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The probiotic supplementation reduced inflammation in polycystic ovary syndrome: A randomized, double-blind, placebo-controlled trial

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ABSTRACT

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders that plaques women today. One of the underlying factors associated with the development of PCOS is elevated inflammation. However, the application of available nutraceutical therapies remains relatively unknown and thus is the focus of the current study. In this manner, we evaluated the effects of probiotic supplement on clinical and immunological parameters of PCOS. Our randomized-control trial, four strains of *Lactobacillus* for a probiotic group and the equivalent dosage with maltodextrin was used as the placebo. To further understand the impacts of immunological parameters towards inflammation in PCOS, we measured interleukin (IL) 6, 10, TNF-α, hs-CRP and clinical manifestations before and after the trial. The probiotic supplementation resulted in a significant increase in IL-10 levels compared with the placebo, after the intervention. However, in both groups, there was a significant decrease in hs-CRP and IL-6 levels. Probiotic supplementation does not make any significant changes in the TNF-α levels. This study observed that *Lactobacillus* supplementation modulates inflammation in PCOS patients.

1. Introduction

Polycystic ovary syndrome is a heterogeneous endocrinopathy disorder in 5–10% of women in their reproductive ages (Deligeoroglou, Kouskouti, & Christopoulos, 2009). The observed clinical and metabolic component of this disease are obesity, menstrual abnormalities, hirsutism, hyperandrogenism, increasing LH (luteinizing hormone) to FSH (follicle-stimulating hormone) ratio, and hyperinsulinemia (Norman et al., 1995). The exact etiology of the polycystic ovary syndrome (PCOS) has not been studied well; however, it appears that the inflammatory pattern, which can be detected by the C-reactive protein (CRP) level and is one of the underlying causes that enhances the progress of this disorder (Giallauria et al., 2008). More specifically, the elevation of CRP levels serves as an indicator for low-grade chronic inflammation and is also positively correlated with the insulin resistance (Toulis et al., 2011). The PCOS patients display 96% higher levels of CRP levels than seen in healthy subjects (Deligeoroglou et al., 2012; Toulis et al., 2011). Moreover, the central obesity that occurs as a result of hyperandrogenemia is regarded to be a vital link between the low-grade inflammation and hyperandrogenemia in this syndrome (Deligeoroglou et al., 2009). Without taking the obesity into consideration, about 50–70% of the PCOS patients have insulin resistance. This percentage increases to 95% for the PCOS patients suffering from obesity (Setji & Brown, 2014).

The exact pathophysiology of this syndrome remains unknown, however hyperandrogenism has been reported to be a central mediator for the development of this syndrome (Setji & Brown, 2014). A novel "microgendermo" hypothesis has suggested a bidirectional correlation

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between sex steroid hormone and gut microbiota components (Flak, Neves, & Blumberg, 2013). Dysbiosis of gut microbiota by a high-fat-sugar diet induces an increase in testosterone levels in PCOS women (Tremellen & Pearce, 2012). Tremellen and Pearce have been shown the rolls of the lipopolysaccharide of gram-negative bacteria to make low-grade-inflammation through "leaky gut" syndrome (Tremellen & Pearce, 2012). In this regard, Guo and colleges raise questions upon the effects of gut microbiota modulation for the treatment of PCOS (Guo et al., 2016). In their study, *Lactobacillus* and fecal microbiota from healthy rats were transplanted into letrozole-induced PCOS rat model which have increased estradiol and estrone levels (Guo et al., 2016). The *Lactobacillus* administration raises the gut barrier, resulting in short-chain fatty acid metabolites that display unique effects to reduce insulin resistance, inflammation, and modulate androgen level (Hulston, Churnside, & Venables, 2015).

It is hypothesized that insulin influences the androgen synthesis in ovarian theca cells, which is considered as the pathogenic cause of the hyperandrogenemia in PCOS (Moghetti et al., 2000). However the full benefits of metformin, an anti-diabetic agent, on PCOS woman with hyperandrogenism disturbance and anovulation problems are unclear (Morin-Papunen et al., 2003). Current treatments for PCOS is the utilization of oral contraceptive pills such as Cyproterone acetate (2 mg), especially for whom have acne, hirsutism, and hyperandrogenism (Morin-Papunen et al., 2003). Further studies have reported that IL-6 is a crucial factor in regulating CRP, insulin resistance, and obesity in PCOS (González, Rote, Minium, & Kirwan, 2009; Kaya et al., 2010). Inflammatory markers such as hs-CRP, TNF-α have been observed to be elevated in the follicular phase of PCOS when compared to control (Puder et al., 2005). Furthermore, probiotics have been observed to display regulatory effects on IL-6, CRP, and insulin resistance in an inflammatory disease, including in PCOS subjects (Shoaei, Heidari-Beni, & Tehrani, 2015). However, the association of oral contraceptives with probiotics have not been studied and is the focus of the current study. We have observed that together oral contraceptives with probiotics significantly reduces inflammation associated with PCOS by reducing CRP and IL-6 as well as weight in PCOS patients.

2. Methods

2.1. Subjects and methods

Our double-blind placebo-controlled trial evaluated the efficacy of probiotics in decreasing the inflammation in PCOS based upon criteria from the Rotterdam trial (Franks, 2006). Inclusion criteria for the current study (Rotterdam criteria) requires the presence of more than two of the following symptoms: oligo/anovulation, hyperandrogenism (symptoms like hirsutism, acne, male pattern alopecia, increasing the free testosterone levels), polycystic ovaries based upon ultrasound evaluation (Franks, 2006). In the current study, we recruited 60 Iranian women between 18 and 45 years old that resided in Tehran and suffered from PCOS. A Stratified blocked randomization was used to assign participants to four blocks based on the subjects' BMI and age. The study exclusion criteria were thyroid dysfunction, hyperprolactinemia, diabetes, history of premature menopause, smoking, and Cushing's syndrome. Individuals who displayed symptoms from the flu or was diagnosed with cancer, autoimmune disease, or who made the significant changes in their routine diet and physical activity during the trial were excluded. Also, all subjects with a history of smoking, professional athletes, and those who were taking any medication other than Cyproterone acetate or any supplement or herbal medicine were also excluded from the study. Lastly participants who had any form of oral antibiotic therapy less than a month from the initiation of the trial were omitted. The subjects were given two capsules per day each capsule consisting of 1×10^9 colony forming units (CFU) of each *lactobacillus* strains (equal to 500 mg) made by Zist Takhmir company under the supervision of Tehran University of Medical Sciences. Two maltodextrin capsules from the same company with the identical texture, color, and size given to the placebo group per day. The trial was conducted for 12 weeks. The four strains of *Lactobacillus* are: *Acidophilus*, *Lactobacillus Plantarum*, *Lactobacillus Fermentum*, and *Lactobacillus Gasseri* were in equal quantity in each capsule. The same company made the same capsule considering the shape and color. All participants were administered the oral contraceptive Cyproterone acetate (ethinylestradiol-anti androgenic therapy) following the 3rd day of completion of their menstrual cycle. The following were carried out at the beginning and the end of the study;

1 A transvaginal ultrasound 7.0-MHz curved-array probe applied for all participants between the 3rd and 5th days of their menstrual cycles to assess the ovaries polycystic phenomenon (> 10, 2–8 mm follicle). The same ultrasound procedure was repeated between the 12th to 14th days of period to evaluate a dominant follicle (Atiomo, Pearson, Shaw, Prentice, & Dubbins, 2000).
2 The anthropometric measurements (body weight, waist, hip, abdominal circumference, height, and BMI) recorded for all subjects at the beginning and end of the study. Body weight measured by Seca scales with an accuracy of 100 g (Seca, Homburg, Germany) and Seca stadiometer with a precision 0.1 cm was determined. Flexible body tape was utilized to measure the waist circumference with an accuracy 0.1 cm on the average waistline between ribs and iliac bone. The same tape measure was used to determined abdominal and hip circumference.
3 The history of menstrual cycles was recorded for each subject for the six months before the starting date of the study as well as during the study.
4 Blood samples were collected at the beginning and end of the trial. The samples were analyzed at Laboratory of Immune Deficiency, Tehran University of Medical Sciences. Five (ml) of venous blood samples were obtained after 12-h of overnight fasting. All blood sample were immediately centrifuged at 1500–2000 for 5 min, then serum samples were collected and stored at −80 °C. The cytokines (IL-6, IL-10, TNF-α) were measured by ELISA (IBM, Germany) following protocols from the manufacturer.
5 High-sensitivity C-reactive protein (hs-CRP) was quantified by a latex-enhanced immunonephelometric assay that has a reported sensitivity of 0.01 mg/L and an intra-assay coefficient of variation of 8.7% (Behring Nephelometer II, Dade Behring, Inc., Newark, DE, USA)

All patients were also evaluated in their early follicular phase (days 3–5) of the menstrual cycle (Moghetti et al., 2000). Every two weeks after randomization all participants received a phone call to evaluate the dose of their capsule consumption during their trials and any possible side-effects of treatment.

Investigators obtained a signed written informed consent from all participants before the study. The study was approved by the ethics committee of National Nutrition and Food Technology Research Institute of Iran and was registered in the Iranian Registry of Clinical Trials (number: IRCT2016081429362N1).

2.2. Statistical analysis

Analysis of Covariance (ANCOVA) used to adjust the mean of some clinical, metabolic and immunological parameters after the trial. For other variables which measured two times in each group, the differences were evaluated using the independent samples *T*-test (for inter-group analysis) and paired samples *t*-test (for intra-group analysis). Blinded-duplicate sampling and (during intervention) decreases systematic error and inter-assay variability. All statistical analyses were done by SPSS (v24.0 for Windows, SPSS, Chicago, IL). P-values < .05 were considered statistically significant.

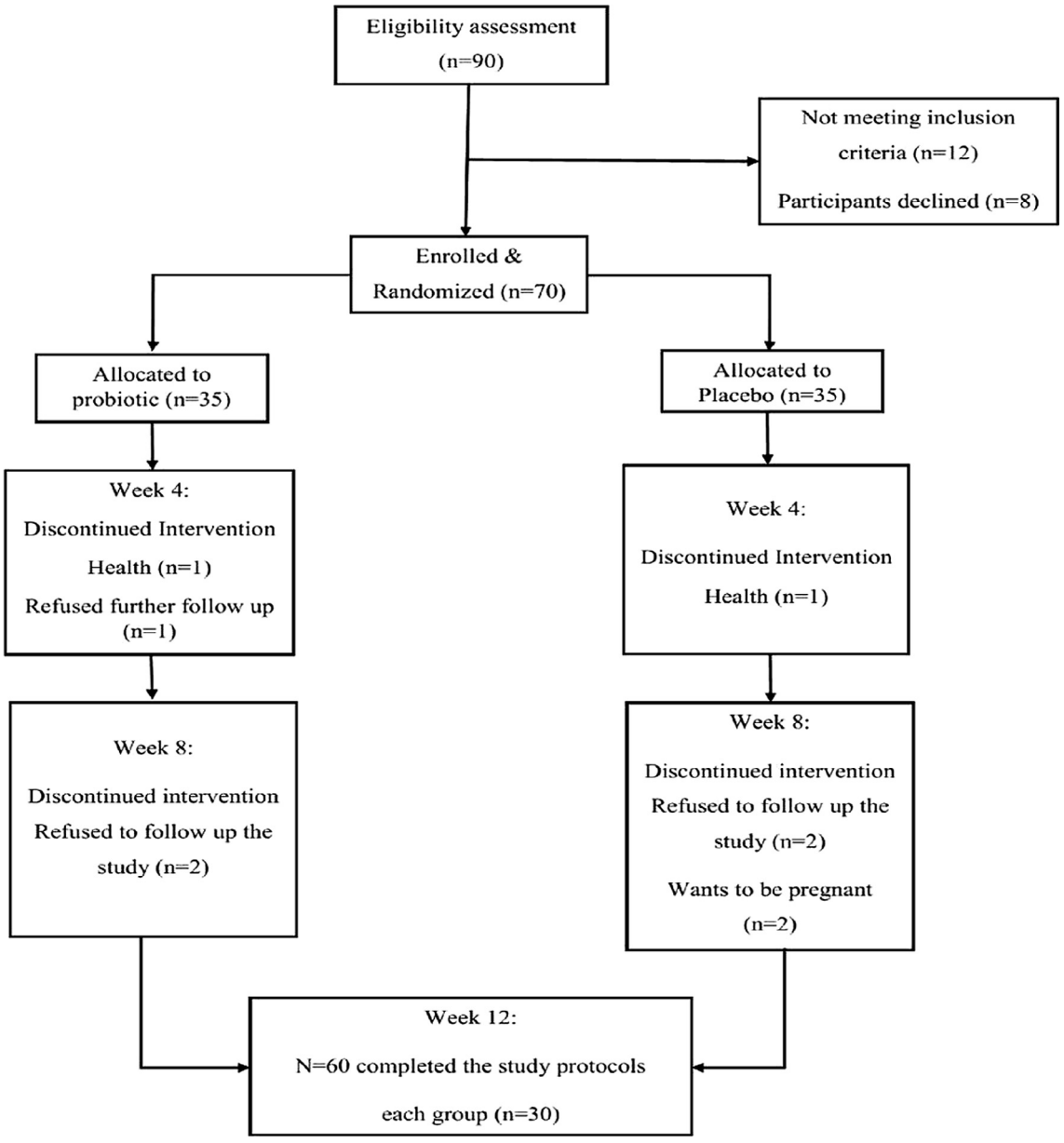

Fig. 1. Study design and allocation of participants in the trial.

3. Results

3.1. Baseline characteristics

In this study, ninety PCOS women aged from 18 to 40 years were screened. Seventy women included and completed the protocol of the study. At week 4, three women were removed from the study due to lack of participation in follow-up studies. At week 8, four additional patients were removed from the study due to lack of adherence to medication protocol. Two other patients withdrew due pregnancy. At the end of the study, sixty PCOS women, mean age of 29.51 ± 5.58 years, who completed the 12-week follow concluded the stud (Fig. 1). There were no significant differences regarding mean age, weight, BMI, waist, abdominal circumference, hip circumference, menstrual cycles days between probiotic and placebo groups at the baseline of the study (Table 1).

Table 1
Baseline characteristics of patients in two groups at baseline.

Characteristic	Probiotic (n = 30)	Placebo (n = 30)
Age (y)	30.06 ± 1.06	28.96 ± 0.98
Weight (kg)	71.47 ± 1.77	68.47 ± 1.34
BM1 (kg/m^2)	26.84 ± 0.53	25.97 ± 0.42
Waist (cm)	86.06 ± 1.10	82.33 ± 0.97
Abdominal circumference (cm)	97.76 ± 1.76	91.93 ± 1.38
Hip circumference (cm)	105.65 ± 0.71	105.34 ± 0.44
Menstrual cycle (days)	50.6 ± 3.12	48.43 ± 1.73
Height (m)	1.63 ± 0.01	1.62 ± 0.01
Diagnosis period (month)	3.0 ± 0.19	3.23 ± 0.14

3.2. Immunological characteristics

As shown in Table 2, the serum IL-10 level significantly increases in the in probiotics group in comparison to the baseline (P = .001). TNF-α serum levels in the probiotic group were observed to display a

Table 2
Serum levels of cytokines (IL-6, IL-10, TNF-α) and hs-CRP in the two study groups before and after intervention.[a]

Parameters		Baseline	Week 12	Changes	P-value
IL-6 (pg/ml)	Probiotics	1.53 ± 0.07	1.03 ± 0.06	−0.5 ± 0.07	0.001[f]
	Placebo	1.69 ± 0.06	1.52 ± 0.41	−0.17 ± 0.05	0.003
	P-Value		0.001		
IL-10 (pg/ml)	Probiotics	2.44 ± 0.15	3.24 ± 1.17	0.79 ± 0.13	0.001
	Placebo	2.21 ± 0.12	2.24 ± 0.66	0.03 ± 0.06	0.59
	P-Value		0.001		
TNF-α (pg/ml)	Probiotics	0.02 ± 0.001	0.02 ± 0.007	−0.002 ± 0.001	0.05
	Placebo	0.02 ± 0.001	0.02 ± 0.007	−0.002 ± 0.001	0.05
	P-Value		0.73		
Hs-CRP (mg/l)	Probiotics	7.29 ± 0.57	4.26 ± 2.32	3.55 ± 0.31	0.001
	Placebo	7.46 ± 0.4	6.22 ± 2.09	1.24 ± 0.23	0.001
	P-Value		0.73		

[a] n = 30 for all values.
[f] $p < .05$ vs. baseline consider significant with ANCOCA test.

substantial increase (P = .03). Interestingly, in both groups, there was a significant reduction in hs-CRP and IL-6 levels ($P < .05$).

3.3. Anthropometric indicators

A significant reduction in weight was observed in the in the probiotics group, (p = .001). The randomization in our study was based on BMI and age. Surprisingly both groups showed a reduction in BMI. In both groups, there was no significant change in abdominal circumference ($p > .05$) (see Table 3).

4. Discussion

This study uncovered immunological and inflammatory changes after three months' treatment of polycystic ovary syndrome with the Cyproterone acetate and probiotics supplementation. Our data suggested beneficial effects of *Lactobacillus* supplementation on anthropometric indexes, and cytokines balance in PCOS patients. The present study showed a substantial increase in IL-10 levels in the probiotic after twelve weeks of the trial. There is no significant change in TNF-α observed by probiotic supplementation. Also, the considerable reduction in both hs-CRP and IL-6 irrespective of probiotics supplementation after 12-weeks of the intervention in both groups was observed.

Previous findings reported by Morin-Papunen et al., illustrated a significant increase in hs-CRP levels in both obese and non-obese PCOS patients who were prescribed Cyproterone acetate regime and thus is considered as a significant consequence from this form of therapy (Morin-Papunen et al., 2003). While addressing the hormone imbalance as one the primary concerns in PCOS management, elevated inflammatory factors could detrimentally affect the Cyproterone therapy. The utilization of probiotics as an adjuvant therapy to mitigate the inflammatory reaction could offer a significant benefit to patients. Previous reports have deciphered a potential mechanism when they observed a significant reduction in hs-CRP in patients treated with probiotics in comparison with placebo in patients with type 2 diabetes (Mazidi, Rezaie, Ferns, & Vatanparast, 2017). This concept is supported by our findings in the current study that suggested a considerable reduction of hs-CRP in both probiotic (Cyproterone, probiotic) and placebo (Cyproterone, placebo) group. To further characterize the impact of Cyropterone upon influencing CRP levels, alternative third-generation oral contraceptives pills have also been reported to increase CRP levels in healthy women (Cauci et al., 2008). However our findings also contradict previous reports by Shoaei and colleges that observed no significant effect on a hs-CRP levels of PCOS women and maybe due to the unequal formulation of their probiotic which consisted of two strains both *Lactobacillus* and *Bifidobacterium* and one strain of *Streptococcus* (Shoaei et al., 2015). The precise effects of *Lactobacillus* on hs-CRP levels of the PCOS remains controversial due to the many different species of *Lactobacillus* and vary in their modulatory effects on the immune system, (Deligeoroglou et al., 2012; Setji & Brown, 2014).

However in regards to correlations between levels of inflammation and disease states, Makedos et al. have demonstrated an association of obesity and PCOS, with chronic inflammation (Makedos, Goulis, Papanikolaou, & Panidis, 2010). To help explain the mitigating factors that explain the cause of inflammation in central obesity and PCOS, the mononuclear cells (MNCs) have been reported to generate the reactive oxygen species (ROS) Evans, Goldfine, Maddux, & Grodsky, 2002. In further investigation the site of inflammation has been reported to be associated with effects of abdominal fat on reactive oxygen species (ROS) induction González, Rote, Minium, & Kirwan, 2006a. Aggregation of oxidative stress increases lipid, protein, and DNA oxidation which causes cellular damages (González et al., 2006a). The resultant ROS elevates the transcription of nuclear factor kappa B (NF)-kB and activates the transcription of Tumor Necrosis Factor Alpha (TNF-α) as a trigger of insulin resistance and further inflammation (Evans et al., 2002; González et al., 2006a). Our findings suggest no significant

Table 3
Anthropometric measures in the two study groups before and after the trial.

Parameters		Baseline	Week-12	Changes	P-value
Weight (kg)	Probiotics	71.47 ± 1.77	69.58 ± 1.62	1.88 ± 0.41	0.001
	Placebo	68.47 ± 1.34	68.18 ± 1.19	0.29 ± 0.31	0.37
	P-Value		0.001		
BMI (kg/m^2)	Probiotics	26.84 ± 0.53	26.16 ± 0.51	0.68 ± 0.15	0.001
	Placebo	25.97 ± 0.42	25.87 ± 0.38	0.09 ± 0.12	0.44
	P-Value		0.02		
Abdominal Circumference (cm)	Probiotics	97.76 ± 1.76	98.13 ± 1.59	−0.37 ± 1.12	0.74
	Placebo	91.65 ± 1.40	92.93 ± 1.56	−1.27 ± 0.81	0.12
	P-Value		0.009		

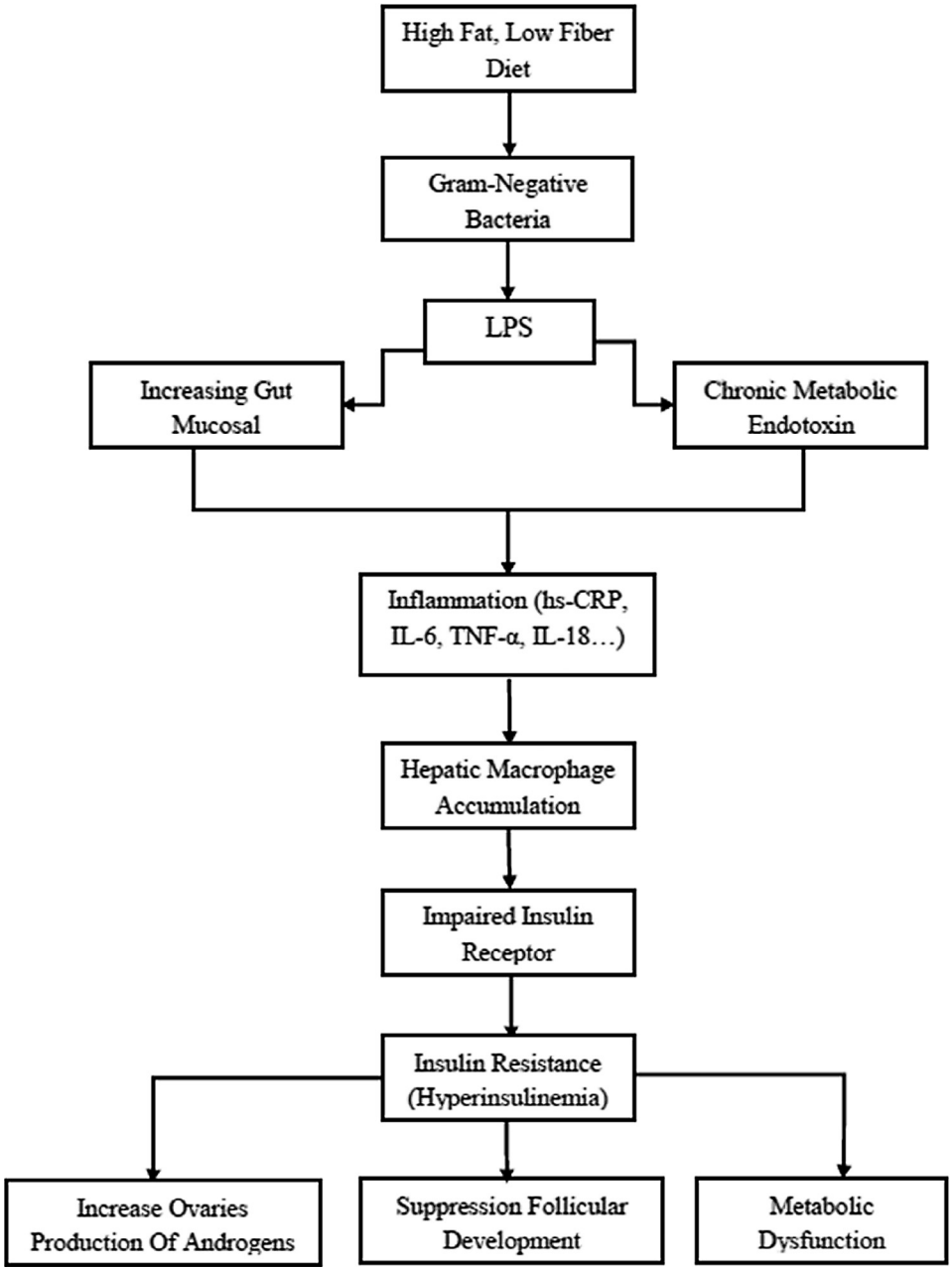

Fig. 2. Leaky gut" hypothesis in the pathogenesis of PCOS and its likely effects on increasing the IL-6 and TNF-α confirmed its key role of gut microbiota by increasing circulating lipopolysaccharide (LPS).

change in TNF-α with *Lactobacillus* supplementation in comparison to the placebo group. Furthermore, the correlation between ROS formation and insulin resistance in PCOS is independent of obesity (González et al., 2006a). Also, there is a direct correlation between ROS generation and hyperandrogenism in PCOS (González, Rote, Minium, & Kirwan, 2006b). Moreover, there was a significant relationship between (ROS) formation, TNF-α and IL-6 generation, and insulin resistance (IR) in PCOS Victor et al., 2016. There are several mechanisms by which pro-inflammatory cytokines (IL-6 and TNF- α) are associated with ROS generation through hyperglycemia in IR-PCOS subjects (Klinke et al., 2011). As well, some studies that demonstrate a direct correlation between IL and 6 concentration, insulin resistance, and fasting glucose in PCOS woman in compared to control (Tarkun, Çetinarslan, Türemen, Cantürk, & Biyikli, 2006). Alternatively, the meta-analysis highlighted the intrinsic effects of IL-6 levels in the pathogenicity of PCOS and thus serves as a biomarker for monitoring and treatment PCOS in patients (Peng et al., 2016). Our study indicated a significant reduction of IL-6 in both groups compared to their baseline. We tested whether Cyproterone acetate, would diminish the hyperandrogenism and modulation of the hormonal profile. Our hypothesis was therefore to evaluate the effects of microflora and related "leaky gut" theory in the pathogenesis of PCOS and its likely impact on increasing the IL-6 and TNF-α as illustrated in Fig. 1 (Tremellen & Pearce, 2012). In summation probiotics supplement is serves as functional food to decrease the risk of ROS accumulation (Zanoni et al., 2008).

The results from the current study, indicate that 12 weeks of treatment with Cyproterone acetate and probiotic results in a significant reduction in weight and BMI when compared to the placebo group. These findings are in agreement with previous reports by Sanchez et al. who reported that *Lactobacillus* therapy serves as a positive modulator for weight management in obesity (Sanchez et al., 2014). They discovered that *Lactobacillus rhamnosus*, CGMCC1.3274, (LPR) supplementation over 24 weeks in association with a calorie restricted diet helped obese women lose weight (Sanchez et al., 2014). However, we observed a modest reduction in weight irrespective of calorie restriction and thus we postulate these findings may be related to the change in gut

permeability. In contrast to these findings, Leber et al., reported that patients with metabolic syndrome due not display significant weight and BMI reduction when given a *Lactobacillus* Casei Shirota supplementation (Leber et al., 2012). Together these findings indicate that selective *Lactobacillus* strains may have modulate a positive effect on weight loss in PCOS patients.

To the best of our knowledge we are the first to explore the impact of probiotics in association with Cyproterone acetate on the immunological consequences of PCOS. Our observation that IL-10 is significantly elevated in the response to probiotics in PCOS with Cyproterone acetate treatment. The elevation of IL-10 levels by probiotic supplement may help explain a potential mechanism for how probiotics reduce inflammation. In addition, short term *Lactobacillus* supplementation had no measurable effects upon circulating levels of TNF-α in comparison to the placebo group. This indicates that the increase in IL-10 levels was not due to TNF- α, but rather by an alternative signaling mechanism such as hs-CRP reduction.

In conclusion, the present study presented positive effects of *Lactobacillus* supplementation to reduce the inflammation through IL-6, hs-CRP reduction and increasing IL-10. These findings are congruent with a substantial decrease in weight and BMI of women with PCOS. A critical limitation of this study was the trial duration. Future work will investigate alternative strains of probiotics in comparison to *Lactobacillus*. The findings from the current study also support that PCOS is a syndrome with the broad spectrum of possible treatments and they need to be assessed upon the immunological effects (see Fig. 2).

Conflict of interest

There are no conflicts of interest for authors.

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