ORIGINAL ARTICLE

Comparison of the impact of intermittent fasting diet alone or in conjunction with probiotic supplementation versus calorie-restricted diet on inflammatory, oxidative stress, and antioxidant capacity biomarkers in women with polycystic ovary syndrome: A randomized placebo-controlled trial

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Background: The objective of this study was to compare the effects of early time-restricted eating (eTRE) and eTRE plus probiotic supplementation to daily caloric restriction (DCR) alone in terms of biomarkers of oxidative stress (OS), antioxidant capacity, inflammation, and blood pressure (BP) in obese women with polycystic ovary syndrome (PCOS). **Materials and Methods:** The research was conducted as a randomized, parallel, placebo-controlled clinical trial with an 8-week follow-up period. Participants were randomly assigned to one of three groups: 14:10 eTRE with probiotic supplementation (n = 30), 14:10 eTRE with placebo supplementation (n = 30), or DCR with placebo supplementation (n = 30). At the beginning and 8 weeks of the intervention, systolic blood pressure (SBP) and diastolic BP, inflammation, and OS parameters were evaluated. **Results:** A total of 90 participants (mean age, 30.49 years and mean weight, 81.45 kg) were enrolled in this trial. After 8-week intervention, we observed SBP significantly decreased in both the eTRE + probiotic group (−0.31 mmHg [95% confidence interval (CI): −0.55, −0.07]) and the eTRE + placebo group (−0.24 mmHg [95% CI: −0.43, 0.04]), with no significant differences observed between groups. Moreover, C-reactive protein (CRP) levels were significantly reduced in all groups (P < 0.005). Total antioxidant capacity (TAC) also showed notable improvement in both the eTRE + probiotic group (P = 0.012) and the DCR group (P = 0.032). However, there were no significant differences between the three groups regarding BP, OS, TAC, and CRP markers. **Conclusion:** It was not found that eTRE alone or eTRE with probiotics intervention resulted in improving BP, inflammatory, OS, and antioxidant capacity biomarkers than a standard DCR diet among obese women with PCOS. The present study did not reveal significant improvements in BP, inflammatory markers, OS, or antioxidant capacity with either eTRE alone or eTRE combined with probiotics compared to a standard DCR among obese women diagnosed with PCOS.
Trial Register no: IRCT20121110011421N5.

Key words: Intermittent fasting, oxidative stress, polycystic ovary syndrome, probiotics

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INTRODUCTION

Polycystic ovary syndrome (PCOS), the most prevalent polygenic endocrine disorder in women of childbearing age, is detected by hyperandrogenism, ovarian enlargement, androgenic alopecia, hirsutism, menstrual irregularity, acne, oligomenorrhea or anovulation, abortion, and infertility.[1] Up to 70% of women with PCOS remain undiagnosed worldwide.[2] The quality of life of these patients is affected by disease-related symptoms, especially those symptoms related to psychological aspects.[3] Due to the involvement of visceral adipose tissue in insulin resistance and the inflammatory process by producing inflammatory cytokines, and according to the bidirectional association between PCOS and abdominal obesity, this tissue plays a key role in the development of PCOS-related inflammation, oxidative stress (OS), and insulin resistance.[4] Hence, since obesity and insulin resistance are common in patients with PCOS, dietary interventions might have been taken into consideration as the first line of management for this disorder.[5]

Each of three different types of intermittent fasting (IF) diets including IF, the 5:2 diet, and time-restricted eating (TRE) might alleviate PCOS by stimulating AMP-activated protein kinase, decreasing insulin resistance and OS, and improving circadian rhythm.[6] On the other hand, regarding the crucial role of gut microbiota in the pathogenesis of PCOS, probiotics could be used as a probable treatment option for PCOS.[7] Probiotics including species of *Lactobacillus* and *Bifidobacterium* improved the management of PCOS by regulating sex hormones and reducing insulin resistance, inflammation, and OS through the production of short-chain fatty acid metabolites.[8]

A recent clinical trial conducted by Łagowska and Kapczuk[9] demonstrated no beneficial effects of *Lactobacillus rhamnosus* on PCOS. However, another clinical trial suggested that probiotic supplementation for 8 weeks significantly improved sexual function and body satisfaction in patients with PCOS.[10] Regarding IF diets, a recent systematic review concluded that there was conflicting evidence about the effects of the TRE diet on PCOS.[11] Due to conflicting results of investigations about the effects of low-calorie diets or probiotic supplementation on PCOS, the present clinical trial aimed to evaluate the effects of IF alone or with probiotic supplementation versus a daily calorie restriction (DCR) on inflammatory, OS, and antioxidant capacity biomarkers in women with PCOS.

MATERIALS AND METHODS

Ethical considerations

This study was confirmed by the Tehran University of Medical Sciences ethics committee (ID: IR.TUMS.MEDICNE.REC.1401.425). Before participation in the study, all subjects provided written informed consent. IRCT20121110011421N5 is the registration number of the study on http://www.IRCT.ir, according to the principles of the Declaration of Helsinki [Supplementary Table 1].

Study participants

This research comprises three research groups and is a randomized, parallel, placebo-controlled clinical trial with an 8-week follow-up period. The data collection for PCOS outpatients was executed between October 2022 and July 2023, with the participants being drawn from the Arash Hospital of Tehran University of Medical Sciences. Eligible participants were adult women aged 18–40 years with a body mass index (BMI) of 25–35 kg/m^2, who were newly diagnosed with PCOS by a gynecologist using the Rotterdam criteria[12] without medical treatment. Cases were not included if they had taken antibiotics, probiotic supplements, or foods containing probiotics, were on a special or prescribed diet, had night shift jobs, did not use a smartphone, or were unwilling to participate in the study. During the study, women were excluded from the study if they had used antibiotics, had an allergic reaction to any supplement, contracted COVID-19, showed poor adherence to the intervention, or were on medications impacting appetite, weight, hormonal balance, carbohydrate, or lipid metabolism. In addition, individuals with diagnoses such as diabetes mellitus, hypertension, liver or kidney disorders, cancer, severe acute or chronic infections, serious gastrointestinal conditions, Cushing's disease, adrenal disorders, acromegaly, gigantism, or eating disorders were also disqualified.[6]

Intervention

This trial compared the effects of early TRE (eTRE) alone and in combination with probiotic supplementation against DCR in patients with PCOS. Participants were randomly assigned (1:1:1) to one of three groups for 8 weeks. One group (n = 30) followed a 14:10 eTRE (10-h eating window between 8:00 a.m. and 6:00 p.m.) with probiotic supplementation. Another group (n = 30) followed a 14:10 eTRE (10-h eating window between 8:00 a.m. and 6:00 p.m.) with placebo supplementation. The last group (n = 30), serving as the control group, followed a DCR diet (energy restricted to 25% of required calories) with placebo supplementation.

Time-restricted eating protocol

The eTRE group was directed to eat *ad libitum* between 8:00 AM and 6:00 PM, then abstain from any caloric intake from 6:00 PM to 8:00 AM the following day (14 h fast: 10 h eat). As part of the 14:10 program, subjects were asked not to change the components of their regular diet. After the fast start, participants were instructed to eat a fasted snack

consisting of 200 kcal of mixed nuts (4 g carbohydrate, 5 g protein, and 18 g fat) in case of low energy or a severe headache.[13,14] No recommendations were made regarding physical exercise, calorie consumption, or macronutrient composition as part of the TRE intervention.

Probiotics supplementation

A probiotic capsule containing 1×10^9 colony-forming units of *L. rhamnosus* and *Lactobacillus roteri*, two different types of probiotics, was given to subjects in one of the eTRE groups. The placebo capsule contained 130 mg of starch without bacteria. Following breakfast, each participant took a probiotic or placebo supplement and sipped a glass of water. Since there is no evidence-based guidance on the appropriate dosage of this probiotic supplement for individuals with PCOS, we used the dosage from a previous study on the treatment of vulvovaginal candidiasis patients.[15]

Control group (calorie restriction protocol)

In the control group's DCR, a dietitian calculated the daily caloric needs of each participant. The basal metabolic rate was determined individually using the Harris–Benedict formula.[16] The total daily energy requirement for each person was then calculated, taking into account their physical activity level and the thermogenic effect of food. Eventually, 25% of the calculated caloric requirement for baseline body weight was subtracted.[17] Following that daily calorie intake was divided into six meals, consisting of three main meals and three snacks. The diet's macronutrient distribution was roughly 30% fat, 55% carbohydrates, and 15% protein. Patients with obesity and PCOS are commonly managed by DCR, characterized by a consistent reduction in daily intake of calories. Therefore, the control group was chosen to follow this calorie-restricted dietary strategy.

Sample size calculation

By consideration of a type I error of 5% ($\alpha = 0.05$), a power ($1-\beta$) of 80%, according to the mean weight loss of 2.5 kg (a minimum clinically important difference) as the primary outcome and the standard deviation of 3.87 and 3.04,[18] the sample size was computed to be 25 for each group based on the two-sided *t*-test. To conduct the study, 90 participants (30 per group) were needed, considering a 5% dropout rate during the period.

Randomization and blinding

The project coordinator employed computer-generated random numbers to carry out stratified permuted block randomization with a block size of six. Participants were stratified based on their BMI into 25–30 kg/m² and 30–35 kg/m². There was a similar color, flavor, and taste in placebos and probiotic supplements produced by TakGen Zist Pharmaceutical Company (Tehran, Iran). The allocation of placebo versus probiotic supplements was blinded for patients, researchers, and laboratory staff. However, they were not blinded to the dietary intervention.

Adherence

To ensure participant adherence to the diet, we used weekly phone calls and daily reminder messages about when to start and stop eating. In addition, participants met individually with a nutritionist each month. They were asked to complete a 24 h dietary recall for 3 days every 2 weeks. Non-compliant patients were those who failed to follow dietary instructions three times a week for more than 2 consecutive weeks, missed more than two consecutive phone sessions, or ate inappropriate meals more than three times a week.[19,20] In addition, to assess participants' adherence to the supplement intake, we utilized the same weekly phone calls, interim visits, and monitoring of the number of empty probiotic packages.

Assessment of variables

At baseline, a comprehensive questionnaire was administered to gather initial data from patients, encompassing variables such as age, education level, employment status, marital status, alcohol consumption habits, smoking history, utilization of herbal medicines, and medical history encompassing various diseases. Physical activity was quantified through a 24 h physical activity recall every 2 weeks. Food intake (macronutrients and micronutrients) was also measured using a 24 h food recall for 3 days at baseline, 2, 4, 6, and 8 weeks after the intervention. Finally, dietary data were calculated using Nutritionist IV software (First Databank, San Bruno, CA, USA) modified for Iranian foods.

Patient weights were measured using standard methods, whereas they wore light clothing and wore no shoes. Tape measures were used to measure height to the nearest 0.1 cm using a nonstretched tape measure. BMI was calculated by dividing weight in kilograms by the square of height in square meters. Blood pressure (BP) measurements were taken using a mercury sphygmomanometer (Riester, Germany) after patients rested for 10 min in a sitting position. Each participant's BP was measured twice, with a 10-min interval between measurements. The average of these two measurements was recorded as the patient's BP.

At the beginning and end of the trial, fasting blood samples (10 ml) were collected and immediately centrifuged at 3000 rpm for 10 min to separate serum. C-reactive protein (CRP) was measured by immunoturbidimetry (Pars Azmoon). Total antioxidant capacity (TAC) and total oxidant status (TOS) were analyzed with a standard colorimetric kit (ZellBio GmbH, Germany). Oxidative stress index (OSI) was computed indirectly through the following

formula: OSI = ([TOS, μmol/l]/[TAC, μmol/l] ×100). It was found that all inter- and intra-assay CVs for all outcomes measurements were below 5%.

Statistical method

The analyses were all conducted based on the intention-to-treat method of multiple imputations. The normality of data distributions was assessed using both Q-Q plots and the Shapiro–Wilk test. Descriptive statistics for quantitative variables were presented as mean ± standard deviation, with *P* values derived from one-way analysis of variance or the Kruskal–Wallis Test. Qualitative variables were expressed as numbers (percentages), with *P* values determined through the Chi-square test. A paired *t*-test and Wilcoxon rank-sum test were used for the analysis within groups. A general linear model was used to analyze the differences between the three groups after adjusting for baseline values. To mitigate alpha error and correct for multiple comparisons, the Benjamin–Hochberg method was applied to *q* values.[21] Statistical significance was set at $P < 0.05$. SPSS software (Version 24, SPSS Inc., Chicago, IL, USA) facilitated statistical analysis.

RESULTS

Ninety participants were enrolled after screening 380 individuals. Ninety participants were randomly assigned to the three groups and included in the intent-to-treat population. Sixteen participants dropped out due to changes in medication, refusal to participate, noncompliance with the study protocol, antibiotic use, stomach pain, pregnancy, or inability to continue with the study procedures. A total of 74 (82.2%) of the randomized participants completed the intervention after 8 weeks. A flowchart showing the sample composition and dropouts during the 8-week follow-up can be found in Figure 1. The probiotics or placebo did not cause any side effects among the participants. No adverse events or other problems have been reported regarding safety or tolerability.

The baseline demographic characteristics of the participants are depicted in Table 1. Overall, there were no significant differences observed in demographic characteristics such as age, weight, height, BMI, and waist circumference among the three groups (all $P > 0.05$). Similarly, marital status and education level did not differ significantly across the groups ($P > 0.05$). However, notable differences were observed in one of the clinical parameters. For instance, the age of onset of menstruation differed significantly between groups ($P = 0.003$). Other clinical parameters such as the interval between menstrual cycles, menstrual status, hirsutism, and acne did not exhibit significant differences among the groups (all $P > 0.05$). Moreover, between the three groups, there were no significant differences in terms of physical activity, macronutrient intake, and micronutrient intake [Supplementary Table 2].

As compared to week 8, systolic blood pressure (SBP) significantly decreased in the TRE group +

Table 1: Baseline characteristics of study subjects

Variables	TRE + probiotic (*n*=30)	TRE + placebo (*n*=30)	DCR + placebo (*n*=30)	*P*	*P*[a]
Age (years)*	30.00±5.55	30.43±5.66	31.06±5.78	0.765	0.765
Weight (kg)*	79.42±10.84	80.85±13.34	84.10±12.55	0.339	0.669
Height (cm)*	163.56±5.48	164.20±5.80	165.16±5.97	0.558	0.669
BMI (kg/m^2)*	29.65±3.44	29.85±3.73	30.68±3.54	0.525	0.669
WC (cm)*	95.90±11.06	96.36±8.99	99.06±8.74	0.395	0.669
Marital status, *n* (%)					
Single	11 (36.7)	9 (30.0)	10 (33.3)	0.674	0.735
Married	19 (63.3)	21 (70.7)	19 (63.3)		
Divorced	0	0	1 (3.3)		
Education, *n* (%)					
Under diploma	1 (3.3)	1 (3.3)	0	0.382	0.669
Diploma	4 (13.3)	8 (26.7)	10 (33.3)		
Collegiate	25 (83.3)	21 (70.0)	20 (66.7)		
Age of onset of menstruation (years)†	12.00 (11.75–13.00)	13.00 (11.75–14.00)	13.00 (13.00–14.00)	0.003	0.036
The interval between menstrual cycles (days)†	42.50 (30.00–90.00)	60.00 (44.25–120.00)	38.00 (30.00–120.00)	0.059	0.236
Menstrual status, *n* (%)					
Regular	11 (36.7)	3 (10.0)	7 (23.3)	0.051	0.236
Irregular	19 (63.3)	27 (90.0)	23 (76.6)		
Hirsutism (mF-G scores)†	15.99 (12.00–18.25)	16.00 (12.7–18.00)	13.00 (12.00–17.00)	0.229	0.669
Acne (score)†	4.48 (0.01–10.00)	6.00 (1.50–12.00)	3.50 (0.01–10.50)	0.466	0.669

*Quantitative variables are reported as mean±SD and *P* values obtained from one-way ANOVA; †Quantitative variables are reported as median (IQR) and *P* values obtained from the Kruskal–Wallis test; [a]*P*-values were adjusted for multiple comparisons using the Benjamini and Hochberg method[21] to control for the false discovery rate. Qualitative variables are reported as, *n* (%) and *P* values obtained from the Chi-square test. BMI=Body mass index; WC=Waist circumference; mF-G score=Modified Ferriman–Gallwey; SD=Standard deviation; IQR=Interquartile range; TRE=Time-restricted eating; DCR=Daily calorie restriction; ANOVA=Analysis of variance

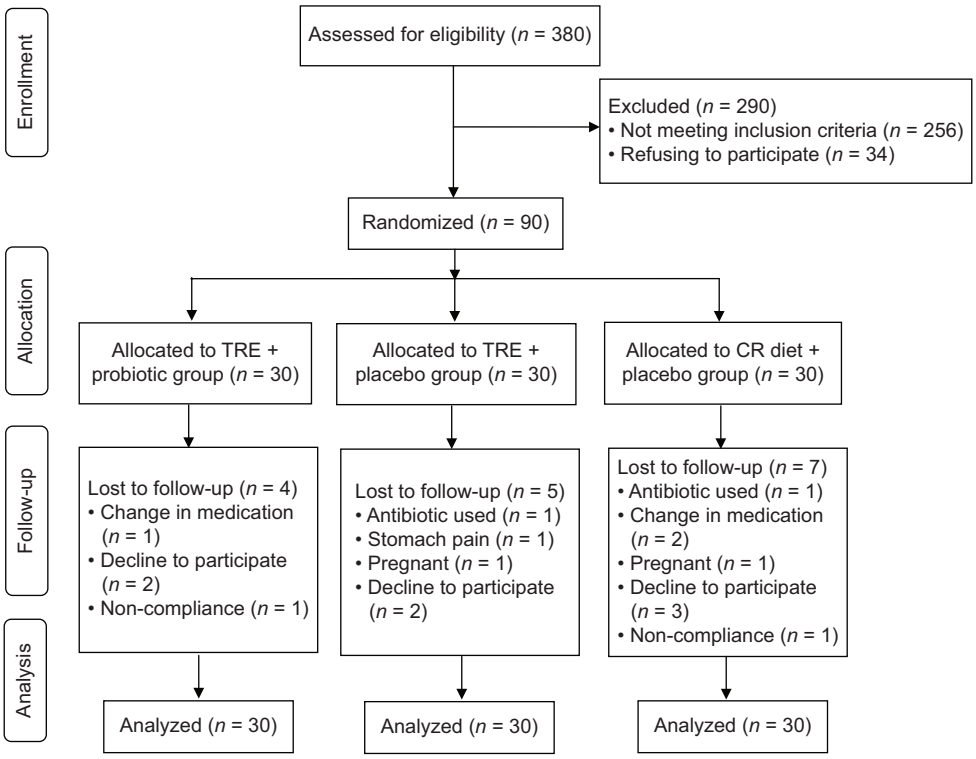

Figure 1: Summary of patient flow diagram

probiotic (−0.31 mmHg [95% confidence interval (CI): −0.55, −0.07]) and TRE group + placebo (−0.24 mmHg [95% CI: −0.43, 0.04]) with no difference between groups. Furthermore, all groups had lower CRP levels ($P < 0.005$). Similarly, TAC showed significant improvement in the TRE + probiotic group ($P = 0.012$) and DCR + placebo group ($P = 0.032$). While some parameters showed significant changes within groups, there were no significant differences between groups at week 8. Notably, there were no significant differences in SBP, diastolic blood pressure (DBP), CRP, TAC, TOS, and OSI between the intervention groups at week 8 (all $P > 0.05$). These findings suggest that while TRE, with or without probiotic supplementation, can lead to improvements in certain physiological parameters such as SBP, CRP, and TAC, there were no significant differences between the groups in terms of BP and OS markers after 8 weeks of intervention. Changes in BP, inflammation, oxidant, and antioxidant parameters are illustrated in Tables 2 and 3.

DISCUSSION

This randomized, placebo-controlled trial investigated the comparative effects of IF alone or in combination with probiotic supplementation versus a calorie-restricted (CR) diet on biomarkers linked to inflammation, OS, and antioxidant capacity among women diagnosed with PCOS. The findings revealed notable reductions in SBP and CRP levels in both IF groups, regardless of probiotic supplementation, alongside enhanced TAC, particularly evident in the IF with probiotic and CR with placebo groups. However, no statistically significant disparities emerged between the intervention groups by the study's conclusion concerning SBP, DBP, CRP, TAC, TOS, and OSI. These results suggest that while IF positively impacts select physiological markers, the adjunctive use of probiotics did not yield discernible additive benefits over the 8-week study duration.

Women afflicted with PCOS often confront a myriad of metabolic challenges, including heightened levels of androgens, escalated inflammation, and augmented OS.[22,23] Moreover, in individuals with PCOS, inflammation has been linked to dysfunction in pancreatic beta cells, insulin resistance, the initiation of atherosclerosis, and compromised ovarian function.[24] This inflammatory cascade is further exacerbated by an imbalance in antioxidant levels.[25] The modulation of OS and inflammatory markers has been demonstrated to correlate with reductions in hyperandrogenemia and improvements in atherogenic profiles, suggesting a potential avenue for therapeutic intervention in managing the metabolic complications associated with PCOS.[26,27]

In comparison to week 8, the results of the current study demonstrate a reduction in SBP in both the eTRE group with probiotic supplementation and the TRE group with placebo, with statistical significance. Importantly, the findings also reveal no significant discrepancies in SBP and DBP between the intervention groups at week 8 ($P > 0.05$).

Table 2: A paired *t*-test was used to compare pre- and post-tests on blood pressure, inflammation, oxidant, and antioxidant parameters

Variable	TRE + probiotic (*n*=30)			TRE + placebo (*n*=30)			DCR + placebo (*n*=30)		
	Baseline	End of trial	*P*[a]	Baseline	End of trial	*P*[a]	Baseline	End of trial	*P*[a]
SBP (mmHg)	11.00 (10.00–11.50)	10.4 (10.00–11.00)	0.010	11.00 (10.0–12.00)	10.60 (10.00–12.00)	0.018	11.00 (10.00–11.50)	11.00 (10.15–11.13)	0.062
DBP (mmHg)	7.00 (8.00–8.00)	7.50 (7.55–8.00)	0.493	8.00 (7.00–8.50)	7.53 (7.00–8.11)	0.098	7.75 (7.00–8.00)	7.50 (7.00–8.00)	0.338
CRP (mg/L)	6.15 (4.45–10.65)	5.65 (2.89–7.30)	<0.001	6.65 (4.60–9.65)	4.69 (2.17–7.67)	<0.001	6.45 (4.64–8.30)	4.75 (3.74–6.62)	0.007
TAC (µmol/L)	424.61 (367.59–495.46)	430.04 (389.47–554.53)	0.012	461.72 (388.38–533.80)	471.40 (389.47–537.83)	0.486	483.25 (406.17–527.09)	494.47 (444.36–553.74)	0.032
TOS (µmol/L)	0.97 (0.85–1.35)	0.94 (0.79–1.14)	0.143	1.21 (0.93–1.47)	0.93 (0.81–1.16)	0.018	1.10 (0.89–1.34)	0.97 (0.87–1.04)	0.009
OSI	2.25 (1.79–3.69)	2.23 (1.61–2.95)	0.065	2.68 (2.05–3.03)	2.25 (1.73–2.72)	0.012	2.41 (1.64–2.92)	2.02 (1.57–2.29)	0.004

[a]Obtained from the Wilcoxon test comparing baseline and endpoint values within each group. Values are expressed as median (IQR). SBP=Systolic blood pressure; DBP=Diastolic blood pressure; CRP=C-reactive protein; TAC=Total antioxidant capacity; TOC=Total oxidant status; OSI=Oxidative stress index; IQR=Interquartile range; TRE=Time-restricted eating; DCR=Daily calorie restriction

These outcomes echo the notable improvements observed in previous studies investigating the effects of dietary interventions on BP, underscoring the potential efficacy of eTRE in mitigating cardiovascular risk factors. While the study did not replicate the dramatic reductions reported in certain interventions, the observed modest decreases in SBP underscore the potential benefits of TRE, particularly when combined with probiotic supplementation, in managing BP levels. Possible mechanisms underlying these effects may involve the modulation of insulin levels[28] and natriuresis, consistent with existing literature on the physiological impacts of dietary interventions on BP regulation.[29] Furthermore, previous research by Sutton *et al*. demonstrated significant reductions in SBP and DBP following a 5-week eTRE intervention, highlighting the potential for short-term dietary modifications to influence BP parameters.[30] Moreover, studies by Bhutani *et al*., Eshghinia and Mohammadzadeh, Varadi *et al*., and Wei *et al*. have also reported improvements in BP with various IF regimens, indicating the broader impact of dietary modifications on cardiovascular health.[31-34]

Increased CRP concentrations are positively correlated with insulin resistance and the incidence of type 2 diabetes mellitus (T2DM), making high CRP levels a potential cause of the long-term outcomes of PCOS.[35,36] Toulis *et al*. found that CRP levels in individuals with PCOS were significantly higher compared to the control group.[37] Generally, most studies have reported that IF does not substantially affect hs-CRP, tumor necrosis factor-alpha (TNF-a), or interleukin-6 (IL-6).[31] For instance, in the study by Sutton *et al*., eTRE did not impact any inflammatory markers, with morning fasting levels of hs-CRP, cortisol, and IL-6 all unchanged.[30] Similarly, a previous trial involving TRE reported a decrease in interleukin-1 beta (IL-1b) but no significant changes in IL-6 or TNF-a.[38]

Conversely, probiotic supplementation has shown varied effects on inflammation. In PCOS patients, a 12-week course of probiotics reduced hs-CRP levels.[39] An 8-week regimen also reduced serum hs-CRP in individuals with major depressive disorder and critically ill patients.[40,41] However, results are inconsistent; for example, an 8-week probiotic supplement did not affect CRP levels in PCOS patients,[40] and synbiotic supplementation for 6 weeks did not alter serum CRP in subjects with low serum enterolactone concentrations.[42] Previous research indicated that consuming probiotic yogurt for 6 weeks improved antioxidant status in individuals with T2DM,[43] and a 12-week probiotic course benefited patients with multiple sclerosis by affecting several systemic inflammatory markers.[44] Nevertheless, a meta-analysis among T2DM subjects found no effect on CRP concentrations.[45] Furthermore, an 8-week probiotic intervention in women

Table 3: The effects of intermittent fasting diet alone or in combination with probiotic supplementation in comparison with calorie-restricted diet on blood pressure, inflammation, oxidant, and antioxidant parameters

Status	Variable	TRE + probiotic versus TRE + placebo				TRE + probiotic versus DCR + placebo				TRE + placebo versus DCR + placebo				P^a	P^b
		Difference	95% CI		P^a	Difference	95% CI		P^a	Difference	95% CI		P^a		
			Lower	Upper			Lower	Upper			Lower	Upper			
Adjusted*	SBP (mmHg)	−0.12	−0.39	0.13	0.346	−0.18	−0.44	0.08	0.184	−0.05	−0.31	0.21	0.702	0.393	0.838
	DBP (mmHg)	0.01	−0.17	0.19	0.883	0.03	−0.14	0.22	0.691	0.02	−0.16	0.20	0.804	0.922	0.945
	CRP (mg/L)	0.11	−1.69	1.92	0.902	−0.26	−2.07	1.54	0.775	−0.37	−2.20	1.44	0.684	0.917	0.945
	TAC (μmol/L)	11.05	−26.33	48.44	0.562	−14.90	−52.41	22.60	0.436	−25.95	−62.97	11.05	0.169	0.387	0.838
	TOS (μmol/L)	0.02	−0.09	1.15	0.676	0.04	−0.07	0.16	0.484	0.01	−0.10	0.14	0.782	0.780	0.838
	OSI	0.05	−0.25	0.35	0.744	0.18	−0.12	0.48	0.246	0.13	−0.17	0.43	0.403	0.489	0.945
Adjusted†	SBP (mmHg)	−0.08	−0.34	0.17	0.510	−0.05	−0.32	0.21	0.714	0.03	−0.22	0.29	0.788	0.805	0.899
	DBP (mmHg)	0.02	−0.15	0.20	0.776	0.09	−0.10	0.28	0.352	0.06	−0.12	0.24	0.494	0.633	0.899
	CRP (mg/L)	0.37	−1.42	2.18	0.683	0.38	−1.53	2.30	0.694	<0.01	−1.83	1.85	0.992	0.899	0.899
	TAC (μmol/L)	12.45	−24.89	49.80	0.513	−9.53	−48.81	29.74	0.634	−21.98	−59.44	15.47	0.250	0.510	0.838
	TOS (μmol/L)	0.04	−0.07	0.17	0.454	0.08	−0.04	0.21	0.199	0.03	−0.08	0.16	0.551	0.437	0.838
	OSI	0.07	−0.23	0.38	0.632	0.23	−0.08	0.56	0.154	0.16	−0.14	0.47	0.310	0.346	0.899

*Adjusted based on baseline value; †Adjusted based on baseline value, age of onset of menstruation, and baseline weight; [a]Obtained from GLM and adjusted based on baseline value; [b]*P*-values were adjusted for multiple comparisons using the Benjamini and Hochberg method[21] to control for the false discovery rate. SBP=Systolic blood pressure; DBP=Diastolic blood pressure; CRP=C-reactive protein; TAC=Total antioxidant capacity; TOC=Total oxidant status; OSI=Oxidative stress index; TRE=Time-restricted eating; DCR=Daily calorie restriction; GLM=General linear models; CI=Confidence interval

with gestational diabetes mellitus significantly improved biomarkers of inflammation and OS,[46] although another study found no significant change in hs-CRP levels in women with PCOS who received probiotics for 8 weeks.[47]

The current study found that all groups, including those undergoing TRE with and without probiotic supplementation, as well as the control group, had lower CRP levels ($P < 0.005$). Although there were significant changes within each group, there were no significant differences between the groups at week 8. In particular, CRP levels were not significantly different between the TRE group and the TRE with probiotic supplementation group at week 8 ($P > 0.05$). These findings are consistent with previous studies indicating that while TRE and probiotic supplementation can lower CRP levels, the combination does not provide a significantly greater benefit than either intervention alone within the study period.

The current study compared interventions for OS and antioxidant capacity biomarkers in women with PCOS yielded insightful results. TAC demonstrated significant improvement in both the TRE with probiotic and DCR with Placebo groups ($P = 0.012$ and $P = 0.032$, respectively). However, while some parameters exhibited significant changes within groups, there were no significant differences between the groups at week 8. Notably, at week 8, there were no significant differences in TAC, TOS, and OSI between the intervention groups ($P > 0.05$).

Moreover, emerging evidence underscores the link between increased OS, driven by reactive oxygen species (ROS), and the development of insulin resistance and hyperandrogenism, key features of PCOS.[48] Sutton *et al*.'s investigation revealed that compared to the control arm, 5 weeks of early eTRE reduced plasma levels of 8-isoprostane, a marker of OS to lipids, by approximately 14%.[30] Notably, both sequence and period effects were statistically significant for 8-isoprostanes. However, the relative improvement observed was primarily attributed to the worsening observed in the control arm, suggesting that in that study, eTRE may have mitigated the deterioration of 8-isoprostane levels through adherence to the study foods.

Conversely, the findings from studies examining probiotic supplementation present conflicting results. For instance, a 12-week probiotic intervention in women with PCOS demonstrated beneficial effects on both TAC and malondialdehyde levels.[39] In addition, Kullisaar *et al*. noted that specific strains of *Lactobacillus fermentum* exhibited antioxidant properties, such as increasing Glutathione levels.[49] Furthermore, Songisepp *et al*. reported a significant enhancement in total antioxidant status following a 3-week probiotic supplementation regimen in healthy subjects.[50]

The absence of significant differences between intervention groups in our study may be attributed to several factors. First, the inclusion of a CR diet as the control group, known to be effective in managing PCOS outcomes,[51] might have minimized the discernible disparities between intervention arms. This aligns with previous findings suggesting the efficacy of caloric restriction in mitigating OS and improving antioxidant capacity.[52] Second, while individual intervention groups demonstrated notable improvements in TAC, the lack of divergence between groups suggests that neither IF with probiotic supplementation nor calorie

restriction alone conferred a discernible advantage over the other in terms of OS modulation. This finding resonates with the complexity of PCOS pathophysiology, where multiple factors beyond dietary interventions may influence oxidative balance.[53] Furthermore, the multifactorial etiology of PCOS, encompassing genetic predispositions and hormonal dysregulation, may have contributed to the variability in treatment responses across intervention groups.[54] Thus, while our study underscores the individual efficacy of dietary interventions in managing OS markers, the absence of significant intergroup disparities underscores the intricate interplay of multifaceted factors in PCOS management.

The present study possesses several strengths, foremost among them being its rigorous methodology, including randomization and the inclusion of an appropriate control group, which enhances the internal validity of the findings. In addition, the discovery that various weight loss strategies, including TRE and calorie restriction alone, yield comparable outcomes in individuals with PCOS underscores the versatility and effectiveness of these interventions in clinical practice. Moreover, the study benefits from its homogeneous population of PCOS patients, facilitating a more targeted and nuanced analysis of intervention effects. However, these strengths are juxtaposed with several limitations that warrant consideration. First, the reliance on self-reported measures of adherence and physical activity may introduce recall bias and limit the accuracy of data interpretation. Similarly, the study's short duration and small sample size constrain the statistical power and generalizability of the findings, underscoring the need for larger, longer-term clinical trials to bolster the robustness of conclusions. Moreover, the impact of external factors such as the COVID-19 pandemic on participant recruitment and adherence further complicates the interpretation of results and underscores the need for caution in extrapolating findings to broader populations.

CONCLUSION

The present study did not reveal significant improvements in BP, inflammatory markers, OS, or antioxidant capacity with either eTRE alone or eTRE combined with probiotics compared to a standard DCR among obese women diagnosed with PCOS.

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Conflicts of interest

There are no conflicts of interest.

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Supplementary Table 1: CONSORT 2010 checklist of information to include when reporting a randomized trial*

Section/topic	Item number	Checklist item	Reported on page number
		Title and abstract	
	1a	Identification as a randomized trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	1, 2
		Introduction	
Background and objectives	2a	Scientific background and explanation of the rationale	3
	2b	Specific objectives or hypotheses	3, 4
		Methods	
Trial design	3a	Description of trial design (such as parallel and factorial) including allocation ratio	5
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	5
Participants	4a	Eligibility criteria for participants	6
	4b	Settings and locations where the data were collected	6, 7
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	9
Outcomes	6a	Completely defined prespecified primary and secondary outcome measures, including how and when they were assessed	9
	6b	Any changes to trial outcomes after the trial commenced, with reasons	No changes
Sample size	7a	How sample size was determined	8
	7b	When applicable, explanation of any interim analyses and stopping guidelines	8
		Randomization	
Sequence generation	8a	The method used to generate the random allocation sequence	7, 8
	8b	Type of randomization; details of any restriction (such as blocking and block size)	7
Allocation concealment mechanism	9	The mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	7
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	7
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	8
	11b	If relevant, a description of the similarity of interventions	10
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	10
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	10
		Results	
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned received intended treatment, and were analyzed for the primary outcome	11
	13b	For each group, losses and exclusions after randomization, together with reasons	11
Recruitment	14a	Dates defining the periods of recruitment and follow-up	11, 12
	14b	Why the trial ended or was stopped	11, 12
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	11, 12
Numbers analyzed	16	For each group, the number of participants (denominator) included in each analysis and whether the analysis was by originally assigned groups	11, 12

Contd...

Supplementary Table 1: Contd...

Section/topic	Item number	Checklist item	Reported on page number
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% CI)	11, 12
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	11, 12
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing prespecified from exploratory	11, 12
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	Not applicable
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	15
Generalizability	21	Generalizability (external validity and applicability) of the trial findings	12-16
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	12-16
Other information			
Registration	23	Registration number and name of trial registry	5, 6
Protocol	24	Where the full trial protocol can be accessed, if available	No
Funding	25	Sources of funding and other support (such as the supply of drugs), role of funders	No

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 explanation and elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomized trials, noninferiority and equivalence trials, nonpharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: For those and for up-to-date references relevant to this checklist, see www.consort-statement.org. CI=Confidence interval

Supplementary Table 2: Dietary intake and physical activity of participants

Nutrients	TRE + probiotic (*n*=30)	TRE + placebo (*n*=30)	DCR + placebo (*n*=30)	*P*[b]	*P*[c]
Energy (kcal/day)					
Before	2133.99±326.45	2175.10±417.55	2173.51±306.65	0.698	0.978
After	1845.68±223.68	1843.29±325.00	1729.75±203.31	0.138	0.864
P[a]	0.001	0.001	0.001		
Carbohydrate (g/day)					
Before	299.05±52.13	312.56±67.48	302.83±56.96	0.953	0.978
After	258.18±38.89	259.92±59.53	241.21±42.75	0.287	0.978
P[a]	0.001	0.001	0.001		
Protein (g/day)					
Before	89.83±25.92	86.62±17.09	88.87±20.81	0.815	0.978
After	78.72±18.03	79.82±22.85	70.51±14.36	0.116	0.864
P[a]	0.027	0.094	0.001		
Lipid (g/day)					
Before	70.42±15.19	69.72±18.47	73.01±15.85	0.342	0.978
After	60.24±12.89	58.02±12.41	58.79±11.68	0.779	0.978
P[a]	0.003	0.005	0.001		
Cholesterol (mg/day)					
Before	259.30±193.68	218.90±111.06	218.82±154.79	0.674	0.978
After	220.36±145.79	236.16±168.44	226.07±114.07	0.902	0.978
P[a]	0.456	0.688	0.614		
Polyunsaturated fat (g/day)					
Before	24.12±5.48	23.31±6.56	25.38±6.42	0.371	0.978
After	20.75±5.31	19.62±4.30	20.00±4.18	0.758	0.978
P[a]	0.003	0.005	0.001		
Saturated fat (g/day)					
Before	14.47±4.06	16.20±5.50	16.18±3.88	0.144	0.864
After	13.41±3.93	13.20±4.63	13.55±4.67	0.954	0.978
P[a]	0.160	0.001	0.016		
Vitamin C (mg/day)					
Before	161.74±54.27	158.36±46.50	132.10±49.38	0.073	0.864
After	138.83±70.46	148.73±65.88	124.11±48.61	0.247	0.978
P[a]	0.144	0.572	0.360		
Vitamin E (mg/day)					
Before	24.11±5.57	25.24±6.62	26.06±7.05	0.525	0.978
After	22.12±4.89	21.56±4.82	21.37±5.82	0.340	0.978
P[a]	0.082	0.004	0.002		
Dietary fibers (g/day)					
Before	25.78±6.26	28.49±10.09	27.78±9.37	0.786	0.978
After	23.91±5.55	25.43±8.79	23.33±6.94	0.569	0.978
P[a]	0.430	0.111	0.020		
Selenium (mg/day)					
Before	0.07±0.02	0.08±0.03	0.09±0.03	0.348	0.978
After	0.07±0.01	0.08±0.03	0.07±0.02	0.899	0.978
P[a]	0.793	0.077	0.029		
Zinc (mg/day)					
Before	10.19±2.05	10.68±2.11	10.30±1.91	0.619	0.978
After	9.38±2.01	9.31±2.19	8.62±1.66	0.115	0.864
P[a]	0.157	0.016	<0.001		
Magnesium (mg/day)					
Before	322.81±40.81	337.11±83.82	337.87±79.44	0.978	0.978
After	297.21±59.16	294.53±68.11	290.20±31.51	0.533	0.978
P[a]	0.056	0.028	0.001		
Chromium (μg/day)					
Before	0.10±0.03	0.11±0.04	0.11±0.05	0.891	0.978

Contd...

Supplementary Table 2: Contd...

Nutrients	TRE + probiotic (*n*=30)	TRE + placebo (*n*=30)	DCR + placebo (*n*=30)	*P*[b]	*P*[c]
After	0.09±0.02	0.10±0.04	0.09±0.03	0.757	0.978
P[a]	0.191	0.110	0.068		
Physical activity MET (min/week)					
Before	769.75±777.62	553.06±605.08	822.73±903.40	0.570	0.978
After	662.09±605.21	622.81±696.55	729.81±791.71	0.863	0.978
P[a]	0.383	0.616	0.660		

[a]Obtained from paired *t*-test comparing baseline and endpoint values within each group; [b]Obtained from one-way ANOVA, [c]*P*-values were adjusted for multiple comparisons using the Benjamini and Hochberg method[1] to control for the false discovery rate. MET=Metabolic equivalent; ANOVA=Analysis of variance; TRE=Time-restricted eating; DCR=Daily calorie restriction