

# Effects of Green Cardamom Supplementation on Obesity and Diabetes Gene Expression Among Obese Women With Polycystic Ovary Syndrome; A Double Blind Randomized Controlled Trial

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## Research

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# Abstract

## Background

Polycystic ovary syndrome (PCOS) is an endocrine disease in which related obesity, metabolic disorders and is considered as one of the main causes of infertility in women. This trial was investigated the effects of green cardamom on obesity and diabetes genes expression among obese women with PCOS.

## Methods

194 PCOS women were randomly divided two groups: intervention (n= 99; 3 g/day green cardamom) and control groups (n=95). All of them were given low calorie diet. Anthropometric, glycemic and androgen hormones were assessed before and after 16 weeks' intervention. Fat mass and obesity-associated (*FTO*), Peroxisome proliferative activating Receptor- $\gamma$  (*PPAR- $\gamma$* ) Carnitine Palmitoyltransferase 1A (*CPT1A*), Acetyl-CoA Carboxylase Beta (*ACAB*), Leptin Receptor (*LEPR*), Gherlin, and lamin A/C (*LAMIN*) genes in PBMC were measured in each group using reverse transcription-polymerase chain reaction (RT-PCR) method.

## Results

Anthropometric indices were significantly decreased after intervention in both two studied groups. Glycemic indices and androgen hormones were significantly improved in the intervention group. The expression level of *FTO*, *CPT1A*, *LEPR*, and *LAMIN* were significantly down-regulated ( $P<0.001$ ), as well as, *PPAR- $\gamma$*  was significantly up-regulated in the intervention group after intervention with green cardamom ( $P<0.001$ ).

## Conclusion

This current study showed that the administration of green cardamom is a beneficial approach for improving of anthropometric, glycemic and androgen hormones, as well as, obesity and diabetes genes expression in PCOS women under low calorie diet.

This trial was registered with the Iranian Clinical Trials Registry (registration number: IRCT20200608047697N1). 1 August, 2020; <https://www.irct.ir/trial/48748>

# Background

Polycystic ovary syndrome (PCOS) is a common and complex endocrine disease in women and is considered as one of the main causes of infertility in the reproductive years [1]. Women with PCOS often refer to medical care for menstrual disorders, clinical manifestations of hyperandrogenism, and infertility. PCOS is associated with hyperandrogenism, hyperinsulinemia, hypothalamic–pituitary–ovarian axis changes, ovulation disorders and menstruation irregular menstruation, and these women also suffer from mood swings, anxiety, and depression [2]. These patients are often prone to metabolic disorders characterized by weight gain and obesity, insulin resistance, type 2 diabetes, and cardiovascular disease [3]. The etiology of the PCOS is not fully understood. However, some studies have found insulin resistance to be effective in its pathogenesis [4, 5]. Additionally, oxidative stress and an increase in inflammatory cytokines have also been reported to contribute to the PCOS development [2].

Recent studies have found that genetic factors play a key role in the development of obesity and insulin resistance in patients with PCOS [6]. For instance, the fat mass and obesity associated (*FTO*) gene increases the number of fat cells, especially in the abdomen area, as well as, hyperandrogenism tend to an increase in PCOS' incidence [7, 8]. Peroxisome proliferative activating receptors (*PPARs*) are part of the nuclear hormone receptors [9]. *PPAR- $\gamma$*  gene isoforms play an important role in regulating metabolism, hormones related to reproduction and ovarian function, therefore, *PPAR- $\gamma$*  gene plays an important role in maintaining normal ovarian function [9, 10].

Green cardamom consists of the whole dried fruit of *Elettaria cardamomum* (Linn), which belongs to the ginger family [11]. Green cardamom as a seasoning contains several polyphenols including flavonoids (lutolin), flavonols (quercetin and camperfor) and anthocyanins which play antioxidant and anti-inflammatory roles [12]. Green cardamom might affect insulin sensitivity, inflammation and liver stasis by suppressing oxidative stress [13]. So far, several studies have been conducted on the benefits of green cardamom, such as antimicrobial, anti-cancer, anti-inflammatory, and antioxidant activities in animal models [13, 14]. Interventional studies with green cardamom in humans show a reduction in metabolic and inflammatory diseases, for instance obesity, diabetes and pre-diabetes, cardiovascular disease, and hypertension [15, 16].

Considering that most studies in this field have been conducted mostly in cell and animal models and there are few human studies in other fields other than polycystic ovary syndrome, this current randomized clinical trial was aimed to evaluate effects of green cardamom supplementation on obesity and diabetes gene expression among obese women with PCOS.

## Materials And Methods

### Study design

This randomized, double-blind, placebo-controlled clinical trial study was conducted to evaluate the effects of green cardamom supplementation on obesity and diabetes gene expression among obese women with PCOS referring to gynecology and female infertility clinics. CONSORT statement for randomized clinical trials [17] was used to design of the current study. According to previous study [18] with 95% power and 5% significance, sample size was considered 70 subjects for each group. We entered 100 people in each group for more reassurance and possible dropouts. The trial was ethically approved by the Ethics Committee of Kermanshah University of Medical Sciences (Ethical NO: IR.KUMS.REC.1399.375) and registered with the Iranian Clinical Trials Registry (registration number: IRCT20200608047697N1). After explaining the objectives of the study, a written consent form was completed for all subjects.

### Participants, recruitment, and randomization

Study subjects were recruited from gynecology and female infertility clinics in Kermanshah, western Iran. Inclusion criteria Women with PCOS are diagnosed according to Rotterdam criteria if there are at least two factors: 1) oligomenorrhea or amenorrhea; 2) biochemical or clinical signs of increased androgens in the blood; and 3) having polycystic ovaries based on ultrasonography report, as well as, age 18-45 years, body mass index (BMI)  $\geq 30 \text{ kg/m}^2$ , and willingness to cooperate in this research. We did not include pregnant and lactating women, women with diseases such as autoimmune diseases, gastrointestinal, liver, thyroid and unstable cardiovascular diseases, severe depression (due to inability to answer questions), mental illness, severe respiratory disease (asthma and chronic bronchitis), consumption of any vitamins, minerals, other dietary supplements, allergies to green cardamom, green cardamom tea, and green cardamom products. Furthermore, we did not include women receiving medications for mentioned diseases that might interfere with green cardamom. Initially, 219 subjects were assessed. Subsequently, twenty subjects were excluded on account of the coronavirus exposure, inaccessible remote residence, and becoming pregnancy. Finally, subjects were randomly divided into two groups of placebo (n= 99) and intervention group (n= 100) using the random number table method. (Figure 1)

### Intervention

All studied subjects underwent a weight loss diet that reduced their daily calorie intake by 400- 500 kcal per day based on their adjusted ideal weight. According to previous studies, the dose of cadmium powder was three grams per day. This dose of cardamom improved lipid profile, increased total antioxidant status, decreased systolic and diastolic blood pressure, improved inflammatory markers and liver enzymes, and no toxicity was observed with this dose [16, 18]. Therefore, patients in the intervention group were given three 1000 mg cardamom capsules of Karen Company three times a day to reduce possible gastrointestinal side effects with a meal. On the other hand, patients in the placebo group received three placebo tablets containing starch powder three times a day with the same shape, color and size of cardamom supplement. The duration of intervention was four months according to the time of supplementation in genetic studies. Each supplement packs were coded by a representative's company that the researcher and the subjects were not aware of the content of the packs.

### Measurements

All subjects were asked to provide demographic information including age and marital status. Furthermore, we collected their anthropometric indices, dietary intake, physical activity, biochemical indices, and expression of obesity and diabetes genes before and after intervention.

### Anthropometric indices

In current study, height was measured with a wall-mounted stadiometer (Seca, Hamburg, Germany) while their shoulders, hips and heels were in contact with the wall. Weight and body fat mass of the subjects were measured by bioelectrical impedance analysis using body analyzer device (Inbody Co, Seoul, Korea) in standing position with light clothing and without shoes. The non-stretched and

flexible tape was used to measure waist circumference (WC) at the level of the iliac crest with a precision of 0.1 cm [19]. BMI was calculated by dividing weight (kg) to height square (m).

#### Dietary assessment

Three day food record (two days of weekdays and the weekend) was completed to evaluate the dietary intake and macronutrients, additionally, the amount of vitamin D intake through diet before and after sixteen week intervention by trained dietitian. The energy and nutrients of their dietary intake were calculated by NUTRITIONIST IV software using the United States Department of Agriculture Food Composition Table, which was modified for Iranian foods [20].

#### Physical activity

The International Physical Activity Questionnaire (IPAQ) - short form before and after the intervention was completed from all studied subjects by an interviewer. The validity and reliability of the questionnaire had previously been confirmed in Iran [21].

#### Biochemical indices

At the beginning of the follicular phase (third day of the menstrual cycle), 10 cc of fasting venous blood was collected after 12 hours of fasting overnight from all subjects. The blood samples were centrifuged, and serum was stored at -80 °C until analysis. Fasting blood sugar (FBS) was measured by enzymatic method (Pars Azmoon Co., Iran). Fasting insulin concentrations and vitamin D3 were measured by Electrochemiluminescence (ECL) method. Glycated hemoglobin (Hb) A<sub>1C</sub> was analyzed by Ion exchange chromatography. The Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) was calculated using the following formula:

HOMA-IR: [Fasting serum insulin ( $\mu$ U/ml) \* fasting glucose (mmol/l)]/22.5 [22].

Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels were measured by radioimmunoassay (RIA) technique by a LKB gamma counter. Testosterone, prolactin, thyroid stimulating hormone (TSH), Androstenedione, and dehydroepiandrosterone (DHEA) were measured in these subjects by Monobinde kit and SGHB by ILB kit using ELISA device. Measurement of ghrelin by ELISA method using Human Acylated Ghrelin kit made (SPI Bio Company, France). Leptin was measured based on ELISA with dual antibody method (Sandwich) prepared by the company DRG.

#### Expression of obesity and diabetes genes

Blood samples were stored in coated vials of Ethylenediaminetetraacetic acid (*EDTA*) to evaluate the expression of *FTO*, *PPAR-γ*, Carnitine Palmitoyltransferase 1A (*CPT1A*), Acetyl-CoA Carboxylase Beta (*ACACB*), Leptin Receptor (*LEPR*), Gherlin (*GHRL*), and lamin A/C (*LAMIN*) genes. Using Ficoll-Histopaque solution gradient (Ficoll-paque, Miltenyi Biotec GmbH, and Germany), peripheral blood mononuclear cells (PBMC) were separated during density gradient centrifugation (Ficoll-paque, Miltenyi Biotec GmbH, and Germany). Using Trisor Regaent kit (YTzol pure RNA, Iran) total RNA from PBMC cells was extracted. One microgram of the extracted RNA was applied for complementary DNA synthesis (cDNA) by Prime Script-RT Reagent kits (Takara Bio Ink. Tokyo, Japan). Dedicated primers purchased from Metabion (Metabion, Germany) are presented in Table 1. Data were normalized to the rate of 18SrRNA expressing as housekeeping control gene and then calculated based on fold change formula. All samples were done in three versions.

#### Statistical analysis

Data from this current trial were analyzed by SPSS (SPSS Inc. Chicago, IL, USA version 19). Kolmogorov-Smirnov test was applied to the data normality. Basic characteristics of studied subjects described by mean± standard deviation (SD), percent frequency, and chart. To compare qualitative variables was used Chi square test. Mann-Whitney U, and Independent sample T Test were used to evaluate the quantitative variables difference between studied two groups. Quantitative variables difference within studied groups was analyzed by paired samples t-test or Wilcoxon. A significance level of less than 0.05 was considered.

## Results

In current study 199 subjects with PCOS were included in which 5 of them were excluded during intervention because of infected with the coronavirus (n=4) and pregnancy (n=1). (Figure 1) Ultimately, 194 subjects with PCOS were completed period of the study intervention (intervention group: 99 subjects, placebo group: 95 subjects).

Mean of age in intervention and placebo groups were  $32.99 \pm 5.57$  and  $33.81 \pm 5.42$  years, respectively, that there was no difference between two studied groups ( $P=0.073$ ). Moreover, there was no difference between two studied groups in term of physical activity, marital status, weight, BMI, WC, and BFM. Table 2 are presented basic characteristics of two studied groups.

Mean of the calorie and nutrient had no difference between two studied groups before and after intervention. According to the given low calorie diet to all subjects, the calorie and nutrient had difference within each of studied groups before and after intervention. (Table 3)

PCOS were improved in 54.1% of intervention group and 35.5% of placebo group in which were significantly different between two studied groups ( $P=0.031$ ).

Table 4 showed that the mean of weight, BMI, WC, BFM, and FBS were significantly decreased after intervention in both two studied groups. Also, we observed that  $\text{HbA}_{1c}$ , insulin, HOMA-IR, leptin, androstenedione, DHEA, and LH were significantly decreased in intervention group after intervention with green cardamom, as well as, FSH were significantly increased in this group.

Figure 2 indicated the expression level of the obesity and diabetes genes in both two studied groups. Among of the measurement the obesity and diabetes genes, the expression level of *FTO*, *CPT1A*, *LEPR*, and *LAMIN* were significantly down-regulated in intervention group after intervention with green cardamom ( $P<0.001$ ). Furthermore, *PPAR- $\gamma$*  was significantly up-regulated in this group ( $P<0.001$ ).

## Discussion

This parallel trial reflected that the green cardamom supplementation could improve glycemic indices and androgen hormones among women with PCOS under low calorie diet. In addition, intervention with the green cardamom was related to down-regulated the expression level of *FTO*, *CPT1A*, *LEPR*, and *LAMIN*, while *PPAR- $\gamma$*  was significantly up-regulated in this group after intervention in PCOS women. PCOS is a complex disease with genetic and environmental components, and genes related to obesity and insulin metabolism appear to be involved in the etiology of this syndrome [23]. Insulin resistance and hyper insulinemia affect 65-70% of women with PCOS and obesity also accelerates the clinical manifestations of this syndrome in susceptible women [1].

In current study, weight, BMI, WC, and BFM were significantly decreased in both two studied groups under low calorie diet. Furthermore, all glycemic indices including FBS,  $\text{HbA}_{1c}$ , insulin, and HOMA-IR were significantly improved after during sixteen weeks' intervention. Yaghooblou et al. [24] in their trial on pre-diabetic women observed that after 2 months intervention with three gram cardamom, weight, BMI, WC, insulin sensitivity were significantly decreased compared to control group, however, other glycemic indices including FBS, insulin, and HOMA-IR had not changed after intervention. Another trial by Aghasi et al. [25] showed that  $\text{HbA}_{1c}$ , insulin, and HOMA-IR were significantly decreased after green cardamom supplementation. Since, we gave both groups the low calorie diet for ethical consideration, therefore, it seems changes in anthropometric indices after intervention in both groups are normal. Green cardamom is rich in flavonoids and isoflavones that are contributed in reducing insulin resistance by decreasing adipose tissue storage [12, 13].

Our results indicated that after intervention with the green cardamom, endocrine outcomes including leptin, androstenedione, DHEA, and LH were significantly reduced in the intervention group, as well as, FSH were significantly increased in this group. A literature review on 33 studies showed decreasing of LH, prolactin, insulin and testosterone after administration herbal medicine on women with PCOS [26]. Khorshidi et al. [6] in a study on overweight and obese PCOS women reported that after quercetin supplementation, the level of LH, testosterone, and SHBG were significantly decreased. Obesity, especially abdominal obesity, as well as insulin resistance exacerbate hyperandrogenism. Obesity is mainly associated with increased levels of free fatty acids (FFA), which increase FFA, reducing insulin sensitivity [27]. Finally, abdominal obesity and insulin resistance synergistically affect the production of androgen hormones [1]. On the other hand, increasing adipose tissue causes the production of the hormone leptin. Leptin is a hormone encoded by the obesity gene (*LEPR*) on human chromosome 7 [28]. High levels of this hormone are seen in women, which prevents the conversion of androgens to estrogen and subsequent follicular atresia [1, 28]. Therefore, it seems that the green cardamom with anti-inflammatory properties and reduced fat storage has beneficial effects in improving the status of androgen hormones.

This present study was demonstrated that *FTO*, *CPT1A*, *LEPR*, and *LAMIN* down-regulated and *PPAR- $\gamma$*  up-regulated after intervention with the green cardamom in the PCOS women. Limited data are available evaluating the effects of the green cardamom on the obesity and diabetes genes expression. (Figure 3)

Results of a meta-analysis by Liu et al. showed that the expression level of *FTO* gen was related to higher risk of PCOS in which the *FTO* gene appears to be involved in the pathogenesis of the PCOS by increasing fat mass and eventually obesity [29]. In animal models the expression level of *CPT1A* was associated with increasing BMI, WC and hypertriglyceridemia [30, 31]. *LEPR*, as single-transmembrane-domain receptor has been shown that *LEPR* polymorphism was related to obesity, insulin resistance, and dyslipidemia and the serum leptin in PCOS women is high due to high level of adipose tissue [32, 33]. Excess of inflammatory markers production in adipose tissue is mediated by *LAMIN* gene (maps on the long arm of chromosome 1) through macrophages in which lead to diabetes development [34]. In randomized clinical trial by Nasri et al. [35] was seen administration of omega three fatty acids with anti-inflammatory properties could up-regulated *PPAR-γ* in PCOS women ( $P=0.005$ ). Heshmati et al. [36] in their study showed that 4.5 g/day curcumin supplementation was related to *PPAR-γ* coactivator 1a gene up-regulation in PCOS women ( $P=0.011$ ). Similarly, Daneshi et al. [16] reported that 3 g/day cardamom supplementation in overweight and obese with non-alcoholic fatty liver disease patients could increase the level of Irisin in which can improve *PPAR-γ* coactivator 1a secretion. *PPAR-γ* gene modulate in regulating metabolism, hormones related to reproduction and ovarian function [9, 10]. Green cardamom due to its anti-inflammatory and antioxidant properties plays an important role in reducing inflammation and improving insulin resistance [13].

## Conclusion

In conclusion, this is the first study was evaluated effect of the green cardamom supplementation on the obesity and diabetes genes expression in PCOS women. This study demonstrated that intervention with the green cardamom improved of anthropometric indices, glycemic indices, and sexual hormones, as well as, among the obesity and diabetes genes expression, the expression level of *FTO*, *CPT1A*, *LEPR*, *LAMIN*, and *PPAR-γ* genes were improved after the administration of green cardamom in PCOS women.

## Abbreviations

Acetyl-CoA Carboxylase Beta (*ACACB*); body mass index (BMI); Carnitine Palmitoyltransferase 1A (*CPT1A*); dehydroepiandrosterone (DHEA); Ethylenediaminetetraacetic acid (*EDTA*); fat mass and obesity associated (*FTO*); Fasting blood sugar (FBS); Follicle-stimulating hormone (FSH); Gherlin (*GHRL*); Glycated hemoglobin (Hb) A<sub>1C</sub>; Homeostatic Model Assessment for Insulin Resistance (HOMA-IR); International Physical Activity Questionnaire (IPAQ); lamin A/C (*LAMIN*); Leptin Receptor (*LEPR*); luteinizing hormone (LH); peripheral blood mononuclear cells (PBMC); Peroxisome proliferative activating receptors (*PPARs*); Polycystic ovary syndrome (PCOS); Standard deviation (SD); thyroid stimulating hormone (TSH); waist circumference (WC)

## Declarations

**Ethical approval:** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This study was approved by the Ethics Committee of Kermanshah University of Medical Sciences (ethics approval number: IR.KUMS.REC.1399.375) and registered with the Iranian Clinical Trials Registry (registration number: IRCT20200608047697N1). 1 August, 2020; <https://www.irct.ir/trial/48748>

**Informed consent:** Written informed consent was obtained from each studied subject after explaining the purpose of the study. The right of subjects to withdraw from the study at any time and subject's information is reserved and will not be published.

**Consent for publication:** not applicable.

**Availability of data and materials:** Data will be available upon request from the corresponding author.

**Competing interests:** On behalf of all co-authors, the corresponding author states that there is no conflict of interest.

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**Author contributions:** SM and SCh contributed in conception and design of the research; NE, MG, and EM contributed to data collection; SM and SCh contributed to the acquisition and analysis of the data; SM and SCh contributed to the interpretation of the data; SM, SCh, and YP contributed to draft the manuscript. All authors are in agreement with the manuscript and declare that the content has not been published elsewhere

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## Tables

Table 1. Primers sequences for RT-PCR amplification

Sequence (5' → 3')	Gene name and symbol
F : 5'-ACTTGGCTCCCTTATCTGACC-3'	<i>FTO</i>
R : 5'-TGTGCAGTGTGAGAAAGGCTT-3'	
F : 5'-TCCAGTTGGCTTATCGTGGTG-3'	<i>CPT1A</i>
R : 5'-TCCAGAGTCGATTGATTTTGC-3'	
F : 5'-CAAGCCGATCACCAAGAGTAAA-3'	<i>ACACB</i>
R : 5'-CCCTGAGTTATCAGAGGCTGG-3'	
F : 5'-GATGCCAGCGACTTGACTC-3'	<i>PPAR-γ</i>
R : 5'-ACCCACGTCATCTTCAGGGA-3'	
F: 5'-GAATGTCATGTGCCTGTGCC-3'	<i>LEPR</i>
R: 5'-GGGCTGGACCACGAAATCTT- 3'	
F : 5'-GTTGGGATCAAGTTGTCAGG-3'	<i>GHRL</i>
R: 5'-TGGGAGAACAGAGGTGGC- 3'	
F : 5'-ACCAAAAAGCGCAAATGGAG-3'	<i>LMNA</i>
R: 5'-GGTAAGTCAGCAAGGGATCATCT- 3'	
F : 5'-ACCCGTTGAACCCCATTGTG A-3'	<i>18s rRNA</i>
R : 5'-GCCTCACTAAACCATCCAATCGG-3'	
F, forward; R, reverse	

Table 2. Basic characteristics of studied subjects with PCOS

Variables	Intervention (n=99)	Placebo (n=95)	P*
Age, year	32.99±5.57*	33.81±5.42	0.073
Total MET, MET minute/ week	351.39±502.01	294.76±456.84	0.271
Marital status, married %	85.9	82.1	0.303
<b>Anthropometric indices</b>			
Weight, kg	86.26±10	87.38±10.75	0.506
WC, cm	113.01±12.55	113.57±7.89	0.683
BMI, kg/m <sup>2</sup>	34.78±3.39	35.18±5.16	0.989
Body fat, %	46.12±2.72	47.13±3.11	0.151
<b>Biochemical indices</b>			
FBS, mg/dl	106.2±11.88	104.88±10.87	0.33
HA1C, %	6.78±2.26	6.97±2.12	0.425
Insulin, µU/ml	27.12±3.92	26.02±5.14	0.06
HOMA-IR	7.09±1.28	6.72±1.41	0.617
Leptin, ng/ml	28.62±9.35	30.71±8.45	0.324
TSH, µIU/mL	2.86±0.24	2.89±0.25	0.704
Gerlin, pmol/l	0.42±0.03	0.39±0.04	<0.001
Androstenedione, ng/ml	2.09±1.81	1.97±0.32	0.121
DHEA, ng/dL	321.27±62.38	363.39±76.01	0.198
Prolactin, ng/ml	8.44±4.22	7.78±4.09	0.647
Testosterone, ng/ml	1.24±0.21	1.35±0.21	0.99
LH, IU/L	6.31±1.41	5.94±2.28	<0.001
FSH, IU/L	1.82±0.74	1.32±0.84	0.283
SHBG, nm/l	27.77±10.63	33.12±10.17	0.726
25(OH) D3, ng/mL	20.03±11.23	18.7±11.26	0.371

\*Mean ± SD

P1 was obtained Chi square, Mann-Whitney U, and Independent sample T Test.

**Table 3. Energy and nutrients intake of subjects with poly cystic ovarian syndrome**

Variables	Intervention (n=99)			Placebo (n=95)			P2	P3
	Before	After	P1	Before	After	P1		
Energy (kcal/day)	3424.24±769.51*	2216.61±590.47	<0.001	3101.48±748.6	2189.48±633.14	<0.001	0.5	0.801
Protein (g/day)	121.03±37.11	84.81±24.01	<0.001	106.68±32.09	84.22±25.21	<0.001	0.158	0.91
Carbohydrate (g/day)	500.17±140.71	348.57±102.75	<0.001	460.17±138.14	342.79±113.71	<0.001	0.704	0.591
Fat (g/day)	106.82±33.75	56.13±18.54	<0.001	93.91±28.19	55.89±20.92	<0.001	0.193	0.639
Vitamin D (IU/day)	2.77±5.37	2.32±2.38	0.402	1.93±3.81	2.11±2.34	0.098	0.043	0.66

\*All presented values are means ± SD.

P1; P values denote significance of within-group changes.

P2: P values denote significance of between-group difference in the baseline.

P3: P values denote significance of between-group difference after intervention.

\*Significant difference within group throughout the study (P < 0.05, paired samples t-test or Wilcoxon).

\* Significant difference between groups throughout the study (P < 0.05, independent samples t-test or U Mann Whitney).

**Table 4. Anthropometric indices, glycemic indices, androgen hormones of subjects with poly cystic ovarian syndrome**

Variables	Intervention (n=99)		P1	Placebo (n=95)		P1	P2
	Before	After		Before	After		
<b>Weight, kg</b>	86.26±10	79.65±10.98	<0.001	87.38±10.75	81.48±12.40	<0.001	0.156
<b>WC, cm</b>	113.01±12.55	106.30±9.33	<0.001	113.57±7.89	108.18±9.88	<0.001	0.366
<b>BMI, kg/m<sup>2</sup></b>	34.78±3.39	32.13±4.46	<0.001	35.18±5.16	32.86±5.95	<0.001	0.015
<b>Body fat, %</b>	46.12±2.72	44.48±2.53	<0.001	47.13±3.11	44.76±3.06	<0.001	0.036
<b>FBS, mg/dL</b>	106.2±11.88	99.28±15.78	<0.001	104.88±10.87	100.68±9.97	<0.001	0.012
<b>HbA1C, %</b>	6.78±2.26	5.59±1.99	<0.001	6.97±2.12	6.67±1.92	0.202	0.821
<b>Insulin, pmol/L</b>	27.12±3.92	23.49±4.35	<0.001	26.02±5.14	26.56±5.93	0.647	0.001
<b>HOMA-IR</b>	7.09±1.28	5.72±1.32	<0.001	6.72±1.41	6.63±1.63	0.315	0.025
<b>Leptin, ng/ml</b>	28.62±9.35	20.46±6.74	<0.001	30.71±8.45	29.22±7.67	0.262	0.143
<b>Gerlin, pmol/l</b>	0.42±0.03	0.42±0.03	0.769	0.39±0.04	0.41±0.03	0.013	0.07
<b>TSH, µIU/mL</b>	2.86±0.21	2.88±0.27	0.469	2.89±0.25	2.89±0.34	0.787	0.724
<b>Androstenedione, ng/ml</b>	2.09±1.81	1.68±0.25	<0.001	1.97±0.32	1.85±0.24	0.013	<0.001
<b>DHEA, ng/dL</b>	321.27±62.38	282.97±64.54	<0.001	363.39±76.01	383.06±48.71	0.109	0.033
<b>Prolactin, ng/ml</b>	8.44±4.22	8.54±3.87	0.884	7.78±4.09	7.98±3.65	0.747	0.594
<b>Testosterone, ng/ml</b>	1.24±0.21	1.19±0.3	0.188	1.35±0.21	1.35±0.25	0.968	0.016
<b>LH, IU/L</b>	6.31±1.41	3.36±1.41	<0.001	5.94±2.28	5.95±1.84	0.774	<0.001
<b>FSH, IU/L</b>	1.82±0.74	2.77±1.42	<0.001	1.32±0.84	1.43±0.95	0.768	<0.001
<b>SHBG, nm/l</b>	27.77±10.63	29.42±10.51	0.261	33.12±10.17	34.79±10.59	0.251	0.829
<b>25(OH) D3, ng/mL</b>	20.03±11.23	20.52±10.08	0.587	18.7±11.26	22.15±12.79	0.06	0.68

\*All presented values are means ± SD.

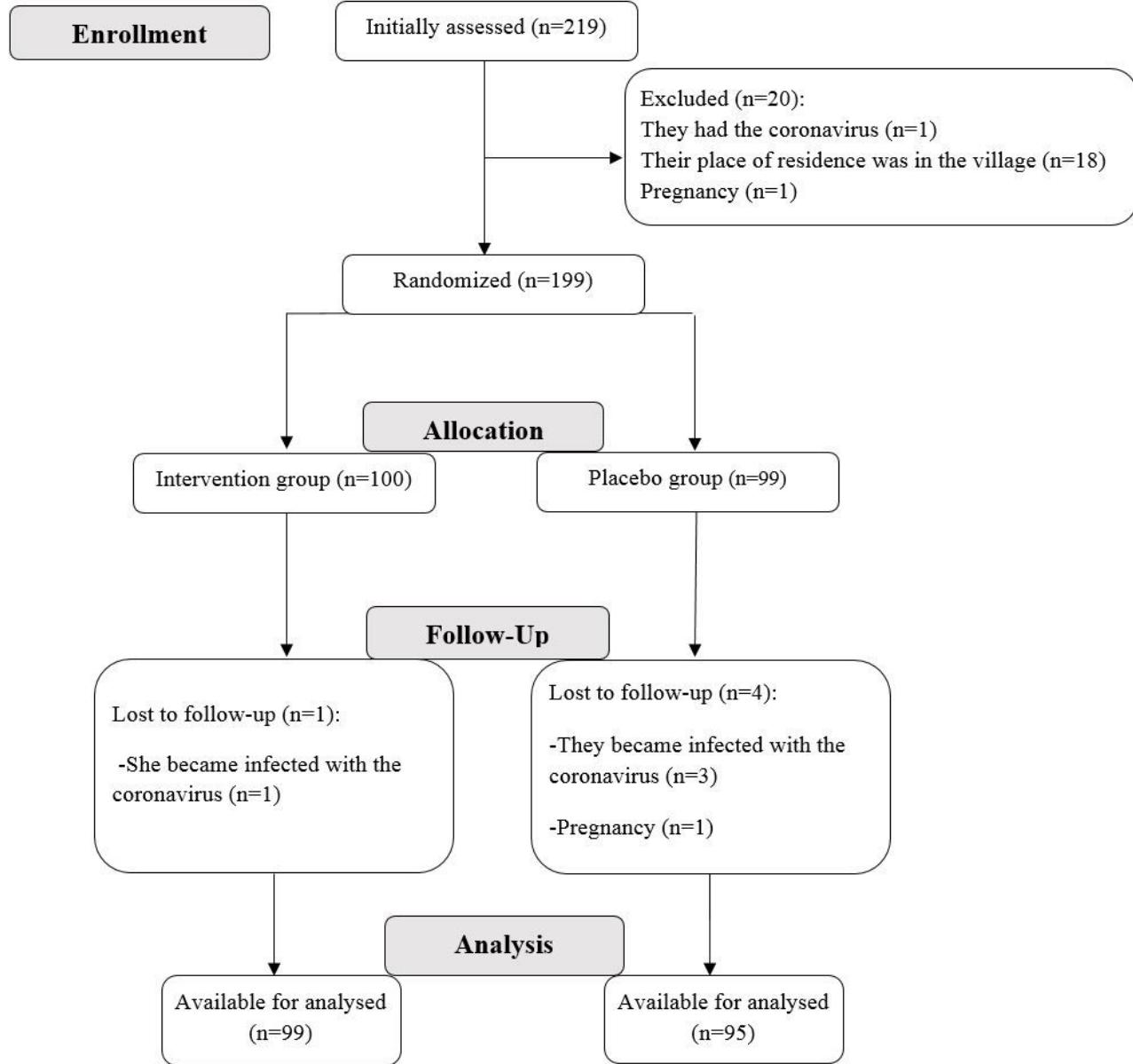
P1; P values denote significance of within-group changes.

P2: P values denote significance of between-group difference after intervention.

\*Significant difference within group throughout the study (P < 0.05, paired samples t-test or Wilcoxon).

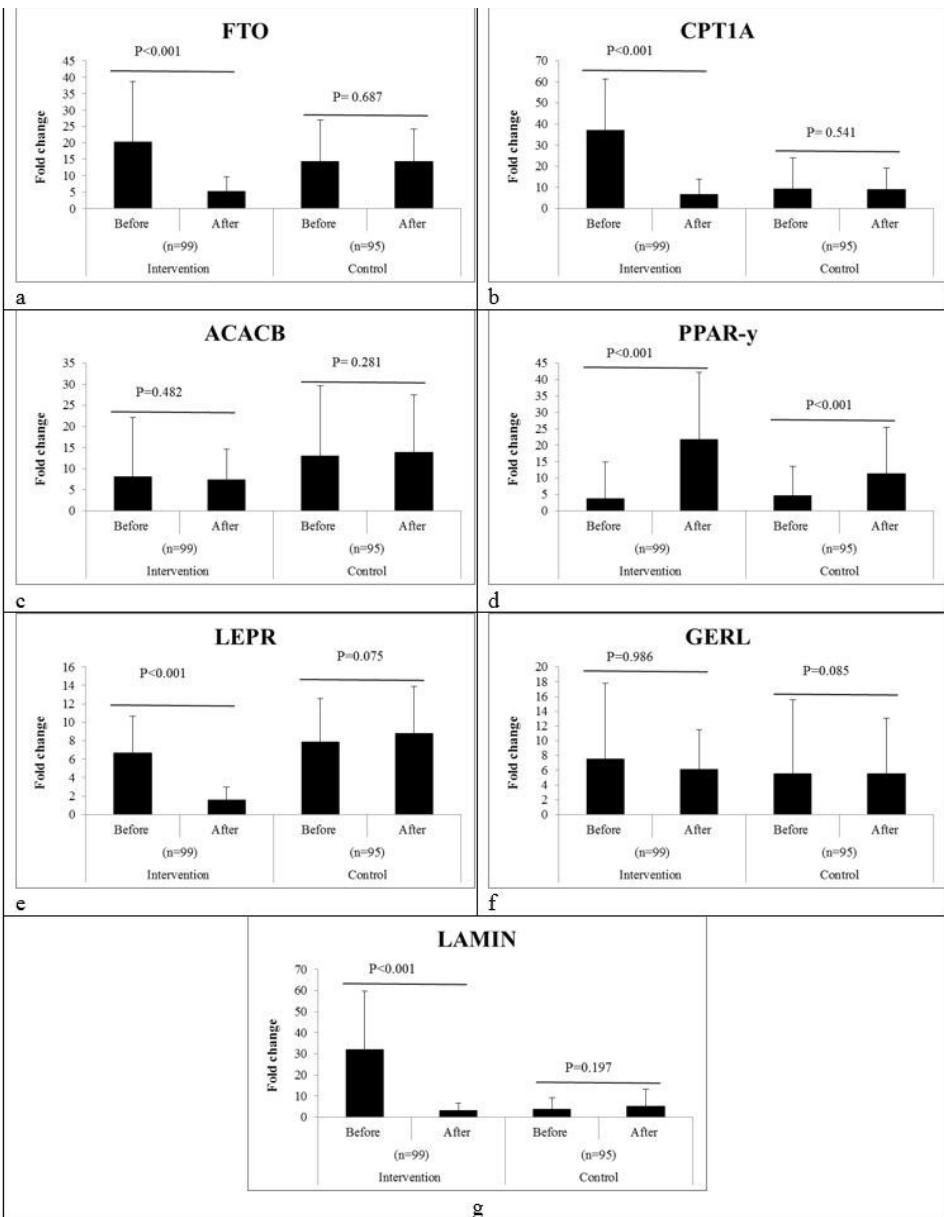
\* Significant difference between groups throughout the study (P < 0.05, independent samples t-test or U Mann Whitney).

## Figures



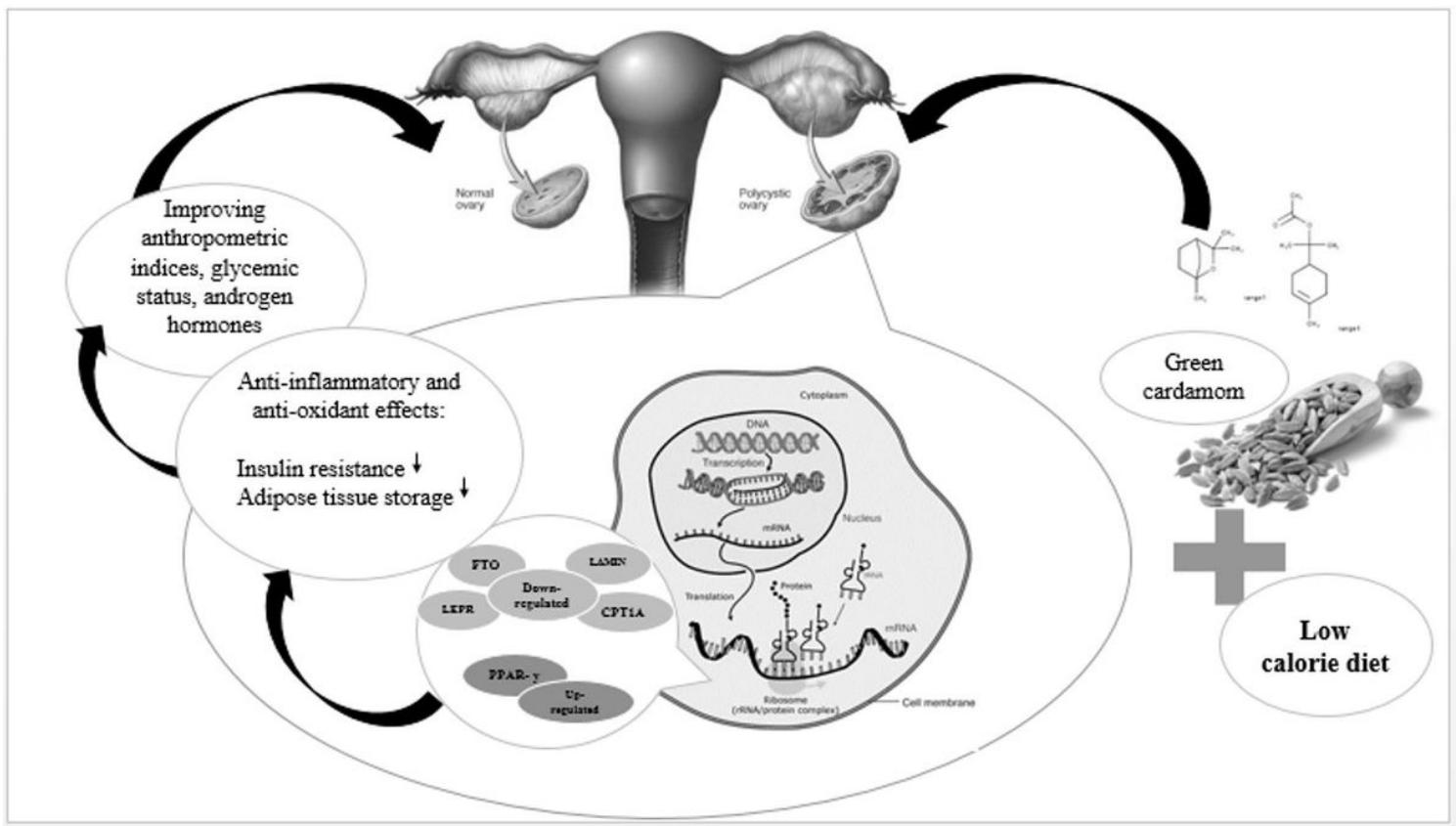
**Figure 1**

CONSORT flow chart of study



**Figure 2**

Changes of the obesity and diabetes genes including: a) FTO, b) CPT1A, c) ACACB, d) PPAR- $\gamma$ , e) LEPR, f) GERL, and g) LAMIN before and after intervention with green cardamom



**Figure 3**

The effect of the green cardamom plus low calorie diet on the obesity and diabetes genes among women with PCOS