Original Article

Effects of Ramadan Fasting on Glucose Homeostasis, Lipid Profiles, Inflammation and Oxidative Stress in Women with Polycystic Ovary Syndrome in Kashan, Iran

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Abstract

Background: To our knowledge, no reports are available indicating the effects of Ramadan fasting on metabolic parameters, inflammatory factors and oxidative stress in polycystic ovary syndrome (PCOS). The current study was designed to evaluate the effects of Ramadan fasting on metabolic status among women with PCOS.

Methods: This cross-sectional study was conducted on twenty seven PCOS patients who had fasted for a mean period of 16.5 hours a day during the 29 days of the month of Ramadan in Kashan, Iran. Fasting blood samples were collected at the beginning of the study and after 29 days of the study to quantify related variables. To identify within-group differences (before and after Ramadan), paired-samples t-tests were used.

Results: Plasma nitric oxide (NO) levels in PCOS women after Ramadan fasting were significantly higher compared to the baseline values (70.63 ± 15.78 vs. 59.94 ± 13.87 μmol/L, $P = 0.003$). Post-Ramadan levels of plasma glutathione (GSH) increased significantly in comparison with pre-Ramadan (974.95 ± 414.20 vs. 746.96 ± 205.93 μmol/L, $P = 0.011$). In addition, a trend toward a significant effect of Ramadan fasting on reducing serum high sensitivity C-reactive protein (hs-CRP) concentrations (2001.07 ± 1686.08 vs. 2962.72 ± 2845.21 ng/mL, $P = 0.072$) was seen. We did not observe any significant effect of Ramadan fasting on glucose hemostasis parameters, lipid profiles or total antioxidant capacity (TAC).

Conclusion: In conclusion, Ramadan fasting in women with PCOS for 4 weeks had beneficial effects on NO and GSH levels, but did not affect glucose hemostasis parameters, lipid profiles or TAC.

Keywords: Fasting, metabolic parameters, PCOS Ramadan

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Introduction

Polycystic ovary syndrome (PCOS), the most common endocrine disorder of reproductive age women, is often associated with insulin resistance, dyslipidemia and other metabolic conditions.[1] The prevalence is different based on population characteristics and methodology used. It affects 6%–25% of reproductive aged women.[2] PCOS is associated with several long-term complications including increased classical cardiovascular risks.[3]

Several dietary strategies including low glycemic index diet,[4] dietary approaches to stop hypertension (DASH) eating plan and weight loss diet have been used in the management of PCOS.[5] Muslims fast daily during the month of Ramadan; however, the effect of this fasting on human health has not been studied extensively. People in Ramadan abstain from eating and drinking from dawn until sunset for one month per lunar year and eat only two main meals and a few snacks at night (from sunset until dawn). It is important to know whether abstaining from food and drink has any significant effects on metabolic status, inflammation and oxidative stress among PCOS women. Some earlier studies have reported improvements in metabolic profiles after Ramadan in healthy adults,[6] healthy Algerian young men and women volunteers[7] and diabetic patients.[8] On the other hand, some investigators did not observe any significant change in these same parameters across the time of Ramadan[9–10] or did find significant increases in total cholesterol.[11]

In the current study, we hypothesized that Ramadan fasting might affect the metabolic status of women with PCOS. We are aware of no study that has examined the effect of Ramadan fasting on glucose homeostasis, lipid concentrations or biomarkers of inflammation and oxidative stress in patients with PCOS. This study aimed to investigate the effect of Ramadan fasting on metabolic profiles of women with PCOS.

Materials and Methods

Participants

This cross-sectional study was conducted in accordance with the guidelines of the Declaration of Helsinki during the period of Jan 2014 to August 2014 in Kashan, Iran. A diagnosis of PCOS was established according to the Rotterdam criteria[12]: those with two

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of the following criteria were considered as having PCOS: oligo- and/or anovulation, hyperandrogenism and polycystic ovaries. Patients with PCOS who were 18–40 years old were included in the study. We did not include those with pregnancy or lactation, current treatment of infertility, established diagnosis of diabetes mellitus, and those taking insulin sensitizing treatment within 3 months of study enrollment as well as those with cardiovascular or cerebrovascular disease. Furthermore, all patients received oral medroxyprogesterone acetate 5 mg twice a day for 10 days each month between day 15 and day 25 of the menstrual cycle. Individuals meeting the inclusion criteria were offered study participation. Those who provided written informed consent were recruited in the study. A total of 27 women with PCOS expressed their willingness to participate. In the current study, the primary outcome variable was homeostasis model of assessment-insulin resistance (HOMA-IR). We calculated our study sample size to be at 95% confidence level, based on the standard deviation (SD) of 3 for the mean of HOMA-IR based on a previous study[13] with the error equal to 1.25. The sample size calculated was 23. However, we assumed that some patients might be lost. Therefore, the sample size was increased by 15% to a total of 27 persons. These patients had fasted for a mean period of 16.5 hours a day during the 29 days of the month of Ramadan. The study was approved by the Research Ethics Committee of Kashan University of Medical Sciences.

Study design

Subjects were requested not to change their usual physical activity or routine dietary pattern throughout the study. All participants provided three dietary records (one weekend day and two week days) and three physical activity records to make sure that they maintained their usual diet and physical activity at the beginning of the study and day 30 of Ramadan. In the current study, physical activity was defined as metabolic equivalents (METs) in hours per day. To quantify the METs for each patient, we multiplied the times (in hour per day) reported for each physical activity by its related METs coefficient by standard tables.[14] To determine macro- and micro-nutrient intakes of participants, we applied Nutritionist IV software (First Databank, San Bruno, CA) modified for Iranian foods.

Assessment of anthropometric and biochemical variables

A trained nutritionist at gynecology clinic performed the anthropometric measurements 1 day before and 1 day after Ramadan. Body weight and height were measured in an overnight fasting state, without shoes and in a minimal clothing state by the use of a digital scale (Seca, Hamburg, Germany). BMI was determined as weight in kg divided by height in meters squared. At 1 day before and 1 day after Ramadan, 10 mL venous blood samples were taken after overnight fasting at Kashan reference laboratory from each woman who passed one cycle of menstrual period during this month. Fasting plasma glucose (FPG) and lipid profiles were assessed on the day of blood collection. Fresh blood samples were immediately centrifuged (Hettich D-78532, Tuttlingen, Germany) at 3500 rpm for 10 min to separate plasma. Enzymatic kits (Pars Azmun, Tehran, Iran) were used to quantify FPG, serum cholesterol, triglycerides, LDL- and HDL-cholesterol concentrations. The intra- and inter-assay CVs for FPG and lipid concentrations were less than 5%. Serum insulin concentrations were determined using ELISA kit (Monobind, California, USA) with the intra- and inter-assay CVs of 2.9% and 5.8%, respectively. HOMA-IR and β-cell function (HOMA-B) and QUICKI were determined based on suggested formulas.[15] Serum high sensitivity C-reactive protein (hs-CRP) levels were assessed by the use of ELISA kit (LDN, Nordhorn, Germany). To determine the plasma nitric oxide (NO) concentrations, we used Griess method.[16] Plasma total antioxidant capacity (TAC) concentrations were quantified by the method of FRAP method developed by Benzie and Strain.[17] Plasma total glutathione (GSH) levels were assessed according to Beutler, *et al*.[18]

Statistical analysis

To evaluate the normal distribution, we used the Kolmogorov-Smirnov test. All data were reported as mean ± SD. We used paired-samples *t*-tests to identify within group differences (before and after Ramadan). $P < 0.05$ was considered as statistically significant. All statistical analyses were done using the Statistical Package for Social Science version 17 (SPSS Inc., Chicago, Illinois, USA).

Results

The means of weight and BMI of pre- and post-Ramadan were not significantly different between the two groups (Table 1).

Within-group changes were not significant in dietary intakes of energy, carbohydrates, proteins, fats, saturated fatty acids (SFA), polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), cholesterol, total dietary fiber (TDF), magnesium and vitamins C (Table 2).

Plasma NO levels in PCOS women after Ramadan fasting were significantly higher compared with the baseline values (70.63 ± 15.78 vs. 59.94 ± 13.87 μmol/L, $P = 0.003$) (Table 3). Post-Ramadan levels of plasma GSH increased significantly in comparison with pre-Ramadan (974.95 ± 414.20 vs. 746.96 ± 205.93 μmol/L, $P = 0.011$). In addition, a trend toward a significant effect of Ramadan fasting on reducing serum hs-CRP concentrations (2001.07 ± 1686.08 vs. 2962.72 ± 2845.21 ng/mL, $P = 0.072$) was seen. We did not observe any significant effect of Ramadan fasting on glucose hemostasis parameters, lipid profiles or TAC.

Table 1. General characteristics of study participants.

	Day o	Day 30	*P*[1]
Age (y)	27.5 ± 3.6	—	—
Height (cm)	159.4 ± 5.5	—	—
Weight (kg)	72.5 ± 9.6	72.3 ± 9.7	0.439
BMI (kg/m^2)	28.6 ± 3.9	28.4 ± 3.9	0.646

Data are means± SDs. [1]Obtained from paired-samples *t*-test.

Table 2. Dietary intakes of study participants throughout the study.

	Day 0	Day 30	*P*[1]
Energy (kcal/d)	2368 ± 252	2355 ± 153	0.825
Carbohydrates (g/d)	325.3 ± 55.5	325.1 ± 35.8	0.990
Protein (g/d)	88.1 ± 18.5	84.5 ± 14.1	0.308
Fat (g/d)	82.8 ± 14.1	83.2 ± 14.5	0.923
SFA (g/d)	25.1 ± 5.2	25.1 ± 7.2	0.990
PUFA (g/d)	24.7 ± 5.7	26.4 ± 6.0	0.364
MUFA (g/d)	23.3 ± 7.2	22.6 ± 6.5	0.710
Cholesterol (mg/d)	215.9 ± 123.2	225.4 ± 134.3	0.738
TDF (g/d)	19.2 ± 4.3	18.5 ± 4.9	0.625
Magnesium (mg/d)	265.8 ± 67.3	280.8 ± 52.0	0.406
Vitamin C (mg/d)	186.1 ± 92.7	195.1 ± 89.1	0.737

Data are means ± SDs. [1]Obtained from paired-samples *t*-test. MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; TDF, total dietary fiber.

Table 3. Metabolic profiles, inflammation and biomarkers of oxidative stress at study baseline and after 29 days study in women with PCOS.

	Day 0	Day 30	Change	*P*[1]
FPG (mg/dL)	97.55 ±6.36	95.03 ± 9.74	-2.52 ± 9.56	0.183
Insulin (μIU/mL)	16.43 ± 5.53	15.50 ± 5.18	-0.93 ± 6.67	0.474
HOMA-IR	3.99 ± 1.52	3.67 ± 1.50	-0.32 ± -1.82	0.364
HOMA-B	56.81 ± 18.57	55.30 ± 17.29	-1.51 ± 23.07	0.736
QUICKI	0.31 ± 0.01	0.31 ± 0.01	0.002 ± 0.01	0.308
Triglycerides (mg/dL)	100.79 ± 32.72	98.82 ± 49.81	-1.97 ± 31.19	0.745
VLDL-cholesterol (mg/dL)	20.15 ± 6.54	19.76 ± 9.96	-0.39 ± 6.23	0.745
Total cholesterol (mg/dL)	166.37 ± 25.64	167.27 ± 29.98	0.90 ± 26.66	0.862
LDL-cholesterol (mg/dL)	93.53 ± 21.93	90.73 ± 29.53	-2.80 ± 27.63	0.810
HDL-cholesterol (mg/dL)	52.67 ± 6.88	54.90 ± 7.01	2.23 ± 7.68	0.144
Total: HDL-cholesterol ratio	3.18 ± 0.50	3.09 ± 0.58	-0.08 ± 0.53	0.407
hs-CRP (ng/mL)	2962.72 ± 2845.21	2001.07 ± 1686.08	-961.65 ± 2664.97	0.072
NO (μmol/L)	59.94 ± 13.87	70.63 ± 15.78	10.69 ± 17.22	0.003
TAC (mmol/L)	941.57 ± 531.56	848.37 ± 255.41	-93.20 ± 461.61	0.304
GSH (μmol/L)	746.98 ± 205.93	974.95 ± 414.20	227.97 ± 431.93	0.011
MDA (μmol/L)	3.99 ± 1.31	3.51 ± 0.88	-0.48 ± 1.69	0.156

All values are means ± SDs. [1]Obtained from paired-samples *t*-test. FPG = fasting plasma glucose; GSH = total glutathione; HOMA-B = homeostasis model of assessment- estimated b cell function; hs-CRP = high-sensitivity C-reactive protein; MDA = malondialdehyde; NO = nitric oxide; PCOS = polycystic ovary syndrome; QUICKI = quantitative insulin sensitivity check index; TAC = total antioxidant capacity.

Discussion

Our findings revealed that Ramadan fasting among women with PCOS led to improve NO and GSH levels; however, it did not affect glucose hemostasis parameters, lipid profiles or TAC concentrations. To the best of our knowledge, the current study is the first examining the effects of Ramadan fasting on glucose homeostasis, inflammation and biomarkers of oxidative stress in women with PCOS. Several nutritional changes must be taken into account including changes in meal frequency, food composition, energy intake and sleep duration occur during Ramadan. In addition, the quality of ingested nutrients differs during Ramadan compared with the rest of the year. All these instances may affect weight change. However, the number of meal frequency in our study was less than the rest of the year (average: 5 vs. 6), we did not observe weight change in patients. Previous studies suggested that fat oxidation during Ramadan fasting may lead to an adaptive mechanism for weight maintenance in women.[19–20]

Women with PCOS are susceptible to several complications including impaired insulin metabolism, inflammation and dyslipidemia.[21] The findings of the current study demonstrated that Ramadan fasting for 4 weeks in PCOS women did not affect glucose hemostasis parameters or lipid profiles. Several studies have reported the effects of Ramadan fasting on metabolic profiles with mixed results. Some researchers have demonstrated that Ramadan fasting resulted in decreased fasting serum glucose levels among healthy male subjects[22] and in students,[23] while others showed that serum glucose was unaltered during Ramadan in healthy women[24] and in obese adolescents.[25] In addition, no significant difference in insulin metabolism parameters was observed in normal-weight and obese men[26] and in patients with cardiovascular risk factor during the Ramadan fast.[13] Similarly, some studies showed most lipid profiles not to change during Ramadan in healthy volunteers[27–28] while other studies indicated improvement.[6] The discrepancies in our results with other studies on the metabolic profiles in Ramadan are possibly due to differences in protocols, diurnal variations due to the time differences in blood sampling before and during Ramadan, choice of the sampling day during Ramadan, hydration status of the study subjects, differences in the eating and nutritional customs and habits of different Islamic populations, difference in climates depending on the location of the study and variations in the seasonal occurrence of Ramadan.

The findings of the present study indicated that Ramadan fasting for 4 weeks in women with PCOS was associated with a significant rise in plasma NO levels, but did not influence serum hs-CRP levels. In agreement with our study, NO levels in patients with cardiovascular diseases after Ramadan fasting have significantly increased compared with the baseline values.[29] Furthermore, Shariatpanahi, *et al*.[30] showed that Ramadan fasting in subjects with metabolic syndrome resulted in a significant decrease in hs-CRP levels. In addition, a significant reduction in CRP levels was seen following Ramadan fasting in healthy volunteers of normal weight.[28] However, a significant increase in hs-CRP levels was found among ethnic obese adolescents at the end of Ramadan.[25] The accurate mechanism by which fasting may affect inflammatory factors is unclear. It seems that Ramadan fasting through effects on circadian pattern of cortisol, melatonin and core temperature results in decreased hs-CRP levels. A study by Varady, *et al*.[31] observed that Ramadan fasting modified cortisol and melatonin levels and core temperature, which in turn could explain the effect of morning-to-evening change on hs-CRP concentrations. In addition, Ramadan fasting may decrease inflammatory cytokines by suppressing the expression of pro-inflammatory markers and decreasing the circulating levels of leukocytes.[32]

The current study revealed that Ramadan fasting in subjects with PCOS was associated with a significant increase in plasma GSH concentrations, but it did not affect plasma TAC or MDA concentrations. In agreement with our finding, Ramadan fasting significantly increased GSH concentrations in diabetic patients.[33] In addition, at 6 weeks post-fasting, GSH higher by 30.2% in hypertensive patients.[34] Ramadan fasting among patients with cardiovascular diseases (CVDs) was also associated with decreasing biomarkers of inflammation and oxidative stress.[32] In another study by Zangeneh, *et al*.,[35] it was seen that Ramadan fasting decreased stress neurohormones in patients with PCOS. Oxidative stress occurs when the cellular antioxidant defense mechanisms such as TAC, GSH and antioxidant enzymes are deficient or cannot effectively remove the free radicals and reactive oxygen species (ROS) that are formed inside the body.[36] Free radicals and ROS oxidize polyunsaturated fatty acids in the membrane phospholipids which in turn results in production of oxidized lipid molecules including MDA which are released into the blood and excreted in urine and other body fluids.[37] The mechanism by which fasting decreases oxidative stress needs to be elucidated. The high intake of fresh vegetables and fruits rich in antioxidant during Ramadan fasting could be a possibility.[38]

Our study had some limitations. This study included relatively few subjects. Therefore, conclusions regarding the relative beneficial effects of Ramadan fasting in PCOS women need to be confirmed in large scale studies. Due to limited funding, we did not examine the beneficial effects of Ramadan fasting on other biomarkers of systemic inflammation as well as on other biomarkers of oxidative stress. Although comparison of the findings with a control group would provide more information, we could not recruit a control group in the study. In addition, filling a food frequency questionnaire (FFQ) at the beginning and at the end of 29 days could be very beneficial to show the differences between nutrients intake at the beginning and the end of Ramadan. In the current study, we did not use a FFQ for dietary assessment at the beginning and at the end of 29 days.

In conclusion, Ramadan fasting in women with PCOS for 4 weeks had beneficial effects on NO and GSH levels, but did not affect glucose hemostasis parameters, lipid profiles or TAC.

Conflicts of interest

None of the authors had any personal or financial conflict of interest.

Author contributions

Zatollah Asemi contributed to conception, design, statistical analysis and drafting of the manuscript. Mansooreh Samimi and Mohsen Taghizadeh contributed to conception, data collection and manuscript drafting. Ahmad Esmaillzadeh contributed to conception, design and statistical analysis. All authors read and approved the final version of the paper.

Guarantor

Ahmad Esmaillzadeh is the guarantor of this work.

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