Study Protocol

Effects of three medical nutrition therapies on nutritional metabolism and intestinal flora in overweight/obese with polycystic ovary syndrome (PCOS): Study protocol for a randomised controlled trial

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Background and Objectives: Observational studies have shown that energy restriction could be beneficial for controlling bodyweight in patients with polycystic ovary syndrome (PCOS). We aim to compare the effects of a high-protein diet (HPD), a high-protein and high-dietary fiber diet (HPHFD), and a calorie-restricted diet (CRD) on metabolic health and gut microbiota in overweight/obese PCOS patients. **Methods and Study Design:** We will enroll a total of 90 overweight/obese PCOS patients into this eight-week open-label randomised controlled trial. Participants will be randomly assigned to three groups: CRD group (energy coefficient 20 kcal/kg.day, water ≥1500 mL, 0.8-1.2 g/kg protein, carbohydrate energize 55-60%, and fat energize 25-30%), HDP group (energy coefficient 20 kcal/kg.day, water ≥1500 mL, and 1.5-2.0 g/kg protein) and HPHFD group (based on the high protein diet with 15 g more dietary fiber supplement). The primary outcome is body weight, body fat percentage, and lean body mass. The secondary outcomes will include changes in blood lipids, inflammation, glucose tolerance, blood pressure, and gut microbiota compositions. Between-group differences in adiposity measurements at baseline will be compared using one-way analysis of variance (ANOVA) or Kruskal-Wallis test when appropriate. Within-group difference after 8-week intervention will be compared using paired t-test or Wilcoxon signed rank test. Between-group differences in adiposity measurements after 8-week diet intervention will be compared using linear mixed model and ANCOVA. The gut microbiota will be analyzed using 16S amplicon sequencing and the sequencing data will be analyzed using the standardized QIIME2 piperline.

Key Words: nutrition therapy, polycystic ovary syndrome, weight loss, nutritional metabolism, intestinal microecological metabolism

INTRODUCTION

Polycystic ovary syndrome (PCOS) is one of the most common endocrine and metabolic disorders in women of reproductive age, with a 10-15% prevalence of PCOS global, with hyperandrogenism, insulin resistance, ultrasound showing polycystic ovary and polycystic, infrequent menstruation or amenorrhea, hirsutism or acne as its common main characteristics. If PCOS is not diagnosed and treated in time, it may increase the risk of infertility, diabetes and tumor.[1] Obesity, especially visceral adiposity, amplifies and worsens all metabolic and repro-

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ductive outcomes in PCOS.[2] There is broad consensus that the lifestyle management focused on diet-weight loss and concurrent exercise are central to the therapy of PCOS.[3]

According to an international evidence-based guideline, multicomponent lifestyle intervention is important in overweight/obese PCOS patients with a goal of ≥5% weight loss.[4] Crosignani et al[5] reported that 75% overweight patients with PCOS had a modest weight loss of 5% after interventions including energy restriction (1200 kcal/day) and aerobic exercise for 40 weeks which improves many of the features of PCOS.[6,7] Systematic reviews and meta-analyses of randomised clinical trials have shown that exercise intervention improves body composition, clinical and biochemical manifestations of hyperandrogenism and insulin resistance in women with PCOS.[8-11] Overall, these researches generally support the critical role of diet and exercise in improving both weight-loss and metabolic outcomes in the PCOS population.

The Chinese Guidelines on Medical Nutritional Therapy for Overweight/Obesity (2021) has suggested several medical nutrition therapies, including the energy-restricted diet named CRD, and the high protein diet (HPD) and the high-protein and high-dietary fiber diet (HPHFD) on the basis of CRD, as effective approaches of weight management for overweight/obese population, thereby reducing the inflammation, metabolic syndrome components, and cardiovascular disease risk factors.[12] However, which diet is more beneficial to weight loss, metabolic changes and gut microbiota in PCOS patients have not been confirmed in China to date. Recent study has indicated that modifying the gut microbiota, altering bile acid metabolism and/or increasing IL-22 concentrations may be of great value for the treatment of PCOS.[13] Therefore, it is essential to understand the efficacy of diet and exercise therapy in reducing obesity in PCOS in face of the major amplifications of the metabolic and clinical abnormalities associated with obesity in women with PCOS. Therefore, we conducted a randomised clinical trial to investigate the changes of body composition, related metabolic profiles and gut microbiota after the medical nutrition therapy for weight loss, such as CRD, HPD HPHFD, as well as exercise intervention among overweight/obese patients with PCOS in China.

METHODS AND ANALYSIS

Ethics approval and consent to participate

This study was approved by the institutional review board of the Peking University First Hospital (No. 2018-157). All participants provide written informed consent.

Aim and hypothesis

The main goal of the present study (an 8-week, open-label, randomized clinical trial) is to determine the effect of CRD, HPD or HPHFD, combined with exercise intervention, on the anthropometric assessments and metabolic factors including hormones, inflammatory markers, and gut microbial composition in overweight/obese patients with PCOS, which may provide reliable evidence for the nutritional treatment of the clinical highly heterogeneous disease. The primary hypothesis is that all these medical nutrition therapies will help loss weight, improve the symptoms of PCOS, decrease concentrations of inflammatory markers, and favorably affect the diversity of the intestinal microbiota. In addition, compared with the CRD group, the HPD group (with whey protein) and HPHFD group (with whey protein and diet fiber) were better for muscle maintenance, while the HPHFD group with dietary fiber was more conducive to improving the composition of gut microbiota.

Study design

This is an 8-week open-label randomised controlled design, which has been registered in the Chinese Clinical Trial Registry as ChiCTR2100054961.

Study population

The study sample is planned to be a total of 90 adult volunteers with a diagnosis of PCOS. Participants are planned to be recruited through publicity by outpatient admission department of Peking Univesity First Hospital, as well as fliers, newspaper advertisements, and the internet posts that describe the study indicates that meals will be nutritious and free for 8-week. To be eligible in the trial, subjects must fulfil all of the inclusion criteria and none of the exclusion criteria, as shown below.

The subject will be included if she: 1) aged 18-45 years; 2) provides written informed consent; 3) meets the revised 2003 criteria for PCOS (any 2 out of 3)14 , and exclusion of other aetiologies (congenital adrenal hyperplasias, androgen-secreting tumours, Cushing's syndrome): oligo- and/or anovulation, clinical and/or biochemical signs of hyperandrogenism, and ultrasound appearance of polycystic ovaries with the exclusion of other known causes of hyperandrogenemia and ovulatory dysfunction including 21-hydroxylase deficiency, congenital adrenal hyperplasia, Cushings syndrome, androgensecreting tumors, thyroid disease, and hyperprolactinemia; 4) meets any one of the following three criteria for overweight and obesity in Asian populations 15: body mass index (BMI) ≥24 kg/m^2, waist circumference ≥80 cm, and body fat percentage ≥30%; 5) could tolerance slight hunger; 6) agree to avoid pregnancy during the study perioid; 7) agrees to follow the study protocol.

The subject will be excluded if she: 1) is sexually active without any contraceptive measures; 2) has a history of any chronic kidney disease, heart disease, or cerebrovascular disease; 3) has a history of acute infectious diseases or chronic consumptive diseases, such as tuberculosis infection, malignant tumor, and HIV infection; 4) is currently undergoing any treatment of thyroid diseases; 5) has a history of serious liver diseases, such as liver cirrhosis and liver cancer; 6) has ongoing gout or acute gout attack; 7) has mental diseases or cognitive impairment; 8) has eating disorders such as bulimia nervosa, anorexia; 8) has difficulties in commucating with doctors via Internet or telephone; 9) has participated in other clinical trials within 3 months before baseline screening; 10) has used any antibiotics, probiotics, or hormone drugs that may affect the intestinal flora in the past month.

Sample size calculation

As the primary outcomes will be repeatedly measured and three groups of patients will be entering this trial, the number of enrolled study participants was determined to be90, taking into account hospital attendance, the number of dietitians, study period, and possible drop-out before completion.

Randomization

Age-stratified and BMI-stratified randomization will be conducted using a computer-generated random number list by a statistian. The eligible participants will be randomly assigned to one of the three groups of different dietary: HPD, HPHF, and CRD. Laboratory staff performing the measurements will be masked to group allocation. Dietitians will be aware of the diet assignment of each participant, but they will not participate in laboratory investigation and data analysis. Notably, though participants will not be informed of the allocated treatment, blinding them is not feasible owing to the obvious difference in the meals provided.

Interventions and quality control

The administration of the three medical nutrition therapies will be conducted according to the Chinese Guidelines on Medical Nutritional Therapy for Overweight/Obesity (2021).[12] CRD (energy coefficient 20 kcal/kg.day, water ≥1500 mL, 0.8-1.2 g/kg protein, carbohydrate energize 55-60%, and fat energize 25-30%), HPD (energy coefficient 20 kcal/kg.day, water ≥1500 mL, and 1.5-2.0 g/kg protein), and HPHFD, which was based on the high protein diet with 15g dietary fiber supplement additionally. For CRD, we set breakfast as 0.5-1 fist sized cooked staple food (0.5-1 tael raw rice and noodles), 250 mL low-fat milk, one egg, and 100 g leafy vegetables; extra meals include 100 g fruit; lunch was set as 0.5-1 fist sized cooked staple food (0.5-1 tael raw rice and noodles), 100 g low-fat lean meat, and leafy vegetables. On this basis, HPD added 40 g whey protein; HPHFD added 40g whey protein and 10-15 g dietary fiber in extra meals. In addition to the above medical nutrition therapies, all subjects will be instructed to keep 150-minute moderate-intensity exercise per week. It is worth noting that CRD can be achieved through a balanced combination of natural foods, while both the HPD and the HPHFD require nutritional supplements, which are 40 g whey protein supplement (Guangdong Junjoy Medical Nutrition Co., Ltd.China) and 12 g soluble dietary fiber supplement (Nutrasumma, 83.3% inulin and 16.7% xylooligosaccharide) per day, respectively.

On the first day after the screening period the 8-week intervention phase will begin, during which participants will be provided with food and exercise recommendations and nutritional supplements for the next two month. We will visit all patients according to the timeline in Table 1. In order to ensure the adherence of the participants, they were required to take photos of each meal before eating and send them to the dietitian, who would estimate the nutritional value and assess the intake and percentage of consumed nutrients from the recommended amounts. Participants were also asked to return their diet diary for outpatient visits at week 4 and 8, and receive dietary supplements for the next month. When they fail to abide by protocols, we will supervise and remind them timely. If the participant continues to deviate from the study protocol or can't take photos on time to record his diet more than three times a week, he or she will be withdrawn from the trial.

Table 1. Schedule of study procedure

	Screening period	Intervention and follow-up period		
Visit number	Visit 0	Visit 1	Visit 2	Visit 3
Visit time and window	Week -1	Week 0	Week 4	Week 8
Informed consent	√			
Inclusion/exclusion criteria	√			
Biochemistry test		√	√	√
Fasting insulin		√	√	√
sex hormone		√	√	√
CRP and IL-6		√	√	√
body composition analysis		√	√	√
Androgen binding globulin, Mullerian hormone		√	√	√
Inflammatory factor		√	√	√
Fecal flora		√	√	√
formulate a scheme		√		
write out 4W test sheet		√		
Submit 4W test sheet			√	
write out 8W test sheet			√	
Submit 8W test sheet				√
Distribution of nutritional supplements for the first month		√		
Collect the diary of diet and exercise for 0-4 weeks and distribute nutritional supplements for the second month			√	
Collect the diary of diet and exercise for 4-8 weeks				√

4W: 4-week; 8W: 8-week.

Reasons for the participant to be discontinued from the study:

(1) Withdrawal of informed consent;
(2) Lack or incomplete compliance with the diet and/or exercise intervention;
(3) Non-attendance at any study visits;
(4) Meet the exclusion criteria after enrollment;
(5) Any serious adverse event during the period of this trial.

Data collection and methods

Tools

The following clinical information will be obtained:

(1) Demographic information
(2) Anthropometric measures: Weight, height, BMI, body fat mass, fat free mass, body fat percentage, basal metabolic rate, visceral fat area, skeletal muscle index;
(3) Imaging data: vagina or abdominal color Doppler ultrasound;

In the blood serum, we will evaluate markers of:

(1) Metabolic indices: total protein, albumin, prealbumin, creatinine, estimated glomerular filtration rate, uric acid, total cholesterol, low density lipoprotein cholesterol, high density lipoprotein cholesterol, triglycerides, glucose, and insulin;
(2) Inflammation: high-sensitivity C reactive protein, homocysteine, tumor necrosis factor alpha (TNF-alpha), and interleukins IL-6 and IL-8;
(3) Coagulation factor: prothrombin time, prothrombin time ratio, prothrombin activity, international normalized ratio, activated partial thromboplastin time, Activated partial thrombin time ratio, fibrinogen, thrombin time;
(4) Sex hormones: luteinizing hormone, follicle stimulating hormone, estradiol, prolactin, testosterone, progesterone, sex hormone-binding globulin, free testosterone, anti-Mullerian hormone

In the stool, we will conduct these following analyses: gut microbiota (taxonomic and functional analysis) and concentrations of short-chain fatty acids.

Anthropometric measurement

The measurement of weight, BMI, fat mass, muscle mass, and total body water will be performed using a segmental multifrequency bioimpedance analyzer (InBody720, Seoul, Korea). All anthropometric data will be documented after an overnight fast. Patients will wear light clothing without metal objects (i.e. belt, jewelry) when weighing. In addition, participants will be required to wear clothes of a similar weight on each visit. All anthropometric measurements will be taken at week 0, week 4 and week 8.

Sampling

Biochemical measurement

Overnight fasting venous blood will be collected by nurses in the laboratory department of Peking University First Hospital between 8:00 and 10:00 a.m.. To obtain plasma, blood will be centrifuged at 1500 g for 10 minute.

Gut microbiota analysis

Participants will be instructed to collect stool samples after an overnight fasting to avoid the influence of diet. Every participant will receive detailed instructions on the method for material collection. A plastic holder will be used to collect faeces into a sterilized screw-capped collection container. Participants will be asked to store the sample in a fridge and send it to the clinic within 24 hours of collection. The samples will be stored in 2 mL Eppendorf tubes containing 600 μL DNA/RNA later solution (Zymo Research, Freiburg, Germany) to prevent the degradation processes and will be frozen at -80 °C until the microbiota analyses begins. Stool collection will be administered on the weekend in every week during study period.

DNA was extracted from the stool using Omega E.Z.N.A. Stool DNA Kit (Omega Bio-tek, Inc., USA) following the manufacturacer's instruction and will be amplified using primers flanking the V3-V4 hypervariable region of the 16S rRNA gene. Sequencing will be performed using an Illumina Miseq PE300 sequencing platform (Illumina, Inc., CA, USA) at the Beijing Allwegene Technology Company and the raw sequencing data will be processed using the pipeline tools QIIME and PEAR (v0.9.6). Sequences will be clustered into operational taxonomic units (OTUs) at a similarity level of 97% use Uparse algorithm of Vsearch (v2.7.1) software and OTU representative sequences were aligned based on SILVA138 rRNA database with BLAST software, while the E-value threshold was set to 1e-5.

An overview of the study design and assessments to be

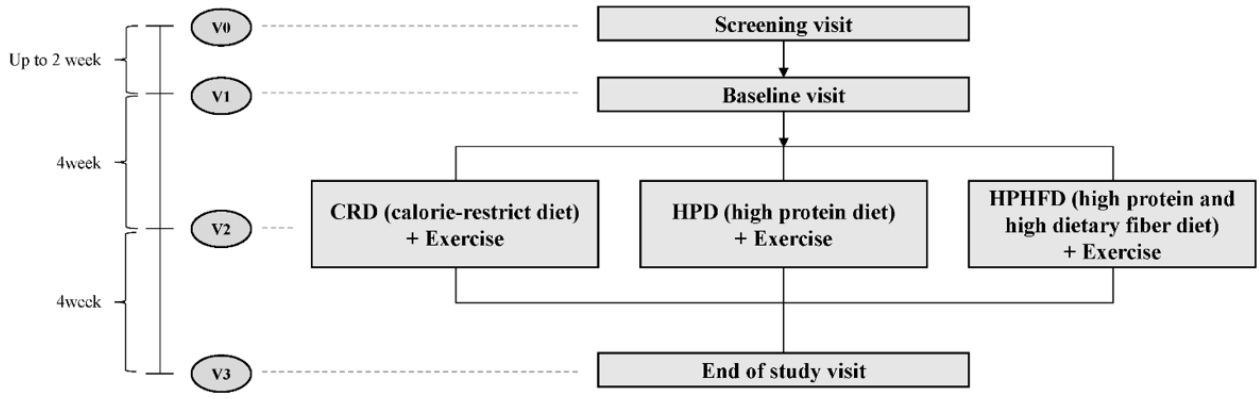

Figure 1. Study protocol flow diagram. V0: Screening visit; V1: Baseline visit; V2: Visit 2; V3: End of study visit.

conducted during the study and their timing is presented in Figure 1 (study design) and Table 1 (assessment point times and examined variables).

Data management and analysis plan

Data analysis principles

Primary outcomes

The primary outcome are the changes in weight, height, BMI, body fat mass, fat free mass, body fat percentage and other anthropometric measures after 8-week intervention.

Secondary outcomes

Secondary outcomes are the between-group differences in metabolic indices (including total protein, albumin, prealbumin, creatinine, estimated glomerular filtration rate, uric acid, total cholesterol, low density lipoprotein cholesterol, high density lipoprotein cholesterol, triglycerides, glucose, and insulin), inflammation factors (including high-sensitivity C reactive protein, homocysteine, TNF-alpha, and interleukins IL-6 and IL-8), coagulation factor (Prothrombin time, prothrombin time ratio, prothrombin activity, international normalized ratio, activated partial thromboplastin time, Activated partial thrombin time ratio, fibrinogen, thrombin time), and sex hormones (luteinizing hormone, follicle stimulating hormone, estradiol, prolactin, testosterone, progesterone, sex hormone-binding globulin, free testosterone, anti-Mullerian hormone) from blood samples, ang gut microbiota from stool samples after 8-week intervention.

Monitoring data collection

All team members of this study will be trained by qualified, experienced professionals in areas related to the planned measurements to ensure accurate execution of procedures, data collection, and adherence to the study. Standard procedures for data collection will be conducted as mentioned above. After each visit, researchers will check the quality and correctness of the data collection by completing a structured online spreadsheet. Evaluation of the electronic document will be performed once a month by a supervisor.

Statistical analysis

Baseline anthropometric measures, metabolic indices, inflammation factors, and physical activity level will be presented using descriptive statistics. We will test the normality assumption and homogeneity of variance by Kolmogrov–Smrinov test for study variables. For the comparison of variables between groups at baseline, we will use independent t-test, $\chi 2$ tests, or Fisher's Exact Tests. In order to compare the difference between groups at each time point and delta value between the visits (Δ(Week 4–Week 0) and Δ(Week 8–Week 0) between the two groups, unpaired t-test, Mann-Whitney U test or generalized linear model will be performed. To compare the difference between baseline and the data from end point (Week 8), we will apply paired t-test or Wilcoxon signed rank test. To examine the difference between groups over the visits, we used a mixed-model analysis of variance (ANOVA) or Friedman test as a nonparametric alternative to the repeated measures. Correlations will be evaluated by Spearman rank correlation analysis. Correction for multiple testing will be performed based on the false discovery rate or Bonferroni correction. In all above analyses, the $p<0.05$ and false discovery rate <0.05 will be considered statistically significant.

The analysis of the gut microbiota will include alpha diversity, beta diversity, ordination techniques, and taxonomic and functional differences. Alpha diversity will be measured by means of the Chao1 (richness) and Shannon (richness and evenness) indices. Originally observed count data (without data pre-processing) will be used for all indices. Comparison within the groups (week1-week8) and between the groups at the end of the study (at week8) will apply the non-parametric Wilcoxon signed-rank test or Mann-Whitney (or Kruskal-Wallis) test, respectively. We will measure beta-diversity using means of the Bray-Curtis dissimilarity metric and the weighted UniFrac distance metric. Based on the Bray-Curtis dissimilarity and UniFrac distances, principal coordinate analysis will help visualize the between-group differences in gut microbial composition. The permutational multivariate analysis of variance (PERMANOVA) will be conducted on the Bray-Curtis and weighted UniFrac dissimilarity matrices to assess the group-level (at week8) differences. Difference of Taxonomic differential abundance between groups will be tested by comparing the fractional abundances. We will conduct the analysis of alpha and beta diversity, ordination analysis, and taxonomic differential abundance using the QIIME and R (version 3.6.0). The PERMANOVA + add-on package of PRIMER 7 (version 7.0.13) will be used as analysis tools to compare beta diversity. The software package PICRUSt will be used to predict the metagenome functional content using the output of the 16S rRNA analysis pipeline. Functional abundance differences will be analyzed and visualized using STAMP.[16] For multiple test correction, FDR-adjusted *p* values (q values) will also be reported.

Project Management

Timeline of the study

This study will include four visits, which can be seen in Figure 1:

(1) V0: Screening Visit to screen and enroll eligible patients into the study. During the period, informed consent will be obtained for subsequent procedures.
(2) V1: Baseline Visit up to 1 week after V0 to randomize patients to one of three groups of the study. At this point, participants will complete baseline clinical examination, as well as the formulation of nutrition and exercise interventions.
(3) V2: Visit 2, during the intervention period, after four weeks (±2 days) from Baseline Visit;
(4) V3: End of Study Visit to complete all procedures in this study, after 8 weeks (±2 days) from Baseline Visit.

Data safety monitoring

The long-term safety and tolerability of medical nutrition therapy will be assessed Based on anthropometric measurements, metabolic profiles and self-reported symptoms recorded at the visits during the intervention period (V2 and V3). Participants will be asked to reported any sus-

pected adverse events in written form and to report them at the next visit.

Patient and public involvement

Patients or the public WERE NOT involved in the design, or conduct, or reporting, or dissemination plans of our research

DISCUSSION

PCOS is caused by abnormal androgen secretion contributing to the formation of small fluid-filled sacs in the ovaries, which worsens women's life quality by interfering their physiology and psychology in reproductive age. Obesity-related inflammation may have a potential impact on ovarian physiology due to dysregulation of adipokine secretion and impaired insulin sensitivity.[17] In overweight/obese adolescents, increased visceral fat is also associated with hormonal changes that impair hypothalamus and pituitary functions and directly affect ovarian function. For instance, higher BMI values in the PCOS population was positively correlated with total and free testosterone, free androgen index, total cholesterol, low-density lipoprotein cholesterol, triglyceridemia, fasting glucose, homeostatic model assessment for insulin resistance, fasting insulin, estradioland androstenedione while negatively correlated with circulating concentrations of sex-hormone-binding globulin, inhibin B and high-density lipoprotein cholesterol.[18,19] The α-diversity of gut microbiota in PCOS patients decreased, such as Lactobacillus, Rumen and Clostridium decreased, and Ptelleria increased; dietary fiber (fructooligosaccharides, inulin) can selectively promote the growth and reproduction of gut probiotics, changing the structure of gut microecology by increasing the formation of short-chain fatty acids, thereby helping to loss weight.[20]

As the recommendation of Chinese Guidelines on Medical Nutritional Therapy for Overweight/Obesity (2021), energy restriction as well as dietary fiber and protein supplements may be effective interventions forweight-loss.[12] Numerous studies proved that incorporating a CRD, as well as exercise training, play a key role in losing weight among PCOS patients.[8,21-26] Protein intake induces satiety and enhances a feeling of well-being and also self-esteem for overweight/obese women with PCOS who have tried to lose weight. In addition, diets rich in fiber have been shown to interact directly with gut microbes, impacting gut microbiota composition, diversity and richness.[27,28] Specifically, the consumption of dietary fiber promotes extensive metabolic interactions between bacterial species present in the gastrointestinal microbiota, which was known as cross feeding. Therefore, gut microbiota regulation should, therefore, be considered as a potential adjuvant clinical treatment for PCOS.[29] The present study investigates whether the 3 medical nutrition therapies in the Chinese guideline are effective for weight loss, hyperandrogenemia, inflammatory factors, and intestinal flora in overweight/obese PCOS patients and how much difference there is among these therapies, which may provides a theoretical basis for the selection of weight-loss therapies in PCOS patients. Clinical trials such as this can facilitate evidence-based recommendations of specific medical nutrition therapies for clinical severity of PCOS syndrome.

To the best of our knowledge, this is the first study protocol of an open-label, randomised controlled trial to evaluate which medical nutrition therapy offers additional benefits over conventional energy-restricted therapy for weight loss, and the treatment of metabolic complications and gut dysbiosis in China, and further research is needed.

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AUTHOR DISCLOSURES

The authors declare that they have no competing interests.

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REFERENCES

1. Escobar-Morreale HF. Polycystic ovary syndrome: definition, aetiology, diagnosis and treatment. Nat Rev Endocrinol. 2018;14:270-84. doi:10.1038/nrendo.2018.24.
2. Glueck CJ, Goldenberg N. Characteristics of obesity in polycystic ovary syndrome: Etiology, treatment, and genetics. Metabolism. 2019;92:108-20. doi: 10.1016/j.metabol.2018.11.002.
3. Teede HJ, Misso ML, Costello MF, Dokras A, Laven J, Moran L, Piltonen T, Norman RJ. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. Fertil Steril. 2018;110:364-79. doi: 10.1016/j.fertnstert.2018.05.004.
4. Teede HJ, Misso ML, Costello MF, Dokras A, Laven J, Moran L, Piltonen T, Norman RJ. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. Hum Reprod. 2018;33:1602-18. doi:10.1093/humrep/dey256.
5. Crosignani PG, Colombo M, Vegetti W, Somigliana E, Gessati A, Ragni G. Overweight and obese anovulatory patients with polycystic ovaries: parallel improvements in anthropometric indices, ovarian physiology and fertility rate induced by diet. Hum Reprod. 2003;18:1928-32. doi: 10.1093/humrep/deg367.
6. Moran LJ, Pasquali R, Teede HJ, Hoeger KM, Norman RJ. Treatment of obesity in polycystic ovary syndrome: a position statement of the Androgen Excess and Polycystic Ovary Syndrome Society. Fertil Steril. 2009;92:1966-82. doi: 10.1016/j.fertnstert.2008.09.018
7. Teede H, Deeks A, Moran L. Polycystic ovary syndrome: a complex condition with psychological, reproductive and metabolic manifestations that impacts on health across the lifespan. BMC Med. 2010;8:41. doi: 10.1186/1741-7015-8-41.
8. Shang Y, Zhou H, Hu M, Feng H. Effect of diet on insulin resistance in polycystic ovary syndrome. J Clin Endocrinol Metab. 2020;105:dgaa425. doi: 10.1210/clinem/dgaa425.
9. Lim SS, Hutchison SK, Van Ryswyk E, Norman RJ, Teede HJ, Moran LJ. Lifestyle changes in women with polycystic

ovary syndrome. Cochrane Database Syst Rev. 2019;3: Cd007506. doi: 10.1002/14651858.CD007506.pub4.
10. Kataoka J, Tassone EC, Misso M, Joham AE, Stener-Victorin E, Teede H, Moran LJ. Weight management interventions in women with and without PCOS: A systematic review. Nutrients. 2017;9:996. doi: 10.3390/nu9090996.
11. Domecq JP, Prutsky G, Mullan RJ, Hazem A, Sundaresh V, Elamin MB et al. Lifestyle modification programs in polycystic ovary syndrome: systematic review and meta-analysis. J Clin Endocrinol Metab. 2013;98:4655-63. doi: 10.1210/jc.2013-2385.
12. Guideline for medical nutrition treatment of overweight/obesity in China (2021). Chinese Journal of the Frontiers Medical Science. 2021;13:1-55. doi: 10.12037/YXQY.2021.11-01. (In Chinese)
13. Qi X, Yun C, Sun L, Xia J, Wu Q, Wang Y et al. Gut microbiota-bile acid-interleukin-22 axis orchestrates polycystic ovary syndrome. Nat Med. 2019;25:1225-33. doi: 10.1038/s41591-019-0509-0.
14. Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod. 2004;19:41-7.
15. WHO Expert Consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Lancet. 2004;363:157-63. doi: 10.1016/s0140-6736(03)15268-3.
16. Parks DH, Tyson GW, Hugenholtz P, Beiko RG. STAMP: statistical analysis of taxonomic and functional profiles. Bioinformatics. 2014;30:3123-4. doi: 10.1093/bioinformatics/btu494.
17. Aboeldalyl S, James C, Seyam E, Ibrahim EM, Shawki HE, Amer S. The role of chronic inflammation in polycystic ovarian syndrome-A systematic review and meta-analysis. Int J Mol Sci. 2021;22:2734. doi: 10.3390/ijms22052734.
18. Cassar S, Teede HJ, Moran LJ, Joham AE, Harrison CL, Strauss BJ, Stepto NK. Polycystic ovary syndrome and anti-Müllerian hormone: role of insulin resistance, androgens, obesity and gonadotrophins. Clin Endocrinol (Oxf). 2014;81: 899-906. doi: 10.1111/cen.12557.
19. Li L, Feng Q, Ye M, He Y, Yao A, Shi K. Metabolic effect of obesity on polycystic ovary syndrome in adolescents: a meta-analysis. J Obstet Gynaecol. 2017;37:1036-47. doi: 10.1080/01443615.2017.1318840.
20. Visser JA. The importance of metabolic dysfunction in polycystic ovary syndrome. Nat Rev Endocrinol. 2021;17: 77-8. doi: 10.1038/s41574-020-00456-z.
21. Thomson RL, Buckley JD, Noakes M, Clifton PM, Norman RJ, Brinkworth GD. The effect of a hypocaloric diet with and without exercise training on body composition, cardiometabolic risk profile, and reproductive function in overweight and obese women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2008;93:3373-80. doi: 10.1210/jc.2008-0751.
22. Shishehgar F, Mirmiran P, Rahmati M, Tohidi M, Ramezani Tehrani F. Does a restricted energy low glycemic index diet have a different effect on overweight women with or without polycystic ovary syndrome? BMC Endocr Disord. 2019;19:93. doi: 10.1186/s12902-019-0420-1.
23. Panidis D, Farmakiotis D, Rousso D, Kourtis A, Katsikis I, Krassas G. Obesity, weight loss, and the polycystic ovary syndrome: effect of treatment with diet and orlistat for 24 weeks on insulin resistance and androgen levels. Fertil Steril. 2008;89:899-906. doi: 10.1016/j.fertnstert.2007.04.043.
24. Moini A, Arabipoor A, Hemat M, Ahmadi J, Salman-Yazdi R, Zolfaghari Z. The effect of weight loss program on serum anti-Müllerian hormone level in obese and overweight infertile women with polycystic ovary syndrome. Gynecol Endocrinol. 2019;35:119-23. doi: 10.1080/09513590.2018.1499084.
25. Toscani MK, Mario FM, Radavelli-Bagatini S, Wiltgen D, Matos MC, Spritzer PM. Effect of high-protein or normal-protein diet on weight loss, body composition, hormone, and metabolic profile in southern Brazilian women with polycystic ovary syndrome: a randomized study. Gynecol Endocrinol. 2011;27:925-30. doi: 10.3109/09513590.2011.564686.
26. Mehrabani HH, Salehpour S, Amiri Z, Farahani SJ, Meyer BJ, Tahbaz F. Beneficial effects of a high-protein, low-glycemic-load hypocaloric diet in overweight and obese women with polycystic ovary syndrome: A randomized controlled intervention study. J Am Coll Nutr. 2012;31:117-25. doi: 10.1080/07315724.2012.10720017.
27. Holscher HD. Dietary fiber and prebiotics and the gastrointestinal microbiota. Gut Microbes. 2017;8:172-84. doi: 10.1080/19490976.2017.1290756.
28. So D, Whelan K, Rossi M, Morrison M, Holtmann G, Kelly JT, Shanahan ER, Staudacher HM, Campbell KL. Dietary fiber intervention on gut microbiota composition in healthy adults: a systematic review and meta-analysis. Am J Clin Nutr. 2018;107:965-83. doi: 10.1093/ajcn/nqy041.
29. Giampaolino P, Foreste V, Di Filippo C, Gallo A, Mercorio A, Serafino P et al. Microbiome and PCOS: State-of-art and future aspects. Int J Mol Sci. 2021;22:2048. doi: 10.3390/ijms22042048.