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A low glycemic index, energy-restricted diet but not *Lactobacillus rhamnosus* supplementation changes fecal short-chain fatty acid and serum lipid concentrations in women with overweight or obesity and polycystic ovary syndrome

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Abstract. – OBJECTIVE: To evaluate if changes in fecal short-chain fatty acids (SCFA) content with an energy-restricted diet and with/without 12 × 10^9 CFU/day of *Lactobacillus rhamnosus* supplementation affect the abundance of selected gut bacteria and lipid profile in overweight and obese women with PCOS.

PATIENTS AND METHODS: This randomized controlled trial involved 40 overweight and obese women with a mean age of 28.8 ± 4.8 years diagnosed with PCOS. The subjects were randomly assigned to an energy-restricted diet group (D group; n = 21) or energy-restricted diet + *Lactobacillus rhamnosus* supplementation group (DP group; n=19). SCFA, selected gut bacteria (*Akkermansia muciniphila*, *Bifidobacterium longum*, and *Faecalibacterium prausnitzii*) abundance, lipid profile and anthropometric parameters were evaluated at baseline and after twenty weeks of intervention.

RESULTS: The energy-restricted diet significantly reduced body weight, BMI, fat mass, acetic and butyric acids, and improved the lipid profile (total cholesterol, low-density lipoprotein cholesterol, and triglycerides) of both groups. Changes in the molar ratio of SCFA towards the correct ratio were also observed. All the results were independent of *Lactobacillus rhamnosus* supplementation, with no significant differences between the two groups.

CONCLUSIONS: Twenty weeks of probiotic supplementation has no additional beneficial effects on selected gut bacteria abundance, SCFA levels, or lipid profile beyond the effect of an energy-restricted diet in overweight and obese women with PCOS.

Key Words:
Short-chain fatty acids, Obesity, Lipids, Diet.

Introduction

Polycystic ovary syndrome (PCOS) is a complex and heterogeneous endocrine disorder of women of reproductive age, characterized by the excessive synthesis of testosterone and other androgens, polycystic ovary revealed by ultrasound imaging, menstrual disorders, visceral obesity, and insulin resistance[1]. The prevalence of PCOS ranges from 4% to 21% worldwide, which indicates that it has become a major public health issue worldwide[2]. PCOS also increases the risk of metabolic disorders, such as type-2 diabetes mellitus, gestational diabetes, endometrial cancer, and other pregnancy-related complications. Other complications of PCOS include abnormal lipid metabolism, including increased cholesterol, triglycerides (TG), and low-density lipoprotein cholesterol (LDL-cholesterol), and high-density lipoprotein cholesterol (LDL-cholesterol)[1].

The dysbiosis of the gut microbiome may play a causal role in PCOS, as perturbations in gut bacteria alter the fatty acid metabolism in adipose tissue and the liver, gut peptide YY modulation, and secretion of glucagon-like peptide-1. Additionally, bacteria produce various amino acids that are precursors for fatty acid synthesis, which in turn may lead to obesity and lipid profile changes[3]. Microbially-derived short-chain fatty acids (SCFAs), primarily acetate, propionate, and butyrate, are produced by enteric microbes as end-products of anaerobic fermentation of undigested microbially accessible dietary carbohydrates. They have a variety of important roles in the gut, such as shaping the gut environment, colon physiology, and an energy source for host colonocytes[4].

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It has been suggested that modulation of the gut microbiome may be a potential treatment option for PCOS and could be achieved through changes in dietary habits and probiotic supplementation[1,5,6]. It has been demonstrated that probiotic supplementation is effective in treating obesity, as well as carbohydrate metabolism and hormonal disorders. Furthermore, Hulston et al[7] demonstrated that weight loss therapy was more effective for obese women supplemented with *Lactobacillus rhamnosus* GG. However, no study has combined probiotic supplementation with an energy-restricted reduction diet, which appears to be a valid adjuvant treatment for patients with PCOS. Moreover, no previous studies have assessed changes in gut bacterial SCFA in overweight and obese women with PCOS as a result of nutritional intervention and probiotic supplementation.

Therefore, this study evaluated how SCFA content changes due to a weight reduction diet, whether it is associated with a change in the abundance of selected gut bacteria and can improve the lipid profile of overweight and obese women with PCOS. Furthermore, the benefits of *Lactobacillus rhamnosus* supplementation were also assessed.

Patients and Methods

Participants

This study was registered in the clinical trials database (https://register.clinicaltrials.gov: NCT03902301) and followed the Declaration of Helsinki and Good Clinical Practice guidelines. It was approved by the Poznan Medical Ethics Committee (No. 268/18). All participants provided written informed consent before trial initiation. This study involved 84 women with PCOS diagnosed based on the Rotterdam criteria[8] and treated at the University Hospital of Obstetrics and Gynecology between June 2018 and December 2020. Overweight or obese patients of reproductive age (18-45 years old) diagnosed with PCOS and with no previous ovarian surgeries were included. Women on antibiotics or probiotics within 6 months before the study started, pharmacological agents or hormones that may affect the course of the menstrual cycle within 3 months, medications that may affect carbohydrate metabolism within 4 weeks were excluded. We also excluded women who were taking weight loss supplements, anti-inflammatory or nutraceuticals that are considered prebiotics (e.g., omega 3 fatty acids and polyphenols) or probiotics including probiotic yogurt, kefir, and other fermented foods, had a history of thyroid disorders, hyperprolactinemia, Cushing's syndrome, liver and kidney disease, cardiovascular disease and digestive disease (e.g., irritable bowel syndrome, ulcerative colitis, Crohn's disease, celiac disease), or who were pregnant or breastfeeding".

Study Design

Participants were randomly assigned to one of two nutritional intervention groups:

1. Weight reduction diet with a low glycemic index (D group);
2. Weight reduction diet with a low glycemic index + *Lactobacillus rhamnosus* supplementation (DP group).

Anthropometric parameters, fecal SCFA levels, selected gut bacteria (*Akkermansia muciniphila, Bifidobacterium longum,* and *Faecalibacterium prausnitzii*) abundance, and lipid profile were evaluated at baseline. The course of the nutritional intervention was monitored every four weeks (after 4, 8, 12, 16, 20 weeks) by assessing changes in anthropometric parameters [body weight, waist circumferences (WC), and fat mass (FM)]. At each visit, the patients received a new weight reduction diet and supplements. Diets (conventional weight reduction diets with low glycemic index) were hypocaloric with a 600 kcal deficit that should cause an approximately 0.5 kg weight loss per week. A registered dietitian prepared the weight reduction diets in the five-meal system for each participant. Specific meals for every day of the week as well as information on the selection of food, seasonal meals, food preparation, and serving sizes (in grams and household measurements) were indicated. The dietary plans were consistent with the nutritional recommendations of Moran et al[9], the Polish Diabetes Association[10], and the current nutritional standards for the given age group[11]. The target macronutrient composition was 50% of energy from carbohydrates with a low and medium glycemic index[12], 15-20% of energy from protein, and 25-30% of energy from fat. The energy and nutrient intake of each food item was calculated using Dieta 6.0 software (Institute of Food and Nutrition, Warsaw, Poland). The International glycemic index (GI) and glycemic load (GL) table was used to calculate GI values[12]. Glycemic load was calculated by multiplying the carbohydrate content of each food

per serving by the GI value and dividing by 100. Additionally, a list of food items with a high GI was prepared and all participants were forbidden to consume any of these foods. To reduce drop-outs and increase adherence to the intervention, the participants were contacted regularly and the phone calls (after 2, 6, 10, 14, 18 weeks of nutritional intervention) monitored adverse events and addressed any concerns. Nineteen women randomized to the DP group took 12×10^9 CFU/day probiotic supplements containing *Lactobacillus rhamnosus* (6×10^9 CFU/g each) in the form of capsules (Bayer, Poland), one at breakfast and one at lunch for twenty weeks. The participants were carefully instructed on how to store the probiotics, with any empty and unused packets returned to measure compliance. The participants also received brief daily cell phone reminders to take the supplements. At the end of the nutritional intervention (after twenty weeks), fecal SCFA level, selected gut bacteria (*Akkermansia muciniphila, Bifidobacterium longum,* and *Faecalibacterium prausnitzii*) abundance and the lipid profile were evaluated.

Assessment of Anthropometric Parameters and Body Composition

Height and body weight were measured using an anthropometer coupled with a WPT 200 OC verified medical scale (Rad Wag). During measurements, all participants wore underwear without shoes and had fasted overnight and voided their bladder. The results were rounded to the nearest 0.1 kg and 0.5 cm, respectively. Body mass index (BMI) was calculated as weight (kilograms) divided by height (meters) squared. Waist circumference (WC) was measured with a flexible tape measure at the uppermost lateral border of the hip crest (ilium) and recorded to the nearest millimeter. Fat mass (FM %) was estimated by air displacement plethysmography using a calibrated BODPOD® (COSMED, Rome, Italy). The women were instructed to wear tightfitting compression shorts and a swimming cap, as well as to remove all metal, including jewelry and watches. All women were instructed to sit in the chamber, breath normally, and minimize any movement for body mass measurements to the nearest 0.01 kg.

Blood Sampling and Biochemical Analysis

Biochemical indices were evaluated at baseline and after the twenty-week intervention in separate arms. For subjects with irregular menstrual cycles, blood samples were collected between days 2 and 5 (in the early follicular phase), and random samples were collected from amenorrheic subjects between 6.00 a.m. and 9.00 a.m. following overnight fasting. Subjects were instructed to abstain from caffeine and alcohol during the 24-hours before blood sampling, and refrain from strenuous exercise on the day of sampling. Blood samples for the biochemical measurements were allowed to clot at room temperature for 30 min, then, the serum was separated by centrifugation and stored at -80°C until further analysis. The concentrations of total cholesterol, LDL-cholesterol, HDL-cholesterol, and TG were analyzed using commercial kits (Thermo Fisher Scientific, Waltham, MA, USA) and standard enzymatic methods with a Konelab 20i fully automated analyzer (Thermo Electron Corporation, Vantaa, Finland). All parameters' levels were determined in a duplicate.

Relative Bacterial Abundance

The relative abundance of three bacterial species *Akkermansia muciniphila (A. muciniphila), Bifidobacterium longum (B. longum),* and *Faecalibacterium prausnitzii (F. prausnitzii)* was evaluated by real-time PCR (qPCR). The bacterial DNA was isolated from the feces using a QIAamp Fast DNA Stool Mini Kit (Qiagen, Shanghai, China) and the quality and quantity were checked using a DS-11 spectrophotometer (DeNovix). The PCR reaction mixture contained 0.3 µM of specific primers, 15 µM specific probe (Table I; except for total bacteria, where 0.064 µM and 0.04 µM were used), TaqPath ProAmp Master Mix (Applied Biosystems, Waltham, MA, USA), and 50 ng of template DNA. Quantitative PCR amplification was performed using the Light Cycler 480 (Roche, Basilea, Switzerland) and the following cycling conditions: preincubation at 95°C for 5 min, followed by 40 cycles at 95°C for 15 s (denaturation) and 60°C for 60 s (annealing and extension). The abundance of total bacteria was used to normalize the data and the specific bacterial taxa were quantified using the second derivative maximum method (Roche, Basilea, Switzerland). All samples were analyzed in duplicate.

Fecal SCFA Analysis

Acetic, propionic, and butyric acid concentrations were measured using the method described by Scortichini et al[17]. Thawed stool samples were homogenized, then, 100 mg of feces were

Table I. Primers and probe sets used for qPCR.

Primers and probe sequences	Target group	Reference
Bac339: TCCTACGGGAGGCAGCAGT Bac780: GGACTACCAGGGTATCTAATCCTGTT Uni514p: [6FAM]CGTATTACCGCGGCTGCTGGCAC[BHQ1]	Total bacteria	13
AM1129: CAGCACGTGAAGGTGGGGAC AM1437: CCTTGCGGTTGGCTTCAGAT AM1345p:[6FAM]CGCCGTAGCTGATGCGCCATTACT[BHQ1]	*Akkermansia muciniphila*	14
R_long_IS: ATCGCGCCAGGCAAAA Fm_long_IS: GGGTTGCTGGTGTGGAAGA Pm_long_IS: [6FAM]ATCCGGGAGCCCAAAAGCGC[BHQ1]	*Bifidobacterium longum*	15
FP226: GATGGCCTCGCGTCCGATTAG FP405: AATCCGAAGACCTTCTTCCTCC FP260dp:[6FAM]TAAYGGCCCACCAAGGCRACGAT[BHQ1]	*Faecalibacterium prausnitzii*	16

weighed. The stool sample was acidified with 0.25 ml of sulfuric acid (50%) and shaken for 3 minutes. 50 µl of internal standard (IC6, concentration 330 µM) was added to the acidic solution and the whole was extracted with 1 ml of diethyl ether. The sample was centrifuged for 5 min at 2800 × g and the organic phase was collected in another vial. The extraction was repeated three times. Finally, 0.5 µl of the harvested organic phase was injected into a gas chromatograph (GC) for analysis. The individual acids were quantified by gas chromatography equipped with a flame ionization detector (Agilent 7890 series II Agilent Technologies, Santa Clara, CA, USA) using a BPX 70 column (BPX70, 25 m × 0.22 mm ID × 0.25µm, SGE Analytical Science, Ringwood, Australia). Acids were identified by mass spectrometry (Agilent 5975C, Agilent Technologies, Santa Clara, CA, USA). Peak integration was performed using MSD ChemStation (Agilent Technologies, Santa Clara, CA, USA). Acid concentrations are expressed in [µmol/g].

Statistical Analysis

The sample size was determined according to the IR with HOMA-IR (a primary outcome) in a previous study of PCOS women who received probiotic supplements[18] and was 21 patients per group with S1=0.6, S2=Z 0.05, d = 0.049 for HOMA-IR and test strength of 80% and $\alpha = 0.05$. To allow for a 15% dropout, 24 patients per group was determined as the final minimal sample size. The distribution of the data was assessed using the Kolmogorov-Smirnov test before statistical analysis. Independent *t*-tests were used to determine differences before nutritional intervention between the two groups and repeated measures ANOVA was used to determine the effects of the intervention on SCFA, selected gut bacteria, and lipid profile. Adjustment for changes in baseline values of SCFA, gut bacteria, and lipid profile of baseline anthropometrical parameters was performed by analysis of covariance (ANCOVA). A *p*-value of 0.05 was considered significant. Statistical analyses were conducted using the STATISTICA software (TIBCO Software Inc. 91 (2017), version 13).

Results

Initially, 84 participants were screened for inclusion, and 28 subjects were excluded as they did not meet the inclusion criteria. As shown in Figure 1, during the intervention phase, five participants withdrew from the study due to pregnancy (3 from the D group and 2 from the DP group) and eleven declined to participate. Forty subjects with a mean age of 28.8 ± 4.8 years (21 from the D group and 19 from the DP group) completed the study. No side effects were reported following the probiotic supplementation in PCOS women.

Before the nutritional intervention, the mean body weight, BMI, FM, WC, selected gut bacteria, SCFA, and lipid profile of study participants were not statistically different between Dgroup and DPgroup (Table II). At the end of the twenty weeks, the intervention resulted in a significant reduction in body weight, BMI, and FM in both groups, with a significant decrease in acetic and butyric acid. Additionally, there was a significant reduction in cholesterol, LDL-cholesterol, and se-

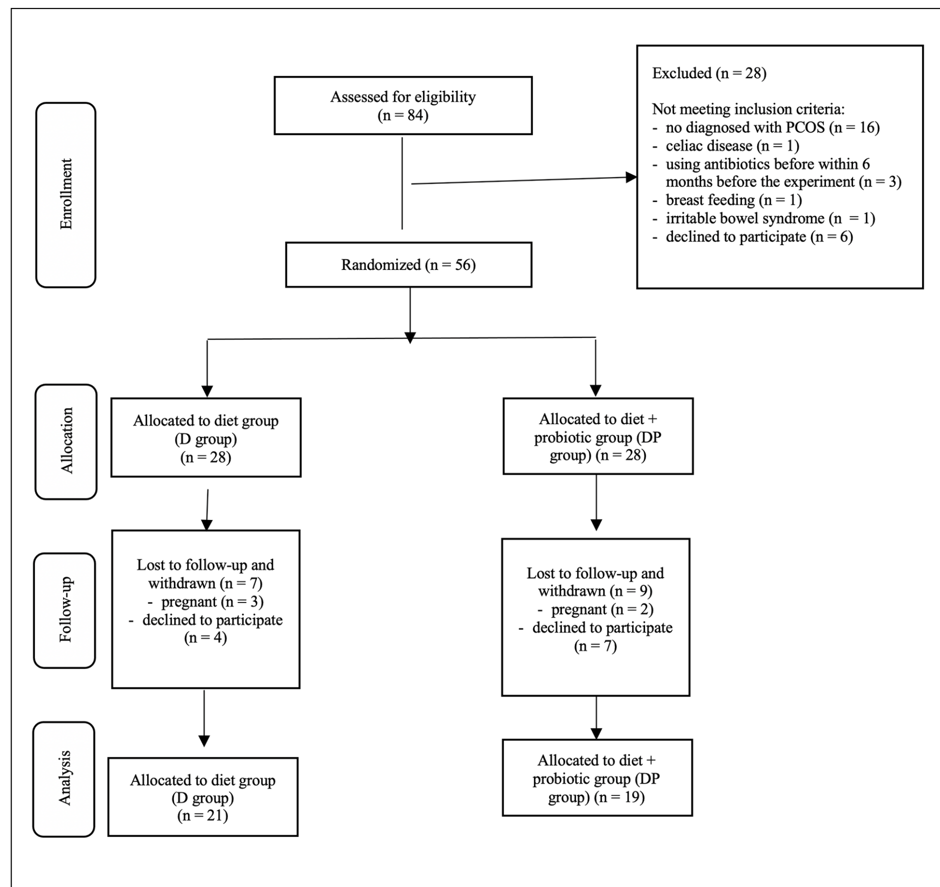

Figure 1. Summary of patient flow through the study.

rum TG, with a significant increase in HDL-cholesterol. There were also changes in the molar ratio of SCFA towards the correct ratio. All observed results were independent of *Lactobacillus rhamnosus* supplementation. Furthermore, when the analyses were controlled for baseline age and anthropometric parameters, no significant changes were observed (Table III).

Discussion

This study evaluated the effects of probiotic supplementation with *Lactobacillus rhamnosus* in combination with an energy-restricted diet on SCFA levels, lipid profile, and selected gut bacteria abundance in overweight and obese women with PCOS. Twenty weeks of weight loss diet reduced the levels of acetic and butyric acid, with no differences observed in the selected gut bacterial abundance. Significant changes in lipid profile were also observed in the study subjects with comparable results in both groups. Furthermore, additional probiotic supplementation did not strengthen this effect.

Gut microbiota decomposes organic materials to produce three major types of SCFA, including acetate, propionate, and butyrate. SCFAs affect the regeneration of the intestinal epithelium as well as lipid and carbohydrate metabolism, possessing anti-inflammatory, anti-cancer, and neuroprotective effects. The approximate molar ratio of acetate to propionate to butyrate is 60:25:15[19-21] Changes in SCFA are possibly associated with the food pattern, the gut microbiota, and the host [22-25]. The gut bacteria selected for analysis in this study were those most often reported by other authors as participating in the synthesis of SCFA, acetate and propionic synthesized by *A. muciniphila* and *B. longum*[26,27] and butyric acid produced by *F. prausnitzi*[28]. It has been previously emphasized that changes in gut microbiota depend primarily on the diet composition, mainly dietary fiber intake, which is prebiotic. In our study, after twenty weeks of weight loss diet with or without supplementation of *Lactobacillus rhamnosus*, the abundance of *A. muciniphila*, *B. longum*, and *F. prausnitzii* were not significantly different between the groups. It should be emphasized that the weight reduction diets were consistent with

Table II. Fecal SCFA, serum lipid concentrations and selected gut bacteria abundance in PCOS women before and after a twenty week energy-restricted diet alone or with *Lactobacillus rhamnosus* supplementation.

	D group (n = 21)		DP group (n = 19)		*p**-value			*p*# value
	Study baseline	End of the twenty weeks of intervention	Study baseline	End of the twenty weeks of intervention	Time	Group	Time x Group	Baseline values D group *vs.* DP group
Body weight (kg)	98.7 (18.6)	89.3 (17.4)	92.7 (19.1)	83.8 (14.6)	0.024	0.146	0.949	0.339
BMI (kg/m^2)	33.9 (5.73)	30.7 (5.48)	33.2 (6.42)	30.0 (4.93)	0.014	0.567	0.992	0.689
FM (%)	47.5 (5.43)	41.8 (6.80)	46.5 (4.62)	40.3 (5.18)	< 0.000	0.331	0.851	0.467
WC (cm)	104.6 (16.1)	93.2 (13.3)	103.6 (16.8)	95.4 (14.3)	0.278	0.946	0.656	0.876
Acetic acid (μmol/g)	25.2 (10.9)	20.0 (7.96)	24.4 (8.36)	19.8 (8.63)	0.019	0.820	0.895	0.877
Propionic acid (μmol/g)	7.25 (3.39)	7.20 (3.60)	7.41 (4.12)	6.57 (3.75)	0.591	0.775	0.631	0.852
Butyric acid (μmol/g)	5.38 (3.38)	3.98 (1.02)	4.89 (2.50)	4.04 (1.12)	0.029	0.673	0.589	0.679
Molar ratio of SCFA	66:20:14	63:23:14	66:20:14	64:22:15	-	-	-	-
A. muciniphila	0.0199 (0.072)	0.0288 (0.076)	0.0222 (0.064)	0.0141 (0.026)	0.659	0.977	0.549	0.673
B. longum	0.0003(0.001)	0.0005 (0.002)	0.0009 (0.004	0.0015 (0.005)	0.606	0.304	0.821	0.476
F. prausnitzii	0.1494 (0.153)	0.1492 (0.149)	0.1453 (0.104)	0.1480 (0.103)	0.966	0.927	0.959	0.976
Total cholesterol (mg/dL)	204.1 (26.8)	178.9 (27.9)	208.4 (44.1)	173.7 (31.0)	< 0.001	0.951	0.514	0.556
LDL-cholesterol (mg/dL)	136.2 (67.3)	99.5 (26.3)	121.7 (34.8)	101.6 (26.7)	0.003	0.593	0.452	0.665
HDL-cholesterol (mg/dL)	53.5 (11.8)	59.0 (12.3)	48.4 (16.8)	58.6 (15.7)	0.015	0.410	0.482	0.180
TG (mg/dL)	148.8 (67.8)	113.3 (47.1)	138.0 (49.8)	94.9 (33.3)	0.001	0.209	0.738	0.625

All values are mean (SD). *p** values represent the time, group, and time × group interaction (computed by analysis of the repeated-measures analysis of variance ANOVA). *p*# obtained from an independent *t*-test. D group: diet group, DP group: diet + probiotic group, BMI: body mass index, FM: fat mass, WC: waist circumferences, SCFA: short-chain fatty acids, LDL-cholesterol: low density lipoprotein cholesterol, HDL-cholesterol: high density lipoprotein cholesterol, TG: triglycerides.

Table III. Adjusted changes in microbiota and lipid profile variables in PCOS women.

	D group (n = 21) Change	DP group (n = 19) Change	p^* value
Acetic acid (μmol/g)	-5.14 (3.04)	-4.61 (2.33)	0.953
Propionic acid (μmol/g)	-0.05 (1.04)	-0.85 (1.08)	0.898
Butyric acid (μmol/g)	-1.40 (0.83)	-0.85 (0.57)	0.827
A. muciniphila	0.002 (0.022)	-0.015 (0.019)	0.168
B. longum	0.0002 (0.001)	0.0005 (0.002)	0.421
F. prausnitzii	-0.0002 (0.043)	0.0028 (0.029)	0.168
Total cholesterol (mg/dL)	-25.1 (7.94)	-34.6 (6.21)	0.127
LDL- cholesterol (mg/dL)	-36.7 (14.12)	-22.3 (3.81)	0.542
HDL- cholesterol (mg/dL)	5.7 (2.49)	10.2 (2.45)	0.072
TG (mg/dL)	-35.4 (7.82)	-43.2 (8.69)	0.332

the nutritional recommendations of Moran et al[9], the Polish Diabetes Association[10], and current nutritional standards for the age group[11]. However, the positive energy balance of the overweight and obese women with PCOS in our study was associated with an excessive food intake, thus also of dietary fiber (data not shown). It can therefore be suggested that the recommended dietary fiber intake for women with PCOS over a twenty-week intervention may be insufficient to produce a significant increase in the abundance of *A. muciniphila, B. longum,* and *F. prausnitzii.* Nonetheless, the present results are in one with other studies reporting no change in selected bacteria despite weight loss[29,30]. The authors of these studies indicate that it is not the ratio of *Firmicutes* to *Bacteroidetes* that is important, rather the amount of SCFA produced. We also observed a significant decrease in acetic and butyric acid despite the lack of change in the abundance of the selected gut bacteria. Given that most evidence suggests that increased SCFA production benefits the host by exerting antiobesity and antidiabetic effects, some *in vitro* and *in vivo* studies have indicated that overproduction or accumulation of SCFAs in the bowel may also lead to obesity due to increased energy accumulation[31,32]. Schwiertz et al[33] showed that the total amount of SCFA was higher in obese than in the lean subjects. Zhang et al[34] reported significantly greater SCFA levels in control compared to women with PCOS but provided no information regarding their weight status. As in our study and a few others[35,36], the calorie-controlled diet decreased the amount of SCFA in obese and overweight patients. The reduction in straight SCFA reduces saccharolytic and increased proteolytic fermentation, respectively[37,38]. However, the lack of valid references for the analytical method used means that it was not possible to determine whether the major straight SCFA were elevated before treatment, then normalized, or were normal and reduced to subnormal amounts after treatment. Taking into account our results and those of other authors, future studies should focus on the assessment of SCFA levels in various population groups, both healthy and unhealthy (including with PCOS), in order to determine the desired SCFA level. The next step is then to determine in which population groups, depending on body weight and health condition, will increases or decreases in SCFA be beneficial.

Apart from assessing changes in the number of selected intestinal bacteria and the amount of SCFA, we also estimated changes in lipid profile. A significant improvement in lipid profile was expected in the DP group due to cholesterol integration with the cell wall of the probiotic bacteria in the intestinal tissue preventing the absorption of cholesterol[39,40]. Furthermore, Trautwein et al[41] suggested that probiotic intake may decrease triglycerides and VLDL-cholesterol levels *via* increased SCFA production, especially propionate, which in turn could inhibit the fatty acid synthesis in the liver, thereby decreasing triglyceride secretion and serum triglycerides. Acetate enters the peripheral circulation to be metabolized by peripheral tissues and is a substrate for cholesterol synthesis. Thus, the significant decrease in acetate in this study may have contributed to reduced cholesterol, LDL-cholesterol, and TG observed. PCOS treatment recommendations suggest that a modest weight loss of 5-10% significantly mitigates the reproductive, metabolic, and

psychological features of PCOS. The nutritional intervention resulted in the required reduction in body weight (9.5% in the D group and 9.6% in the DP group), which may also have improved the lipid profile.

To the best of our knowledge, this is the first report to evaluate the effects of combined probiotic supplementation and an energy-restricted diet on SCFA levels, the abundance of selected gut bacteria, and lipid profile in overweight and obese women with PCOS. A major strength of this study was the intervention time, which was longer than in other reports of eight or twelve weeks[42,43]. Nonetheless, our study had several limitations. First, the small sample size impedes statistical analysis and data interpretation, so further studies with a larger sample of women with diverse PCOS phenotypes and body mass are necessary. The second limitation is the probiotic dose, all subjects received the same dose but due to the lack of data, dose optimization was not performed. Further studies are needed to assess SCFA levels in both healthy individuals and those with PCOS of various body weights so that SCFA reference ranges can be established. Also, to fully assess the relationship between SCFA changes and the microbiota, it is necessary to perform a complete analysis of the patients' microbiota, which would be costly.

Conclusions

Twenty weeks of probiotic supplementation has no additional beneficial effects in overweight and obese women with PCOS on selected gut bacteria abundance, SCFA levels, or lipid profile beyond the effect of a weight loss diet only.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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