Eight-hour Time-restricted Feeding Improves Endocrine and Metabolic Profiles in Women With Anovulatory Polycystic Ovary Syndrome

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Research

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Abstract

Background: Time-restricted feeding (TRF) is a form of intermittent fasting, which is beneficial for weight loss and cardiometabolic health. Polycystic ovary syndrome (PCOS) is one of the most common reproductive endocrine and metabolic diseases affecting women of childbearing age. It is associated with an increased prevalence of metabolic syndrome, cardiovascular disease and type 2 diabetes. While limited data are available on the effects of TRF in PCOS. Thus, we sought to investigate the effects of TRF in women with anovulatory PCOS.

Methods: Eighteen women aged between 18 and 31 with anovulation of PCOS participated in a 6-week trial and were divided into two consecutive periods: (1) 1-week baseline period; and (2) 5-week TRF period. Fifteen participants completed the study. Changes in body weight, body mass index (BMI), Waist-to-Hip Ratio, skeletal muscle mass, body fat mass (BFM), body fat percentage (BF%), visceral fat area (VFA), luteinizing hormone (LH), follicle-stimulating hormone (FSH), LH/FSH, total testosterone (TT), sex hormone-binding globulin (SHBG), free androgen index (FAI), fasting glucose, fasting insulin (FINS), homeostasis model assessment-insulin resistance (HOMA-IR), area under the curve (AUC) for insulin (AUCInsulin), area under the curve (AUC) for glucose (AUCGlucose), AUCInsulin/AUCGlucose Ratio, lipids, uric acid, alanine aminotransferase (ALT), aspartate aminotransferase, high-sensitivity C-reactive protein (hsCRP), insulin-like growth factor (IGF-1), menstrual cycle and eating behaviors were evaluated.

Results: Significant changes in body weight, BMI, BFM, BF%, VFA, TT, SHBG, FAI, FINS, HOMA-IR, AUCInsulin, AUCInsulin/AUCGlucose Ratio, ALT, hsCRP and IGF-1 were found after the TRF period. An improvement in menstrual cycle irregularity was detected in 73.3% (11/15) patients.

Conclusion: The diet of TRF may be beneficial to anovulatory PCOS on weight loss (especially reducing body fat), improving menstruation, hyperandrogenemia, insulin resistance and inflammation.

Trial registration Clinicaltrial.gov, NCT04580433, registrered October 8, 2020, https://clinicaltrials.gov/ct2/show/NCT04580433

Background

Polycystic ovary syndrome (PCOS) is one of the most common reproductive endocrine and metabolic disorders that affects up to 10% women of childbearing age [1]. It manifests as a broad range of clinical symptoms, including menstrual disorders, infertility and hyperandrogenism. Although the etiology of PCOS remains unknown, hyperinsulinemia seems to play a crucial role in the development of PCOS. Obesity, hormonal disorders, and insulin-resistance (IR) are associated with compensatory hyperinsulinemia [2], and low-grade chronic inflammation often coexists with IR and hyperandrogenism affecting the metabolic phenotype of PCOS [3,4]. In recent years, dietary interventions in PCOS have become popular in both reproductive and endocrine research. Since up to 60% of women with PCOS are overweight or obese [5], the International Evidence-based Guideline for the Assessment and Management of PCOS also emphasizes the importance of diet in PCOS and recommend dietary and exercise

interventions as the first-line management inthis population [6]. To date, several dietary strategies have been proposed for the treatment of PCOS, such asthe low glycemic index diet, dietary approaches to stop hypertension diet, the Mediterranean diet, low carbohydrate diet, pulse-based diet, ketogenic diet, low-starch/low-dairy diet,and vegetarian diets [4,7-13].

Typically, Intermittent fasting (IF) is the practice of alternate eating and fasting. IF is an umbrella term for three different types of diets: alternate-day fasting, the 5:2 diet, and time-restricted feeding (TRF). TRF is generally defined as fasting for 12–20 h; most people eat three times a day over a 12 h period [15]. TRF allows to *ad libitum* feeding within a large window of time each day without any calorie counting. Emerging evidence has suggested that TRF was beneficial for losing body weight, ameliorating IR, regulating metabolism, and improving cardiometabolic health [16-17]. To the best of our knowledge, except for a very limited report on dawn-to-sunset Ramadan fasting, Muslims abstain from eating or drinking from sunrise to sunset [18], there has been no persuasive study on the possible role of IF in the PCOS population. Considering that PCOS is often accompanied by severe metabolic disorders such as obesity and insulin resistance, and hyperinsulinemia, which play key roles in inducing androgen excess, TRF may also have a good effect on the endocrine and metabolic profiles in PCOS. In addition, anovulation is a common characteristic in women with PCOS and it is unclear whether TRF can improve menstrual disorders. Thus a 6-week trial, with 2 consecutive periods: (1) 1-week baseline period; and (2) 5-week TRF period, was implemented to explore the effect of TRF on women with anovulatory PCOS and propose a basis for its inclusion in the treatment of PCOS.

Materials And Methods

Participants

PCOS outpatients were recruited from the Department of Endocrinology, Shengjing Hospital of China Medical University in 2020 and data collection was completed in January 2021. The inclusion criteria were: age 18-40 years; BMI≥24kg/m^2; anovulation; and a diagnosis of PCOS based on the Rotterdam diagnostic criteria. The exclusion criteria were: use of medication therapy that impacting on carbohydrate or lipid metabolism (oral contraceptive pills, insulin-sensitizers, anti-epileptics, anti-psychotics, statins, and fish oil) in the recent 6 months; body weight fluctuations of more than 5% in the past 3 months; in preparation for pregnancy, pregnant or lactating; perimenopausal; night-shift workers; fasting for more than 16 hours a day; hypotension; patients with other diseases (such as congenital adrenal hyperplasia, Cushing syndrome, androgen-secreting tumors, hyperprolactinemia, diabetes, thyroid diseases, severe serious cardiovascular, gastrointestinal, and kidney or liver diseases); alcohol intake of more than 100g per week; smoking within the past 3 months and engaging in high-intensity exercise.

Study Design

The protocol for this study was approved by the Medical Research and New Technology Ethics Committee of Shengjing Hospital of China Medical University (reference: 2020PS682K). The study

followed a pre-post non-randomized design and it was pre-registered on ClinicalTrials.gov (NCT04580433). The trial consisted of a 1-week baseline weight stabilization period followed by a 5-week TRF intervention period. After signing the informed consent, the following data were obtained to determine eligibility: (1) Height, weight, and age, (2) Blood pressure, (3) Menstrual cycle, and (4) Medical history. The eligible participants were then invited to attend a baseline assessment visit where they completed the following assessments: (1) Body composition analysis, and (2) Three Factor Eating Questionnaire Revised 21 Item (TFEQ-R21) questionnaire [23]. They also underwent a standard 75 g oral glucose tolerance test and blood samples for measurement of plasma glucose and insulin were drawn prior to and at 60 and 120 min after glucose ingestion. During the baseline (week 0-1), participants continued with their usual diets, exercise, and sleep habits to keep their weight stable. During the TRF period (week 2-6), they were asked to not change the composition of their usual diets but were instructed to eat freely from 8 a.m. to 4 p.m. daily and fast from 4 p.m. to 8 a.m. the next day. Participants were provided with a food diary and were instructed to record their daily food intake from start to finish and use the Boohee software, a diet and fitness app in China to calculate the corresponding calories. Daily dietary calorie intake was required to be approximately consistent with the baseline for as much as possible (fluctuations of no more than 10%). During the 16-hour fasting, only water or calorie-free beverages were allowed, and participants were encouraged to drink enough water throughout the intervention period. We contacted participants via phone at the end of each week to review the protocol, monitor any adverse events, and provide support and guidance to promote compliance with interventions. All the baseline assessments were repeated at the follow-up visit on the 6th week when the diaries and data of time to return to normal menstrual cycle were collected. All the examinations were conducted in the morning while the participants were fasting.

Anthropometric Measurements

For each participant, body weight and height were measured to calculate the BMI [weight (kg) divided by height squared (m^2), kg/m^2]. Height was measured to the nearest 1 cm using a wall-mounted stadiometer (Seca 711; Seca, Hamburg, Germany). Body weight was determined to the nearest 0.1 kg using a multi-frequency bioelectrical impedance analyzer InBody 770 scanner (In-body Bldg, Seoul, Korea), with high resolution touch screen, frequency 1,5, 50, 260, 500, 1000kHz and measurement time 60 seconds, with the subjects in a standing position after shoes, coats and sweaters were removed, according to the manufacturer's instructions. Waist-to-Hip Ratio (WHR) was measured by InBody 770 scanner, waist circumstance and hip circumstance were measured by the same nurse. The WHR was calculated using these measurements.

Body Composition

Body composition such as skeletal muscle mass (SMM) (kg), body fat mass (BFM) (kg), body fat percentage (BF%) and visceral fat area (VFA) (cm^2) were evaluated by InBody 770 scanner.

Blood Sampling and Analysis

The participant's fasted blood samples were collected before and after the intervention. Blood samples were taken from antecubital vein and collected into BD Vacutainers Tubes (SSTTM II Advance, REF 367953). Then, samples were centrifuged for 10 min at 3600 rpm at 4 °C. The obtained serum and plasma were aliquoted and stored at –80 °C until analysis. Insulin 0min, 60min, 120min (μU/mL) were measured by radioimmunological assay, luteinizing hormone (LH) (mIU/mL) and follicle-stimulating hormone (FSH) (mIU/mL) were measured by chemiluminescent immunoassay, total testosterone (TT) (ng/mL) was measured by electrochemiluminescent immunoassay, insulin-like growth factor 1 (IGF-1) (ng/mL) and sex hormone-binding globulin (SHBG) (nmol/L) was measured by immunochemiluminescent on Beckman Coulter Unicel Dxl 800. Total cholesterol (TC) (mmol/L) was measured by cholesterol oxidase method, triglycerides (TG) (mmol/L) were measured by deionization & enzyme method, and low density lipoprotein-cholesterol (LDL-C) (mmol/L) was measured by selective solubilization method, alanine aminotransferase (ALT) (U/L) and aspartate aminotransferase (AST) (U/L) were measured by NADH method, uric acid (UA) (umol/L) (umol/L), Glucose 0min, 60min, 120min (mmol/L) and high-sensitivity C-reactive protein (hs-CRP) (mg/L) were measured separately by uricase-PAP method, hexokinase method and rate nephelometry method on ci 16200 Abbott Architect analyzer. Homeostasis model assessment-insulin resistance (HOMA-IR) was calculated according to the formula "FINS (μU/mL) × FG (mmol/L)/22.5". Free androgen index (FAI) (%) was calculated according to the formula "TT (ng/mL) × 100/SHBG (nmol/L)".

Questionnaires

The TFEQ-R21 were completed during pre- and post- visits. The TFEQ-R21 covers 3 eating behavior domains: the cognitive restraint scale (6 items) assesses control over food intake to influence body weight and body shape; the emotional eating scale (6 items) measures the propensity to overeat in relation to negative mood states; the uncontrolled eating scale (9 items) assesses the tendency to lose control overeating when feeling hungry or when exposed to external stimuli. It contains 20 questions based on a 4-point Likert-Scale (1 = definitely false, 2 = mostly false, 3 = mostly true, 4 = definitely true) and one question scored between 1–8 on eating restraint (1 = no restraint when eating, 8 = extreme restraint when eating). Higher scores indicate greater cognitive restraint, uncontrolled eating, or emotional eating.

Statistical Analysis

The distribution of continuous data was tested with the Anderson-Darling test. Continuous variables were presented as mean ± standard deviation (mean ± SD) (normally distributed) or median (25th–75th percentile) (non-normally distributed) and analyzed using Student's test for matched pairs (normally distributed) or Wilcoxon matched pairs signed-rank test (non-normally distributed) to compare parameters before and after 5 weeks of the TRF period. All data analyses were performed in GraphPad Prism (version 8.0.1; GraphPad Software). Significance was considered at a value of $P<0.05$. For responses with missing values, the values were not included in the analyses.

Compliance with ethical standards

Medical Research and New Technology Ethics Committee of Shengjing Hospital affiliated to China Medical University (reference: 2020PS682K).

Results

Participants

25 participants were recruited to participate in the investigation, 7 women were excluded: 2 for current PCOS pharmacological therapy, 2 for preparation for pregnancy, 1 for BMI < 24kg/m^2 and 2 were adolescents. While fifteen participants completed the study and three dropouts could not be contacted. Thus 15 subjects concluded the study **(Fig. 1)**. Participants were between 18 and 31 years with anovulation (the menstrual cycle was delayed by 3 months-3 years) and normotensive but mainly insulin resistant (Patients at the beginning of the study had a HOMA-IR higher than 2.3). All PCOS participants were diagnosed hyperandrogenemia within one month. Three participants caught a cold during the follow-up and the values of hsCRP of them were excluded, but by the time of the blood collection, the treatments were already completed, and the participants were free from symptoms. One participant was not in fasting state at the follow-up, so we did not test her blood for glucose and insulin analysis. Feeling hungry every day was reported by two participants (2/15, 13.3 %), several days per week by three participants (3/15, 20.0%), once a week or less by ten participants (7/15, 46.7%), and never by three participants (3/15, 20.0%). They did not have any discomfort, except for one participant complained that she had irregular defecation in the first week of TRF. Besides, TRF did not influence their sleep. Seven participants (7/15, 46.7%) participants found it easy or very easy to adhere to TRF rules, six participants (6/15, 40.0%) found it neither easy nor difficult, while two participants (2/15, 13.3 %) found it difficult or very difficult. All participants said that they wanted to continue and 4 participants (4/15, 26.7%) said that they would recommend TRF to others.

Anthropometric and body composition measurements

The study revealed significant reductions in body weight (P<0.001), BMI (P<0.001), BFM (P<0.001), BF% (P=0.012) and VFA (P=0.015). There were no significant differences in WHR and SMM. The results are presented in **Table 1**.

Table 1. Anthropometric measurements and body composition			
	Pre	**Post**	***P*-value**
Body weight (kg)	74.70 (69.80–97.50)	73.40 (68.40–95.50)	<0.001*
BMI (kg/m^2)	29.75±4.31	28.57±4.41	<0.001*
WHR	0.93±0.05	0.92±0.05	0.050
SMM (kg)	25.41±3.96	24.77±3.98	0.062
BFM (kg)	35.28±10.03	32.89±9.91	<0.001*
BF%	40.65 (39.83–47.63)	39.65 (38.38–45.98)	0.012*
VFA (cm^2)	164.8±39.45	154.7±41.42	0.015*

BMI: Body mass index; WHR: Waist-to-Hip Ratio; SMM: Skeletal muscle mass; BFM: Body fat mass; BF%: Body fat percentage; VFA: Visceral fat area.

The comparison across timepoints has been assessed using Paired t test and Wilcoxon test.

Results are expressed as mean ± SD or median (25th–75th percentile).

*P<0.05.

Metabolic parameters

Multivariate analyses of insulin resistance (both AUCInsulin/AUCGlucose and HOMA-IR) were performed. Significant decreases were observed in FINS (p=0.017), HOMA-IR (p=0.025), AUCIns (p=0.007), AUCInsulin/AUCGlucose (p=0.001), while there were no significant changes in FG, AUCGlucose, TG, TC and LDL-C. **(Fig. 2)**. The data are reported in **Table 2.**

Table 2. Metabolic parameters			
	Pre	**Post**	***P*-value**
FG (mmol/L)	5.08 (4.76–5.60)	4.97 (4.76–5.66)	0.614
FINS (μU/mL)	15.60 (13.45–25.00)	12.30 (10.30–17.30)	0.017*
AUC Ins(mU/L*min)	15974±6158	11694±5230	0.007*
AUC Glu (mmol/L*min)	963.8±140.9	988.3±190.7	0.516
AUC Ins/AUC Glu	16.49±5.90	11.84±4.80	0.001*
HOMA-IR	3.45 (2.91–5.64)	2.73 (2.27–3.85)	0.025*
TG (mmol/L)	1.23 (0.94–1.68)	1.05 (0.70–1.67)	0.715
TC (mmol/L)	4.57±0.75	4.43±0.66	0.328
LDL-C (mmol/L)	2.76±0.61	2.75±0.62	0.984

FG: Fasting glucose; FINS: Fasting insulin; HOMA-IR: Homeostasis model assessment-insulin resistance; AUCIns: AUCInsulin area under the curve (AUC) for insulin; AUCGlu: AUCGlucose area under the curve (AUC) for glucose; AUCIns/AUCGlu: AUCInsulin/AUCGlucose; TG: Triglycerides; TC: Total cholesterol; LDL-C: high density lipoprotein-cholesterol.

The comparison across timepoints has been assessed using Paired t test and Wilcoxon test.

Results are expressed as mean ± SD or median (25th–75th percentile).

*P<0.05.

Menstruation and endocrine parameters

At the end of the study, an improvement in menstrual cycle irregularity was detected in 73.3% (11/15) participants. For androgen profiles, there was a significant increase in SHBG (P<0.001) and decrease in TT (P=0.048) and FAI (P=0.001). **(Fig. 3)**. There were no significant changes in LH, FSH and LH/FSH. The data are reported in **Table 3**.

Table 3. Endocrine parameters			
	Pre	**Post**	***P*-value**
TT (ng/mL)	1.00 (0.77–1.26)	0.91 (0.61–1.09)	0.048*
SHBG (nmol/L)	19.00 (11.70–25.10)	22.70 (15.20–33.90)	<0.001*
FAI (%)	21.91±11.17	16.20±9.56	0.001*
LH (mIU/mL)	13.09±4.83	10.67±5.22	0.176
FSH (mIU/mL)	5.66 (5.06–7.09)	5.21 (4.32–5.51)	0.252
LH/FSH	2.21 (1.72–2.52)	1.81 (1.43–3.07)	0.515

LH: Luteinizing hormone; FSH: Follicle-stimulating hormone; TT: Total testosterone; SHBG: Sex hormone-binding globulin; FAI: Free androgen index.

The comparison across timepoints has been assessed using Paired t test and Wilcoxon test.

Results are expressed as mean ± SD or median (25th–75th percentile).

*P<0.05.

Eating behaviors

According to TFEQ-R21, cognitive restraint, uncontrolled eating and emotional eating did not change over TRF period. The data are reported in **Table 4**.

The comparison across timepoints has been assessed using paired t test

Results are expressed as mean ± SD.

*P<0.05.

Other parameters

Compared to baseline, there were significant decreases in hsCRP (p=0.040) and ALT (p=0.027) and a significant rise in IGF-1 levels (p=0.006). There were no significant

Table 4. Eating behaviors			
	Pre	Post	*P*-value
Cognitive restraint	15.00±4.29	17.80±2.62	0.067
Emotional eating	13.13±5.19	12.20±3.78	0.332
Uncontrolled eating	19.13±4.16	18.67±2.44	0.669

changes in UA and AST. The data are reported in **Table 5**.

Table 5. Other parameters			
	Pre	Post	*P*-value
hs-CRP (mg/L)	4.91±3.21	2.86±1.55	0.040*
IGF-1 (ng/mL)	157.5±31.94	210.3±51.84	0.006*
UA (umol/L)	418.2±76.75	407.7±122.6	0.656
AST (U/L)	25.50(15.75–38.00)	18.00(16.00–31.00)	0.113
ALT (U/L)	47.67±37.95	32.58±26.11	0.027*

hs-CRP: High-sensitivity C-reactive protein; IGF-1: Insulin-like growth factor 1; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; UA: Uric acid.

The comparison across timepoints has been assessed using Paired t test and Wilcoxon test.

Results are expressed as mean ± SD or median (25th–75th percentile).

*P<0.05.

Discussion

This is the first study to investigate the implications of TRF in individuals with chronic anovulation of PCOS on anthropometric, body composition, endocrine and metabolic profiles. Five weeks of TRF improved menstruation, hyperandrogenemia profiles (such as TT, SHBG, FAI), body weight, BMI, body composition profiles (such as BFM, BF% and VFA), hyperinsulinemia and insulin resistance profiles (such as FINS, HOMA-IR, AUCInsulin and AUCInsulin/AUCGlucose), decreasing ALT, hsCRP and increasing IGF-1 in a group of 15 young women with anovulatory PCOS.

Hyperandrogenemia is one of the main features of PCOS which often leads to irregular menstruation. Compared with normal weight PCOS patients, overweight PCOS patients showed significantly higher TT and FAI [19]. In addition, compared with PCOS patients with peripheral obesity, PCOS patients with visceral obesity had a lower circulating SHBG concentrations, and thus these women tended to have a higher FAI. In turn, hyperandrogenemia can also induce abdominal adipose accumulation [20]. In our present study, all the patients were anovulation women with diagnosed or previously diagnosed hyperandrogenemia. After TRF, more than half has their normal menstrual cycles restored. It is promising that TRF can significantly ameliorate SHBG and FAI and then exert beneficial effects on the recovery of the normal menstrual cycle [21]. Abnormal LH/FSH ratio is common in patients with PCOS. A prior study has shown that TRF had a negative effect on LH pulsation during ovarian development in prepubertal gilts. [22]. In addition, TRF has been shown to reduce gonadotropin concentration in humans [23], whereas we did not find any significant differences in LH, FSH and LH/FSH. This needs further large-scale investigations.

Obesity is closely related to PCOS. A higher BMI is associated with a greater prevalence of menstrual irregularity, hyperandrogenemia and hirsutism [24]. The visceral fat of obese and non-obese PCOS patients is higher than that of non-PCOS women, and it is positively correlated with cardiometabolic diseases, indicating that obesity plays an important role in PCOS [25]. Accumulating evidence shows that TRF may produce a small but statistically significant 1–4% of weight loss [26]. Previous meta-analyses also have shown that TRF was more likely to control weight and improve body composition [15,27]. In active females, resistance training combined with TRF may result in a greater loss of fat mass than resistance training with a usual diet [28], TRF group also reported a 18% decrease in visceral fat mass [28]. Nonetheless, in our study, participants who maintained dietary intake of past habits and a low level of exercise also reduced their body weight, BMI, BFM, BF% and VFA in the TRF period, while only TRF for a short time did not significantly decrease WHR and change lean mass.

Elevated blood glucose concentration is a pivotal factor in the diagnosis of metabolic disease. Consistently high concentrations of glucose can damage blood vessels and lead to increased heart disease risk and insulin resistance [26]. Hyperglycemia can worsen inflammation and in PCOS patients, glucose ingestion may induce an inflammatory response that is independent of obesity [29]. While previous findings on the effects of TRF on fasting glucose were equivocal, and most of these trials reported no change in fasting glucose as a review mentioned [26], it is worth noting that while Martens et al. [30] reported no change in fasting glucose, they did find a significant decrease in AUCGlucose during the 8h TRF. A five-day TRF (10am–5pm) reduced night-time glucose in participants who were overweight [31]. A four-day TRF (8am–2pm) also lowered the mean glucose levels at night-time but did not lower glucose during the awake period. In summarizing the results of the entire day, TRF reduced mean 24h glucose levels by 4 ± 1 mg/dL [32]. In our study, the blood tests were all conducted in the morning and we did not detect significant differences in FG and AUCGlucose. Thus, it is necessary to use 24h glucose monitors in future TRF studies in PCOS patients to monitor the changes of glucose level. Insulin resistance and compensatory hyperinsulinemia also play a key role in the hyperandrogenemia of PCOS, which in turn has significant adverse effects on chronic anovulation [33]. Specifically, hyperinsulinemia

cooperates with LH to promote androgen secretion in membrane cells and adrenal cells [34]. Hyperinsulinemia reduces SHBG, thus increases circulating (active) androgen, stimulates pulse production in the hypothalamus, increases LH synthesis, and ultimately enhances the role of adrenocorticotropin, thus results in an increase in adrenal androgen secretion [35]. A review reported that isocaloric TRF seemed to be more beneficial in reducing FINS and IR when compared to *ad libitum* TRF [26]. Isocaloric TRF may improve fasting insulin levels independent of weight loss, especially in those who have prediabetes [36]. Our *ad libitum* study observed significant reductions in FINS, HOMA-IR, AUCInsulin and AUCInsulin /AUCGlucose in a short time TRF (8am-4pm), which suggested that TRF without limiting energy may also ameliorate hyperinsulinemia and improve IR.

Dyslipidemia is present in 70% of patients with PCOS, regardless of BMI [37]. The effects of TRF in plasma lipids were not consistent. Additionally, no significant differences were found in TC, TG and LDL-C in the present study.

Polycystic ovaries show persistent chronic inflammation with a larger number of infiltrating inflammatory cells. These cells induce insulin resistance, stimulate androgen production, and disrupt the hypothalamic-pituitary-ovarian axis. The increased number of lymphocytes can be a factor triggering chronic inflammation and altered hormone secretion [4, 14]. A meta-analysis showed that CRP levels were higher in PCOS patients than in healthy women, independent of obesity [38]. We found that short time TRF may reduce hsCRP and thus improve the state of chronic inflammation in overweight PCOS patients. IGF-1 plays an important role in glucose metabolism [39]. Low circulating levels of IGF-1 in healthy adults are associated with reduced β-cells function [40]. The increased level of IGF-1 after the reduction diet had a cardioprotective effect [41]. We found a higher IGF-1 in PCOS patients after TRF, which was contrary to the results of a 5-day TRF in overweight humans [32]. While it is still difficult to clarify the effect of IGF-1 in PCOS, since IGF-1 is increasingly linked to the disturbed follicular development in PCOS [42]; a higher IGF-1 in PCOS women may be related to the increased vascularity that underlies the increased blood flow [43]. Abnormal serum ALT is associated with impaired insulin sensitivity in young women with PCOS in a manner that is independent from the contribution of age and total adiposity [44]. Our study found TRF may decrease ALT levels in PCOS. Future larger sample size studies are needed.

There were several limitations in the current study. Firstly, this was a non-randomized intervention without a control group with a small number of participants, which limited our ability to detect a significant difference. Secondly, our study was of a short duration (five weeks) which limited the physiological changes that could be induced. Because ofthe strict inclusion criteria, the variability of the cohort may have limited the generalizability of the results to a broader cohort of patients with PCOS. Thirdly, the intervention was conducted in a free-living population, and participants failed to receive standardized diets. Although the participants were asked to record calories to retain their the energy intake from foods, the estimated value can only be a reference. Fourthly, adherence to the 8h TRF may have been affected since participants may be more likely to adhere if they feel in control. Thus, well-designed studies are needed to examine the safety, applicability, and usefulness of TRF for PCOS patients.

Conclusions

Eight-hour TRF has beneficial effects on improving menstruation, hyperandrogenemia, and reducing weight especially body fat, decreasing insulin resistance and inflammation in women with anovulatory PCOS. TRF may be suitable for PCOS with appropriate counseling and patient management.

Abbreviations

PCOS: Polycystic ovary syndrome; IF: Intermittent fasting; TRF: Time-restricted feeding (TRF); TFEQ-R21: Three Factor Eating Questionnaire Revised 21 Item; BMI: Body mass index; WHR: Waist-to-Hip Ratio; SMM: Skeletal muscle mass; BFM: Body fat mass; BF%: Body fat percentage; VFA: Visceral fat area; FG: Fasting glucose; FINS: Fasting insulin; IR: Insulin-resistance; HOMA-IR: Homeostasis model assessment-insulin resistance; AUCIns: AUCInsulin area under the curve (AUC) for insulin; AUCGlu: AUCGlucose area under the curve (AUC) for glucose; AUCIns/AUCGlu: AUCInsulin/AUCGlucose; LDL-C: Low density lipoprotein-cholesterol; TC: Total cholesterol; TG: Triglycerides; TT: Total testosterone; SHBG: Sex hormone-binding globulin; FAI: Free androgen index; LH: Luteinizing hormone; FSH: Follicle-stimulating hormone; hs-CRP: High-sensitivity C-reactive protein; IGF-1: Insulin-like growth factor 1; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; UA: Uric acid; SD: standard deviation.

Declarations

Ethics approval and consent to participate

All patients provided written informed consent before the beginning of the study. The study was approved by the Medical Research and New Technology Ethics Committee of Shengjing Hospital affiliated to China Medical University (reference: 2020PS682K).

Consent for publication

Written informed consent has been obtained from the patients to publish this paper.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Author Contributions

Conceptualization and formal analysis, L.C.Z., X.C.,and H.B.; methodology, L.C.Z.; data curation, L.C.Z., X.C., Z.H., Z.J.Q., S.W.J.; funding acquisition, H.B.; L.C.Z. was a major contributor in writing the manuscript. All authors read and approved the fnal manuscript.

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References

1. Bozdag, G.; Mumusoglu, S.; Zengin, D.; Karabulut, E.; Yildiz, B. O., The prevalence and phenotypic features of polycystic ovary syndrome: a systematic review and meta-ana lysis. Hum Reprod 2016, 31 (12), 2841-2855, doi:10.1093/humrep/dew218.
2. Barrea, L.; Arnone, A.; Annunziata, G.; Muscogiuri, G.; Laudisio, D.; Salzano, C.; Pugliese, G.; Colao, A.; Savastano, S., Adherence to the Mediterranean Diet, Dietary Patterns and Body Composition in Women with Polycystic Ovary Syndrome (PCOS). Nutrients 2019, 11 (10), doi:10.3390/nu11102278.
3. Barrea, L.; Marzullo, P.; Muscogiuri, G.; Di Somma, C.; Scacchi, M.; Orio, F.; Aimaretti, G.; Colao, A.; Savastano, S., Source and amount of carbohydrate in the diet and inflammation in women with polycystic ovary syndrome. Nutr Res Rev 2018, 31 (2), 291-301, doi:10.1017/s0954422418000136.
4. Parr, E. B.; Devlin, B. L.; Radford, B. E.; Hawley, J. A., A Delayed Morning and Earlier Evening Time-Restricted Feeding Protocol for Improving Glycemic Control and Dietary Adherence in Men with Overweight/Obesity: A Randomized Controlled Trial. Nutrients 2020, 12 (2), doi:10.3390/nu12020505.
5. Lim, S. S.; Davies, M. J.; Norman, R. J.; Moran, L. J., Overweight, obesity and central obesity in women with polycystic ovary syndrome: a systematic review and meta-analysis. Hum Reprod Update 2012, 18 (6), 618-37, doi:10.1093/humupd/dms030.
6. Teede, H. J.; Misso, M. L.; Costello, M. F.; Dokras, A.; Laven, J.; Moran, L.; Piltonen, T.; Norman, R. J., Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. Hum Reprod 2018, 33 (9), 1602-1618, doi:10.1093/humrep/dey256.

7. Shang, Y.; Zhou, H.; Hu, M.; Feng, H., Effect of Diet on Insulin Resistance in Polycystic Ovary Syndrome. J Clin Endocrinol Metab 2020, 105 (10).doi:10.1210/clinem/dgaa425.
8. Porchia, L. M.; Hernandez-Garcia, S. C.; Gonzalez-Mejia, M. E.; López-Bayghen, E., Diets with lower carbohydrate concentrations improve insulin sensitivity in women with polycystic ovary syndrome: A meta-analysis. Eur J Obstet Gynecol Reprod Biol 2020, 248, 110-117, doi:10.1016/j.ejogrb.2020.03.010.
9. Zhang, X.; Zheng, Y.; Guo, Y.; Lai, Z., The Effect of Low Carbohydrate Diet on Polycystic Ovary Syndrome: A Meta-Analysis of Randomized Controlled Trials. Int J Endocrinol 2019, 2019, 4386401, doi:10.1155/2019/4386401.
10. Kazemi, M.; Hadi, A.; Pierson, R. A.; Lujan, M. E.; Zello, G. A.; Chilibeck, P. D., Effects of Dietary Glycemic Index and Glycemic Load on Cardiometabolic and Reproductive Profiles in W omen with Polycystic Ovary Syndrome: A Systematic Review and Meta-analysis of Randomized Controlled Trials. Adv Nutr 2020, doi:10.1093/advances/nmaa092.
11. Lie Fong, S.; Douma, A.; Verhaeghe, J., Implementing the international evidence-based guideline of assessment and management of polycystic ovary syndrome (PCOS): how to achieve weight loss in overweight and obese women with PCOS? J Gynecol Obstet Hum Reprod 2020, 101894, doi:10.1016/j.jogoh.2020.101894.
12. Kazemi, M.; McBreairty, L. E.; Chizen, D. R.; Pierson, R. A.; Chilibeck, P. D.; Zello, G. A., A Comparison of a Pulse-Based Diet and the Therapeutic Lifestyle Changes Diet in Combination with Exe rcise and Health Counselling on the Cardio-Metabolic Risk Profile in Women with Polycystic Ovary Syn drome: A Randomized Controlled Trial. Nutrients 2018, 10 (10), doi:10.3390/nu10101387.
13. Pohlmeier, A. M.; Phy, J. L.; Watkins, P.; Boylan, M.; Spallholz, J.; Harris, K. S.; Cooper, J. A., Effect of a low-starch/low-dairy diet on fat oxidation in overweight and obese women with polycystic ovary syndrome. Appl Physiol Nutr Metab 2014, 39 (11), 1237-44, doi:10.1139/apnm-2014-0073.
14. Paoli, A.; Mancin, L.; Giacona, M. C.; Bianco, A.; Caprio, M., Effects of a ketogenic diet in overweight women with polycystic ovary syndrome. J Transl Med 2020, 18 (1), 104, doi: 10.1186/s12967-020-02277-0.
15. Moon, S.; Kang, J.; Kim, S. H.; Chung, H. S.; Kim, Y. J.; Yu, J. M.; Cho, S. T.; Oh, C. M.; Kim, T., Beneficial Effects of Time-Restricted Eating on Metabolic Diseases: A Systemic Review and Meta-Analysis. Nutrients 2020, 12 (5), doi:10.3390/nu12051267.
16. Cho, Y.; Hong, N.; Kim, K. W.; Cho, S. J.; Lee, M.; Lee, Y. H.; Lee, Y. H.; Kang, E. S.; Cha, B. S.; Lee, B. W., The Effectiveness of Intermittent Fasting to Reduce Body Mass Index and Glucose Metabolism: A Systematic Review and Meta-Analysis. J Clin Med 2019, 8 (10), doi:10.3390/jcm8101645.
17. Harris, L.; Hamilton, S.; Azevedo, L. B.; Olajide, J.; De Brún, C.; Waller, G.; Whittaker, V.; Sharp, T.; Lean, M.; Hankey, C.; Ells, L., Intermittent fasting interventions for treatment of overweight and obesity in adults: a systematic review and meta-analysis. JBI Database System Rev Implement Rep 2018, 16 (2), 507-547, doi:10.11124/jbisrir-2016-003248.

18. Asemi, Z.; Samimi, M.; Taghizadeh, M.; Esmaillzadeh, A., Effects of Ramadan Fasting on Glucose Homeostasis, Lipid Profiles, Inflammation and Oxidative Stress in Women with Polycystic Ovary Syndrome in Kashan, Iran. Arch Iran Med 2015, 18 (12), 806-10.
19. Lazúrová, I.; Lazúrová, Z.; Figurová, J.; Ujházi, S.; Dravecká, I.; Mašlanková, J.; Mareková, M., Relationship between steroid hormones and metabolic profile in women with polycystic ovary syndrome. Physiol Res 2019, 68 (3), 457-465, doi:10.33549/physiolres.934062.
20. Milutinović, D. V.; Nikolić, M.; Veličković, N.; Djordjevic, A.; Bursać, B.; Nestorov, J.; Teofilović, A.; Antić, I. B.; Macut, J. B.; Zidane, A. S.; Matić, G.; Macut, D., Enhanced Inflammation without Impairment of Insulin Signaling in the Visceral Adipose Tissue of 5α-Dihydrotestosterone-Induced Animal Model of Polycystic Ovary Syndrome. Exp Clin Endocrinol Diabetes 2017, 125 (8), 522-529, doi:10.1055/s-0043-104531.
21. Moran, L. J.; Pasquali, R.; Teede, H. J.; Hoeger, K. M.; Norman, R. J., Treatment of obesity in polycystic ovary syndrome: a position statement of the Androgen Excess and Polycystic Ovary Syndrome Society. Fertil Steril 2009, 92 (6), 1966-82, doi:10.1016/j.fertnstert.2008.09.018.
22. Booth, P. J.; Cosgrove, J. R.; Foxcroft, G. R., Endocrine and metabolic responses to realimentation in feed-restricted prepubertal gilts: associations among gonadotropins, metabolic hormones, glucose, and uteroovarian development. J Anim Sci 1996, 74 (4), 840-8, doi:2527/1996.744840x.
23. I'Anson, H.; Foster, D. L.; Foxcroft, G. R.; Booth, P. J., Nutrition and reproduction. Oxf Rev Reprod Biol 1991, 13, 239-311.
24. Fauser BC, Tarlatzis BC, Rebar RW, Legro RS, Balen AH, Lobo R, et al. Consensus on women's health aspects of polycystic ovary syndrome (PCOS): the Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. Fertil Steril. 2012;97(1):28–38 e25, doi: 10.1016/j.fertnstert.2011.09.024.
25. Jena, D.; Choudhury, A. K.; Mangaraj, S.; Singh, M.; Mohanty, B. K.; Baliarsinha, A. K., Study of Visceral and Subcutaneous Abdominal Fat Thickness and Its Correlation with Cardiometabolic Risk Factors and Hormonal Parameters in Polycystic Ovary Syndrome. Indian J Endocrinol Metab 2018, 22 (3), 321-327, doi:10.4103/ijem.IJEM_646_17.
26. Gabel, K.; Varady, K. A., Current research: effect of time restricted eating on weight and cardiometabolic health. J Physiol 2020, doi:10.1113/JP280542.
27. Pellegrini, M.; Cioffi, I.; Evangelista, A.; Ponzo, V.; Goitre, I.; Ciccone, G.; Ghigo, E.; Bo, S., Effects of time-restricted feeding on body weight and metabolism. A systematic review and meta-analysis. Rev Endocr Metab Disord 2020, 21 (1), 17-33, doi:10.1007/s11154-019-09524-w.
28. Tinsley, G. M.; Moore, M. L.; Graybeal, A. J.; Paoli, A.; Kim, Y.; Gonzales, J. U.; Harry, J. R.; VanDusseldorp, T. A.; Kennedy, D. N.; Cruz, M. R., Time-restricted feeding plus resistance training in active females: a randomized trial. Am J Clin Nutr 2019, 110 (3), 628-640, doi:10.1093/ajcn/nqz126.
29. Gonzalez F. Infammation in polycystic ovary syndrome: underpinning of insulin resistance and ovarian dysfunction. Steroids. 2012;77(4):300–5, doi:10.1016/j.steroids.2011.12.003.

30. Martens, C. R.; Rossman, M. J.; Mazzo, M. R.; Jankowski, L. R.; Nagy, E. E.; Denman, B. A.; Richey, J. J.; Johnson, S. A.; Ziemba, B. P.; Wang, Y.; Peterson, C. M.; Chonchol, M.; Seals, D. R., Short-term time-restricted feeding is safe and feasible in non-obese healthy midlife and older adults. Geroscience 2020, 42 (2), 667-686, doi:10.1007/s11357-020-00156-6.
31. Parr, E. B.; Devlin, B. L.; Radford, B. E.; Hawley, J. A., A Delayed Morning and Earlier Evening Time-Restricted Feeding Protocol for Improving Glycemic Control and Dietary Adherence in Men with Overweight/Obesity: A Randomized Controlled Trial. Nutrients 2020, 12 (2), doi:10.3390/nu12020505.
32. Jamshed, H.; Beyl, R. A.; Della Manna, D. L.; Yang, E. S.; Ravussin, E.; Peterson, C. M., Early Time-Restricted Feeding Improves 24-Hour Glucose Levels and Affects Markers of the Circadian Cl ock, Aging, and Autophagy in Humans. Nutrients 2019, 11 (6), doi:10.3390/nu11061234.
33. Laganà, A. S.; Sapia, F.; La Rosa, V. L.; Vitale, S. G., Comment on "Inositols: from physiology to rational therapy in gynecological clinical practice". Expert Opin Drug Metab Toxicol 2016, 12 (12), 1527, doi:10.1080/17425255.2016.1248598.
34. Muscogiuri, G.; Palomba, S.; Laganà, A. S.; Orio, F., Current Insights Into Inositol Isoforms, Mediterranean and Ketogenic Diets for Polycystic Ovary Syndrome: From Bench to Bedside. Curr Pharm Des 2016, 22 (36), 5554-5557, doi:10.2174/1381612822666160720160634.
35. Muscogiuri, G.; Palomba, S.; Laganà, A. S.; Orio, F., Inositols in the Treatment of Insulin-Mediated Diseases. Int J Endocrinol 2016, 2016, 3058393, doi:10.1155/2016/3058393.
36. Sutton, E. F.; Beyl, R.; Early, K. S.; Cefalu, W. T.; Ravussin, E.; Peterson, C. M., Early Time-Restricted Feeding Improves Insulin Sensitivity, Blood Pressure, and Oxidative Stress Even without Weight Loss in Men with Prediabetes. Cell Metab 2018, 27 (6), 1212-1221.e3, doi:10.1016/j.cmet.2018.04.010.
37. Daghestani, M. H., Evaluation of biochemical, endocrine, and metabolic biomarkers for the early diagnosis of polycystic ovary syndrome among non-obese Saudi women. Int J Gynaecol Obstet 2018, 142 (2), 162-169, doi:10.1002/ijgo.12527.
38. Escobar-Morreale, H. F.; Luque-Ramírez, M.; González, F., Circulating inflammatory markers in polycystic ovary syndrome: a systematic review and metaanalysis. Fertil Steril 2011, 95 (3), 1048-58.e1-2, doi:10.1016/j.fertnstert.2010.11.036.
39. LeRoith, D.; Yakar, S., Mechanisms of disease: metabolic effects of growth hormone and insulin-like growth factor 1. Nat Clin Pract Endocrinol Metab 2007, 3 (3), 302-10, doi:10.1038/ncpendmet0427.
40. Thankamony, A.; Capalbo, D.; Marcovecchio, M. L.; Sleigh, A.; Jørgensen, S. W.; Hill, N. R.; Mooslehner, K.; Yeo, G. S.; Bluck, L.; Juul, A.; Vaag, A.; Dunger, D. B., Low circulating levels of IGF-1 in healthy adults are associated with reduced β-cell function, increased intramyocellular lipid, and enhanced fat utilization during fasting. J Clin Endocrinol Metab 2014, 99 (6), 2198-207, doi:10.1210/jc.2013-4542.
41. Szczuko, M.; Zapałowska-Chwyć, M.; Drozd, A.; Maciejewska, D.; Starczewski, A.; Wysokiński, P.; Stachowska, E., Changes in the IGF-1 and TNF-α synthesis pathways before and after three-month

reduction diet with low glicemic index in women with PCOS. Ginekol Pol 2018, 89 (6), 295-303, doi:10.5603/GP.a2018.0051.

42. Buyalos, R. P., Insulin-like growth factors: clinical experience in ovarian function. Am J Med 1995, 98 (1a), 55s-66s, doi:10.1016/s0002-9343(99)80060-6.
43. Abd El Aal, D. E.; Mohamed, S. A.; Amine, A. F.; Meki, A. R., Vascular endothelial growth factor and insulin-like growth factor-1 in polycystic ovary syndrome and their relation to ovarian blood flow. Eur J Obstet Gynecol Reprod Biol 2005, 118 (2), 219-24, doi:10.1016/j.ejogrb.2004.07.024.
44. Targher, G.; Solagna, E.; Tosi, F.; Castello, R.; Spiazzi, G.; Zoppini, G.; Muggeo, M.; Day, C. P.; Moghetti, P., Abnormal serum alanine aminotransferase levels are associated with impaired insulin sensitivity in young women with polycystic ovary syndrome. J Endocrinol Invest 2009, 32 (8), 695-700, doi:10.1007/bf03345743.

Figures

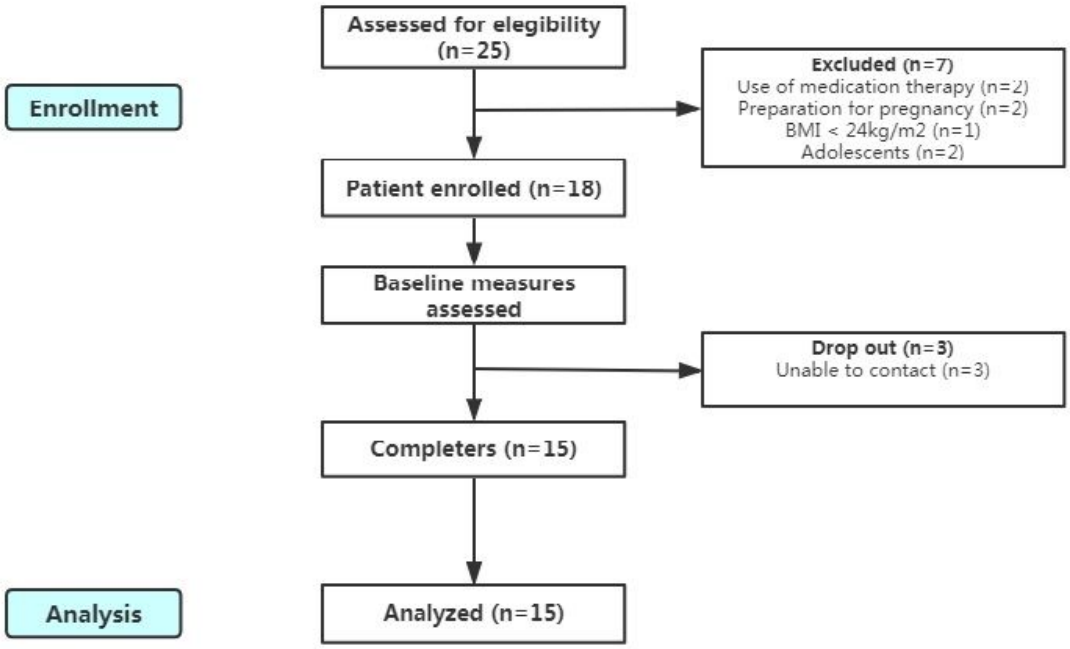

Figure 1

Flow chart from the study design

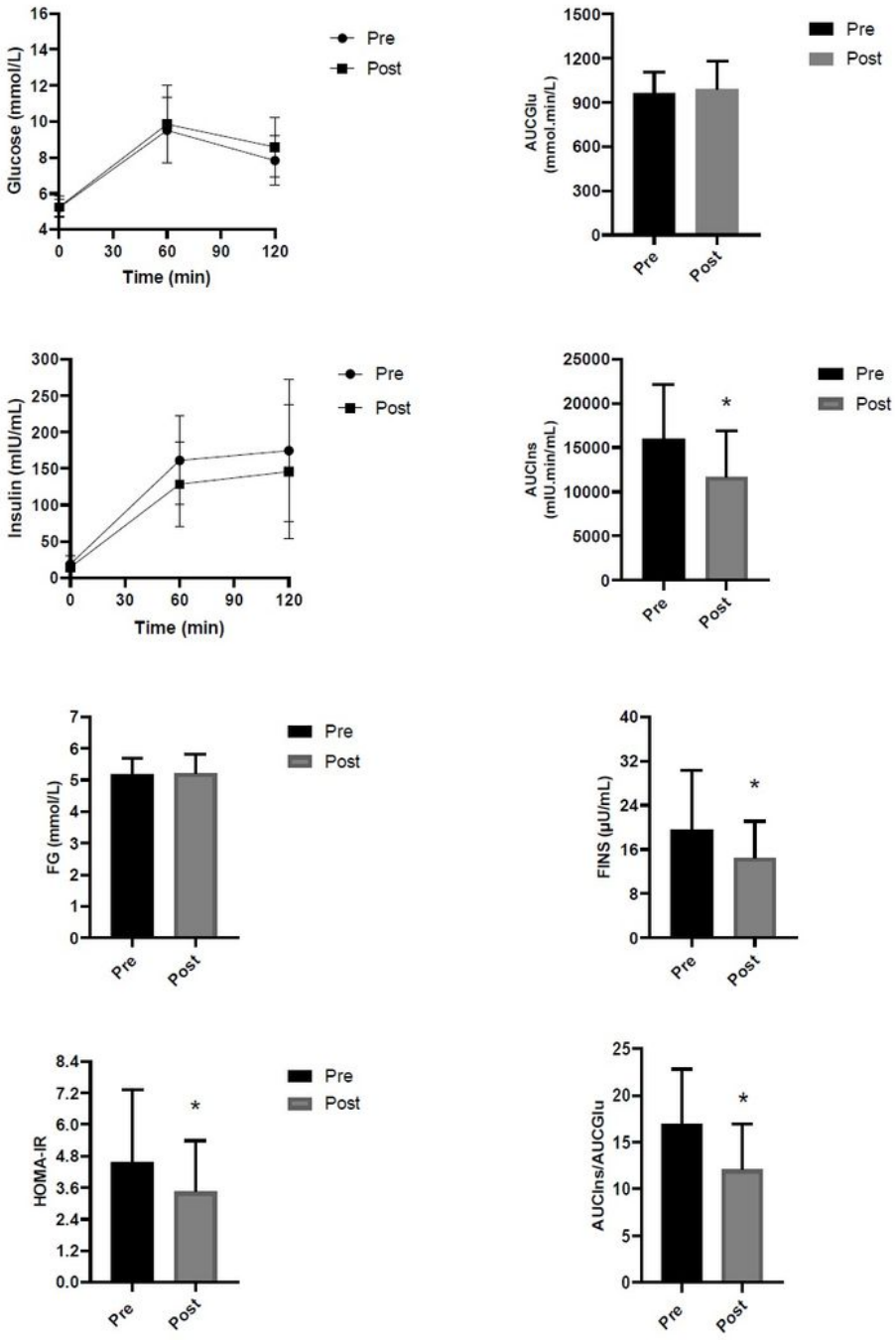

Figure 2

Changes in glucose and insulin metabolism variables after TRF period FG: Fasting glucose; FINS: Fasting insulin; HOMA-IR: Homeostasis model assessment-insulin resistance; AUCIns: AUCInsulin area under the curve (AUC) for insulin; AUCGlu: AUCGlucose area under the curve (AUC) for glucose; AUCIns/AUCGlu: AUCInsulin/AUCGlucose. Standard deviation is represented in the figure. *P<0.05

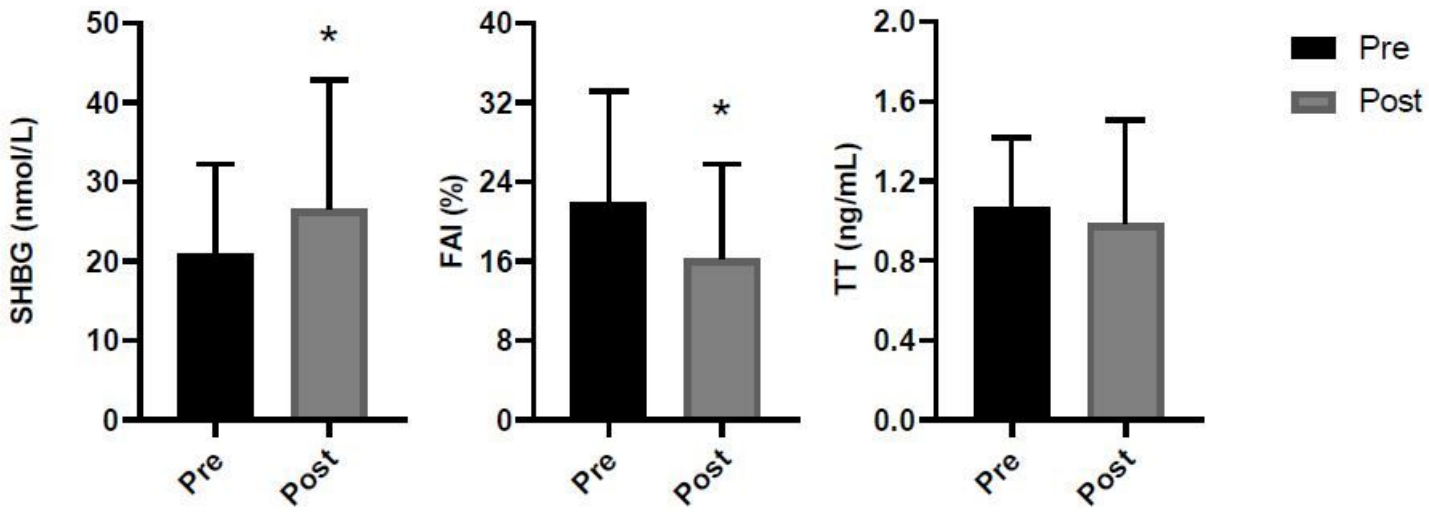

Figure 3

Changes in androgen variables after TRF period TT: Total testosterone; SHBG: Sex hormone-binding globulin; FAI: Free androgen index. Standard deviation is represented in the figure. *P<0.05.