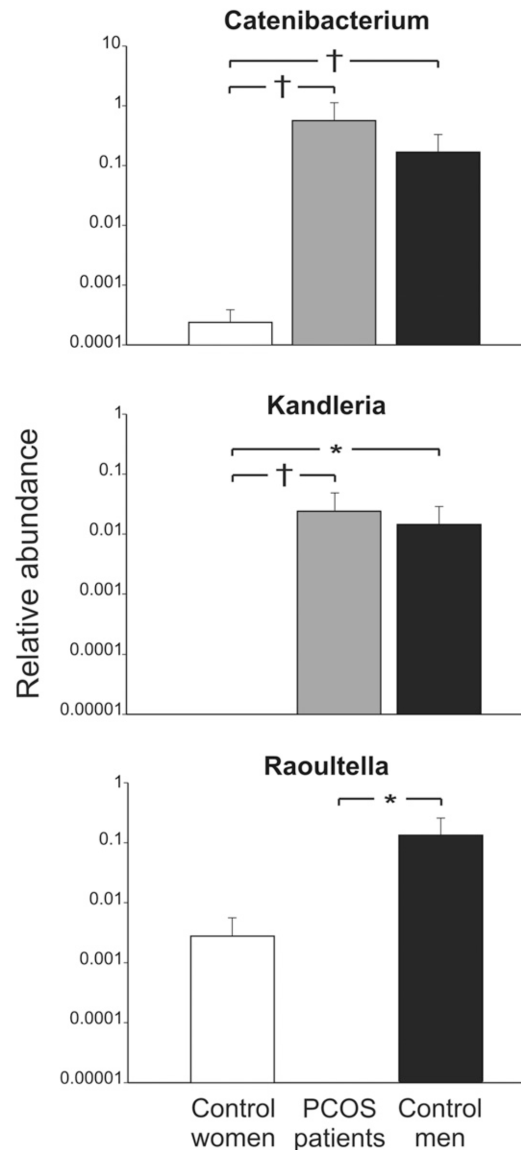

Figure 2. Differences in bacterial relative abundance at the genus level among groups. Columns are means plus SEM. White columns are control women, gray columns are patients with PCOS, and black columns are men. Only bacterial genera showing statistically significance differences ($P < 0.0125$) in at least one of the comparisons are shown. Missing columns indicate that the genus was not detected in this particular category. *$P < 0.0125$; †$P < 0.010$.

We then investigated which genera of the gut microbiota changed in abundance with obesity regardless of the study group being analyzed. The *Anaerofustis, Atopobium,* and *Scardovia* genera were more abundant, and the *Alloprevotella* and *Neisseria* genera were less abundant, in obese than in nonobese persons (Fig. 3). Moreover, obese compared with nonobese participants more frequently had *Anaerofustis* (47.6% vs 9.1%; $P = 0.006$), *Klebsiella* (61.9% vs 31.8%; $P = 0.047$), *Mogibacterium* (19% vs 0%; $P = 0.048$), and *Scardovia* (42.9% vs 9.1%; $P = 0.013$), whereas the presence of *Alloprevotella* (59.1% vs 14.3%; $P = 0.003$),

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Table 2. Correlations Between the Relative Abundance of Bacterial Genera, Markers of Weight Excess and Adiposity, Circulating Sex Hormones, and Biochemical Markers of Intermediate Metabolism in All Participants as a Whole

Markers of Weight Excess and Adiposity

Genera	BMI (kg/m^2) r	BMI P Value	Fat Mass (kg) r	Fat Mass (kg) P Value	Fat Mass (% of Total Body Weight) r	Fat Mass (% of Total Body Weight) P Value	Waist-to-Hip Ratio r	Waist-to-Hip Ratio P Value
Alloprevotella	−0.542[a]	<0.001	−0.369[b]	0.016	−0.312[b]	0.045	−0.250	0.105
Anaerofustis	0.409[a]	0.006	0.420[a]	0.006	0.344[b]	0.026	0.304[b]	0.048
Atopobium	0.378[b]	0.013	0.342[b]	0.027	0.267	0.087	0.293	0.057
Neisseria	−0.375[b]	0.013	−0.274	0.080	−0.322[b]	0.037	−0.411[a]	0.006
Scardovia	0.449[a]	0.003	0.442[a]	0.003	0.245	0.118	0.344[b]	0.024

Circulating Sex Hormone Concentrations

Genera	Total T (nmol/L) r	Total T P Value	Free T (pmol/L) r	Free T P Value	E_2 (pmol/L) r	E_2 P Value	Free E_2 (pmol/L) r	Free E_2 P Value	Free T/Free E_2 (nmol/pmol) r	Free T/Free E_2 P Value	Androstenedione (nmol/L) r	Androstenedione P Value
Catenibacterium	−0.169	0.278	−0.100	0.524	−0.069	0.659	−0.123	0.430	−0.037	0.813	0.247	0.110
Kandleria	−0.023	0.883	0.059	0.705	0.047	0.765	0.069	0.659	0.021	0.895	0.301[b]	0.049
Raoultella	0.317[b]	0.038	0.316[b]	0.039	−0.219	0.159	−0.138	0.379	0.308[b]	0.045	−0.131	0.404

Markers of Intermediate Metabolism

Genera	Glucose (mmol/L) r	Glucose P Value	Insulin (pmol/L) r	Insulin P Value	ISI r	ISI P Value	Total Cholesterol (mmol/L) r	Total Cholesterol P Value	HDL (mmol/L) r	HDL P Value	LDL (mmol/L) r	LDL P Value	Triglycerides (mmol/L) r	Triglycerides P Value
Atopobium	0.256	0.097	0.368[b]	0.015	−0.421[a]	0.005	0.237	0.126	−0.142	0.364	0.251	0.105	0.263	0.088
Catenibacterium	−0.406[a]	0.007	−0.230	0.137	0.269	0.081	−0.237	0.125	0.285	0.064	−0.325[b]	0.034	−0.143	0.361
Raoultella	0.352[b]	0.021	−0.067	0.668	0.006	0.971	−0.091	0.563	−0.215	0.167	−0.103	0.511	0.050	0.748
Scardovia	0.053	0.737	0.388[b]	0.010	−0.425[a]	0.005	0.271	0.079	0.043	0.783	0.101	0.518	0.318[b]	0.038

Abbreviations: E_2, estradiol; HDL, high-density lipoprotein cholesterol; ISI, insulin sensitivity index; LDL, low-density lipoprotein cholesterol; T, testosterone.

[a] $P < 0.01$.

[b] $P < 0.05$.

Gordonibacter (86.4% vs 52.4%; P = 0.017), *Neisseria* (81.8% vs 42.9%; P = 0.009), *Propionibacterium* (50% vs 19%; P = 0.034), and *Pseudomonas* (54.5% vs 19%; P = 0.017) was more frequent in nonobese than in obese participants.

The interactions between study group and the effects of obesity revealed statistically significant differences in the abundance of two bacterial genera, *Atopobium* and *Neisseria*. Obesity exerted a different effect in control women and in women PCOS and men; it was associated with an increase in the abundance of *Atopobium* in control women but not in women with PCOS and men (Fig. 4). Moreover, obesity was associated with a decrease in the abundance of *Neisseria* in control women, an association that was not found in women with PCOS or in men (Fig. 4).

Given the impact of obesity on the findings described above, we analyzed the correlations between the relative abundance of bacterial genera with several metabolic parameters. When all participants were considered as a whole, irrespective of group, sex, and obesity, BMI showed positive correlations with the abundance of *Anaerofustis, Atopobium,* and *Scardovia* and negative correlations with that of *Alloprevotella* and *Neisseria* (Table 2). Fat mass, expressed as total kilograms or percentage of total body weight, showed positive correlations with the abundances of *Anaerofustis, Atopobium,* and *Scardovia* and negative correlations with those of *Neisseria* and *Alloprevotella* (Table 2). Fasting glucose levels showed positive correlations with abundance of *Raoultella* and negative correlations with abundance of *Catenibacterium*, whereas insulin showed positive correlations, and the insulin sensitivity index showed negative correlations, with the abundance of *Atopobium* and *Scardovia* (Table 2).

Discussion

Our study showed that the diversity and composition of the gut microbiota were influenced by sex and

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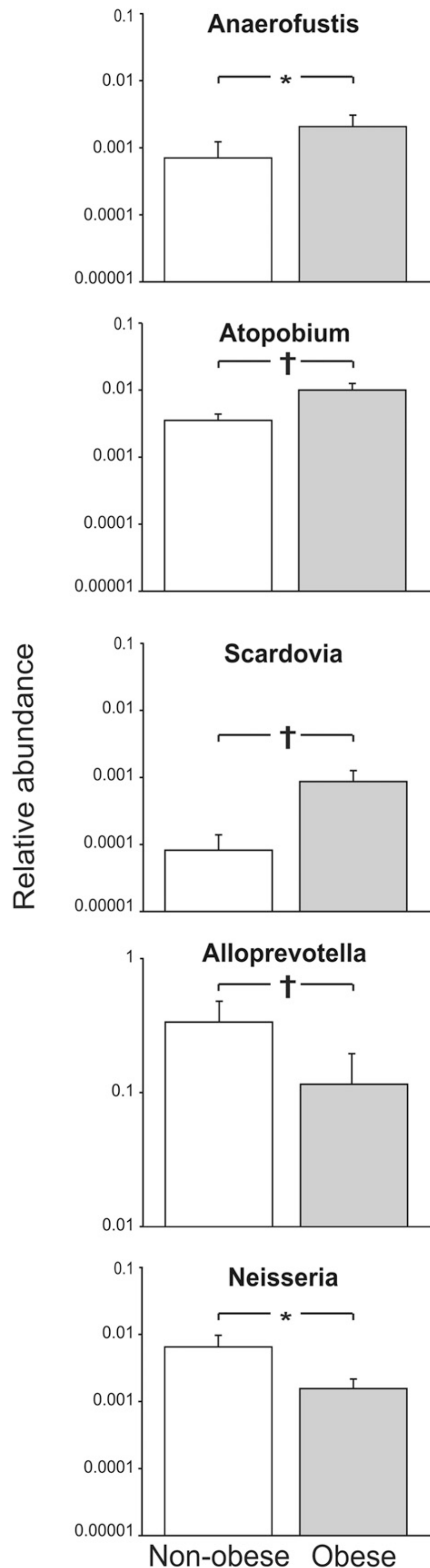

Figure 3. Differences in bacterial relative abundance at the genus level among nonobese and obese participants. Columns are means plus SEM. White columns are nonobese participants and gray columns are obese participants. Only bacterial genera showing statistically significant differences ($P < 0.0125$) in the comparisons are shown. *$P < 0.0125$; †$P < 0.010$.

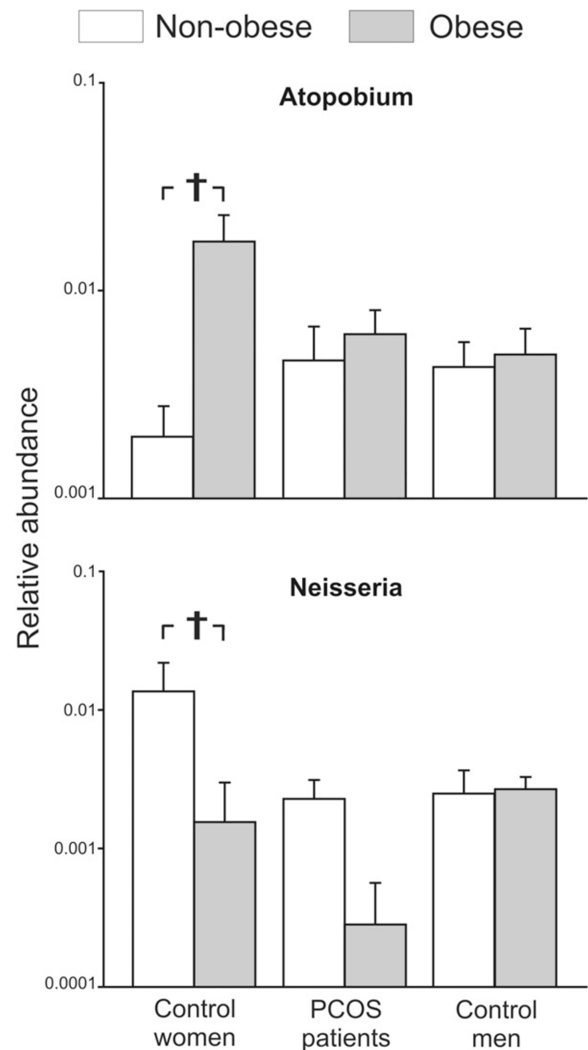

Figure 4. Interactions between study group and obesity in bacterial relative abundance at the genus level. Columns are means plus SEM. White columns are nonobese participants and gray columns are obese participants. Only bacterial genera showing statistically significant differences ($P < 0.0125$) in the comparisons are shown. †$P < 0.010$.

obesity and that sex hormones may be related to these differences.

Indexes of bacterial α diversity in the gut microbiota were reduced in women with and without androgen excess when compared with men. Such an effect of sex was not found in a recent study; that study showed no differences in α bacterial diversity between men and women (13). However, the fact that the women included in that study were postmenopausal (13) may have obscured the sexual dimorphism in the diversity of gut microbiota and possible influences of sex hormones suggested in the analysis of our young adults. Moreover, because α diversity showed positive correlations with testosterone levels and ratio of free testosterone to free estradiol and negative correlations with estradiol concentrations, our study further suggests that androgens

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and estrogens might be involved in the sexual dimorphism reported here. However, being derived from a cross-sectional study, our data cannot clarify the causality of these associations, and the possibility exists that gut microbes participate in the regulation sex hormones and, conversely, that sex hormones modify microbial diversity (12).

Contrary to the results in α diversity, the changes in β bacterial diversity of gut microbiota found in our young adults was heavily influenced by obesity, exemplifying the complex interactions among sex, sex hormones, and obesity on gut bacterial diversity. This effect was particularly important in the subset of women with PCOS. Even though the current literature on the effect of obesity on bacterial diversity yielded conflicting results (22–25), our study addressed the role of sex and sex hormones on such an effect; this issue is of paramount importance because gonadal dysfunction, consisting of androgen excess in women and androgen deficiency in men, is prevalent in obese persons (26, 27). Moreover, the negative effect of obesity on the bacterial diversity of gut microbiota might be explained, at least in part, by the ecological theory, which predicts that species-rich communities, with different species specializing to each potentially limiting resource, are less susceptible to invasion because they use limiting resources more efficiently (22). Excess nutrient loading may result in decreased ecosystem diversity as a small number of species overgrow and outcompete everything else (22).

In relative agreement with our finding of decreased β diversity in obese patients with PCOS, Lindheim *et al.* (28) and Torres *et al.* (29) reported reductions in indexes of α diversity in women with PCOS compared with control women. The fact that we did not find such a reduction in indexes of α diversity in our patients might be related to differences in the criteria used to diagnose PCOS—all patients had hyperandrogenism in our series compared with only 60% in previous studies (28, 29)—and in the characteristics of the populations analyzed because the aforementioned studies did not include obese participants (28, 29).

At the phyla level, our study showed that the abundance of candidatus Saccharibacteria was higher in obese patients than in nonobese patients. This phylum plays a role in the degradation of various organic compounds as well as glucose, butyrate, oleic acid, and amino acids (30). However, studies in mice reported a reduced abundance of this phylum in response to a high-fat diet (31, 32). Such an effect was unlikely to influence our present results because all the participants maintained similar diets in the days preceding sampling.

Sex also influenced the composition of gut microbiota at the genera level, with women showing reduced abundance of *Raoultella* genus when compared with men. Moreover, the *Kandleria* genus was not present in control women, and the *Megasphaera*, *Paraprevotella,* and *Butyricimonas* genera were less frequent in control women than in men. Most of these genera have not been related to sex before in human studies, with the notable exception of *Butyricimonas*, which was also decreased in women in earlier studies (13).

The influence of sex on the composition of gut microbiota is in conceptual agreement with animal data that also demonstrate the remarkable interaction of these effects with host genetics and environmental factors, such as diet (12, 33). Accordingly, in our study *Paraprevotella* showed the strongest positive correlation with testosterone and negative associations with estradiol concentrations among the genera that correlated with sex hormones and sex. In addition, androgenized rats are characterized by an increase in the abundance of the *Paraprevotella* genus (34). But similar to what has been suggested for bacterial diversity, evidence suggests that gut microbiota may catalyze modifications of steroid hormones (35) and might play some role in the regulation of sex hormone concentrations in the body (36).

The analysis of bacterial composition at the genus level revealed an abnormality characteristic of women with PCOS. These patients presented increased abundance of the *Catenibacterium* and *Kandleria* genera compared with both female and male controls. Moreover, the abundance of *Kandleria* correlated positively with circulating androstenedione concentrations when all participants were considered as a whole, suggesting an association with androgen levels. *Catenibacterium* are gram-positive anaerobic bacteria that use glucose to produce acetic, lactic, butyric, and isobutyric acids (37). Several studies reported an increase in the abundance of *Catenibacterium* in infectious and autoimmune disorders (38, 39), suggesting a link with the proinflammatory response. Moreover, the abundance of this genus in human microbiota increased with western-style and carbohydrate-rich diets (40, 41), whereas in ob/ob mice, feeding with prebiotics decreased the abundance of *Catenibacterium* in parallel with amelioration of low-grade inflammation and fat-mass accumulation (42). *Kandleria* are gram-positive anaerobic bacteria recently reclassified on the basis of phylogenetic information from the genus *Lactobacillus* and placement in family *Erysipelotrichaceae* (43). *Kandleria* metabolizes glucose producing lactic acid (43) and have not been related to metabolism or sexual hormones before in human studies. To the best of our knowledge, no data have been published linking differences in the *Raoultella* genus with PCOS and/or obesity.

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Obesity increased the abundance of *Anaerofustis, Atopobium,* and *Scardovia* and decreased the abundance of *Alloprevotella* and *Neisseria.* Of these, *Atopobium* increased specifically in obese control women compared with their nonobese counterparts. Even though the abundance of *Atopobium* has been reported to be decreased in fecal samples of patients with diabetes who presented with higher BMI than the controls (44) and in obese children (45), the abundance of this genus increased in male mice with high-fat diet–induced obesity, in agreement with our present results (46). A similar model of obesity in the rat resulted in a decrease in the abundance of *Alloprevotella* (47) similar to the increase found in our obese participants.

Only a few human and animal studies studied gut microbiota genera in PCOS (28, 29, 48–50). In mice, letrozole-induced PCOS resulted into a reduction in overall species and phylogenetic richness, where the abundance of the *Alistipes* genus was decreased and that of the *Allobaculum* and *Blautia* genera were increased (49). A similar model in rats resulted into reduced *Lactobacillus*, *Ruminococcus*, and *Clostridium* abundances and increased *Prevotella* levels (50). Moreover, correction of this dysbiosis improved the menstrual pattern in these animals (50).

In humans, available data suggest an association between the abundance of certain gut microbiota genera and PCOS. In Europeans, PCOS is associated with a reduced relative abundance of the phylum Tenericutes, two unclassified genera from the order ML615J-28 (belonging to the phylum Tenericutes), and the family S24-7 (belonging to the phylum Bacteroidetes) (28). However, as many as 40% of the women diagnosed with PCOS lacked androgen excess (28) in a phenotype that usually associates much milder metabolic disorders compared with the classic PCOS phenotype that motivated the diagnosis in our series, explaining the different outcomes of both studies. In addition, patients with PCOS were younger than controls in this study (28) and therefore, such results may have been influenced by age. In the Chinese population nonobese and obese control women and women with PCOS showed differences in relative abundance in 28 operational taxonomic units (48). However, most differences in abundance in these studies (28, 48) pertained to unclassified bacterial genera, making difficult a direct comparison with our present results.

Among the advantages of our present study, we highlight the nontargeted approach to the analysis of the gut microbiome, which may have permitted the finding of associations of gut microbiota with sex, sex hormones, and PCOS with genera that might have been missed by targeted approaches. But we also acknowledge that our experiments are not free from limitations, especially with regard to the cross-sectional design used, the limited sample size analyzed, and the high interindividual variability observed. We tried to compensate for these limitations by comparing only rigorously defined and homogeneous groups of participants of similar age and BMI. Similarly, host specificities or diet-related differences may explain variations observed in the composition of gut microbiota, yet we tried to minimize these confounding factors by excluding from analysis the fecal samples from the three participants who had used antibiotics and by standardizing the diet of all these persons for 3 days before sampling. However, we cannot definitely exclude a putative impact of differences in the diet occurring before these 3 days on the microbiota of these individuals. The data presented were obtained for participants living in a specific geographical region, which does not necessarily reflect the environment in other locations. Similarly, we included only patients with hyperandrogenic phenotypes of PCOS and, accordingly, our results should not be extrapolated to nonhyperandrogenic patients with PCOS. Finally, a more detailed structural and functional analysis of intestinal microbiota is required to better define lifestyle- or diet-related effects. Discrimination at the species or even strain level might be of interest for some bacterial groups in order to detect subtle variations in dominant groups.

In summary, our findings indicate that the diversity and composition of gut microbiota are influenced by the combined effects of sex, sex hormone concentrations, and obesity. Intervention studies designed to determine the causality of these associations may permit targeting gut microbiota as putative diagnostic and therapeutic targets for PCOS, obesity, and associated metabolic comorbidities.

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