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Effects of Probiotic Supplementation on PCOS Outcomes

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by

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Abstract

Polycystic ovarian syndrome (PCOS) is a multifactorial metabolic, endocrinologic, and gynecologic condition affecting up to 5 million women in the United States. It is a disease characterized by oligo- or anovulation, hyperandrogenism, and/or polycystic ovaries. Phenotypic presentation can include irregular menstrual cycles, obesity, hirsutism, insulin resistance, or difficulty conceiving. If untreated, PCOS can result in a sequelae of chronic disease burden. Current standard of care consists of metformin for blood sugar control, oral contraceptives for menstrual regularity, and spironolactone for androgen imbalance, but newer research is identifying gut dysbiosis as a possible contributing etiology to disease development and symptomology. This literature review aims to investigate the effectiveness of probiotic supplement regimens in improving the gut microbiome and subsequent outcomes for patients with PCOS, specifically inflammatory, anthropometric, androgen, and blood sugar markers. A comprehensive literature review was performed using PubMed and Clinical Key databases. A variety of keywords and similar articles were used to identify studies that were further screened for inclusion and exclusion criteria. Studies were excluded if they were systematic reviews, published over 10 years ago, utilized non-human subjects, had poor study design, or looked at conditions outside of PCOS. There were 16 articles that met final criteria and were included in this review. Results showed significant improvement in androgen and inflammatory markers but were inconsistent in anthropometric and blood sugar findings. Further studies with longer duration and increased generalizability are needed to more adequately compare probiotics to standard of care in the treatment of PCOS.

Keywords: PCOS, probiotics, synbiotics, microbiome, testosterone, androgens, anthropometrics, glycemic control

Introduction

Polycystic ovarian syndrome (PCOS) is a multifactorial metabolic, endocrinologic, and gynecologic condition often diagnosed based on Rotterdam criteria in which a patient must meet two of the following three criteria in the absence of other causative endocrinopathies: (1) oligo- or anovulation, (2) clinical and/or biochemical signs of hyperandrogenism, and (3) polycystic ovarian morphology visualized on imaging. Phenotypical presentations of this disease vary but can commonly include obesity, hirsutism, irregular periods, and insulin resistance. Based on the most recent data, the Centers for Disease Control and Prevention (CDC, 2023) estimates that 6-12% of reproductive age women in the United States have been diagnosed with PCOS.

The etiology of PCOS is largely unknown, but it is thought to be a combination of genetic and environmental factors. Recently, there has been more research done on the potential connection between the gut microbiome and PCOS. In theory, dysbiosis of the gut can lead to increased intestinal permeability and release of lipopolysaccharides, as well as abnormal expression of short chain fatty acids, which can further activate toll-like receptors (TLRs) to chronically increase inflammatory markers, thus contributing to obesity and insulin resistance (Duan et al., 2021).

Several studies have identified decreased fecal bacterial diversity and richness in PCOS patients. Findings are consistent with increased levels of pathologic *Escherichia, Shigella, Bacteroides, Parabacteroides, Klebsiella, Enterobacteriaceae, Gammaproteobacteria,* and *Streptococcus* strains in PCOS patients compared to increased levels of beneficial *Firmicutes, Faecalibacterium prausnitzii, Ruminococcaeceae,* and *Akkermansia* in healthy controls (Chu et al., 2020; d'Afflitto et al., 2022; Li et al., 2022; Lindheim et al., 2017; Liu et al., 2017; Zhou et al., 2020). The same studies have identified these fecal discrepancies to be negatively correlated

with ghrelin and positively correlated with testosterone, luteinizing hormone (LH), anti-müllerian hormone, and body mass index (BMI) in PCOS patients. One mouse study found that prebiotic inulin in combination with metformin resulted in decreased inflammatory markers, body weight, and testosterone in PCOS model group, but human studies on complimentary probiotic and standard therapy continue to be lacking (Xue et al., 2019).

Statement of the Problem

PCOS is an increasingly common and underdiagnosed condition that, if unrecognized or undertreated, can increase risk for diabetes, metabolic syndrome, infertility, cardiovascular disease, and endometrial cancer. Standard of care currently focuses on treating insulin resistance (metformin), androgen imbalance (spironolactone), and anovulation (oral contraceptives) but does not address gut dysbiosis as a potential root cause of PCOS and its associated symptoms. A 2021 mixed-method study by Kaur et al. identified that PCOS patients were dissatisfied with the extended time to an official diagnosis, adequacy of information shared with them, and treatment options available. Patients included in this study cited that the internet, rather than their provider, was the primary source of information on their condition, resulting in conflicting and confusing recommendations for symptom management. This literature review aims to study the effectiveness of pre- and probiotic supplementation on PCOS outcomes. Supplementation and nutrition practices aimed at correcting imbalances in the gut microbiome could be a low-cost alternative or conjunctive therapy to standard of care in improving PCOS outcomes and satisfaction.

Research Question

What is the effect of probiotic regimens on metabolic and endocrinologic parameters in women with PCOS compared to standard of care?

Methods

A comprehensive literature review was performed using PubMed and Clinical Key databases. A combination of keywords were used to find pertinent studies relating to the impact of probiotics on PCOS outcomes. Keywords included PCOS, probiotics, synbiotics, microbiome, testosterone, androgens, anthropometrics, and glycemic control. Additional studies were found via PubMed list as Similar Articles or as reference in other studies used. A total of 609 articles were found. Systemic reviews, metanalyses, and studies performed on non-human subjects were excluded. Additionally, many studies were not used due to being greater than 10 years old, looking at conditions other than PCOS, and being duplicate articles. A total of 16 studies met the final criteria and were included in this literature review.

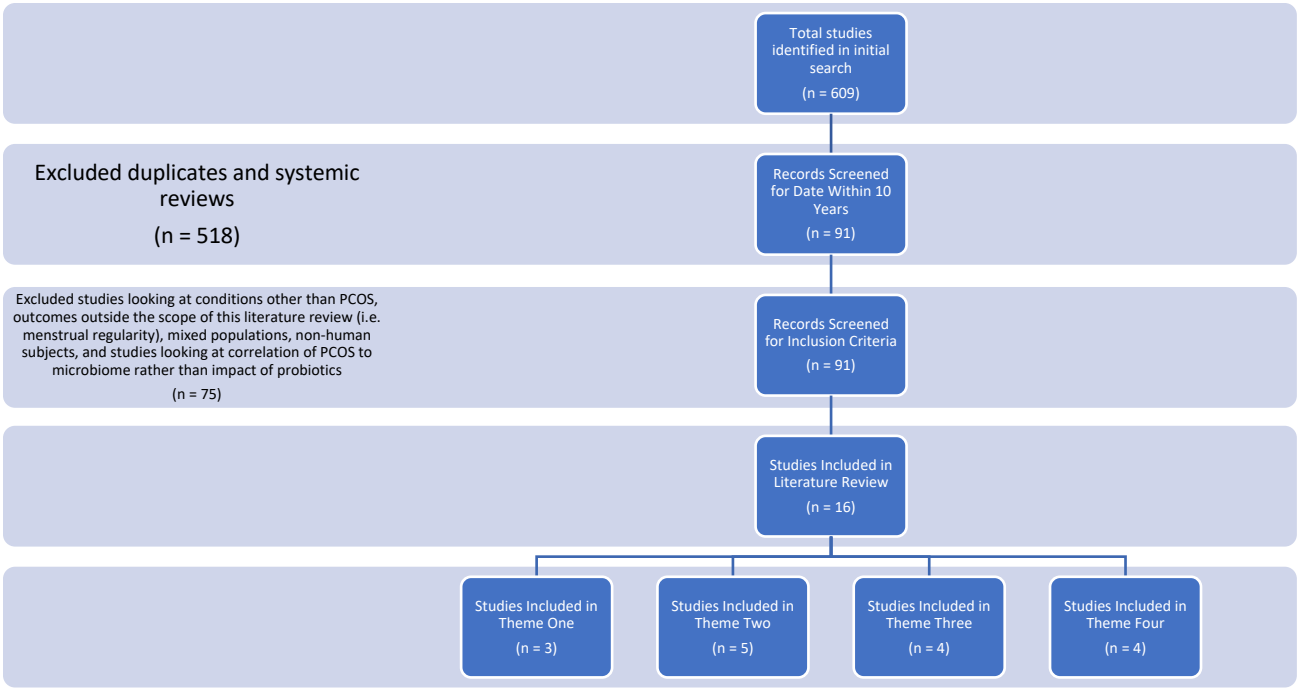

Figure 1: Research Methodology

Literature Review

Theme One: Probiotic Effect on Inflammatory Markers in Women with PCOS

Nasri et al. (2018) conducted a randomized, double-blind, placebo-controlled trial to assess the effect of probiotic supplementation on oxidative stress in PCOS. There were 60 subjects ages 18-40 years recruited from the Kossar Clinic in Arak, Iran. Inclusion criteria included meeting Rotterdam criteria for PCOS. Exclusion criteria included smoking, current supplementation of probiotic or synbiotic, pregnancy, gastrointestinal conditions, and other diagnosed endocrine conditions. The women were randomly assigned to the synbiotic group (n=30) or control group (n = 30), each taking their assigned pill daily for 12 weeks. Three day diet recall, physical activity measured via metabolic equivalents, anthropometrics, and blood samples were collected prior to intervention without significant differences between groups at the starting point. The primary outcome evaluated the level of high sensitivity C-reactive protein (hs-CRP) with secondary outcomes including plasma nitric oxide (NO), total antioxidant capacity (TAC), serum sex hormone-binding globulin (SHBG), hirsutism measured via modified Ferriman Gallaway score (m-FG), free androgen index (FAI), dehydroepiandrosterone sulfate (DHEAS), total glutathione (GSH), malondialdehyde (MDA), and total testosterone. There was a 0% dropout rate resulting in all 60 subjects being included in final analysis. Compliance was assessed at the end of intervention through returning supplement containers. After 12 weeks, more than 90% of capsules were taken by both groups. Results showed a statistically significant decrease in serum hs-CRP (−950.0 ± 2246.6 ng/mL; $p = 0.02$) and increase in plasma NO (5.5 ± 4.8 μmol/L; $p = 0.006$) when compared to control group. Additionally, results showed decreased m-FG, FAI, serum insulin, HOMA-IR, and total testosterone with an increase in SHBG in the synbiotic group compared with control ($p < 0.05$). Based on these results, it was concluded that

synbiotic supplementation has a beneficial effect on inflammatory markers and oxidative stress in women with PCOS. Longer term supplementation at higher doses may result in greater effects and should continue to be studied. Limitations of this study include not testing for a dose-response relationship between the supplement and oxidative markers, small sample size, and duration of study.

Ziaei et al. (2022) conducted a randomized, double-blind, placebo-controlled trial on the effects of inulin-type fructans (prebiotic) on oxidative stress in women with PCOS. The study included 75 women ages 18 to 40 years old who were recruited from Shahid-Beheshti Women's Hospital and Amin Hospital's gynecologist clinics in Iran, as well as from advertisements in the hospital and clinic lobbies. To meet inclusion criteria, participants had to have a body mass index (BMI) between 25 and 35 kg/m^2 and meet Rotterdam criteria for PCOS. Exclusion criteria included being outside of the age and BMI range, smoking, using hormone therapy, weight-loss interventions, diet supplements with pre or probiotics, antacids, or antibiotics in the past three months, or having additional chronic medical conditions. Patients were also excluded if they were presently or hoping to become pregnant in the next 6 months. Participants were randomized into one of three groups: 10 g/day of high-performance inulin (HPI), 10 g/day oligofructose-enriched inulin (OEI), or placebo with 10g/day of maltodextrin. All participants maintained their normal diet and physical activity throughout the 12 week study. Additionally, all supplements were identical in color, appearance, taste, and packaging. To promote compliance with supplementation, participants received 45 packages of supplement at baseline and 45 packages at six week follow-up. Participants also received weekly reminder calls, and used packages were to be returned at follow-ups. Baseline three day diet recall, anthropometrics, and fasting blood samples were collected prior to intervention and after 12 weeks of supplement or placebo. There

were no statistically significant differences between groups at baseline. Serum hs-CRP was considered the primary outcome with secondary outcomes including blood pressure and additional biomarkers of oxidative stress and endothelial dysfunction. After 12 weeks, 68 participants (91%) completed the trial. Reasons for drop out included low compliance rate (n = 2), COVID-19 infection (n = 2), pregnancy (n = 1), issues taking medication (n = 1), and personal reasons (n =1). On statistical analysis, a $p < 0.05$ was considered significant with confidence intervals not defined. Results showed a decrease in hsCRP in both the HPI and OEI groups (OEI -1.12; $p = 0.03$) with changes only in the HPI group being significant when compared to placebo (HPI -0.11 vs placebo + 0.004; $p = 0.007$). Systolic and diastolic blood pressures did not show significant changes in any groups. Levels of NO increased in both intervention groups but were not significant when compared to placebo. Similarly, ET-1 decreased in both intervention groups but non-significantly when compared to placebo. Based on these results, it was concluded that inulin-type fructans, especially high-performance kinds, have beneficial effects on CRP levels in women with PCOS but do not significantly impact blood pressure. Notably, all participants were normotensive at baseline and further studies on supplementation in hypertensive women with PCOS may be warranted. Additional limitations include relatively small sample size that may not be generalizable outside of the Irani area. Fecal gut bacterial analyses should be considered in future studies.

Karamali et al. (2018) conducted a randomized, double-blind, placebo-controlled trial regarding probiotic effect on inflammation and hormonal profiles in women with PCOS. Participants included 60 women ages 18 to 40 who met Rotterdam criteria for PCOS. The study was carried out at the Akbarabadi Clinic in Tehran, Iran. Participants were excluded if they smoked, took probiotics, were pregnant, or had endocrine diseases at baseline. Participants were

randomized into control (n = 30) or probiotic group (n = 30) who received a three strain probiotic daily for 12 weeks. The placebo pill was identical in color, shape, size, packaging, smell, and taste to the probiotic. Prior to intervention, baseline three day diet recall, physical activity metabolic equivalents, anthropometrics, and fasting blood sample were collected. The two groups did not have significant differences at baseline apart from serum testosterone ($p < 0.001$), which was controlled for in statistical analysis. The primary outcome was hs-CRP as a measure of inflammation and oxidative stress. Secondary inflammatory studies included NO, TAC, GSH, and MDA. Hormonal markers including FAI, sex hormone binding globulin (SHBG), serum testosterone, m-FG as a measure of hirsutism, and acne/alopecia scores were also studied. Treatment adherence was evaluated by participants returning remaining supplements to be subtracted from amount provided. Patients were also reminded to take their supplement with daily cell phone calls. After 12 weeks, all 60 subjects completed the study with a 90-100% compliance rate in both groups. Results showed that the probiotic group had a significant improvement in hsCRP levels (-1150.0 +/- 1295.2; $p < 0.01$). Results also showed an increase in TAC (+8.8 vs -98.3 mmol/L; $p = 0.04$) and decrease in MDA (-0.2 vs +0.9 umol/L; $p < 0.001$) in the probiotic group. There were no significant changes in NO or GSH. It was concluded that 12 weeks of probiotic supplementation in women with PCOS had beneficial effects on inflammatory markers including serum hs-CRP, plasma MDA, and plasma TAC but not with GSH or NO. Strengths of this study include study design and compliance rate. Limitations include not disclosing recruitment measures and absence of fecal sample to analyze bacterial changes.

Theme Two: Probiotic Effect on Anthropometrics in Women with PCOS

Chudzicka-Strugata et al. (2021) conducted a randomized, double-blind, placebo-controlled trial on 65 women who met Rotterdam criteria for PCOS and had a BMI > 25. The primary purpose of this study was to evaluate how lifestyle changes along with placebo or synbiotic supplementation impacted BMI. A secondary purpose of the study was to observe testosterone levels between the two groups. Participants were recruited from the Reproductive Endocrinology & Infertility Clinical Services at Poznan University of Medical Sciences in Poland. The average starting BMI in the control group was 34.4 +/- 1.2 kg/m^2 compared to 33.4 +/- 1.1 kg/m^2 in the experimental group. Prior to intervention, 85% of participants were found to have hyperandrogenism. There were no statistically significant differences found between control and experimental groups at baseline. Prior to the start of the study, all participants were off hormonal therapy, antibiotics, laxatives, nutrition supplements, and other probiotics or synbiotics for at least two months. Baseline anthropometrics and hormone levels were collected prior to intervention and three months after intervention. Lifestyle modifications for both groups consisted of a 1400-1800 kcal/day diet based on body composition analysis, individualized food composition advice, 30-40 minutes of walking daily, and in-person dietitian consults biweekly. Only 60% of participants completed the duration of the study with dropout rates being equal amongst the two study groups. Reasons for dropout included not being able to participate in meetings and personal reasons. Results showed a 5% decrease in BMI in the control group compared to an 8% decrease in BMI in the intervention group indicating a statistically significant greater impact in the intervention group ($p = 0.03$). Testosterone levels were not significantly decreased in the control group but were decreased by 32% in the intervention group ($p < 0.0001$). Based on these findings, it was concluded that synbiotic supplementation in addition to lifestyle

modifications led to significant decreases in BMI and serum testosterone. Limitations of this study include small sample size with 40% dropout rate and short window of study. Strengths of the study include design with randomization, placebo control, and double-blind intervention.

Ahmandi et al. (2017) conducted a double-blind, placebo-controlled, randomized, parallel-arm trial to study the impacts of probiotic supplementation on anthropometrics in women with PCOS, in addition to glycemic control and lipid profiles. Participants were recruited from Taleghani and Emam Reza Clinics which are part of the Arak University of Medical Sciences in Arak, Iran. Out of 85 volunteers, 60 met inclusion criteria and were included in the study and final analysis. Inclusion criteria included meeting Rotterdam criteria for PCOS, age 18-50 years, and body mass index (BMI) > 19 kg/m^2. Exclusion criteria included history of additional chronic diseases, taking hormones, antiobesity, or antidepressant medications in the past three months, smoking, or taking other forms of probiotics. Participants were randomized into probiotic (n = 30) or placebo (n = 30) groups. Baseline anthropometrics, fasting blood samples, dietary recall, and physical activity records were obtained prior to intervention. Participants in the probiotic group received a daily supplement containing three viable strains of beneficial bacteria, while the placebo group received a capsule that contained starch but no bacteria. The placebo was identical in color, shape, size, packaging, smell, and taste as the probiotic capsule. Probiotic or placebo was taken daily for 12 weeks in addition to maintaining current diet and exercise practices. To promote compliance, participants were given new supplement packages every four weeks with all unused supplements returned at each visit. Additionally, participants received cell phone messages to take their supplements daily. At the end of the study, there was over 90% compliance in both groups. Results at the end of the study were considered significant with $p < 0.05$. During the intervention phase of the study, there were

two participants in the placebo group who dropped out of the study for personal reasons (n = 2) and two participants in the probiotic group who dropped out for personal reasons (n = 2), indicating an overall 92% completion rate. Results showed that participants in the probiotic group had a significant decrease in weight (-0.5 kg probiotic group vs + 0.1 kg placebo group; $p = 0.004$) and BMI (-0.2 kg/m^2 probiotic group vs +0.03 kg/m^2; $p = 0.004$) compared with placebo. Importantly, there were no significant differences between the two groups in dietary intake or physical activity based on records received throughout the intervention. Secondarily but also notably, probiotic usage was associated with decreased fasting plasma glucose ($p = 0.02$), serum insulin concentrations ($p = 0.01$), and serum triglycerides ($p = 0.02$). Based on these results, it was concluded that probiotic supplementation for 12 weeks had beneficial effects on BMI, weight loss, glycemia, and triglycerides in PCOS patients. Strengths of the study include design and compliance rate. Limitations include a short window of follow-up and not obtaining fecal samples for assessing probiotic effect on fecal short chain fatty acids.

Darvishi et al. (2021) conducted a randomized, double-blind, placebo-controlled clinical trial to observe effects of synbiotic supplementation on obesity values in women with PCOS. Secondary outcomes included metabolic parameters, including serum fasting glucose, insulin, homeostatic model assessment for insulin resistance (HOMA-IR), and cholesterol. Participants consisted of 68 women ages 20-44 years with a BMI of 25-40 kg/m^2 who met Rotterdam criteria for PCOS and were recruited from the Alzahra Hospital in General Gynecology Clinic Department in Tabriz, Iran. Exclusion criteria included thyroid disorders, diabetes, hyperprolactinemia, liver or kidney disease, Cushing's disease, cardiovascular diseases, use of hydrochlorothiazide, insulin, beta blockers, cholesterol medication, fertility treatments, cortisone-like medication, antibiotics, or dietary supplements in the past two months. Participants

were randomized into treatment group (n = 34) or placebo group (n = 34). The treatment group received daily synbiotic supplement containing seven strains of beneficial bacteria and inulin-type prebiotics while the placebo group received a capsule containing starch that was identical in color and form for eight weeks. Prior to intervention, baseline BMI, hip circumference (HC), waist circumference (WC), waist-to-hip ratio (WHR), weight-to-height ratio (WHtR), Validated International Assessment of Physical Activity Questionnaire (IPAQ), three-day diet recall, and fasting blood samples were obtained. Compliance with supplementation was encouraged through weekly phone calls and assessed through returning capsules every two weeks. In statistical analyses, a $p < 0.05$ was considered significant. At the end of the study, there were no dropouts in either group and all subjects were included in final analysis (n = 68). There was a 95% compliance rate with supplementation during the study. There were no statistically significant differences in age, weight, BMI, or physical activity level between the participants at baseline, but there were significant differences in baseline energy and carbohydrate intake. After eight weeks, the synbiotic group showed a significant decrease in WC (2.5%; $p = 0.009$) and WHtR ($p = 0.02$) but subtle decrease in BMI and weight were not shown to be significant. However, when compared to placebo, decreases in weight ($p = 0.009$) and BMI ($p = 0.002$) in the synbiotic group were significant. Comparatively, those in the placebo group had a significant increase in weight and BMI (0.74% with $p = 0.003$ and 0.87% with $p = 0.003$, respectively). Secondary findings showed a significant decrease in blood glucose, insulin, and HOMA-IR in the synbiotic group by 1.35% ($p = 0.026$), 13.92% ($p = 0.001$), and 15.68% ($p = 0.001$), respectively, with a 2.88% increase in HDL compared to placebo ($p = 0.024$). Serum apelin values remained unchanged in both groups. Based on these results, it was concluded that synbiotic supplementation for eight weeks in women with PCOS led to reduced weight, BMI, and central obesity indices in addition

to lowering HOMA-IR, blood glucose, and insulin. Strengths of the study included methodology with double-blind placebo-control trial in addition to having no dropouts. Limitations include short duration of study and not analyzing fecal bacterial flora changes. Results cannot be generalized to women with PCOS who have a BMI < 25 kg/m^2.

Karimi et al. (2020) conducted a double-blind, placebo-controlled trial studying the effects of synbiotic supplementation on anthropometrics in addition to lipid profiles in women with PCOS. Participants were recruited from the Arash Hospital in Tehran, Iran. A total of 120 women were screened with 99 meeting inclusion criteria. Participants were randomly assigned to synbiotic or control group. Inclusion criteria included age between 19-37 years of age, meeting Rotterdam criteria for PCOS, and having a BMI > 25 kg/m^2. Participants were excluded if they had a history of thyroid disorder, hyperprolactinemia, Cushing's syndrome, digestive issues, diabetes, other chronic medical conditions, allergy to capsule, pregnancy or breastfeeding, and current or previous use of antibiotics, multivitamin, or pre-, pro-, or synbiotic in the past three months. Baseline anthropometrics, dietary intakes, and fasting blood samples were obtained prior to intervention and again following 12 weeks of supplementation or placebo. There were no significant differences between the two groups at baseline. The synbiotic group took 500 mg of seven strains of beneficial bacteria with prebiotic inulin daily for 12 weeks, whereas the control group received a capsule identical in appearance, smell, and taste. Participants were instructed to not change their usual diet or exercise routines throughout the duration of the study, which included avoiding sources of other synbiotics such as fermented products. Compliance was encouraged through weekly phone calls. Throughout the 12 weeks, there were 11 patients who dropped out due to non-compliance (n = 3), no drug intake (n = 2), unwillingness to continue (n = 5), and pregnancy (n =1) resulting in a final total of 88 participants (synbiotic n = 44; placebo

n = 44). At the end of the study, there were no significant differences found between the two groups in BMI, WC, HC, and WHR, but the synbiotic group did show a significant decrease in LDL (-4.66 mg/dL; $p = 0.041$) and increase in HDL (+1.80; $p = 0.016$) compared to baseline. Based on these results, it was concluded that 12 weeks of probiotic supplementation in women with PCOS did not impact anthropometric indices but did result in beneficial effects on LDL and HDL levels. Strengths of the study include study design and sample size. Limitations include not measuring compliance to supplement use, not obtaining fecal bacterial samples, and short duration of study.

Łagowska and Kapczuk (2022) performed a randomized clinical control trial to assess the impact of a low glycemic index diet with and without supplementation of the probiotic *Lactobacillus rhamnosus* on anthropometrics along with carbohydrate metabolism and androgen status in women with PCOS. Participants were recruited from the Poznan Medical University Hospital of Obstetrics and Gynecology in Poland. Inclusion criteria included BMI > 26 kg/m^2, being of reproductive age (18-45 years), and having a diagnosis of PCOS. Exclusion criteria included having undergone ovarian surgery, use of antibiotics or probiotics in the past six months, using hormonal drugs in the past three months, using medications affecting carbohydrate metabolism in the past four weeks, current use of weight loss supplements, having digestive issues, or currently being pregnant or breastfeeding. A total of 56 women were randomized into one of two groups: (1) low glycemic index diet with placebo pill (group D) or (2) low glycemic index diet with *Lactobacillus rhamnosus* probiotic supplement (group DP). Participants and researchers, except for the dietitians involved, were blinded to the groups. Prior to intervention, baseline anthropometrics and fasting blood samples in the early follicular phase were obtained. An oral glucose tolerance test was also performed at baseline. Over the course of the study, all

participants followed a weight-reduction diet with low glycemic index prepared by a registered dietitian, which included a 600 kcal/day deficit with minimum daily fiber intake of 25 g/day. In addition, each participant took two capsules of placebo or probiotic daily. Compliance was encouraged through having periodic check-ins where anthropometrics were remeasured at weeks 4, 8, 12, 16, and 20 in addition to having phone calls on other weeks. Participants were asked to return the supplement packaging at each check in to calculate compliance. At the end of the study, there were 16 dropouts due to pregnancy (n = 5) and declining to participate (n = 11). Final analysis consisted of 21 participants in the D group and 19 participants in the DP group. Following intervention, both groups showed a significant decrease in weight (-9.35 kg group D; -8.85 kg group DP), BMI (-3.19 kg/m^2 group D; - 3.17 kg/m^2 group DP), fat mass (-5.73% group D; -6.21% group DP), and waist circumference (-11.33 cm group D; -8.21 cm group DP) but results were not significant between the two groups. Similarly, both groups showed improvements in oral glucose tolerance test, but one was not significant over the other. Based on these results, it was concluded that 20 weeks of supplementing with *Lactobacillus rhamnosus* had no significant beneficial effects on metabolic profiles as compared to a low glycemic index weight loss diet alone. Strengths of this study include longer duration of intervention than similar studies and being a randomized control trial. Limitations include small sample size, not reporting compliance to intervention, not having certain staff being blinded to results, and utilizing a single strain antibiotic. Additionally, it would be beneficial to have another control group who was not following a low glycemic index weight loss diet. The diet followed in this study was high in fiber and likely other sources of pre- and probiotics in which a probiotic supplement may show greater effects in women with PCOS who do not get pre- or probiotics in their diet alone.

Theme Three: Probiotic Effect on Androgen Levels in Women with PCOS

Jamilian et al. (2018) conducted a randomized, double-blind, placebo-controlled trial on 60 women ages 18-40 years who met Rotterdam criteria for PCOS. Participants were recruited from Kosar Clinic in Arak, Iran. Participants were excluded from the study if they were pregnant or had adrenal hyperplasia, rogen-secreting tumors, hyperprolactinemia, thyroid dysfunction, or diabetes prior to the study. The primary aim of the study was to evaluate outcomes of probiotic and selenium co-supplementation on hormonal profiles in women with PCOS with secondary outcomes including mental health indices and inflammatory markers. Participants were randomized into experimental group (n = 30) who received 8 x 10^9 CFU probiotic with 200 μg selenium per day or placebo pill (n = 30) for 12 weeks. Fasting blood samples, beck depression inventory (BDI), general health questionnaire-28 (GHQ-28), depression anxiety and stress scale (DASS), and free androgen index (FAI) were obtained before and after intervention. Mean anthropometrics and macro/micronutrient intake were not statistically different between the two groups prior to intervention. Following intervention, the probiotic and selenium co-supplementation group showed a statistically significant decrease in total testosterone (-0.26 ng/mL; $p = 0.03$) and hirsutism (mF-G -0.43; $p = 0.008$). There was not a statistically significant change in SHBG. The experimental group had a significant improvement in BDI (-0.76; $p = 0.003$), GHQ (-1.15; $p = 0.007$), and DAAS (-1.49; $p = 0.009$) for mental health parameters. Inflammatory and antioxidant markers also showed improvement in the experimental group with hs-CRP decreasing by 0.58 mg/L ($p = 0.004$), MDA levels decreasing by 0.29 μmol/L ($p = 0.03$), and total antioxidant capacity increasing by 84.76 mmol/L ($p < 0.001$). All participants completed the study and were included in final analysis with dropout rate of 0%. Based on these findings, it was concluded that 12 weeks of co-supplementation with probiotics and selenium

resulted in improvement in hormonal, oxidative, and mental health parameters in women with PCOS. Strengths of this study include study design and attrition rate. Limitations include sample size and length of study. Additionally, further studies should include probiotic and selenium groups alone to determine the extent of effect of one supplement over the other.

Ostadmohammadi et al. (2019) performed a randomized, double-blind, placebo-controlled trial on the impacts of vitamin D and probiotic co-supplementation on women with PCOS as this combination of supplements has shown beneficial outcomes in metabolic disorders in other studies. Primary outcomes of this study included hormonal profiles with secondary outcomes including mental health parameters and markers of oxidative stress. Participants included 60 women with PCOS according to Rotterdam criteria who were recruited from the Naghavi Clinic in Iran. Exclusion criteria included pregnancy, lactation, adrenal hyperplasia, androgen-secreting tumors, hyperprolactinemia, thyroid conditions, diabetes, and baseline depression or anxiety. Participants were randomized into an experimental group (n = 30) who received 50,000 IU vitamin D every 2 weeks and 8 x 10^9 CFU/day probiotic capsules. The control group (n = 30) received corresponding capsules identical in appearance, color, shape, size, smell, taste, and packaging but did not have supplements added. The probiotic dose and contents were chosen based on previous studies on diabetic and coronary heart patients as the ideal dosage of probiotics in PCOS has not been determined. At baseline, there were no significant differences in anthropometrics, dietary intake, age, or serum lab values between the two groups. Age of participants ranged from 18-40 years old, and BMI ranged from 17-34 kg/m^2. Baseline fasting blood samples, including serum total testosterone, SHBG, 25-hydroxyvitamin D ((25(OH)D)), hs-CRP, NO GSH, and MDA were obtained, in addition to m-FG score for hirsutism measure, TAC, BDI, GHQ-28, DASS, and PSQI for sleep quality. Compliance with

intervention was controlled through quantifying serum 25(OH)D levels, having participants return medication containers, and having brief daily phone call reminders to take supplement. After 12 weeks of intervention, there were no dropouts, and all 60 participants were included in final analysis. Statistical analyses were performed with 95% confidence intervals and statistical significance defined as $p < 0.05$. Results for the primary outcome of the study showed a significant reduction in total testosterone (-0.19 ng/mL; $p < 0.001$) and hirsutism (-0.95; $p < 0.001$) but not SHBG, acne, or alopecia. Additionally, there were significantly improved BDI (-0.58; $p = 0.04$), GHQ-28 (-0.93; $p = 0.03$), DASS (-0.90; p 0.02), hsCRP (-0.67 mg/L; $p < 0.001$), and MDA levels (-0.25 umol/L; $p = 0.001$) with significant increase in TAC (+82.81 mmol/L; $p < 0.001$) and GSH (+40.42 umol/L; $p = 0.02$) in the experimental group when compared to placebo. It was concluded that the co-administration of vitamin D and probiotics has beneficial outcomes on serum testosterone, hirsutism, mental health parameters, TAC, GSH, and MDA levels but did not impact SHBG, NO, acne, or alopecia. Limitations of this study include not having additional groups who received single vitamin D or probiotic supplement alone to see the impact of one verse the other on these parameters compared to the co-supplementation. Additionally, fecal samples were not collected before, during, or after intervention to analyze changes in microbiome. Strengths of the study include study design, attrition rate, and having parameters to enforce compliance with intervention.

Zhang et al. (2019) performed a two-phase cohort experiment looking at the microbiome composition of women with PCOS and subsequent effect of supplementing with *Bifidobacterium lactis V9*. In the first phase, 64 women, 38 of whom had PCOS and 26 healthy controls, provided blood and stool samples to examine differences between triglycerides, total cholesterol, fasting plasma glucose, peptide YY, ghrelin, luteinizing hormone (LH), follicle stimulating

hormone (FSH), prolactin, estradiol, testosterone, and intestinal microbiotic makeup. Participants were recruited from the Hainan General Hospital in Haikou, Hainan Province of China. Results of significance for this present review include the microbiome of women with PCOS showing lower levels of *Faecalibacterium*, *Lachnospira*, *Bifidobacterium*, and *Blautia* and much higher levels of *Parabacteroides*, *Bacteroides*, *Lactobacillus*, *Oscillbacter*, *Escherichia/Shigella*, and *Clostridium* compared to the control group. It is important to note that *Faecalibacterium prausnitzii* and *Bifidiobacterium,* which the PCOS group was lacking, are beneficial, whereas *Prevotella copri* and *Collinsella aerofaciens*, which the PCOS group had more of, are disease-related microbes. Subsequently, it was found that the PCOS group had significantly lower levels of fecal short-chain fatty acids (acetic, propionic, butyric, and valeric), which are the main beneficial metabolites of the microbiome. Additionally, at baseline, the women with PCOS had significantly higher levels of LH (17.96 vs. 7.22 IU/liter; $p < 0.001$), prolactin (11.79 vs. 9.22 ng/ml; $p < 0.001$), and testosterone (6.02 vs. 0.83 nm/liter; $p < 0.001$) compared to control. In the second phase of the study, 31 of the 38 women from the PCOS group volunteered to be part of the experimental phase, but only 14 of them did not start treatment for PCOS and were included in this phase of the study. In this phase, all 14 subjects took a *Bifidobacterium lactis V9* supplement once daily for 10 weeks while avoiding other probiotic products and antibiotics. Stool samples were collected at weeks 0, 2, 4, and 10. Results showed that supplementation with this probiotic significantly decreased levels of LH and LH/FSH ratio while increasing estrogen, acetic acid, propionic acid, butyric acid, and valeric acid in nine of the subjects (64%) and had fluctuations in the remaining 5 participants. Strengths of this study include analyzing fecal makeup of PCOS patients compared to healthy controls. Limitations include having a small cohort limiting clinical significance of the experimental phase of the study. It was concluded that

a potential mechanism modulating sex hormone levels in PCOS patients includes supplementation of *Bifidobacterium lactis* V9 acting on the gut-brain axis.

Kaur et al. (2022) performed a double-blind, placebo-controlled, parallel arm, randomized clinical trial to examine the effects of multi-strain probiotic in combination with diet and exercise on hormonal profiles, menstrual regularity, weight reduction, and metabolic profiles in women with PCOS. Participants were recruited from the Gynecology Outpatient Department at the Postgraduate Institute of Medical Education and Research in India. Participants included 104 women who met Rotterdam criteria for PCOS and were between the ages of 18 and 40 years. Exclusion criteria included any pre-existing chronic condition, immunosuppression, abnormal thyroid or prolactin levels, use of insulin-sensitizing or glucose-lowering medications, being bedridden, or using oral contraceptives, fertility medications, or other probiotics in the past three months. Participants were randomized into control (n = 52) and experimental group (n = 52). Prior to intervention, baseline demographics, anthropometrics, and fasting blood samples in the early follicular period of the menstrual cycle were collected. There were no significant differences in starting points between the two groups. Both groups followed the same diet plan (55-60% carbohydrate, 20% fat, 20-25% protein) and exercise plans as well as had access to a social media app to engage participants and keep them motivated. The probiotic group received capsules that contained 10 billion CFU multi-strain microbes whereas the placebo group received identical capsules without the beneficial microbes. Participants consumed one capsule daily for the first two months and two capsules per day for the next four months to help alleviate potential gastrointestinal side effects. The intervention was performed for six months with compliance promoted and evaluated through daily call and text reminders as well as dispensing a new bottle of supplement or placebo at monthly follow-up visits. There were four total dropouts in the

intervention group due to relocation (n = 1) and inability to go to scheduled visits (n = 3) and three total dropouts in the placebo group due to relocation (n = 1) and no relief (n = 2). Final compliance rate to supplement was 85% in the probiotic group and 95% in the placebo group. Diet and exercise compliance was 58% and 45% in the probiotic group and 77% and 44% in the placebo group, respectively. Of importance for this review is that the probiotic group showed a significantly reduced testosterone from baseline (-2.02 nmol/L; $p = 0.003$) and compared to placebo (-1.31 nmol/L; $p = 0.043$). Additionally, the probiotic group showed significant improvement in LH/FSH ratio (-1.36; $p < 0.001$) but not in DHEAS. The placebo group also showed improvement in LH/FSH ratio (-1.01; $p < 0.001$) through diet and exercise alone but did not have a significant change in testosterone or DHEA. Based on this and other findings in the study, it was concluded that six months of probiotic supplementation along with diet and exercise significantly improved testosterone, insulin, LH/FSH ratio, insulin resistance, weight, waist circumference, body mass index, menstrual regularity, ultrasonography scans, and plasma lipopolysaccharide levels in women with PCOS. Strengths of this study include longer duration and greater number of participants compared with similar studies. Additionally, this study was able to compare effects of probiotic compared to effects of diet and exercise alone. Limitations include not evaluating stool microbiota changes and relatively low compliance to the diet and exercise plans.

Theme Four: Probiotic Effect on Insulin and Blood Sugar Levels in Women with PCOS

Samimi et al. (2018) conducted a prospective, randomized, double-blind, placebo-controlled trial aimed at studying the effects of synbiotic supplementation on glycemic control and lipid profiles in women with PCOS. The study was performed at Naghavi Hospital affiliated with Kashan University of Medical Sciences in Kashan, Iran. Participants included 60 women

ages 18-40 with PCOS according to Rotterdam criteria. Exclusion criteria included smokers, those who have taken probiotics or synbiotics in the past 3 months, pregnant women, and women with other common causes of hyperandrogenism or anovulation. The women were randomly assigned to take a synbiotic capsule plus 800 mg inulin (n = 30) or placebo (n = 30) every day for 12 weeks. All participants were to continue their normal dietary and exercise routines and document a three-day food diary along with physical activity in metabolic equivalents at weeks 0, 3, 6, 9, and 12. There was not a significant difference between macro- and micronutrient intakes or physical activity between the two groups. At baseline and 12-week marks, anthropometrics, fasting plasma glucose (FPG), serum triglycerides, total cholesterol, VLDL, LDL, HDL, and circulating serum insulin were collected. The homeostatic model of assessment for insulin resistance (HOMA-IR) and quantitative insulin sensitivity check index (QUICKI) were also calculated at these times. Two participants from each group (7%) dropped out of the study due to personal reasons. After 12 weeks, the experimental group had a statistically significantly decline in serum insulin ($p = 0.002$), decrease in HOMA-IR ($p = 0.002$), and increase in QUICKI ($p < 0.001$) compared to placebo. Triglycerides, VLDL, and atherogenic index of plasma were also decreased in experimental group. Results showed a decrease in fasting plasma glucose in the experimental group (-4.6 +/- 1.3), which was a significantly greater reduction than placebo group after adjusting for BMI ($p = 0.04$). Strengths of the study include study design and controlling for compounding factors including regular monitoring of diet and exercise. It was concluded that synbiotic supplementation improved insulin resistance and lipid markers in women with PCOS. Limitations of the study include short duration of study as longer follow-up probiotic administration may have more substantial effects.

Esmaeilineahad et al. (2019) conducted a randomized, controlled, triple-blind, parallel trial on 92 women with PCOS to observe the effects of synbiotic and pomegranate juice co-supplementation on glycemic control in PCOS. Subjects were between 15-48 years of age and were recruited from the Motahari Clinic in Iran. All participants met Rotterdam criteria for PCOS in absence of other androgen-related medical conditions. Exclusion criteria included treatment with corticosteroids, chemotherapy, antibiotics, vitamin/minerals, antioxidants, hormonal pills, and chronic health conditions, in addition to special diet and exercise routines. The primary outcome assessed was insulin resistance based on HOMA-IR with secondary outcomes including fasting blood glucose, insulin, total testosterone, LH, and FSH. The participants were randomly assigned into one of four groups. The synbiotic pomegranate juice (SPJ) group received two liters of pomegranate juice with inulin and lactobacillus weekly for 8 weeks; the pomegranate juice (PJ) group received two liters of pomegranate juice alone weekly for 8 weeks; the synbiotic beverage (SB) group received two liters of synbiotic beverage juice with pomegranate flavoring weekly for 8 weeks; and the control group received two liters of water with pomegranate flavoring weekly for 8 weeks. All drinks were identical in appearance, color, and taste. Anthropometrics, three day diet recall, and fasting blood samples were obtained prior to and after intervention. There were no significant differences in anthropometrics, dietary intake, or physical activity between the groups prior to the intervention. At the end of the intervention, six participants dropped out due to nonadherence resulting in a 93% completion rate (n = 86). At the end of the intervention, HOMA-IR significantly decreased in both the SPJ and SB groups ($p < 0.05$) when compared to baseline and control group. Fasting blood glucose and insulin levels also significantly decreased, resulting in an increase in insulin sensitivity in the SPJ and SB groups when compared to the control group, but only the SB group had a significant

decrease when compared to baseline ($p < 0.05$). Secondary findings included a statistically significant decrease in testosterone, body mass index, weight, and waist circumference in SPJ and SB groups ($p < 0.05$) when compared to control. There were no significant changes in LH or FSH in any group. Based on these findings, it was concluded that a synbiotic regimen with or without the addition of antioxidant-rich fruit juice may promote glycemic, hormonal, and anthropometric control in women with PCOS. Limitations of this study include not knowing the bioavailability of probiotics in fruit juices, not measuring fecal bacterial loads before and after probiotic supplementation, short duration of study, and lower probiotic count in synbiotic supplements. Strengths of the study include having four different groups to determine effect of probiotic against pomegranate juice in addition to the effects together. Furthermore, this study had a larger sample size as compared to similar studies.

Karimi et al. (2018) conducted a randomized, double-blind, placebo-controlled trial to analyze effects of synbiotic supplementation on markers of glycemic control and apelin in women with PCOS. Apelin is an adipokine that is secreted in response to insulin secretion and is suggested to be elevated in obesity, insulin resistance, and inflammation, thereby potentially being correlated with PCOS. In this study, 120 women who met Rotterdam criteria for PCOS were recruited from Arash Women's Hospital in Tehran, Iran. There were 99 women who met inclusion criteria and were included in the study. Inclusion criteria was narrowed down to women between 19-37 years old to account for changes in microbiome after reproductive years. Participants also had a body mass index (BMI) of > 25 kg/m^2 as levels of inflammatory markers and insulin resistance being studied are more prevalent in overweight and obese subjects. Exclusion criteria included being out of the BMI or age range, having a history of chronic health conditions, allergies to probiotic or placebo capsules, or use of antibiotics, multivitamin, or

certain diet and exercise regimens in the past 3 months. Participants were randomly assigned to taking a synbiotic capsule with seven strains of beneficial bacteria or a placebo capsule daily for 12 weeks. To ensure compliance with intervention, participants were given six weeks of supplements at a time and were provided with the second set at the halfway point of the study. Prior to intervention and after 12 weeks of placebo or supplement, baseline weight, height, waist and hip circumference, three day diet recall, BMI, fasting blood sugar (FBS), two hour post prandial blood sugar (PGF-2h), hemoglobin A1c (HbA1c), hs-CRP, serum apelin 36, HOMA-IR, and QUICKI were obtained. Statistical analyses were performed with a confidence interval of 95% and a significant *p*-value of < 0.05. There was an overall 11% drop-out rate with 11 women (12%) in the experimental group and five women (10%) in the placebo group withdrawing from the study or being lost to follow-up. Reasons for drop-out included non-compliance to intervention (n = 3), no drug intake (n = 2), unwillingness to continue (n = 5), and pregnancy (n = 1). There were 88 participants who completed the study and were included in final analysis. At baseline, anthropometrics, age, nutrient intake, menstruation, infertility, family history of diabetes, and markers of insulin resistance were similar, except for PGF-2h levels which were significantly higher in the treatment group than placebo group. Following intervention, there were no significant differences between the two groups in FBS, PGF-2h, HbA1c, HOMA-IR, QUICKI, or hsCRP. The only marker that showed statistically significant decrease was apelin 36 concentrations in the intervention group (27 nmol/L at baseline to 14.4 nmol/L after 3 months; $p < 0.02$) with no significant decrease seen in the control group. Based on these findings, it was concluded that 12 weeks of synbiotic supplementation did not have a significant impact on markers of glycemic control in the fasting state but did have an impact on apelin 36 levels. Apelin should be further studied for its association to PCOS. Strengths of this study include

larger sample size in relation to similar studies, study design, and controlling for compound variables. Limitations include not examining fecal bacterial flora prior to and following intervention. To make findings more generalizable, similar studies should be conducted with more diverse participant characteristics.

Shoaei et al. (2015) conducted a randomized, double-blind, placebo-controlled trial to assess the effects of probiotic supplements on pancreatic beta cell function and C-reactive protein in women with PCOS. Participants included 72 women ages 15-40 years old who met Rotterdam criteria for PCOS and were recruited from infertility centers of two hospitals affiliated with Isfahan University of Medical Sciences in Iran. Exclusion criteria included those with a history of other chronic health conditions, allergy to probiotic capsules, and current or previous use in the past six months of chemotherapy, corticosteroids, antibiotics, multivitamins, or omega-3 supplements. Participants were randomly assigned to taking a probiotic or placebo supplement daily for eight weeks. Baseline anthropometrics, demographics, dietary intake data, physical activity records, and blood samples were collected prior to intervention and did not show any significant differences between the two groups. Interestingly, both groups appeared to have markedly low energy intakes at baseline, with the placebo group consuming an average of 993 kcal/day and the probiotic group consuming an average of 1010 kcal/day. Blood samples included fasting blood glucose, CRP, and serum insulin. From this, HOMA-IR and the QUICKI were calculated. After eight weeks, three participants in the placebo group dropped out due to being unwilling to continue (n =2) and becoming pregnant (n = 1), and four participants in the probiotic group dropped out due to being unwilling to continue (n = 2), becoming pregnant (n = 1), and health problems (n = 1), indicating a 9.7% total dropout rate. After intervention, there was a nonsignificant decrease in fasting blood sugar (-4.15 +/- 2.87 md/dL; $p = 0.2$), serum

insulin (-0.49 +/- 0.18; $p = 0.5$), and HOMA-IR (-0.25 +/- 0.005; $p = 0.7$) in the probiotic group. Only serum insulin levels were considered significant after adjusting for age, BMI, waist circumference, education, marriage status, and physical activity. There was no significant impact on CRP levels in either group. Based on these findings, it was concluded that probiotic supplementation for eight weeks does not have significant beneficial effects on fasting blood sugar, insulin levels, or CRP. However, a key limitation in this study is the short duration of intervention as microbiome changes may not be seen within this time frame. Additionally, oral glucose tolerance tests and hormonal panels could be considered in future studies. Lastly, compliance and noncompliance with intervention were not reported.

Discussion

Inflammatory Markers

The studies included in this section collectively exhibited significant improvement in hs-CRP levels with probiotic supplementation. Nasri et al. (2018) showed improvement in NO levels but not TAC, whereas Karamali et al. (2018) showed an improvement in TAC but not NO. The improvement in NO in the Nasri et al. (2018) study may be associated with the prebiotic fiber addition as Ziaei et al. (2022) also showed an improvement in NO solely looking at prebiotic fibers.

Study	Final Subject Number	Duration of Intervention	Probiotic Composition	Relevant Outcome Measures
Nasri et al. (2018)	n = 60	12 weeks	2 x 10^9 CFU/g *Lactobacillus acidophilus* *Lactobacillus casei* *Bifidobacterium bifidum* + 0.8 g inulin	hs-CRP: -950.0 ng/mL (p = 0.02) NO: +5.5 μmol/L (p = 0.006) TAC: insignificant
Ziaei et al. (2022)	n = 68	12 weeks	10 g/day high-performance inulin (HPI) OR 10 g/day oligofructose inulin (OEI)	hs-CRP: HPI -0.11 mg/L (p = 0.02) OEI insignificant NO: HPI + 2.09 μmol/L (p = 0.04) OEI +2.83 μmol/L (p = 0.03)
Karamali et al. (2018)	n = 60	12 weeks	2 x 10^9 CFU/g *Lactobacillus acidophilus* *Lactobacillus casei* *Bifidobacterium bifidum*	hs-CRP: -1150.0 ng/mL (p < 0.01) NO: insignificant TAC: +8.8 mmol/L (p =0.04)

Table 1: Inflammatory Results

Anthropometrics

Studies showed a mixed effect on probiotic supplementation and anthropometric outcomes. Importantly, three out of four studies that used waist circumference as an outcome measure, which is a marker of central obesity associated with negative health outcomes, found significant improvements with probiotic supplementation (Chudzicka-Strugata et al., 2021; Darvishi et al., 2021; Karimi et al., 2021; Lagowska and Kapczuk, 2022). However, only Chudzicka-Strugata et al. (2021) and Ahmandi et al. (2017) showed reduction in weight and BMI with supplementation alone. Notably, these studies had a longer duration of intervention compared to similar studies, indicating that longer duration of supplementation may be necessary to see significant anthropometric changes. Lagowska and Kapczuk (2022) showed significant

reduction in weight, BMI, fat mass, and waist circumference but only when paired with a low glycemic index diet.

Study	**Final Subject Number**	**Duration of Intervention**	**Probiotic Composition**	**Relevant Outcome Measures**
Chudzicka-Strugata et al. (2021)	n = 39	12 weeks	*Bifidobacterium lactis* *Lactobacillus acidophilus* *Lactobacillus paracasei* *Lactobacillus plantarum* *Lactobacillus salivarius* *Lactobacillus lactis* + fructooligosaccharides and inulin	Weight: -7.46 kg (p =0.02) BMI: -2.68 kg/m^2 (8%) (p = 0.03) WC: -10.7 cm (p = 0.03) Body Fat: insignificant
Ahmandi et al. (2017)	n = 60	12 weeks	2 x 10^9 CFU/g *Lactobacillus acidophilus* *Lactobacillus casei* *Bifidobacterium bifidum*	Weight: -0.5 kg (p = 0.004) BMI: -0.2 kg/m^2 (p = 0.004)
Darvishi et al. (2021)	n = 68	8 weeks	3 x 10^9 CFU/g *Lactobacillus rhamnosus* *Lactobacillus bulgaricus* *Lactobacillus acidophilus* *Bifidobacterium longum* *Streptococcus thermophilus* + fructooligosaccharides and inulin	WC: -2.94 cm (p = 0.009) WHtR: -0.01 (p 0.028) Weight: insignificant BMI: insignificant
Karimi et al. (2020)	n = 88	12 weeks	500 mg/day *Lactobacillus acidophilus* *Lactobacillus casei* *Lactobacillus bulgaricus* *Lactobacillus rhamnosus* *Bifidobacterium longum* *Bifidobacterium breve* *Streptococcus thermophilus*	WC: insignificant HC: insignificant WHR: insignificant BMI: insignificant
Lagowska & Kapczuk (2022)	n = 40	20 weeks	*Lactobacillus rhamnosus*	Weight: -8.85 kg (p BMI: -3.17 kg/m^2 Fat Mass: -6.21% WC: -8.21 cm *Note:* insignificant when compared to low glycemic index diet alone

Table 2: Anthropometric Results

Androgen Levels

Studies agreed that probiotic supplementation resulted in a significant reduction in total testosterone, mFG as a measurement of hirsutism, and LH/FSH ratio. The studies did not seem to show beneficial effects on SHBG or DHEA levels.

Study	Final Subject Number	Duration of Intervention	Probiotic Composition	Relevant Outcome Measures
Jamilian et al. (2018)	n = 60	12 weeks	8×10^9 CFU/day *Lactobacillus acidophilus* *Lactobacillus reuteri* *Lactobacillus fermentum* *Bifidobacterium bifidum*	Total Testosterone: -0.26 ng/mL ($p = 0.03$) mFG: -0.43 ($p = 0.008$) SHBG: insignificant
Ostadmohammadi et al. (2019)	n = 60	12 weeks	8×10^9 CFU/day *Lactobacillus acidophilus* *Bifidobacterium bifidum* *Lactobacillus reuteri* *Lactobacillus fermentum*	Total Testosterone: -0.19 ng/mL ($p < 0.001$) mFG: -0.95 ($p < 0.001$) SHBG: insignificant Acne: insignificant Alopecia: insignificant
Zhang et al. (2019)	n = 14	10 weeks	10×10^6 CFU/day *Bifidobacterium lactis* V9	LH and LH/FSH improved in 64% of subjects
Kaur et al. (2022)	n = 97	6 months	10×10^9 CFU/day *Lactobacillus acidophilus* *Lactobacillus rhamnosus* *Lactobacillus reuteri* *Lactobacillus plantarum* *Lactobacillus casei* *Lactobacillus fermentum* *Bifidobacterium bifidum* + fructooligosaccharides and inulin	Total Testosterone: -2.02 nmol/L ($p = 0.003$) LH/FSH: -1.36 ($p < 0.001$) DHEA: insignificant

Table 3: Androgen Results

Insulin and Blood Sugar Levels

There were inconsistent findings amongst studies on the impact of probiotic supplementation on markers of blood sugar control and insulin resistance in patients with PCOS. Samimi et al. (2018) and Esmaeilineahad et al. (2019) both showed improvement in HOMA-IR,

FPG, and serum insulin levels, whereas Karimi et al. (2018) and Shoaei et al. (2015) did not find significant changes in any biomarkers or equations.

Study	**Final Subject Number**	**Duration of Intervention**	**Probiotic Composition**	**Relevant Outcome Measures**
Samimi et al. (2018)	n = 56	12 weeks	8×10^9 CFU/day each *Lactobacillus acidophilus* *Lactobacillus casei* *Bifidobacterium bifidum* + 800 mg inulin	FPG: -4.1 mg/dL ($p = 0.04$) Serum Insulin: -2.8 µIU/mL ($p = 0.002$) HOMA-IR: -0.7 (p 0.002) QUICKI: +0.01 ($p < 0.001$)
Esmaeilineahad et al. (2019)	n = 86	8 weeks	2×10^8 CFU/g *Lactobacillus casei* *Lactobacillus ramnosus* *Lactobacillus plantrom* + 20 g inulin	HOMA-IR, FPG, insulin: "improved" ($p < 0.05$) *Note: study did not include baseline levels for comparison to results*
Karimi et al. (2018)	n = 88	12 weeks	500 mg/day *Lactobacillus acidophilus* *Lactobacillus casei* *Lactobacillus bulgaricus* *Lactobacillus rhamnosus* *Bifidobacterium longum* *Bifidobacterium breve* *Streptococcus thermophilus* + inulin	FPG: insignificant PGF-2hr: insignificant HbA1c: insignificant HOMA-IR: insignificant QUICKI: insignificant
Shoaei et al. (2015)	n = 65	8 weeks	500 mg/day *Lactobacillus casei* *Lactobacillus acidophilus* *Lactobacillus rhamnosus* *Lactobacillus bulgaricus* *Bifidobacterium breve* *Bifidobacterium longum* *Streptococcus thermophiles*	FPG: insignificant Serum Insulin: insignificant HOMA-IR: insignificant

Table 4: Insulin and Blood Sugar Results

Overall, studies included in this review did an appropriate job controlling for compounding variables. Strengths of this review include several studies with a 0% drop out rate, including Nasri et al. (2018), Karamali et al. (2018), Jamilian et al. (2018), and Ostadmohammadi et al. (2019). All studies, except for Zhang et al. (2019), were randomized clinical control trials. However, Zhang et al. (2019) was the only study that analyzed fecal microbiome samples of women with PCOS and compared them to healthy controls. The Kaur et al. (2022) study had the largest final sample size (n = 97) and was the longest in duration at six months of supplementation. This extended duration of supplementation showed significant improvement in total testosterone, insulin, LH/FSH ratio, insulin resistance, weight, waist circumference, BMI, menstrual regularity, ultrasound scans, and plasma lipopolysaccharide levels when combined with diet and exercise.

One key limitation to note includes a lack of generalizability as most studies were conducted in Iran. Because of this, participants may have a different baseline microbiome from cultural and geographic diet differences, as well as genetic differences, that decreases generalizability to other populations and regions. Additionally, most studies were conducted over a period of 8-12 weeks, which may not be sufficient time to alter the microbiome in such a way as to lead to significant alterations in outcomes studied. There were also a few studies included that looked at effects of probiotics as a co-supplement to other products, such as vitamin D, selenium, pomegranate juice, and various diets, in addition to probiotic alone. This can make it difficult to distinguish which factor is playing a larger role in the outcome.

Future studies should continue to include randomized control trials but with larger and more diverse populations. Studies should have a longer intervention timeframe to assess efficacy more adequately. Additionally, fecal analyses should be included at baseline and at the end of

intervention to examine alterations in the gut microbiome. Lastly, studies should be conducted that analyze the effect of probiotics compared to and in conjunction with standard of care, such as metformin, spironolactone, and contraceptive management.

Conclusion

A total of 16 studies were analyzed to assess probiotic effects on inflammatory, metabolic, anthropologic, and endocrinologic factors in PCOS. Cumulative results showed significant improvement in hs-CRP, total testosterone, hirsutism, LH/FSH ratios, and waist circumference. Impacts on other inflammatory markers, weight, BMI, insulin, and blood sugar levels continue to be inconsistent in research.

Application to Clinical Practice

This research is relevant to clinical practice as probiotic supplementation is a low risk therapeutic measure that may play a larger role with consistency over time in PCOS outcomes. The gut-brain axis and dysbiosis have been studied more heavily in the past several years and are being linked to negative health outcomes. A probiotic supplement, in addition to diet and lifestyle interventions, to improve patients' microbiome may be a helpful step in alleviating the polypharmacy and disease burden of conditions such as PCOS.

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