Influence of Ketogenic Mediterranean with Phytoextracts Diet on Hyperandrogenism Markers in Married Women with Polycystic Ovarian Syndrome

Rabeea Khalid[1], Shaista Jabeen[1*], Sana Farooq[1], Fatima Abid[1]

[1]University Institute of Diet and Nutritional Sciences, Faculty of Allied Health Sciences, The University of Lahore, Pakistan.

* Corresponding author: shaista.jabeen@ahs.uol.edu.pk

Abstract

Objective: To evaluate the influence of ketogenic mediterranean with phytoextracts diet on hyperandrogenism markers in married women with polycystic ovarian syndrome. **Methodology:** A Randomized control trial (pre- post treatment) was conducted among 47 participants with BMI 25.0 – 29.9 and who meet the inclusion criteria. The participants were selected from Mansoora Hospital Gynecology OPD, Lahore. All the participants were advised to take ketogenic mediterranean with phytoextracts diet which is very low caloric ketogenic diet (1000kcal) + phytoextracts for first 3 weeks, low caloric ketogenic diet (1200kcal) + phytoextracts for second 3 weeks and Mediterranean diet (1600kcal) + phytoextracts for last 6 weeks. It was requested that the participants refrain from using any additional dietary supplements. The lab test for hormones LH, FSH and total cholesterol, LDL, HDL and triglycerides levels were collected on 0 week and after 12th week of study. Followup was taken on 0 week and 12th week of both groups. **Results:** Average mean age of participants is 29.30. The mean difference of BMI pre and post treatment is 28.78±1.17 to 25.82±1.25. The mean difference of LDL in pretreatment 123.70±12.94 to post treatment 91.53±3.38 mg/dl and total Cholesterol level pretreatment 206.26±9.26 to post treatment 184.81±7.90, HDL pretreatment 72.55±9.68 to post treatment 82.00±8.19 and triglycerides level pretreatment 204.32±22.56 to post treatment 169.57±12.13 was observed. The difference of hormones level (FSH, LH and LH:FSH) were also observed. The mean difference of hormones in LH pre and post treatment (10.54±0.52 to 6.80±0.51), FSH pre and post treatment (5.18±0.20 to 5.57±0.18), LH:FSH pre and post treatment was (2.03±0.12 to 1.22±0.10). Blood glucose levels (91.51±3.20 to 82.43±2.33), body fat percentage (27.06±3.26 to 19.89±2.39), and ferriman-gallwey score (15.13±2.53 to 14.45±2.38). also presented some reduction. **Conclusion:** ketogenic mediterranean with phytoextracts diet showed significant reduction of BMI in overweight participants ($p<0.05$). A significant reduction in LDL, HDL, total cholesterol, triglycerides and LH were also observed. So, Null hypothesis is rejected and the alternative hypothesis is accepted i.e, ketogenic mediterranean with phytoextracts diet has effect on hyperandrogenism markers among married women with polycystic ovarian syndrome.

Key words: Luteinizing hormone (LH), Follicle-stimulating hormone (FSH), Hyperandrogenism, Insulin resistance, Hypercholesterolemia, Ferriman-Gallwey score.

1. Introduction:

PCOS is a prevalent and complex endocrine disorder that affects five to fifteen percent of women worldwide (1). Females aged 18 to 44 are affected by this endocrine disease (2). Thought to affect between 3% and 10% of the population, the prevalence of polycystic ovarian syndrome in particular subpopulations based on region and race/ethnicity is mostly unclear (3). According to

the Rotterdam criteria, Kumarapeli et al.'s community-based, cross-sectional study in South Asia predicts a prevalence rate of PCOS of up to 6.3% (95% CI: 5.9-6.8%) (4). Patients with PCOS might exhibit a wide range of indications and symptoms, making it challenging to grade the illness precisely. The criteria of the 2003 ESRHE/ASRM Rotterdam consensus meeting are presently used to diagnose PCOS (5), which increased the 1990 NIH classification's scope (6) It is based on at least two of the following characteristics: polycystic ovaries by ultrasonography, hyperandrogenism, and oligo-anovulation. The Androgen Excess Society (AES) established a team of experts to analyze all the information released on PCOS in order to streamline diagnosis in 2006 (7). The AES criteria demand oligo/anovulation, ultrasonographic evidence of polycystic ovaries, and clinical and/or biochemical hyperandrogenism concurrently. PCOS is regarded as a complex condition with a variety of genetic, metabolic, endocrine, and environmental problems despite the fact that its etiology is not yet fully known (8).

Women with PCOS frequently seek treatment for infertility, clinical signs of hyperandrogenism, and monthly irregularities (oligomenorrhea, amenorrhea, and prolonged irregular menstrual flow). Using the Ferriman-Gallwey scoring method, hirsutism, a prevalent clinical manifestation of hyperandrogenism that affects up to 70% of women with PCOS, is assessed. (9). A typical clinical manifestation of hyperandrogenism that affects up to 70% of women with PCOS is hirsutism (10) A modified version of the Ferriman-Gallwey scoring system is used to assess hirsutism (11). This technique is used to assess eleven different parts of the body: the upper lip, chin/face, chest, upper and lower back, belly, arm, forearm, thighs, and legs. A score of 0 is given for terminal hair development, and a score of 4 is given for significant growth. A total of eight or more denotes hirsutism. (12). Over 90% of women who are normally menstruation have polycystic ovaries, as revealed by ultrasound. (13). Additionally, 50% of women have PCOS, which has a milder distribution of unwelcome hair growth (14). Although it is less common in PCOS and less specific than hirsutism, acne can also be a sign of hyperandrogenism. Acne is found in 15% to 30% of adult women with PCOS (15, 16). The variation in hirsutism and acne prevalence may be due to differences in 5'-reductase expression between the sebaceous gland and the hair follicle, which causes the hair follicle to have higher levels of dihydrotestosterone. After presenting with severe acne, more than 40% of the women had PCOS. (17) Certain medical professionals recommend inquiring about menstruation history and examining patients for additional hyperandrogenic symptoms when they present with acne. (18).

Insulin resistance, reversal of the FSH/LH ratio, and obesity, which is a significant clinical feature of PCOS, are common indications of PCOS that are not covered by diagnostic criteria. Women with PCOS have greater visceral and subcutaneous body fat as a result of elevated androgen levels. Obesity has a substantial impact on the metabolic features of PCOS as well (19). Patients with atherogenic lipid profiles—characterized by elevated levels of low-density lipoprotein, triglycerides, and cholesterol and lower levels of high-density lipoprotein—have PCOS (20). But it's important to remember that people who are not obese may also have these metabolic abnormalities (21). It's intriguing to notice how the metabolic profile of PCOS is supported by high carbohydrate intake, low-grade inflammation, insulin resistance, and hyperandrogenism (22).

In example, it is well recognized that acute hyperglycemia increases oxidative stress and inflammation by producing reactive oxygen species (ROS) (23).The ovary's ability to operate normally and the control of the menstrual cycle that it provides are crucial for preserving fertility. A cyst will develop inside an ovary's sac if a female's hormone levels are always out of balance since this will affect how the ovaries function. While androgen, a male hormone, was raised above normal levels in females with PCOS (24).

It is essential to comprehend the intricacies of PCOS in order to facilitate prompt diagnosis and focused treatment approaches, especially with regard to infertility and metabolic issues. However, accurate diagnosis and grading are difficult for doctors due to the variety of clinical presentations. The fundamental causes of PCOS and its effects on women's reproductive health and general well-being require more investigation. Patients with PCOS typically continue to respond to insulin in their ovaries normally. The way that insulin acts on the ovary via the IGF-1 receptor helps to partially explain this mechanism. When insulin reaches high amounts, as in compensatory hyperinsulinemia, this binding takes place. When insulin reaches high amounts, as in compensatory hyperinsulinemia, this binding takes place (25) a distinct second messenger from the traditional one that is active in other tissues when the receptor is phosphorylated at the tyrosine level. Thecal cell proliferation is stimulated by hyperinsulinemia, which also enhances LH-mediated testosterone release and elevates LH and IGF-1 receptor expression (26) Moreover, elevated insulin levels prevent the liver from producing sex hormone binding globulin (SHBG), which results in higher levels of free testosterone (27), and IGF-BP1 production, raising the amount of free IGF-1 (28).

It's interesting to note that insulin resistance, hyperandrogenism, and low-grade inflammation all work together to perpetuate the metabolic profile of PCOS (22). Indeed, acute hyperglycemia is known to produce reactive oxygen species (ROS), which exacerbate oxidative stress and inflammation (29). Compared to normal women, PCOS women have a unique dietary pattern that includes less consumption of extra-virgin olive oil, legumes, seafood, and nuts; it also includes less complex carbs, fiber, and monounsaturated fatty acids; on the other hand, it contains more simple carbohydrates, total fat, and saturated fatty acids. Reduced fat-free mass and an unfavorable body composition are linked to these eating habits (30). There is no clear-cut treatment for PCOS; instead, patient demands and clinical presentation must be taken into account due to the unique variety of this condition.Hormonal contraceptives are recommended as the first line of treatment for monthly irregularities, acne, and hirsutism, regardless of age. When estroprogestinics are contraindicated or when significant hirsutism is present, antiandrogens are recommended.

Because of its adverse effects on the stomach, metformin has long been a part of treatment regimens; nevertheless, there are other options being looked into. One such alternative is inositol. In any case, patients receiving lifestyle adjustments (diet and exercise programs) do not have an increase in weight loss when using metformin. For this reason, the first course of treatment for obese PCOS women should be diet and exercise rather than metformin. If diet and exercise don't work for the patient, they should think about using metformin (31). The most crucial element in

improving the PCOS phenotype is weight loss. Reducing insulin and free testosterone levels along with ovulatory function and pregnancy rates are enhanced by a 5–10% weight loss. Even so, calorie restriction—which prioritizes caloric restriction above macronutrient composition—remains the major treatment for PCOS when it comes to lifestyle modification based on these principles (32, 33). There is a dearth of information regarding diet-related macronutrient change as a treatment strategy (34-36). It is debatable, in fact, whether diet composition in and of itself affects reproductive and metabolic results. Because blood glucose levels are influenced by the consumption of carbs and they control the pancreatic release of insulin, very low carb diets may be more effective than typical hypocaloric diets in promoting satiety, weight reduction, endocrine/metabolic parameters, and fertility in women with PCOS (37). It makes sense that a ketogenic diet (KD) could benefit PCOS patients given the above described circumstances. A ketogenic diet (KD) is a nutritional strategy that limits daily carbohydrate intake to 30 g or 5% of total energy intake, with a corresponding increase in the proportions of protein and fat (38-40). As indicated by the decrease in the respiratory ratio, a decrease in the amount of insulin and glucose in the blood results in a decrease in the oxidation of glucose and an increase in the oxidation of fat (41). Even when calorie restriction is not present, KD for PCOS has significant impacts on AMPK and SIRT-1 activation (42). SIRT1 and AMPK have positive impacts on glucose homeostasis and enhance insulin sensitivity once they are active (43).

The therapeutic role of KD has been investigated for a long time and several works have supported the thesis that physiological ketosis can be useful in many pathological conditions, such as epilepsy, neurological diseases, cancer (with a ketogenic isocaloric diet) (44) and obesity, type 2 diabetes, acne, and the amelioration of respiratory and cardiovascular disease risk factors (with a generally low calorie ketogenic diet) (45-47). This is an important aim, since the use of food as a drug has very relevant social and economic implications, both in economic and social terms. In PCOS, evidence for the effects of KD are scarce: only a small uncontrolled pilot study (48) showed a significant reduction in body weight, free testosterone, LH to FSH ratio, and fasting insulin after a KD regimen, suggesting favourable effects in affected patients. Other data describe several mechanisms consistent with the favourable effects of such diet therapy (49-51). A recent position statement of the Italian Society of Endocrinology suggested a weight-loss program with a very low calorie ketogenic diet for overweight/obese patients with PCOS) not responsive to multicomponent standardized diet to improve insulin resistance, ovulatory dysfunctions and hyperandrogenemia, even if further controlled studies are deemed necessary to confirm the beneficial effects of KD in this clinical context (47)

Thus, aim of the present study was to to evaluate the influence of ketogenic mediterranean with phytoextracts diet on hyperandrogenism markers in married women with polycystic ovarian syndrome. Our hypothesis was that body weight, plasma cholesterol, triglycerides, hyperinsulinemia, and hormonal outcomes would all improve with a modified KD (KEMEPHY diet).

2. Materials and methods

2.1.Study design

This was a single-arm, 12-week study. Body weight, BMI, FBM, glucose, triglycerides, total testosterone, HDL, LDL, and FSH ratios, as well as the Ferriman Gallwey Score, were the end measures. Before the trial started, written informed consent was given by each patient after ethical approval.

2.2.Participants

Using random selection, 47 married, overweight women were enrolled. Inclusion criteria were: Married status and a PCOS diagnosis, per Rotterdam (at least 2 of 3 between oligo/amenorrhea or amenorrhea, clinical signs of hyperandrogenism and polycystic ovary confirmed with ultrasound (5), BMI ≥ 25 kg/m^{2,} between the ages of 20 and 45, wanting to reduce weight, and accepting not to use contraceptives throughout the experimental period. The following conditions were excluded: pregnancy and lactation; hormonal therapy; use of insulin sensitizers within the previous two months; liver, kidney, and heart disease; local treatment for hirsutism; additionally, other etiologies (congenital adrenal hyperplasia, androgen secreting tumors, Cushing syndrome) have been excluded based on the Rotterdam criteria. Three women were excluded for current PCOS pharmacological therapy. One left the study after 2 weeks; therefore 47 subjects (age 29.3 ± 4.0 years; height 163 ± 5.1 cm; weight 77.19 ± 5.09 kg) concluded the study (Fig. 1).

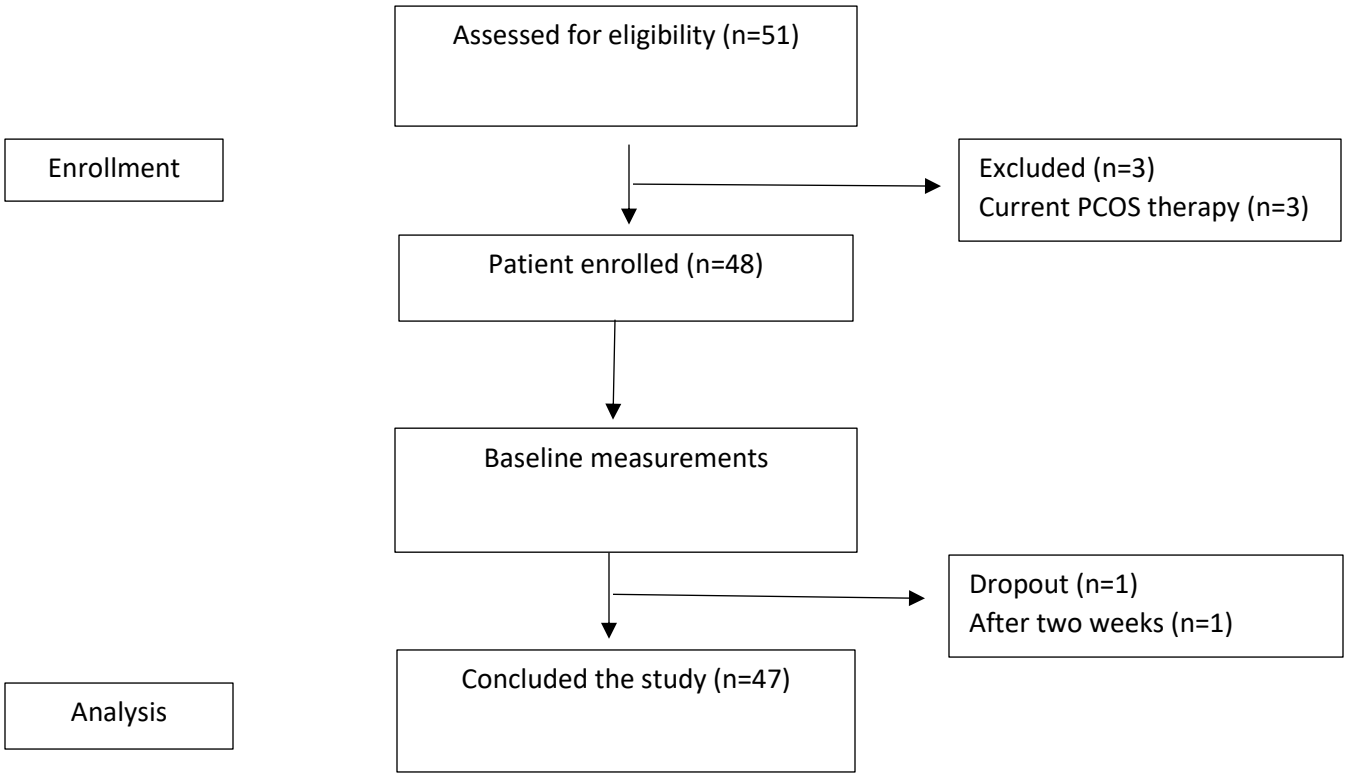


Figure 1. Experimental design.

Participants came to the Mansoora laboratory of the Mansoora hospital for the basal measures prior to beginning the dietary protocol. In addition to a thorough anamnesis and physical examination, the patients had their plasma tested for triglycerides (TGs), glucose, total cholesterol, HDL, LDL, LH, and FSH.

2.3. Anthropometric measurements

Body weight was measured to the nearest 0.1 kg using an electronic scale (Senior 6030 digital scale), and height to the nearest 1 cm using a wall-mounted portable stadiometer. kg/m2 was used to determine the body mass index (BMI). The smallest circumference between the iliac crest and the lowest rib was used to calculate waist circumference (52, 53).

2.4. Body composition analysis

Body composition (fat body mass FBM) was analyzed by using Omron HBF-306C Handheld Body Fat Monitor. Fat body mass was calculated every 15 days during the study period. The participants were scanned by stretching their arms out parallel to the ground while holding the analyzer by the metal electrode plates. The participants were asked to stand straight while a typical electrical current was sent through their hands and arms at a constant frequency of 50 kHz. Using equations supplied by the manufacturer, body fat percentages were computed in the BIA (bioelectrical Impedance Analysis) device and the results were shown on the analyzer screen.

2.5.Blood Analysis

Blood samples were taken from antecubital vein and collected into BD Vacutainers Tubes. After blood sampling, samples were centrifuged. Immediately, after centrifuge pipette separated red-top serum. Then 1ml reagent (cat.no. 704036), Roche Diagnostics) and 10 micro liter serum were added in a plastic tube and drop at 37 °C temperature in dry bath. After 10 minutes of dry bath the tube was dropped into auto chemistry analyzer. The water drop was used and reading was rate at test option for Total cholesterol level. The chemistry analyzer shows the results itself. Similar procedure was done for LDL level. Hormones (LH, FSH) were tested with the help of Chemiluminescence (CL) method. 3cc blood samples was taken from each participant. Auto chemistry analyzer was used for this method. Reagents were already added then the serums were added into serum cup then command was given to the analyzer. Automation performs the entire test by itself. Urinary ketone bodies (KBs), were assessed by using Mission Urinalysis Reagent Strips (Acon laboratories, Inc. USA). Hirsutism was considered positive when the Ferriman Gallwey Score was > 8.

2.6. Dietary Interventioin

A modified KD protocol was used. All the participants took KEMEPHY diet for 12 weeks; 3 weeks VLCKD (very low caloric ketogenic diet), 3 weeks LKD (low caloric ketogenic diet) and 6 weeks Mediterranean diet. Participants also consumed herbal extracts before breakfast and lunch.

The KEMEPHY diet (41, 45, 54-56) is a Mediterranean eucaloric ketogenic protocol (about 1600/1700 kcal/day) with the use of some phytoextracts. Every subject received an explanation of

the diets during a one-on-one appointment. During the initial three weeks of the KEMEPHY program, participants virtually completely abstain from carbs. Each participant received a comprehensive menu that included both approved and prohibited foods, as well as the elements of the ketogenic Mediterranean diet with phytoextracts. The main foods consumed were eggs, beef and chicken, fowl, fish, and unrestricted raw and cooked green vegetables. Herbal extracts, coffee, and infusion tea were permitted beverages. The following foods and beverages were avoided by the subjects: bread, pasta, rice, milk, and yogurt. Additionally, each day, the individuals drank four liquid herbal extracts. In order to lessen some of the more frequently reported mild side effects of ketogenic diets, such as constipation, headache, and halitosis, liquid herbal extracts with draining and toning properties were utilized. (54). Herbal extracts improve glucose management, promote bile production to aid in digesting (choleretic action), and lessen the widely reported feelings of weakness and fatigue during ketosis. (55).

The subjects were told to eat a normal Mediterranean diet consisting of whole grains (bread, pasta, rice, eggs, and poultry), potatoes, meat, fish, eggs, poultry, vegetables, legumes, fruits, whole milk, and wine over the months of the diet. The distribution of macronutrients during the Mediterranean diet phase was as follows on average: CHO made up 58%, proteins 15%, and fats 27% (1600 ± 15 kcal). The recommended daily intake of carbohydrates during the low-carb period was around 25 g, and the daily macronutrient energy distribution was 25 % for carbohydrates, 31 % for proteins, and 44 % for fats (1200 ± 40 Kcal). Proteins made up 36%, Fats 52%, and CHO made up 12% of the total throughout the ketogenic phase (1000 ± 5 Kcal). Kemephy diet composition and Reported beneficial effects of used phytoextracts are presented in table 1 and 2 respectively.

Table 1 KEMEPHY diet composition

Intervention phases	CHO	Proteins	Fats	Calories (Kcal)
Ketogenic Phase	12% (30g)	36% (90g)	Fats 52% (58g)	1000 ± 5
Low Carbohydrate Phase	25% (75g)	31% (93g)	44% (59g)	1200 ± 40
Mediterranean Phase	58% (247g)	15% (64g)	27% (51g)	1600 ± 15

Table 2 Reported beneficial effects of used phytoextracts and relevant references

Phytoextracts	Reported beneficial effects	References
Ginger	Antioxidant	(57)
Licorice	Antioxidant, hypoglycemic	(58)
Mint	Hormonal regulating effects	(59)
Fennel	Effective aid to digestion, Reducing gas, bloating, and stomach cramps.	(60)

2.7. Statistical analysis

A Student's t test was used to compare parameters before and after 12 weeks of the KD, using the software SPSS version 25. All data are expressed as mean ± standard deviation. Normality of data was assessed through the Kolmogorov-Smirnov test. Significance level was considered at a value of $p < 0.05$.

3. Results

Anthropometric and body composition measurements revealed an average weight loss of 7.93 kg (pre 77.19 ± 5.09 vs post 69.26 ± 4.87 kg; $p < 0.0001$) and significant reductions (− 2.96) of BMI (pre 28.78±1.17 vs post 25.82±1.25; $p < 0.0001$) and of FBM (-7.17 kg) (pre 27.06 ± 3.26 kg vs post 19.89 ± 2.39; $p < 0.0001$) with an overall improvement in body composition. Waist circumference decreased in a significant manner (pre 100.04±2.98 vs post 96.72±2.27; $p < 0.0001$). Anthropometric and body composition results are presented as mean ± SD in table. 3.

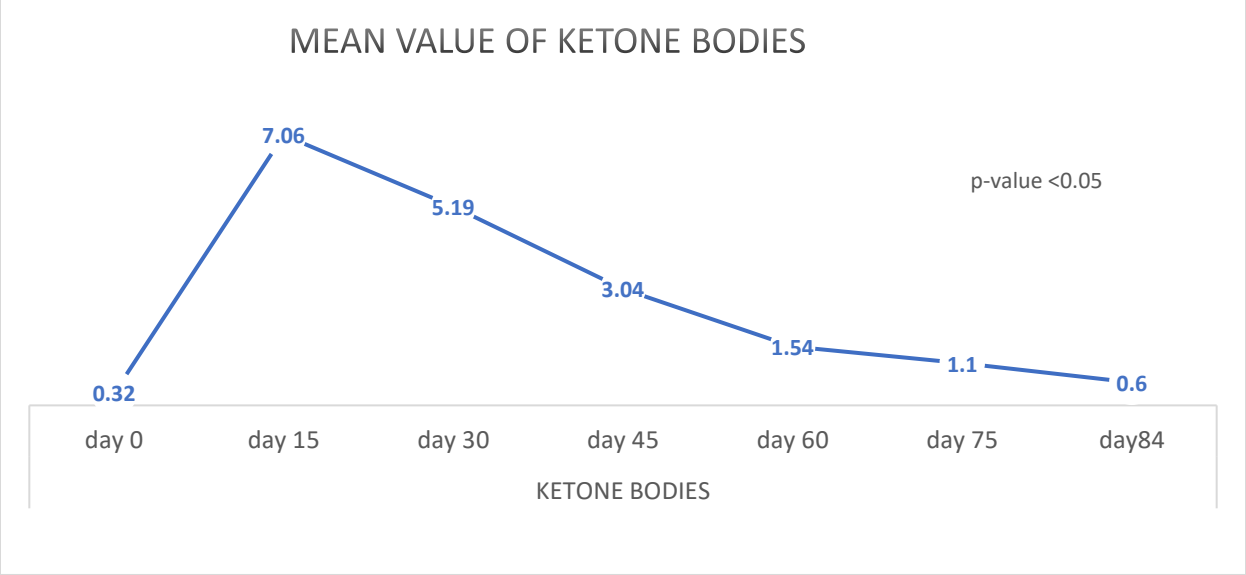
Table. 3. Changes in anthropometric variables after 12 weeks of KEMEPHY diet.

Variables	Pre-treatment	Post-treatment	p-value
Weight (kg)	77.19 ± 5.09	69.26 ± 4.87	<0.00
BMI	28.78±1.17	25.82±1.25	<0.01
Waist circumference (cm)	100.04±2.98	96.72±2.27	<0.00
Body Fat Percentage	27.06 ± 3.26	19.89 ± 2.39	<0.0001

3.1.Metabolic biomarkers

Ketone bodies were measured every 15 days. The mean± SD on day 0 was 0.33±0.35 and it increased to 7.06±1.71 on day 15. It gradually reduced to 5.19±1.84 on day 30, 3.04±1.22 on day 45, 1.54±0.84 on day 60, 1.10±0.65 on day 75 and at the end of study i.e day 84, it was reduced to 0.60±0.31. The significance level was p=0.000. Mean values of ketone bodies are presented in Figure 2.

Fig. 2. Mean values of ketone bodies among married women suffering from Polycystic Ovary Syndrome



At the end of the study, a significant decrease was observed in glucose (pre 91.51± 3.20 mg/dL vs post 82.43±2.33 mg/dL; $p < 0.0001$). There were significant changes in lipid profiles with reductions in triglycerides (pre 204.32±22.56 mg/dL vs post 169.57±12.13 mg/dL; $p < 0.0008$), total cholesterol (pre 206.26±9.26 mg/dL vs post 184.81±7.90 mg/dL; $p < 0.0001$) and LDL (pre 123.70±12.94 mg/dL vs post 91.53± 3.38 mg/dL; $p < 0.0001$) along with a rise in HDL levels (pre 72.55±9.68 mg/dL vs post 82.00±8.19 mg/dL; $p < 0.0001$). The data are reported as mean ± SD in Table 4.

3.2.Endocrine biomarkers

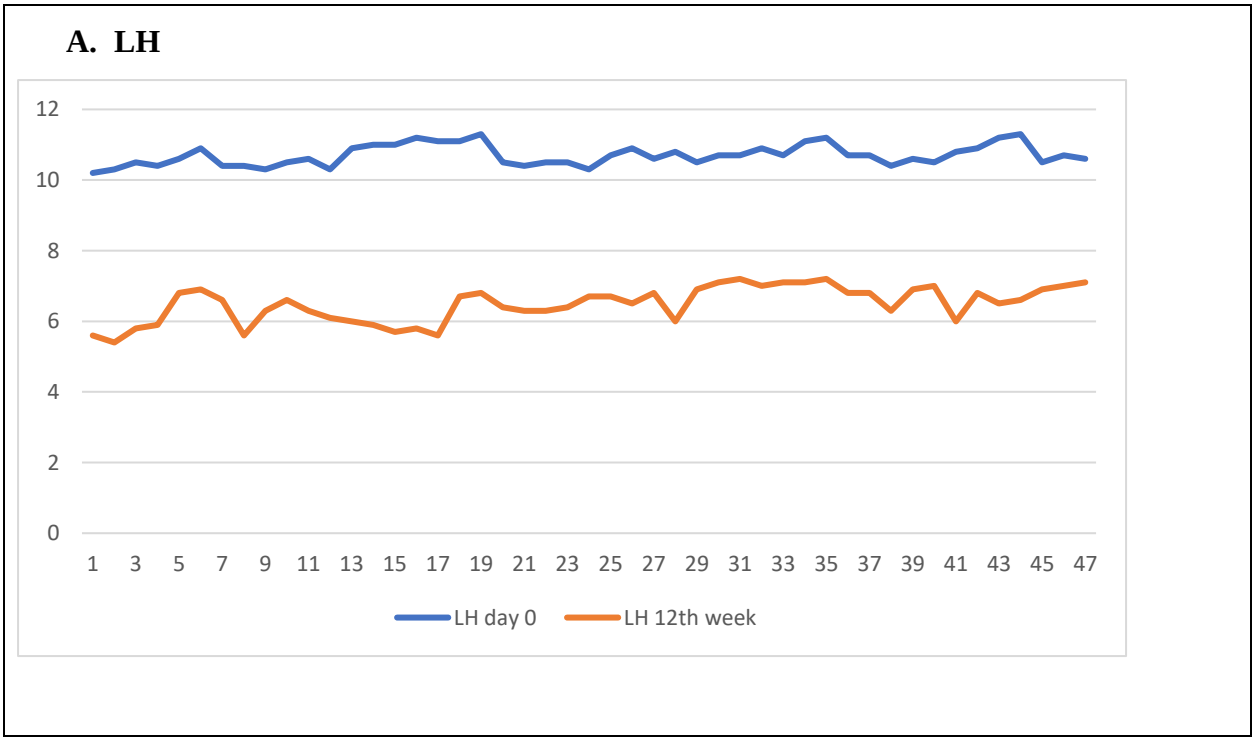
The characteristic reversal of the LH/FSH ratio was observed at the beginning of the study and disappeared after 12 weeks (pre 2.03 ± 0.12 vs post 1.22 ± 0.106; $p < 0.0001$).Compared to basal values, there was also a significant decrease in plasma concentrations of LH (pre 10.54±0.52 vs post 6.80±0.51; $p < 0.0001$). FSH values were found modestly increased, (pre 5.18 ± 0.20 vs post 5.57 ± 0.18; $p = 0.0258$), in accordance with the finding that usually in PCOS patients the concentration of FSH is not affected by the pathology. The Ferriman Gallwey Score was slightly

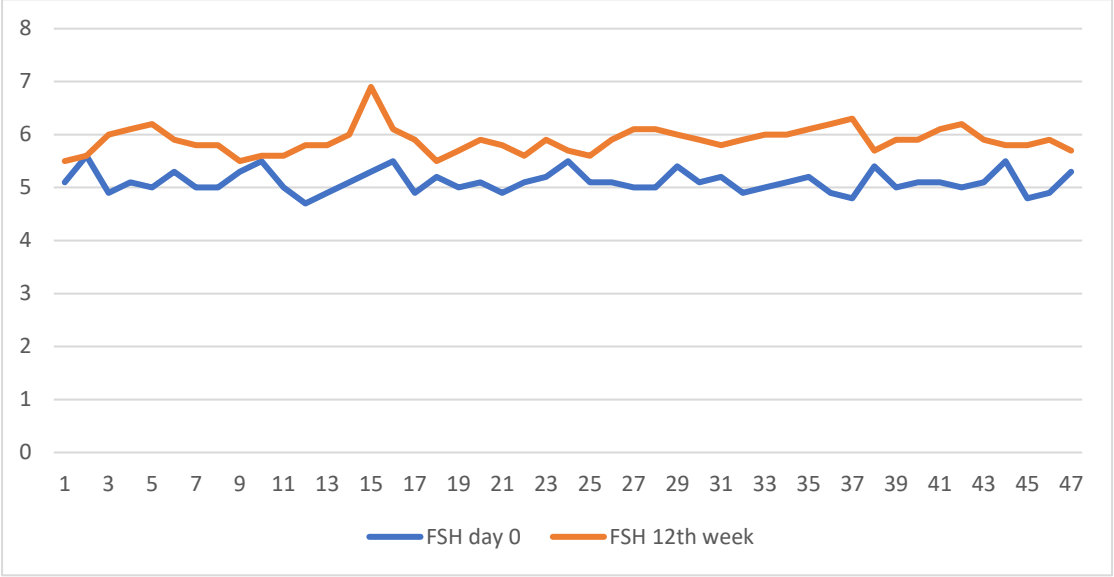
reduced, although not significantly (pre 11.36 ± 1.98 vs post 10.57 ± 1.6). Changes in hormonal variables are presented in Figure 3.

Table 4. Changes in metabolic biomarker after 12 weeks of KEMEPHY diet.

Metabolic biomarkers	Pre-treatment	Post-treatment	P value
Blood Glucose Level (mg/dL)	91.51± 3.20	82.43±2.33	<0.001
LDL (mg/dL)	123.70±12.94	91.53± 3.38	<0.002
HDL (mg/dL)	72.55±9.68	82.00±8.19	<0.0001
Triglycerides (mg/dL)	204.32±22.56	169.57±12.13	<0.000
Total Cholesterol (mg/dL)	206.26±9.26	184.81±7.90	<0.001

Fig. 3. Changes in hormonal variables after 12 weeks of KEMEPHY diet.





B. FSH

4. Discussion

Hyperandrogenism, chronic oligo-ovulation or anovulation, and polycystic ovaries are the three main features of polycystic ovarian syndrome (PCOS). It affects 6%–20% of women around the world who are reproductive age (Sidra et al., 2019). A low-carbohydrate diet that induces physiological ketosis lowers circulating insulin levels, which in turn lower IGF-1 levels, decreasing the stimulation on androgen synthesis in both the ovary and the adrenal glands. This is the rationale for the use of KD in PCOS. Additionally, reducing oxidative stress, low-grade inflammation, and blood cholesterol levels can prevent cardiovascular issues. (38). One of the defining characteristics of PCOS is hyperandrogenism, which appears as increased androgen levels and contributes to clinical symptoms such hirsutism, irregular menstruation, and infertility. The ketogenic Mediterranean diet with phytoextracts (KEMEPHY), among other dietary therapies, have drawn interest as potential therapeutic modalities for treating PCOS. This comprehensive literature review aims to compare the findings from relevant studies investigating the influence of KEMEPHY on hyperandrogenism markers in married women with PCOS to the results obtained from our own research.

Polycystic Ovarian Syndrome (PCOS) is characterized by elevated levels of male hormones (hyperandrogenemia), frequent menstrual irregularities, and difficulties in conceiving (infertility). In addition, a significant number of women diagnosed with PCOS, both lean and overweight, exhibit impaired glucose tolerance and elevated insulin levels. Around 70% of lean women and 95% of overweight women with PCOS experience these metabolic abnormalities. The utilization of a ketogenic diet in the context of polycystic ovary syndrome is supported by theoretical

foundations. This is based on the finding that physiological ketosis brought on by a low-carb diet lowers circulating insulin levels. Consequently, this decrease in insulin levels contributes to lowered levels of insulin-like growth factor 1 (IGF-1), which helps suppress the stimulation of androgen production in both the ovaries and adrenal glands. Furthermore, the reduction in blood cholesterol levels, oxidative stress, and low-grade inflammation associated with a ketogenic diet can help mitigate the risk of cardiovascular issues (38).

In a sample of 14 women with PCOS diagnoses, twelve weeks of LCKD improved nearly all anthropometric, biochemical, and hormonal characteristics. The most prevalent endocrinopathy in women of reproductive age, PCOS is distinguished by a striking phenotypic variability. It is important to note that PCOS is linked to a wide range of long-term metabolic and cardiovascular consequences, and should not merely be viewed as a cosmetic or fertility issue. Although the exact causes are unknown, insulin resistance—which affects 70% of patients and is typically associated with being overweight or obese—is thought to be a significant etiopathogenetic component. In fact, hyperandrogenism promotes the increase in visceral fat, but it also appears to play a significant pathogenetic role in the onset and progression of PCOS in susceptible women. These two factors suggest that abdominal obesity and PCOS are related in a way that combines cause and effect (58).

According to the Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group (10), hirsutism, hyperandrogenemia, and irregular menstruation are more common in people with higher BMIs. In actuality, adipose tissue is an extra-glandular source of androgens, and an excess of it can exacerbate hyperandrogenism. In contrast to adipocytes of the lower body, which characterize gynoid obesity, abdominal adipocytes exhibit higher levels of endocrine activity. They are also more sensitive to catecholamines and less to insulin, which ultimately results in a hyperinsulinemia compensatory with low-grade inflammation, altered lipid profiles, increased androgen production, and low levels of SHBG, all of which favor anovulation. Additionally, metabolic flexibility—the skeletal muscle's capacity to utilize fats or carbs interchangeably based on the availability of energy substrates—is impacted by hyperinsulinemia (59). When hyperandrogenism, high body mass index, and insulin resistance are present, women with PCOS have greater decreased metabolic flexibility (measured by changes in the respiratory quotient following insulin stimulation)(60, 61).

The treatment of PCOS is basically symptomatic: estrogen replacement therapy is the preferred course of action when fertility is not the primary concern, and it is frequently administered for extended periods of time. However, extended usage of oral contraceptives may have harmful effects, and PCOS patients are more likely to have cardiovascular problems. For this reason, selecting an estro-progestinic medication should only be done after each patient has had their cardiovascular risk assessed individually. Using an estro-progesterone product on a PCOS patient with normal weight, normal glucose tolerance, and normal lipid profile is not the same as doing so on an obese PCOS patient with metabolic syndrome (62). Furthermore, the patients do not "heal" from using oral contraceptives, and following the suspension, they frequently experience

anomalies in their cycles. As a result, the majority of them are compelled to undergo ovulation-inducing therapies, typically involving the use of clomiphene, as advised by the guidelines (31). Therefore, using contraception is appropriate when a woman fears becoming pregnant unintentionally; yet, for many women, a different course of treatment would be far more desired and beneficial.

Guidelines (31) recommend hormonal contraceptives as the first line of treatment for irregular menstruation, hirsutism/acne, and PCOS-related acne. In the event that lifestyle modifications are not successful, metformin is recommended as a treatment for women with PCOS, Type 2 diabetes, or impaired glucose tolerance. Metformin is regarded as a second-line treatment for women with irregular menstruation who are contraindicated or have an intolerance to estrogens. In individuals with infertility issues, metformin also appears to enhance the ovarian response to clomiphene that follows, showing similar benefits in patients with very high and non-BMI. Metformin does not, however, come without side effects, which frequently make compliance difficult.

As a result, the discussion about therapy is still ongoing, and patients and medical professionals alike frequently disagree about who should make the treatment decision. Women typically consult gynecologists first when they experience irregular menstruation and infertility, disappointing endocrinologists in the process. Furthermore, a lot of women look for alternative answers and reluctantly accept the idea of ongoing medicalization. Given the suggestions of the same guidelines, which currently hold that dietary and lifestyle modifications are crucial to the management of PCOS, a nutritionist could play a pivotal role in this context.

Numerous dietary theories have been put forth to address the PCOS-related metabolic changes, but no one has yet come to a consensus on the best one to advocate. Additionally, the question remains unanswered as to whether women, regardless of their weight status (normal, overweight, or obese), can derive advantages from an appropriate dietary regimen that targets reducing insulin resistance without restricting calorie intake. Ketogenic diets (KDs) can be regarded as a nutraceutical treatment aimed at enhancing insulin sensitivity in this context. Even though there aren't many data in the literature, they do support the idea that a KD can help normalize the clinical picture in PCOS by reducing hyperinsulinemia and enhancing body composition. After three to five days on a very low-carb diet, when the concentration of KBs starts to increase, hunger significantly declines while maintaining a feeling of wellbeing. When compared to typical hypocaloric diets, which are severely lipid-restrictive and maintain the level of orexigenic hormones for up to 12 months after the diet has been discontinued, the benefit is even more significant.

During a physiological state of ketosis, such as during fasting, the blood pH remains unchanged and ketonemia reaches its maximum levels of 7-8 mmol/L due to the CNS's significant consumption of ketones and the balance between glucagon and insulin (Paoli et al., 2013). Blood ketones greater than 0.5 mmol/L are considered to be in nutritional ketosis. During a long-term chronic kidney disease (LCKD), KB levels are often between 0.5–0.6 and 4. Indeed, our subjects showed shows that ketone bodies elevated during the first 15 days and went upto 7.06. From day 15 to 84, ketone bodies reduced gradually (7.06±1.71 to 0.60±0.31). Therefore, it is critical to distinguish between pathological ketosis, such as that which can develop in diabetes when

uncontrolled rates of ketobolic acid production (KBs) result from hyperglycemia and insulin deficiency, and ketonemia may exceed 20 mmol/L, putting the patient at risk of severe acidosis.

Urinary ketone testing revealed that 61.5% of the women entered a state of ketosis during the first week, with measurable levels of acetoacetate. As the women increased their carbohydrate intake, urinary ketone levels decreased, averaging 2.94 mmol/L by week 3. The study established a positive correlation between serum β-hydroxybutyrate and urine ketone levels, indicating that urinary ketone testing can be an effective and cost-efficient method for estimating ketone production and dietary compliance. The positive correlation between urine and serum ketone production supports the use of urinary ketone testing to assess ketone production and adherence to the diet. They found that as carbohydrate intake increased, urinary ketone levels decreased. This suggests that your results align with the understanding that ketone production is influenced by dietary carbohydrate intake.

However, fasting ketosis causes a loss of protein stores, which particularly impacts muscle mass and creates a condition of general deterioration. On the other hand, the ketogenic diet guarantees a sufficient supply of protein, protecting the tissues, while over time sustaining a state of ketosis due to the restriction of carbohydrates (39). It is crucial to emphasize that a traditional ketogenic diet (KD) often consists of a high-fat, adequate-protein, low-carb diet rather than one that is high in protein. Actually, overabundance of proteins causes gluconeogenesis to increase over time, which impacts KB synthesis. During the early stages of a KD, neo-glucogenesis from amino acids serves as the primary source of glucose to maintain glycemia stability. As this process progresses, the need for amino acids declines and glucose is synthesized from glycerol that is released from adipose tissue through triglyceride hydrolysis. Our food consisted of a low-calorie ketogenic diet, with a high proportion of protein (31-36%) but a typical amount of protein (1.23 g pro/Kg bw) if measured in grams per kilogram of body weight. Such a low-calorie strategy is more practical during a KD (VLCKD and LKD) because ketones are known to decrease hunger, most likely as a direct result of KBs acting on the brain.

The observation that the physiological ketosis brought on by a low carbohydrate intake lowers the levels of circulating insulin and, subsequently, IGF-1, suppressing the stimulus on the production of androgens, both ovarian and adrenal. This observation forms the theoretical basis for the use of KD in PCOS. Cardiovascular problems can also be avoided by reducing oxidative stress, low-grade inflammation, and circulating lipids (38, 39, 45, 46, 63-65). In fact, peripheral inflammation is revealed by elevated CRP, TNF-α, and IL-6 in PCOS patients, as well as greater circulating lymphocytes, monocytes, and eosinophilic granulocytes. Furthermore, a higher number of inflammatory cells that have infiltrated the ovaries exhibit chronic inflammation that is persistent in polycystic ovaries. Given that TNF-a and IL-6 have been shown to cause insulin resistance, boost testosterone synthesis, and interfere with the hypothalamic-pituitary-ovarian axis, it is possible that the elevated lymphocyte count is what causes long-term inflammation and changes in hormone release. Crucially, eating glucose causes an inflammatory response in PCOS patients that is unrelated to weight, and hyperglycemia can exacerbate inflammation. A ketogenic diet has been shown to improve inflammatory indicators in general, possibly as a result of 3-hydroxybutyrate, one of the ketone bodies. participants who were included in the trial saw weight

loss and a notable decrease in FBM. This finding may be explained by AMPK's regulation of mTOR, which plays a protective function for muscle anabolism (Cantó & Auwerx, 2011; Sengupta et al., 2010). In actuality, while utilizing KD, our goal is to maintain lean mass nearly unchanged rather than to achieve hypertrophy, which contrasts with severe calorie restriction diets where loss is typically more noticeable.

Females with menstrual irregularities, who are intolerant or have contraindications to estrogen-based contraceptives, metformin is recognized as a second-line therapy. (31). Contraception is indicated anytime there is a high risk of an unexpected pregnancy, but despite the contraindications, some medical professionals believe that metformin should be tried first before oral contraceptives are given. Metformin does have a number of side effects that usually lower compliance. (57). Therefore, the topic of therapy is still up for debate, and patients and healthcare professionals alike frequently disagree about who is ultimately in charge of selecting a course of action. Regarding infertility-related symptoms, the literature review mentions successful pregnancies in women who had previously struggled with infertility after following an LCKD. In current study the symptoms related to infertility were improved. It is important to note that current study did not assess actual pregnancy outcomes. In current study, acne decreased from 59.6% to 44.7% after the KEMEPHY diet intervention. Additionally, oily skin problems decreased from 91.5% to 78.7% after the intervention. These findings align with the literature review's observation of improved symptoms related to dermatological issues.

Triglycerides and cholesterol were considerably reduced. Significant drops in LH and LH/FSH suggested that the hormonal abnormalities associated with PCOS were abating. Although the improvement in the Ferriman Gallwey Score did not reach statistical significance, we can assume that the duration of 12 weeks was insufficient to witness a decrease in hirsutism scores. This is because the hair cycle can last for several months, depending on the body area, and it is well-known that antiandrogen-based pharmacological therapy requires six to twelve months to achieve a significant reduction in the score.

5. Conclusion:

The current study provides the insight data to explore reproductive hormones, blood lipid levels and body weight of participants with PCOS. Our study's findings point to the potential therapeutic use of KD for PCOS, should be used in conjunction with a more balanced diet plan that always pays special attention to carbohydrate intake. The length of KD is still unknown, however short cycles are thought to be safe and there is no indication of short-term negative effects. Long-term diets are the subject of little research, although knowledge obtained in the fields of epilepsy and GLUT-1 deficient syndrome (66-72), supports a possible use also for prolonged periods. It is conceivable to propose that procedures will be established and then repeated in cycles across time, with intervals of equilibrium regime. Taking into account a few of the study's shortcomings. Firstly, the impact of a KD on other outcomes such as infertility and oligomenorrhea was not evaluated. The experimental setting precluded us from conducting an OGTT for glucose and insulin, which would have provided additional insights into the metabolic effects of a KD. Lastly,

the small sample size and single arm design necessitate further confirmations, which may involve matching a low-calorie ketogenic diet with a low-calorie Mediterranean diet and possibly increasing the number of subjects.

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