ALINEA

NUTRITION

JUNE 2021

www.alineanutrition.com

TABLE OF

CONTENTS

Nasri K, Jamilian M, Rahmani E, Bahmani F, Tajabadi-Ebrahimi M, Asemi Z. The effects of synbiotic supplementation on hormonal status, biomarkers of inflammation and oxidative stress in subjects with polycystic ovary syndrome: a randomized, double-blind, placebo-controlled trial. BMC Endocr Disord. 2018 Apr 12;18(1):21.

What We Know, Think We Know, or Are Starting to Know

Polycystic Ovarian Syndrome (PCOS) is categorised as a female reproductive disorder that is multifactorial in nature, encompassing neuroendocrine, ovarian and metabolic dysfunctions (Nardo 2008). The European Society for Human Reproduction and Embryology and the American Society for Reproductive Medicine [ESHRE/ASRM] established a consensus in 2003 for the pathophysiological characteristics of PCOS as: a) menstrual cycle dysfunction [either amenorrhea, the absence of menstrual cycles, or oligomenorrhea, infrequent cycles (<6-8 per year)]; b) elevated androgens ['hyperandrogenism'], and; c) polycystic ovaries present on ultrasound [(1)].

The multifactorial nature of the syndrome has posed difficulties for defining its pathophysiology. The so-called "Rotterdam criteria" arrived at by the ESHRE/ASRM may encompass a range of phenotype presentations, which led to an expanding of the Rotterdam criteria to encompass the four different phenotypes:

1. Hyperandrogenism with clinical anovulation;
2. Hyperandrogenism with polycystic ovaries present on ultrasound, but with ovulatory cycles;
3. Clinical anovulation with polycystic ovaries on ultrasound, but without hyperandrogenism;
4. Hyperandrogenism, clinical anovulation and polycystic ovaries on ultrasound [(1,2)].

Whether the so-called "Rotterdam Criteria" reflect a spectrum of the same condition is the subject of ongoing debate. Phenotypes with hyperandrogenism as a feature are typically characterised by higher bodyweight, insulin resistance and central adiposity [(2)]. In contrast, phenotypes without hyperandrogenism display normal insulin sensitivity and a metabolic profile similar to woman without PCOS of the same BMI [(2)].

A number of pharmaceutical drugs [clomiphene, metformin], nutritional supplements [inositol], and dietary interventions [high protein, high fibre], exist for PCOS. Recent years have seen the emergence of interest in the effects of synbiotic* supplementation in women with PCOS on markers of metabolic health and hormone levels.

The present study was the first trial to investigated the effects of synbiotic supplementation on hormonal, inflammatory and oxidative stress markers in participants with PCOS.

*Geek Box: Synbiotic

You have no doubt come across the terms 'probiotic' and 'prebiotic' before. To recap, probiotics are defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host"[3]. These are strain-specific bacteria with evidence for human health outcomes from randomised controlled trials. Prebiotics are defined as a "selectively fermented ingredient that allows specific changes, both in the composition and/or activity of the gastrointestinal microbiota that confers benefits" [4]. The key characteristics of prebiotics include resistance to breakdown by gastric acid, resistance to enzymatic breakdown and absorption in the small intestine, and selective fermentation in the large intestine/colon, resulting in selective growth and/or activity of beneficial bacterial populations and associated health benefits. Classification as prebiotic is to date confined to inulin, oligofructose [FOS] and galactooligosaccharides [GOS], for which there is evidence of benefits to human gastrointestinal health. So, what is a 'synbiotic'? The International Scientific Association for Probiotics and Prebiotics [ISAPP] has defined a synbiotic supplement as "a mixture comprising live microorganisms and substrate(s) selectively utilized by host microorganisms that confers a health benefit on the host" [5]. For example, a synbiotic supplement may contain a number of specific probiotic strains of bacteria with an additional dose of FOS. There are two categories of synbiotic supplement: complementary synbiotic and synergistic synbiotic [5]. A complementary synbiotic is one where the included components have previously met the criteria for probiotic and prebiotic, respectively, i.e., there is evidence for their separate effects. A synergistic synbiotic is composed of live microorganisms and a selective substrate, but neither needs to satisfy the criteria for probiotics and prebiotics. Rather, it is intended that synergistic synbiotics work together, and any study investigating a synergistic synbiotic must demonstrate selective utilisation of the substrate by the accompanying microorganisms, and demonstrate a health benefit to the consumer, in the same study. It should be noted that the term 'syn' in synbiotic does not refer to synergy or to symbiosis, but rather denotes 'together', indicating the co-administration of the microorganisms and selective substrate.

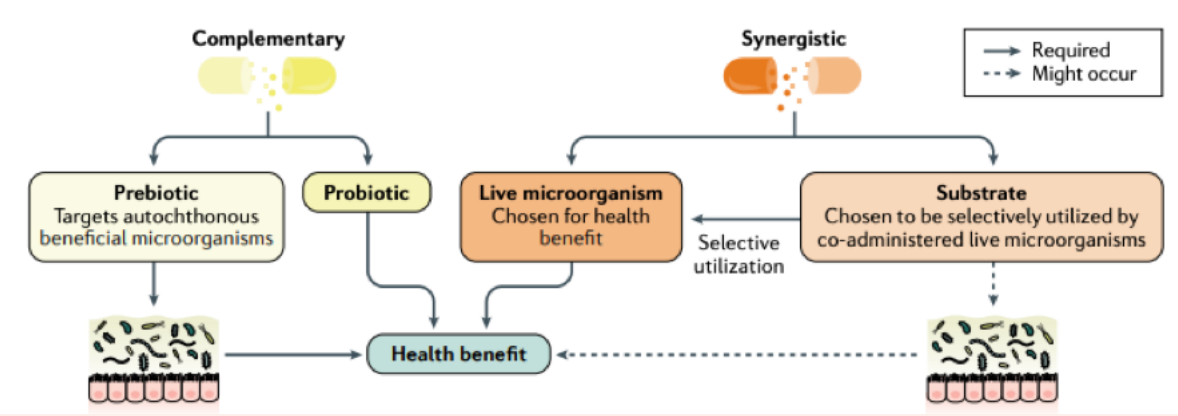

***Figure** from [5] illustrating the differences in definition between complementary and synergistic synbiotic.*

The Study

60 women aged 18-40yrs with a diagnosis of PCOS according to the Rotterdam criteria were enrolled in the study. The study was designed as a randomised, double-blinded, placebo-controlled trial, with 30 women randomly assigned to either the intervention or placebo group. The study lasted 12-weeks. The intervention was a synbiotic supplement consisting of:

- *Lactobacillus acidophilus*
- *Lactobacillus casei*
- *Bifidobacterium bifidum*
- 800mg inulin

Each bacterial strain contained 2 x 109 colony forming units [CFU]. Participants were contacted daily by cell phone to remind them to take the supplement. Compliance was assessed by assessing capsules in returned supplement containers. All participants completed 3-day food records prior to the intervention, and every 3-weeks to the end of the study.

Weight, clinical assessments of hirsutism [dark body hair in bodily regions usually not associated with hair growth in women], and blood measures for hormones, plasma glucose, C-reactive protein [CRP], and oxidative stress markers, were taken before and at the end of the intervention. The study compared differences between pre and post study measures of these outcomes.

Results: All participants completed the study [*n* = 30 in each group]. Based on capsules returned, compliance was >90% in both intervention and placebo groups. Participants in both groups were well matched for age, anthropometric measures [weight, height, BMI], and activity levels. Results for significant differences in outcomes between groups are presented here.

- ***CRP:*** Decreased by 0.95mg/L in the intervention group, while increasing by 0.33mg/L in the placebo group.
- ***Sex Hormone-Binding Globulin:*** SHBG increased by 19.8nmol/L in the intervention group, compared to no difference in the placebo group.
- ***Modified Ferriman Gallwey [hirsutism test]:*** The mFG score decreased by 1.3 in the intervention group, compared to no difference in the placebo group.
- ***Nitric Oxide::*** NO increased by 5.5umol/L in the intervention group, compared to no change in the placebo group.

There were no other significant differences in hormonal measures or markers of oxidative stress.

The Critical Breakdown

Pros: The method of randomisation was described and appropriate [computer-generated randomisation]. Allocation was concealed from researchers and from participants under the analysis was completed. The trial was pre-registered and the inclusion/exclusion criteria, and primary outcomes, all maintained according to the pre-registration. All subjects completed the trial [*n* = 60], providing the required sample size [which was *n* = 25 in each group] for statistical power.

Cons: It is not clear what the placebo consisted of. It is also unclear whether the placebo group also received daily reminders to take the placebo, which could introduce some adherence bias in the intervention group. The study is vague with respect to its primary and secondary outcomes, which are not stated in the paper and in the pre-registration are simply states as "hormonal profiles", which leaves the possibility of selective reporting and emphasis within the findings. Indeed, the results are presented as a list of statistically significant findings with no distinction between outcome measure. The power calculation was based on CRP, so we may presume that was the primary outcome. No data on diet was presented in the paper.

Key Characteristic

The study failed to investigate the effects of the synbiotic supplement on the microbiota. Recall that to be considered a synbiotic, a supplement must be shown to result in both a health benefit *and* selective utilisation by the microbiota, *in the same study*. The present study only reported on the health outcomes of the supplement, but there was no investigation into the microbial effects of the supplement. What populations of bacteria increased? Did the inulin prebiotic result in selective utilisation by the accompanying probiotic microbes? In the absence of this investigation, the supplement does not meet the criteria for a synergistic synbiotic.

Does the supplement meet the criteria for a complementary synbiotic? This would require both the substrate, i.e., insulin, to be established as a prebiotic, and for the accompanying bacterial strains to have an established benefit in the target population. While the probiotics in the study supplement were dosed in sufficient amounts [the minimum is 1 x 109 CFU] and some of the strains have previously shown benefit in women with PCOS [6], the optimal dose for inulin has previously be reported to be 10g per day [7]. The 800mg used in the present study falls short of the individual criteria for an established prebiotic, and thus it does not appear the supplement would meet the definition of a complementary synbiotic.

Interesting Finding

The increase in SHBG is the finding with the greatest magnitude of effect in the study, and potentially the most relevant to the pathophysiology of androgen-dominant PCOS. SHBG is a transporter for sex hormones, and regulates the availability of free testosterone; the higher the SHBG levels, the lower the circulating free testosterone. Elevated androgens in women lowers SHBG levels, resulting in an increase in free testosterone levels [8]. Of note for this androgen-dominant PCOS is also the relationship between the elevated insulin levels observed in this phenotype, as hyperinsulinemia decreases SHBG levels [8,9]. The reference range for normal SHBG levels in non-pregnant females is 18-144nmol/L. Baseline levels in the present study were 37.3nmol/L and 38.3nmol/L in the intervention and placebo groups, respectively. The increase in the intervention group 37.3nmol/L to 57.1nmol/L was statistically significant, but also potentially clinically relevant: the increase in SHBG was accompanied by a minor decrease in total testosterone and significant reduction in the free androgen index, and increases of that magnitude may also correlate with less insulin resistance [9].

Relevance

Despite the use of the term in the study, and while a health benefit was demonstrated in the trial, it appears the investigators failed in fact to establish the supplement as a synbiotic, according to ISAAP criteria [5]. But the health effects shown also warrant further scrutiny. The change in CRP was a minor 0.95mg/L, and the standard deviation was 2.24mg/L, which means that the coefficient of variation was 235% [SD/mean*100]. This level of variation leaves us with little confidence that the change in CRP reflects the effect of the supplement, rather than random between-person variation.

The mFG hirsutism test scores androgen-sensitive body areas for hair growth: a score of 0 indicates the absence of growth, 4 indicates extensive growth, and 8 or higher indicates hirsutism [10]. In this study, baseline mFG scores were 15, indicating high levels of hirsutism in this study population. However, the decrease of 1.3 score points in the intervention group again calls into question the clinical meaningfulness of the finding, irrespective of the statistically significant difference between groups.

There are some associations between lower NO levels in PCOS, which may reflect endothelial dysfunction and potentially oxidative stress [11]. However, there were no significant differences in other oxidative stress markers. The relevance of the nitric oxide finding is unclear as it relates to PCOS pathophysiology.

The finding that warrants most attention is the increase in SHBG: a recent study found that synbiotic supplementation reduced testosterone by 32% over 12-weeks [12]. Whether prebiotics, probiotics, or specific synbiotic combinations may benefit the hormonal milieu of androgen-dominant PCOS remains a promising area of research.

Application to Practice

The primary limitation of the present study is the lack of thorough validation of the formulated supplement as a synbiotic, either complementary or synergistic. Further research will be required to confirm the effects of specific synbiotic formulations. For the androgen-dominant, anovulatory, high insulin PCOS phenotype, the best available evidence for dietary interventions indicates a lower [~30-40%] carbohydrate, low glycaemic and higher protein diet, which collectively may decrease abdominal body fat, decrease androgen levels and increase insulin sensitivity [13-15]. The most efficacious supplemental intervention in this phenotype remains a combination of myo-inositol and d-chiro-inositol at the physiological ratio of 40:1 [myo to d-chiro], which ensures better clinical results, including the reduction of insulin resistance, androgen levels, cardiovascular risk and regularisation of menstrual cycle with spontaneous ovulation [16].

References

1. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertility and Sterility. 2004;81(1):19-25.
2. Pasquali R, Gambineri A. Glucose intolerance states in women with the polycystic ovary syndrome. Journal of Endocrinological Investigation. 2013;36(8):648-653.
3. Hill C, Guarner F, Reid G, Gibson G, Merenstein D, Pot B et al. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nature Reviews Gastroenterology & Hepatology. 2014;11(8):506-514.
4. Gibson G, Hutkins R, Sanders M, Prescott S, Reimer R, Salminen S et al. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nature Reviews Gastroenterology & Hepatology. 2017;14(8):491-502.
5. Swanson K, Gibson G, Hutkins R, Reimer R, Reid G, Verbeke K et al. The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of synbiotics. Nature Reviews Gastroenterology & Hepatology. 2020;17(11):687-701.
6. Askari G, Shoaei T, Tehrani H, Heidari-Beni M, feizi A, Esmaillzadeh A. Effects of probiotic supplementation on pancreatic β-cell function and c-reactive protein in women with polycystic ovary syndrome: A randomized double-blind placebo-controlled clinical trial. International Journal of Preventive Medicine. 2015;6(1):27.
7. Kolida S, Tuohy K, Gibson G. Prebiotic effects of inulin and oligofructose. British Journal of Nutrition. 2002;87(6):193-197.
8. Qu X, Donnelly R. Sex Hormone-Binding Globulin (SHBG) as an Early Biomarker and Therapeutic Target in Polycystic Ovary Syndrome. International Journal of Molecular Sciences. 2020;21(21):8191.
9. Kajaia N, Binder H, Dittrich R, Oppelt P, Flor B, Cupisti S et al. Low sex hormone-binding globulin as a predictive marker for insulin resistance in women with hyperandrogenic syndrome. European Journal of Endocrinology. 2007;157(4):499-507.
10. Sirmans S, Pate K. Epidemiology, diagnosis, and management of polycystic ovary syndrome. Clinical Epidemiology. 2013;:1.
11. Sprung V, Atkinson G, Cuthbertson D, Pugh C, Aziz N, Green D et al. Endothelial function measured using flow-mediated dilation in polycystic ovary syndrome: a meta-analysis of the observational studies. Clinical Endocrinology. 2013;78(3):438-446.
12. Chudzicka-Strugała I, Kubiak A, Banaszewska B, Zwozdziak B, Siakowska M, Pawelczyk L et al. Effects of synbiotic supplementation and lifestyle modifications on women with polycystic ovary syndrome. The Journal of Clinical Endocrinology & Metabolism. 2021;.
13. Mehrabani H, Salehpour S, Amiri Z, Farahani S, Meyer B, 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. Journal of the American College of Nutrition. 2012;31(2):117-125.

References

14. Gower B, Chandler-Laney P, Ovalle F, Goree L, Azziz R, Desmond R et al. Favourable metabolic effects of a eucaloric lower-carbohydrate diet in women with PCOS. Clinical Endocrinology. 2013;79(4):550-557.

15. Goss A, Chandler-Laney P, Ovalle F, Goree L, Azziz R, Desmond R et al. Effects of a eucaloric reduced-carbohydrate diet on body composition and fat distribution in women with PCOS. Metabolism. 2014;63(10):1257-1264.

16. Unfer V, Porcaro G. Updates on the myo-inositol plus D-chiro-inositol combined therapy in polycystic ovary syndrome. Expert Review of Clinical Pharmacology. 2014;7(5):623-631.