Review

The Effect of Nutrient Supplementation in Management of Polycystic Ovary Syndrome Associated Metabolic Dysfunctions: A Critical Review

Günalan et al. The Effect of Nutrient Supplementation in Management of Polycystic Ovary Syndrome Associated Metabolic Dysfunctions

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Abstract
Polycystic ovary syndrome (PCOS) is complex heteregeneous disorder which has several aspects in terms of pathology such as metabolic, endocrine, reproductive and psychological. However, the etiology of PCOS remains poorly understood. Several studies suggest that insulin resistance and hyperandrogenism play a central role in progression of PCOS pathophysiology. Therefore, common treatment strategies of PCOS are based on lifestyle modification which include exercise, diet and nutrient suplementation therapy. Recent studies have recommended some nutrients such as vitamins, minerals and vitamin like nutrients for therapy of PCOS because each one has at least one functional property in PCOS-induced pathways. Therefore, it is claimed that the reason of PCOS can be vitamin or mineral deficiency. This review aims to provide a critical literature survey on nutritional supplementation for the treatment of PCOS associated endocrine and metabolic dysfunctions and discuss the role of nutrients in management of PCOS in view of the clinical trials and experimental studies.
Keywords: Polycystic ovary syndrome, insulin resistance, hyperandrogenism, metabolic dysfunctions, dietary supplements

Introduction

The polycystic ovary syndrome (PCOS) is one of the most common endocrine diseases that affects 5 to 10% in women of adolescent and reproductive age (1-2). PCOS was first defined by Stein and Leventhal in 1935. The basic characteristic trait of PCOS is hyperandrogenism as a result of excessive androgen secretion or activity (3). However, hyperandrogenism is not the only diagnostic criteria for PCOS. According to the Rotterdam criterias, PCOS is defined with the existence of at least two of three criteria that are hyperandrogenism, chronic anovulation and polycystic ovaries on ultrasound findings (4). Later, Androgen Excess and PCOS (AE-PCOS) Society reported that there should be presence of hyperandrogenism and ovarian dysfunction (anovulation and polycystic ovaries) for PCOS (5). Patients with PCOS suffer from various symptoms including menstrual dysfunction, hyperinsulinemia, infertility, glucose intolerance, type 2 diabetes, hirsutism, obesity, acne, metabolic syndrome, increased risk for the development of cardiovascular diseases, endometrium cancer, anxiety, obstructive sleep apnea and abnormalities of lipid profile (6-7).
Although there are extensive studies in the literature, the cause of PCOS remains unclear due to poorly understood interactions between genetic and environmental factors (8). Reproductive neuroendocrine defects, impaired ovarian steroidogenesis, insulin resistance and increased cortisol metabolism related adrenal hyperandrogenism can be among the causes of PCOS (9-11). Recent studies suggest that insulin resistance (IR) contributes to both metabolic and reproductive disturbances. Therefore, IR has a central role in the pathogenesis of PCOS (12). Briefly, insulin is considered as a key hormone for hyperandrogenism in the PCOS pathophysiology via two different pathways: 1-Insulin stimulates androgen production of theca cells with luteinizing hormone (LH) and elevated androgen production leads to hirsutism, acne and anovulatory infertility. 2-Hyperandrogenism associated function of insulin is inhibition of sex hormone binding globulin (SHBG)

synthesis in the liver (13). SHBG is a plasma protein for androgen and estrogens and so decreased SHBG levels can lead to hyperandrogenism in PCOS. At the metabolic perspective, insulin plays a key role in regulating glucose metabolism, blocking of lipolysis and activation of aminoacid transportation (14). Various nutrients have regulatory roles in the insulin signaling pathway and androgen synthesis.

Providing sufficient nutrients and energy for growth and reproduction depend on definition of the optimal nutrient composition. It is clear that nutrition associated signaling pathways play central role in regulation of ovarian follicle growth and ovulation rate (15). Especially, deficiencies of myo-inositol and vitamin D can lead to PCOS pathogenesis related complications (16-18). Therefore, nutritional supplementation may contribute to overcome complications of PCOS such as immature oocyte, insulin resistance, hyperandrogenism and oxidative stress. This review addresses current knowledge about efficacy of nutrients in treatment of PCOS in view of experimental studies and clinical studies.

2. Vitamin Supplements

Vitamin A is a fat soluble vitamin also known as retinol. Vitamin A derived metabolites such as retinoids, retinoic acid and retinol contribute to antioxidant activity, steroid metabolism, oocyte nuclear maturation and inhibition of cumulus cell apoptosis (19, 20). It is known that retinoic acid synthesis related-genes are expressed differentially in theca interna cells isolated from PCOS patients (21). To examine the effects of retinol and retinoids, derivatives of retinol were applied into theca interna cell culture obtained from PCOS and healthy women. All trans retinol treated theca interna cells gave rise to increased dehydroepiandestrone (DHEA) level and mRNA accumulation of cytochrome P450 17α hydrxylase (CYP17) involved in androgen production and retinol biosynthesis (22). On the other hand, in overweight women with PCOS, obesity and abnormal glucose metabolism are associated with elevated retinol binding protein 4 (RBP4) levels (23). Another RBP4 based study reported the measurement of RBP4 expression in isolated subcutenous and omental adipose tissue from women with PCOS. They suggested that elevated 17β estrodiol can contribute to altered gonadal and adrenal steroid profile via upregulation of RBP4 gene (24).

B Group Vitamins: Most of the studies focus on B6, B12 and folic acid in this group due to increasing role of homocysteine (Hcy) in PCOS. In this mechanism, Hcy is an essential aminoacid that derived from dietary methionine and elevated total plasma homocysteine levels lead to increasing risk for cardiovascular and reproductive symptoms in PCOS (25). In addition, other metabolic pathways required for growth of cell and tissue are closely associated with Hcy (26). Folic acid, vitamin 6 and vitamin B12 have significant roles in Hcy regulation. In PCOS physiopathology, a positive correlation has been reported between IR and elevated Hcy levels (27, 28). Kaya et al. demonstrated that insulin resistance, obesity and increased homocysteine level is related to low serum insulin B12 concentrations in women with PCOS (29). In order to reduce elevated levels of serum Hcy, folic acid supplementation for three months produced effective results especially in women without IR. However, dose dependent effect of folic acid suplementation is not known (30). Regular exercise has also been suggested to decrease plasma Hcy concentration in PCOS pathophysiology. According to the study of Randeva et al , regular exercise for a period of months provides significantly lower plasma Hcy level in young obese and overweight women with PCOS (31).

Many women with PCOS have to use insulin sensitizing agents such as metformin for improving insulin sensitivity. Metformin inhibits the binding intrinsic factor (IF)-B_{12} complex and its receptor and also serum vitamin B12 and folic acid levels decrease during metformin therapy (32). On the other hand, metformin provides increase in Hcy level, and so it has a been give rise to the long-term risk of cardiovascular diseases in women with PCOS (33). The interaction between metformin and B group vitamins has been explained by two studies: First report showed that daily administration of folic acid or B group vitamins can be effective in reducing elevated Hcy levels in women with PCOS in short term metformin therapy. However, the authors also suggested that supplements of vitamins have no effects on androgen and lipid levels in PCOS pathophysiology (34). Second report has shown that the use of metformin with folate supplementation for six months has beneficial effect on the vascular endothelium. This treatment provides reduced Hcy levels, thus it can be effective in management of long term complications of PCOS such as cardiovascular disorders (35).

Inositol and its metabolites are known as sugar alcoholes and also belong to B complex vitamins. In addition, inositol has 9 stereoisomers such as myo-, cis-, allo-, epi-, muco-, neo-, scyllo-, D-chiro and L-chiro- forms (36). Inositol derived metabolites have essential roles in insulin sensitivity as second messanger, lipid synthesis, signal transduction, oocyte maturation, oogenesis, cell morphogenesis and cytoskeleton organization (37). According to randomized controlled studies involving inositol supplementation to the women with PCOS, inositol provides improvement in almost all pathological conditions in PCOS such as recovery of reproductive abnormalities, decreased androgen level and improved insulin level (38).

Interestingly, combined treatment of inositol isomers such as myo-inositol (MI) and D-chiro inositol (DCI) should be applied at a certain ratio that is known as plasma physiological ratio (MI/DCI: 40/1) (39). Otherwise, immature oocytes can appear, and efficacy of inositol is decreased in PCOS pathophysiology (40). Some studies have claimed that this pathological conditions may be to reasoned by “DCI paradox” (41). Briefly, MI is found in FSH signaling mechanism and homeostasis of glucose uptake while DCI is prompted to insulin associated

androgen synthesis. Epimerase plays functional role in convertion of MI to CDI depending on insulin level and also intake of inositol isomerase except physiological ratio can lead to decreased MI and increased CDI level. When hyperinsulinemia is occured in PCOS pathogenesis, elevated epimerase activity can lead to abnormalities in FSH signaling pathway, therefore, immature oocytes and hyperandrogenism may develop (42).
Contributions of myo-inositol to treatment in women with PCOS are reviewed in Table 1. (43, 44, 45, 46, 47-50). According to the current literature, treatment of myo-inositol provides healing in hyperandrogenism and insulin resistance associated parameters and also improvement of lipid profile.
Vitamin D is so essential vitamin for skeletal growth, regulation of serotonin synthesis, bone mineral density, dental health, lower extremity functions and regulation of calcium and phosphorus metabolism. In addition, previous studies have reported that vitamin D may be a significant and independent predictor of IR (51). Vitamin D levels decrease in obese patients when compared with non-obese people owing to IR. Regarding to PCOS, a recently published review by Krul-Poel et al. about the role of vitamin D in metabolic disturbances of PCOS confirmed an association between vitamin D and metabolic disturbances (52). And thereby, it is found that women with PCOS (who are obese) have significantly decreased 25-dehydroxyvitamin D level (53). Moreover, a cross sectional study reported that lower D vitamin is linked with insulin resistance as a result of PCOS pathophysiology (54).
Researchers focus on vitamin D supplementation for treatment the PCOS women to show an interaction between vitamin D deficiency and PCOS. A recent study about vitamin D replacement therapy which administration of vitamin D3 for three weeks in 11 PCOS subjects suggested some beneficial effects on insulin resistance, but no changes in androgen level were observed (55). In addition, Kotsa et al. used a vitamin D3 analogues (alphacalcidol) in order to determine the effect of vitamin D in treatment of PCOS. Their findings showed increased the first phase of insulin secretion, decreased serum triglyceride level and increased serum HDL cholesterol profile (56).
Molecular mechanism between vitamin D supplementation and improvement of PCOS is currently unknown. However, a recent report claimed that vitamin D3 replacement to women with PCOS improves some biochemical parameters via increasing of soluble receptor for Advanced Glycosylated Ends (AGEs). Therefore, vitamin D3 inhibits inflammatory progress in pathogenesis of PCOS. Moreover, vitamin D3 treatment plays a vital role in folliculogenesis due to decreasing of elevated anti-mullerian hormone (57). Interestingly, Sfidvajani et al. (2017) have demonstrated that vit D supplementation in women with PCOS did not cause to statistically significant differences in androgen profile when combination with low calorie, however improvement in menstrual frequency appeared (58).
Vitamin E is a lipid soluble vitamin and free radical scavenger which regulates the balance between antioxidant and oxidant systems (59). In addition, new evidence confirmed that vitamin E can improve endometrial thickness in unexplained infertile women that the effects are attributed to its anticoagulant and antioxidant effects (60). On the other hand, cotreatment of coenzyme q10 and vitamin E during 8 weeks in patients with PCOS has been provided improvement in SHBG concentration (61). Another study have shown that vitamin E (400 IU) and omega-3 fatty acid (1000 mg) co-supplementation in women with throughout 12 weeks provide to improvement in IR and androgen levels significantly (62).

3. Supplementation of Vitamin-Like Nutrients in PCOS

Alpha-Lipoic Acid (a-LA) is a free radical scavenger, essential cofactor in citric acid cycle and a regulatory agent of body weight (63, 64). Interestingly, Masharani et al. found that controlled release of α-LA administered to the six non-diabetic women with PCOS is not related with elevation in plasma antioxidant potency or reduction in plasma oxidation metabolites (65). To investigate the role of α-LA and D-chiro-inositol (DCA) in the short term management of PCOS, both of metabolites were given to 46 women (26 women with PCOS and 20 women control subjects) for 180 days. They suggested that some reproductive characteristics improved including menstrual cycles, decreased number of ovarian cysts and increased progesteron level. At the metabolic perspective, insulin resistance significantly improved in PCOS subjects, while impaired lipid metabolism was significantly changed (66).
Bioflavonoids consist of polyphenolic compounds which are found in plants. Properties of flavonoids are antioxidant, antidiabetic, antiestrogenic, anti-inflammatory and antiproliferative effects (67). Bioflavonoids consist of various metabolites and some of them provide improvement of pathogenesis of PCOS at different levels. For instance, Oh et al. reported analysis of six flavonoid classes (anthacyanides, flovan-3-oils, flavanones, flavones, flavonols and isoflavones) in terms of contribution to the treatment of metabolic syndrome in PCOS pathophysiology. Eventually, they suggested that only flavonol consumption was the most effective treatment of metabolic syndrome in PCOS when compared with other groups (68). Romualdi et al. showed that 36 mg/d soy isoflavone genistein treatment to women with PCOS for three months provide significantly improved lipid profile. However, other characteristic traits of PCOS which are hyperinsulinemia, anthropometric measurements, hyperandrogenism and reproductive abnormality did not significantly change (69) On the contrary, in an experimental study on rats, Shah et al. reported improved ovarian and uterine morphological appearences, increased LH level, significantly decreased insulin and decreased testesterone in PCOS following

quercetin treatment, a bioflavonoid with antioxidant activity. They considered that quarcetin is functional in phosphatidylinositole-3-kinase (PI3K) inhibition and therefore PI3K can be beneficial target for novel therapy approach of PCOS (70).

Carnitine is a quaternary ammonium compound which found in fatty acid metabolism, oxidative stress mechanism and glucose metabolism (71). According to a clinical research, non-obese women with PCOS have significantly decreased serum total L-carnitine levels when compared with healthy women (72). Fenkci et al. considered that lower L-carnitine level can be linked with hyperandrogenism and insulin resistance (72). Consistently, some antidiabetic agents which are utilized for PCOS treatment is associated with carnitine metabolism. For instance, Piaglitazone administration during 16 weeks in obese premenoupausal patients with PCOS lead to increased fasting concentration of free carnitine (73). Moreover, Duning et al. have claimed that L-carnitine influences oocyte quality because L-carnitine provides transport of fatty acids and regulation of energy production which have central role at promoting of oocyte maturation (74). Immature oocytes can be source of metabolic and endocrine malfunctions in PCOS (75). A randomized clinical trial in clomiphene resistant women with PCOS have reported that using both clomiphene citrate and L-carnitine provide thicker endometrium, higher oestradiol concentration, higher pregnancy rate and improved lipid profile compared to clomiphene citrate treatment only (76). Another study have demonstrated that L-carnitin supplementation (250 mg per day) for 12 weeks has beneficial effects within mental health and oxidative stress parameters (77).

4. Mineral Supplements

Mineral supplements are among dietary supplements that are expexted to provide improvement of metabolic profile, mental health, ovulation and menstrual cyclicity. Recent studies about PCOS has focused on mineral supplementation in order to remove from pathological situations of PCOS.

Calcium(Ca) is an essential micronutrient and is involved in egg activity, oocyte maturation, progression of follicular development and regulation of cell division in mammalian oocytes (78-80). Furthermore, Ca deficiency can be related to risk of obesity, since insulin signaling pathway is Ca dependent (81). Therefore, it is considered that abnormalities of calcium concentrations can be associated with insulin resistance and promoting of PCOS pathology. Biochemical studies have shown that decreased Ca level is observed in obese women with PCOS, when compared with healthy women. Calcium homeostasis depends on vitamin D receptor (VDR), parathyroid hormone (PTH) and calcium sensing receptor (CaSR). In addition, adiponectin concentration is strongly associated with Ca and vitamin D levels (82). To determine the role of the polymorphisms of Ca homeostasis linked factors in initiating of PCOS, VDR, PTH, CaSR, insulin receptor and adiponectin genes were analyzed and compared with PCOS associated biochemical parameters. Consequently, polymorphisms of VDR are related to increased LH and reduced SHBG levels while gene variant of CaSR is linked to higher HOMA-IR and insulin resistance (83). Combined supplementation of vitamin D 100000 IU/month, Ca 1000 mg/day and metformin 1500 mg/day during 6 months in 100 infertile patients with PCOS is resulted in significanly reduced body mass index (BMI). In addition, menstrual cyclicity, follicular maturation and the pregnancy rate are affected positively, but the alterations are not statistically significant (84).

Chromium is an essential mineral that have essential role in the carbohydrate and lipid metabolism. Since chromium deficiency leads to disorders in glucose homeostasis and insulin resistance, it has been widely studied in the treatment of hyperglycemia, especially type 2 diabetes (85). And also there is evidence to confirm that women with PCOS showed decreased chromium levels which was linked to insulin resistance (86). A pilot study suggested that daily 200 μg chromium supplementation throughout three months, PCOS women showed improved glucose tolerance, and but it did not affect reproductive functions and hormonal disturbances (87). Another study involving 64 women with PCOS showed that daily 200 μg chromium supplementation during eight weeks caused significant decreases in serum insulin levels, HOMA-IR, HOMA-B, triglycerides, VLDL cholesterol, total cholesterol concentration. In addition, Jamillian et al. showed that a significant increasing quantitative insulin sensitivity check index (QUICKI) score in women with PCOS compared with the placebo. However, circulating LDL, HDL, cholesterol levels and fasting plasma glucose level did not alter in treatment group (88).

The effect of chromium within androgen level depends on treatment amount and duration of chromium. According to a double blind randomized clinical study, chromium picolinate (200 μg/day) treatment into 46 patients with clomiphene-citrate-resistant PCOS during 3 months give rise to increasing of insulin sensitivity. However, there is no finding about a relationship between applied chromium and androgen level (89)

On the other hand, Amr et al. high doses of chromium picolinate (1000 μg/day) treatment into adolescent girls with PCOS for 6 months. At the end of the study, improvement of oligo/amenorrhea, decreased number of total follicle, lower free testesterone levels and smaller ovarian volume were obtained by ultrasonographic views and biochemical analyses (90).

Magnesium is the one of the most predominant intracellular cations (91). Magnesium regulates ATP-generating, ATP-utilizing, transphosphorylation reactions, DNA and RNA synthesis, insulin metabolism, ion homeostasis, membrane structure, cytoskeletal function and cell growth (92). In addition, magnesium is associated with entry of calcium into the neuron since magnesium is a calcium antogonist and a voltage-dependent blocker of the

NMDA (N-methyl-D-aspartate) channel (93, 94) This property provides protection for neuron against cell death. Therefore, magnesium supplementation is used generally in neurologic disorders including especially depression related diseases such as PCOS as well hypertension, cardiovascular diseases and diabetes (95, 96). However, only a few studies suggest relationship between serum magnesium level and pathogenesis of PCOS. Lower serum magnesium level and higher Ca/Mg ratios in women with PCOS due to insulin resistance have been reported. No significant correlation between Mg level and steroid hormones was found (97). Effects of magnesium levels in PCOS pathology remains unclear.

Selenium is an effective essential element against oxidative stress and is required for the embryonic gonodal development and function of the reproductive tissues (98). Biochemical studies have shown that women with PCOS have lower selenium level compared with control group. Coskun et al. suggested that accumulation of free radicals is detected in PCOS women due to insufficient selenium level that leads to increased androgen level including luteinizing hormone and total testesterone (99). In this regard, selenium supplementation in the form of IMOD (a herbal immunomodulatory drug) was administered for 21 days to hyperandrogenism-induced PCOS female rats. IMOD reduced TNF-α production and increased antioxidant capacity (100).

Another aspects of selenium intake is related to glucose and fat metabolisms as selenium possesses insulin-like activities (101, 102). There were two clinical research about the effect of selenium supplementation to the PCOS women in terms of insulin resistance. In the first study, 70 PCOS women were randomly divided into two groups as received 200 μg per day selenium supplements (n=35) and placebo (n=35). After 8 weeks of intervention, they presented reduction in serum insulin levels , HOMA-IR, HOMA-B, increased QUICKI about glucose metabolism. And also, selenium intake showed decreased in serum triglycerides and VLDL-C concentrations when compared with placebo (103). Another study include 200-µg selenium supplementation (n = 20) and placebo (n = 20) per day for 8 weeks into 40 infertile women with PCOS. At the end of the study, they measured the insulin and lipid related gene expression levels such as PPAR-γ, GLUT-1 and LDLR from lymphocytes in subjects. Results have shown that selenium supplementation can be candidate for in vitro fertilization due to increased expression levels of PPAR-γ and GLUT-1 and decreased expression levels of LDLR significantly (102).

Zinc is an another essential trace element found in metabolism of lipid, carbohydrates and protein that is responsible for function of over 300 enzymes. It is component of more than 200 enzymes (104). In particular, zinc ions play crucial role in insulin metabolism involving synthesis, storage, secretion, conformational integrity, function and action of insulin and also zinc ions present insulin like effect (105). For this reason, insufficiency of zinc gives rise to diabetes, obesity, glucose intolerance, lipidemia, hyperglycemia and hypertriglyceridemia (106,107). Studies have shown that womens with PCOS have lower zinc level (108). It has been demonstrated that one of the reasons of insulin resistance in PCOS was related to decreased insulin-dependent tyrosine phosphorylation due to a post-receptor defect (109, 110). Therefore, inadequate zinc level could not stimulate insulin receptor tyrosine kinase (IRTK) in patients with PCOS. Zinc level can play an important role in developing of insulin resistance PCOS. Several studies have suggested that zinc supplementation has therapeutic effects for the prevention of type 2 diabetes (111).

On the other hand, pathology of PCOS involves risk of cardiovascular diseases in long term due to altered lipid profiles which including elevated trygliceride level, decreased HDL level and increased LDL level (112). It has been suggested that zinc deficiency in PCOS can be associated with abnormal lipid profiles. The effect of zinc supplementation to women with PCOS has been shown in a recent clinical research (113). In this study, 50mg/d of zinc as zinc sulphate or placebo was applied to 60 women with PCOS for 8 weeks, as an adjunct to their pre-study oral estrogen-progestrone compounds therapy. The results showed significant reduction in levels of serum total cholesterol, LDL-C, triglyceride and TG/ HDL-C ratio in the zinc group (113). Therefore, zinc supplementation can provide an effective adjunctive nutritional therapy with potential for improving lipid metabolism and insulin resistance in women with PCOS.

5. Other Supplements

Melatonin (MT) is a neuroendocrine hormone secreted from the pineal gland and plays a central role in the regulation of circadian rhythm. High concentrations of MT is found in the follicular fluid that affects physiological processes in the ovaries such as folliculogenesis, follicular atresia, ovulation, steroidogenesis in theca cells and corpus luteum (CL) formation due to its powerfull free radical scavenger activity (114-116). Moreover, Wei et al. (2013) have reported that supplementation of MT in low concentration supports nuclear maturation of oocyte *in vitro* (117). Therefore, MT may provide improvement of oocyte quality and increases pregnancy rates (118). Concentration of MT in pre-ovulatory follicular fluid is lower in women with PCOS. Kim et al. have suggested that MT administration may be useful in *in vitro* fertilization strategy and improve clinical outcomes of PCOS (119).

N-acetyl-L-cysteine (NAC) is the acylated form of L-cysteine amino acid and also one of the precursors of glutathione, an antioxidant substance (120). Liu et al. have shown that NAC administration supports oocyte quality with antiaging effect on mouse oocytes (121). On the other hand, NAC regulates insulin receptor function in eryhtrocytes and supports insulin secretion from the pancreatic β cells (122). Fulghesu et al. have

investigated the effects of NAC administration for 5-6 weeks on insulin associated parameters in obese and lean women. They determined a significant decrease in testesterone and androgen levels. In addition, increased peripheral insulin sensitivity appeared in women with PCOS (123). Eventually, both metformin and NAC have important effects on hyperandrogenism, hyperinsulinemia and menstrual cyclicity in women with PCOS. Elnashar et al. have compared the effects of metformin and NAC on insulin and testesterone levels and ovulation success in women with clomiphene citrate resistant PCOS. In fact, clomiphene citrate is used in the first line treatment of PCOS as stimulator of ovulation. However, resistance against to the clomiphene citrate in PCOS women is obstructed to the possibility of pregnancy. As a consequence, it was suggested that metformin has more efficacy in ovulation rates (%51.6) and insulin sensitivity rather than NAC (124). Another clinical comparison trial between receiving metformin (500 mg three times daily) and NAC supplementation (600 mg three times daily) have applied along 24 weeks. Both groups had equal efficacy in terms of decreasing of BMI and free testesterone level, improvement of insulin sensitivity, menstrual cyclicity and lower hirsutism score. On the other hand, metformin administration caused a decrease in total cholesterol level while NAC supplementation led to reduction in both total cholesterol and LDL levels (125).

Omega 3 Fatty Acids are polyunsaturated fatty acids (PUFAs). α-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the most commnly known members in this group. Each fatty acid has distinct metabolic and endocrine properties that PUFAs intake can linked to reduced triglyceride whereas, monounsaturated fatty acids (MUFAs) consumption lead to decreased testesterone level (126). Functions of omega-3 fatty acids can be listed as reduction in oxidative stress, decreased hypertension, improving lipid profiles and anti-inflamatory activity and so have potential role against cardiovascular disease risk (127, 128). In recent years, omega 3 fatty acid is considered as therapeutical agent for healing of PCOS. It has considered that the healing mechanism of omega-3 is associated with regulation of abnormal gene expression in PCOS pathophysiology. For instance, applied different doses (25-100 μg) of omega-3 EPA into granulosa cell culture response to higher IGF-1 (insulin growth factor) expression and lower COX2 (cycloxogenase 2) expression. It is clear that IGF-1 is an essential compound of follicular differentiation and COX-2 contributes to oocyte maturation (129).

The relationship between insulin resistance and omega-3 supplementation have been discussed by various researchers due to inconsistent findings. However, a meta-analysis about the effects of omega-3 in the insulin resistance associated pathology of PCOS have claimed no association between intake of omega-3 and insulin sensitivity (130). Some clinical studies are briefly summarized in **Table 2** (131, 132, 133, 134, 135, 136).

Probiotics are living microbial dietary supplement found in dairy products and have synergism with the gut microbiota (137). Probiotics have beneficial effects in metabolism especially under inflamatory condition (138, 139). According to recent studies, probiotic consumption improves fasting blood glucose and antioxidant status in type 2 diabetes patients (140). In addition, Yadav et al. have shown that probiotic supplemented diet delayed the onset of glucose intolerence, hyperglycemia, hyperinsulinemia and dyslipidemia in diabetic rats (141). Shoaei et al. studied the effects of probiotic supplementation on pancreatic β cell and C reactive protein in PCOS patients using multispecies probiotics throughout 8 weeks. Results of their study showed that reduced fasting blood sugar and serum insulin levels in crude model. Interestingly, CRP levels did not significantly change (142).

On the other hand, etiology of PCOS has two pathological conditions including chronic state of inflamation and insulin resistance (143). Both conditions are associated with dysbiosis of gut microbiota (DOGMA) theory. Background of DOGMA involves an imbalance in gut microbiota ie increasing transition the Gram negative colonic bacteria into the systemic circulation. Therefore, chronic inflamatory response occurred in host. Inflammatory process affects insulin receptor function and PCOS associated pathways such as androgen biosynthesis. Therefore, to overcome the pathophysiological conditions of PCOS, probiotic supplements are recommended by some researchers (144, 145). In this point, Guo et al were applied the fecal microbiota translantation (FMT) and *lactobacillus* transplantation within PCOS rats. At the end of the study, they reported that all of the FMT group rats improved estrous cycle and most of the lactobacillus treated rats decreased androgen biosynthesis (146).

Conclusion

PCOS pathophysiology is associated with various defects which includes neuroendocrine defects, impaired ovarian steroidogenesis, insulin resistance and increased cortisol metabolism related adrenal hyperandrogenism. Although triggering cause of PCOS is currently unknown, androgens and insulin are thought to be two key factors in its pathogenesis. Therefore, treatment of PCOS is required to overcome both hyperandrogenism and hyperinsulinemia. Nutrients act as cofactors in maintaining functions of insulin and androgen receptors. In this study, we focus on the efficacy of nutrient supplementation in management of PCOS since almost all vitamin and mineral deficiencies are seen in PCOS. In this process, published clinical and experimental studies that met specified criteria were extract from the PubMed, Web of Science, EmBASE, Google Scholar data base for last twenty five years as accurately and precisely as possible. Articles were divided into treated nutrient groups which are vitamins, minerals, vitamin like substances and other nutrients and each substance was evaluated in

terms of treated dose, duration and effectiveness about removing PCOS complications. In addition, we summarized that supplementation of different vitamins, minerals and other supplements contribute to remove complications of PCOS (Figure 1).

Figure 1 indicates that interactions between genetic factors and some nutrient deficiencies cause to PCOS pathophysiology related symptoms such as elevated Hcy levels, oxidative stress, hyperandrogenism, hyperinsulinemia. Especially deficiencies of vitamin D, bioflavonoids, calcium, chromium, NAC, probiotics, magnesium, zinc and selenium are associated with insulin resistance. Therefore, the treatment of PCOS women with these supplements provide improvement for hyperinsulinemia and increased insulin sensitivity. On the other hand, inositol, vitamin A, carnitine, omega-3 fatty acids and NAC supplementations affect the hyperandrogenism. At this point, especially inositol and omega 3 supplementation help the recovery of PCOS with regard to metabolic and reproductive parameters. Apart from that, vitamin B6, B12 and folic acid have beneficial effect in abnormal Hcy levels and also vitamin E, α-LA, bioflavonoids, selenium, NAC and melatonin supplements help to remove from oxidative stress of PCOS. Nevertheless, the safety usage and effectiveness of the herbal medicine and nutrient supplementations except for inositol and omega 3 fatty acid have not clear and more study needs to be done about it (147).

A limitation of our study was the huge number of related articles published, but doses, type and combination of supplemented nutrients are extremely different from each other which depend on investigated group, thus it makes the evaluation of results difficult. Another limitation is the dose of nutrients used in those studies, as well as the insufficient diagnostic criteria used for the PCOS. In addition, each women with PCOS requires different supplementation depending appeared signs and physiological abnormalities. For instance, some patients suffer from infertility due to PCOS, others can suffer from endocrine and metabolic dysfunctions. However, most of the nutrient supplementation researches focus on metabolic aspects of PCOS. Therefore, this review mostly focus on the therapeutic affects on the metabolic and endocrine dysfunctions instead of infertility and it is a limiting factor in this study. Actually, molecular large scale up studies can be planned to illuminate the disrupted signaling pathways in PCOS. In this way, nutrients can be used effectively in management all aspects of PCOS via molecular targeting strategy.

In conclusion, vitamin or mineral supplements can exert beneficial effects on PCOS related symptoms such as immature oocytes, hyperinsulinemia, hyperandrogenism, increased BMI, cardiovascular disorders, mental and psycological problems.

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Conflict of interest

All authors declare that they have no conflict of interest.

Ethical approval

This study is a review paper and does not contain any studies with human participants or animals performed by any of the authors. Therefore, ethical considerations are not applicable.

References

1. Knochenhauer ES, Key TJ, Kahsar-Miller M, Waggoner W, Boots LR, Azziz R: Prevalence of the polycystic ovary syndrome in unselected black and white women of the southeastern United States: a prospective study. J Clin Endocrinol Metab 1998, 83:3078-3082.
2. Carreau AM, Baillargeon JP. PCOS in adolescence and type 2 diabetes. Curr Diab Rep 2015, 15:564.
3. Stein JD, Andrews C, Musch DC, Green C, Lee PP. Sight-Threatening Ocular Diseases Remain Underdiagnosed Among Children Of Less Affluent Families. Health Aff (Millwood) 2016, 35:1359-1366.
4. Rotterdam EA-SPcwg. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 2004, 19:41-47.
5. Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Futterweit W, Janssen OE, Legro RS, Norman RJ, Taylor AE, et al. The Androgen Excess and PCOS Society criteria for the polycystic ovary syndrome: the complete task force report. Fertil Steril 2009, 91:456-488.
6. Sheehan MT. Polycystic ovarian syndrome: diagnosis and management. Clin Med Res 2004, 2:13-27.
7. Sirmans SM, Pate KA. Epidemiology, diagnosis, and management of polycystic ovary syndrome. Clin Epidemiol 2013, 6:1-13.
8. Carmina E. Genetic and environmental aspect of polycystic ovary syndrome. J Endocrinol Invest 2003, 26:1151-1159.
9. Doi SA. Neuroendocrine dysfunction in PCOS: a critique of recent reviews. Clin Med Res 2008, 6:47-53.
10. Takayama K, Fukaya T, Sasano H, Funayama Y, Suzuki T, Takaya R, Wada Y, Yajima A. Immunohistochemical study of steroidogenesis and cell proliferation in polycystic ovarian syndrome. Hum Reprod 1996, 11:1387-1392.

11. Yaba A, Demir N. The mechanism of mTOR (mammalian target of rapamycin) in a mouse model of polycystic ovary syndrome (PCOS). J Ovarian Res 2012, 5: 38.
12. Diamanti-Kandarakis E, Dunaif A. Insulin resistance and the polycystic ovary syndrome revisited: an update on mechanisms and implications. Endocr Rev 2012, 33:981-1030.
13. Ehrmann DA. Polycystic ovary syndrome. N Engl J Med 2005, 352:1223-1236.
14. Kahn CR. Banting Lecture. Insulin action, diabetogenes, and the cause of type II diabetes. Diabetes 1994, 43:1066-1084.
15. Yu J, Yaba A, Kasiman C, Thomson T, Johnson J. mTOR controls ovarian follicle growth by regulating granulosa cell proliferation. PLoS One 2011, 6171: e21415.
16. Muscogiuri G, Policola C, Prioletta A, Sorice G, Mezza T, Lassandro A, Della Casa S, Pontecorvi A, Giaccari A. Low levels of 25(OH)D and insulin-resistance: 2 unrelated features or a cause-effect in PCOS? Clin Nutr 2012, 31:476-480.
17. Thomson RL, Spedding S, Buckley JD. Vitamin D in the aetiology and management of polycystic ovary syndrome. Clin Endocrinol (Oxf) 2012, 77:343-350.
18. Jakimiuk AJ, Szamatowicz J. (The role of inositol deficiency in the etiology of polycystic ovary syndrome disorders). Ginekol Pol 2014, 85:54-57.
19. Pu Y, Wang Z, Bian Y, Zhang F, Yang P, Li Y, Zhang Y, Liu Y, Fang F, Cao H, Zhang X. All-trans retinoic acid improves goat oocyte nuclear maturation and reduces apoptotic cumulus cells during in vitro maturation. Anim Sci J 2014, 85:833-839.
20. Deb GK, Dey SR, Bang JI, Lee JG, Kong IK. 9-cis Retinoic acid inhibits cumulus cell apoptosis during the maturation of bovine cumulus-oocyte-complexes. J Anim Sci 2012, 90:1798-1806.
21. Wood JR, Nelson VL, Ho C, Jansen E, Wang CY, Urbanek M, McAllister JM, Mosselman S, Strauss JF, 3rd. The molecular phenotype of polycystic ovary syndrome (PCOS) theca cells and new candidate PCOS genes defined by microarray analysis. J Biol Chem 2003, 278:26380-26390.
22. Wickenheisser JK, Nelson-DeGrave VL, Hendricks KL, Legro RS, Strauss JF, 3rd, McAllister JM. Retinoids and retinol differentially regulate steroid biosynthesis in ovarian theca cells isolated from normal cycling women and women with polycystic ovary syndrome. J Clin Endocrinol Metab 2005, 90:4858-4865.
23. Hahn S, Backhaus M, Broecker-Preuss M, Tan S, Dietz T, Kimmig R, Schmidt M, Mann K, Janssen OE. Retinol-binding protein 4 levels are elevated in polycystic ovary syndrome women with obesity and impaired glucose metabolism. European Journal of Endocrinology 2007, 157:201-207.
24. Tan BK, Chen J, Lehnert H, Kennedy R, Randeva HS. Raised serum, adipocyte, and adipose tissue retinol-binding protein 4 in overweight women with polycystic ovary syndrome: effects of gonadal and adrenal steroids. J Clin Endocrinol Metab 2007, 92:2764-2772.
25. Yarali H, Yildirir A, Aybar F, Kabakci G, Bukulmez O, Akgul E, Oto A. Diastolic dysfunction and increased serum homocysteine concentrations may contribute to increased cardiovascular risk in patients with polycystic ovary syndrome. Fertil Steril 2001, 76:511-516.
26. de la Calle M, Usandizaga R, Sancha M, Magdaleno F, Herranz A, Cabrillo E. Homocysteine, folic acid and B-group vitamins in obstetrics and gynaecology. Eur J Obstet Gynecol Reprod Biol 2003, 107:125-134.
27. Loverro G, Lorusso F, Mei L, Depalo R, Cormio G, Selvaggi L. The plasma homocysteine levels are increased in polycystic ovary syndrome. Gynecol Obstet Invest 2002, 53:157-162.
28. Badawy A, State O, El Gawad S, El Aziz OA: Plasma homocysteine and polycystic ovary syndrome: the missed link. Eur J Obstet Gynecol Reprod Biol 2007, 131:68-72.
29. Kaya C, Cengiz SD, Satiroglu H. Obesity and insulin resistance associated with lower plasma vitamin B12 in PCOS. Reprod Biomed Online 2009, 19:721-726.
30. Kazeroonı T, Asadi N, Dehbashi S, Zolghadri J. Effect of folic acid in women with and without insulin resistance who have hyperhomocysteinemic polycystic ovary syndrome. Int J Gynaecol Obstet 2008, 101:156-160.
31. Randeva HS, Lewandowski KC, Drzewoski J, Brooke-Wavell K, O'Callaghan C, Czupryniak L, Hillhouse EW, Prelevic GM. Exercise decreases plasma total homocysteine in overweight young women with polycystic ovary syndrome. J Clin Endocrinol Metab 2002, 87:4496-4501.
32. de Jager J, Kooy A, Lehert P, Wulffele MG, van der Kolk J, Bets D, Verburg J, Donker AJ, Stehouwer CD. Long term treatment with metformin in patients with type 2 diabetes and risk of vitamin B-12 deficiency: randomised placebo controlled trial. BMJ 2010, 340:c2181.
33. Kilicdag EB, Bagis T, Zeyneloglu HB, Tarim E, Aslan E, Haydardedeoglu B, Erkanli S.Homocysteine levels in women with polycystic ovary syndrome treated with metformin versus rosiglitazone: a randomized trial. Hum Reprod 2005, 20:894-899.
34. Kilicdag EB, Bagis T, Tarim E, Aslan E, Erkanli S, Simsek E, Haydardedeoglu B, Kuscu E. Administration of B-group vitamins reduces circulating homocysteine in polycystic ovarian syndrome patients treated with metformin: a randomized trial. Hum Reprod 2005, 20:1521-1528.

35. Palomba S, Falbo A, Giallauria F, Russo T, Tolino A, Zullo F, Colao A, Orio F. Effects of metformin with or without supplementation with folate on homocysteine levels and vascular endothelium of women with polycystic ovary syndrome. Diabetes Care 2010, 33:246-251.
36. Daughaday WH, Larner J, Hartnett C. The synthesis of inositol in the immature rat and chick embryo. J Biol Chem 1955, 212:869-875.
37. Papaleo E, Unfer V, Baillargeon JP, Chiu TT. Contribution of myo-inositol to reproduction. Eur J Obstet Gynecol Reprod Biol 2009, 147:120-123.
38. Unfer V, Carlomagno G, Dante G, Facchinetti F. Effects of myo-inositol in women with PCOS: a systematic review of randomized controlled trials. Gynecol Endocrinol 2012, 28:509-515.
39. Carlomagno G, De Grazia S, Unfer V, Manna F. Myo-inositol in a new pharmaceutical form: a step forward to a broader clinical use. Expert Opin Drug Deliv 2012, 9:267-271.
40. Dinicola S, Chiu TT, Unfer V, Carlomagno G, Bizzarri M. The rationale of the myo-inositol and D-chiro-inositol combined treatment for polycystic ovary syndrome. J Clin Pharmacol 2014, 54:1079-1092.
41. Carlomagno G, Unfer V, Roseff S. The D-chiro-inositol paradox in the ovary. Fertil Steril 2011, 95:2515-2516.
42. Nestler JE, Unfer V. Reflections on inositol(s) for PCOS therapy: steps toward success. Gynecol Endocrinol 2015, 31:501-505.
43. Nestler JE, Jakubowicz DJ, Reamer P, Gunn RD, Allan G. Ovulatory and metabolic effects of D-chiro-inositol in the polycystic ovary syndrome. N Engl J Med 1999, 340:1314-1320.
44. Baillargeon JP, Iuorno MJ, Jakubowicz DJ, Apridonidze T, He N, Nestler JE. Metformin therapy increases insulin-stimulated release of D-chiro-inositol-containing inositolphosphoglycan mediator in women with polycystic ovary syndrome. J Clin Endocrinol Metab 2004, 89:242-249.
45. Gerli S, Papaleo E, Ferrari A, Di Renzo GC. Randomized, double blind placebo-controlled trial: effects of myo-inositol on ovarian function and metabolic factors in women with PCOS. Eur Rev Med Pharmacol Sci 2007, 11:347-354.
46. Papaleo E, Unfer V, Baillargeon JP, De Santis L, Fusi F, Brigante C, Marelli G, Cino I, Redaelli A, Ferrari A. Myo-inositol in patients with polycystic ovary syndrome: a novel method for ovulation induction. Gynecol Endocrinol 2007, 23:700-703.
47. Genazzani AD, Lanzoni C, Ricchieri F, Jasonni VM. Myo-inositol administration positively affects hyperinsulinemia and hormonal parameters in overweight patients with polycystic ovary syndrome. Gynecol Endocrinol 2008, 24:139-144.
48. Costantino D, Minozzi G, Minozzi E, Guaraldi C. Metabolic and hormonal effects of myo-inositol in women with polycystic ovary syndrome: a double-blind trial. Eur Rev Med Pharmacol Sci 2009, 13:105-110.
49. Minozzi M, Costantino D, Guaraldi C, Unfer V. The effect of a combination therapy with myo-inositol and a combined oral contraceptive pill versus a combined oral contraceptive pill alone on metabolic, endocrine, and clinical parameters in polycystic ovary syndrome. Gynecol Endocrinol 2011, 27:920-924.
50. Morgante G, Orvieto R, Di Sabatino A, Musacchio MC, De Leo V. The role of inositol supplementation in patients with polycystic ovary syndrome, with insulin resistance, undergoing the low-dose gonadotropin ovulation induction regimen. Fertil Steril 2011, 95:2642-2644.
51. Alvarez JA, Ashraf A. Role of vitamin d in insulin secretion and insulin sensitivity for glucose homeostasis. Int J Endocrinol 2010, 2010:351385.
52. Krul-Poel YH, Snackey C, Louwers Y, Lips P, Lambalk CB, Laven JS, Simsek S. The role of vitamin D in metabolic disturbances in polycystic ovary syndrome: a systematic review. Eur J Endocrinol 2013, 169:853-865.
53. Yildizhan R, Kurdoglu M, Adali E, Kolusari A, Yildizhan B, Sahin HG, Kamaci M. Serum 25-hydroxyvitamin D concentrations in obese and non-obese women with polycystic ovary syndrome. Arch Gynecol Obstet 2009, 280:559-563.
54. Joham AE, Teede HJ, Cassar S, Stepto NK, Strauss BJ, Harrison CL, Boyle J, de Courten B. Vitamin D in polycystic ovary syndrome: Relationship to obesity and insulin resistance. Mol Nutr Food Res 2016, 60:110-118.
55. Selimoglu H, Duran C, Kiyici S, Ersoy C, Guclu M, Ozkaya G, Tuncel E, Erturk E, Imamoglu S. The effect of vitamin D replacement therapy on insulin resistance and androgen levels in women with polycystic ovary syndrome. J Endocrinol Invest 2010, 33:234-238.
56. Kotsa K, Yavropoulou MP, Anastasiou O, Yovos JG. Role of vitamin D treatment in glucose metabolism in polycystic ovary syndrome. Fertil Steril 2009, 92:1053-1058.
57. Irani M, Minkoff H, Seifer DB, Merhi Z. Vitamin D increases serum levels of the soluble receptor for advanced glycation end products in women with PCOS. J Clin Endocrinol Metab 2014, 99:E886-890.
58. Jafari-Sfidvajani S, Ahangari R, Hozoori M, Mozaffari-Khosravi H, Fallahzadeh H, Nadjarzadeh A. The effect of vitamin D supplementation in combination with low-calorie diet on anthropometric indices and androgen

hormones in women with polycystic ovary syndrome: a double-blind, randomized, placebo-controlled trial. J Endocrinol Invest 2017.
59. Palamanda JR, Kehrer JP. Involvement of vitamin E and protein thiols in the inhibition of microsomal lipid peroxidation by glutathione. Lipids 1993, 28:427-431.
60. Cicek N, Eryilmaz OG, Sarikaya E, Gulerman C, Genc Y. Vitamin E effect on controlled ovarian stimulation of unexplained infertile women. J Assist Reprod Genet 2012, 29:325-328.
61. Izadi A, Ebrahimi S, Shirzai S, Taghizadeh S, Parized M, Farzadi L, Gargari BP. Hormonal and metabolic effects of coenzyme q10 and/or vitamin E in patients with polycystic ovary syndrome. The Journal of Clinical Endocrinology & Metabolism 2018.
62. Ebrahimi FA, Samimi M, Foroozanfard F, Jamilian M, Akbari H, Rahmani E, Ahmadi S, Taghizadeh M, Memarzadeh MR, Asemi Z. The Effects of Omega-3 Fatty Acids and Vitamin E Co-Supplementation on Indices of Insulin Resistance and Hormonal Parameters in Patients with Polycystic Ovary Syndrome: A Randomized, Double-Blind, Placebo-Controlled Trial. Exp Clin Endocrinol Diabetes 2017, 125:353-359.
63. Biewenga GP, Haenen GR, Bast A. The pharmacology of the antioxidant lipoic acid. Gen Pharmacol 1997, 29:315-331.
64. Lee WJ, Koh EH, Won JC, Kim MS, Park JY, Lee KU. Obesity: the role of hypothalamic AMP-activated protein kinase in body weight regulation. Int J Biochem Cell Biol 2005, 37:2254-2259.
65. Masharani U, Gjerde C, Evans JL, Youngren JF, Goldfine ID. Effects of controlled-release alpha lipoic acid in lean, nondiabetic patients with polycystic ovary syndrome. J Diabetes Sci Technol 2010, 4:359-364.
66. Cianci A, Panella M, Fichera M, Falduzzi C, Bartolo M, Caruso S. d-chiro-Inositol and alpha lipoic acid treatment of metabolic and menses disorders in women with PCOS. Gynecol Endocrinol 2015, 31:483-486.
67. Ross JA, Kasum CM. Dietary flavonoids: bioavailability, metabolic effects, and safety. Annu Rev Nutr 2002, 22:19-34.
68. Oh JS, Kim H, Vijayakumar A, Kwon O, Choi YJ, Huh KB, Chang N. Association between dietary flavanones intake and lipid profiles according to the presence of metabolic syndrome in Korean women with type 2 diabetes mellitus. Nutr Res Pract 2016, 10:67-73.
69. Romualdi D, Costantini B, Campagna G, Lanzone A, Guido M. Is there a role for soy isoflavones in the therapeutic approach to polycystic ovary syndrome? Results from a pilot study. Fertil Steril 2008, 90:1826-1833.
70. Shah KN, Patel SS. Phosphatidylinositide 3-kinase inhibition: A new potential target for the treatment of polycystic ovarian syndrome. Pharm Biol 2016, 54:975-983.
71. Steiber A, Kerner J, Hoppel CL. Carnitine: a nutritional, biosynthetic, and functional perspective. Mol Aspects Med 2004, 25:455-473.
72. Fenkci SM, Fenkci V, Oztekin O, Rota S, Karagenc N. Serum total L-carnitine levels in non-obese women with polycystic ovary syndrome. Hum Reprod 2008, 23:1602-1606.
73. Vigerust NF, Bohov P, Bjorndal B, Seifert R, Nygard O, Svardal A, Glintborg D, Berge RK, Gaster M. Free carnitine and acylcarnitines in obese patients with polycystic ovary syndrome and effects of pioglitazone treatment. Fertil Steril 2012, 98:1620-1626 e1621.
74. Dunning KR, Robker RL. Promoting lipid utilization with l-carnitine to improve oocyte quality. Anim Reprod Sci 2012, 134:69-75.
75. Dumesic DA, Abbott DH. Implications of polycystic ovary syndrome on oocyte development. Semin Reprod Med 2008, 26:53-61.
76. Ismail AM, Hamed AH, Saso S, Thabet HH. Adding L-carnitine to clomiphene resistant PCOS women improves the quality of ovulation and the pregnancy rate. A randomized clinical trial. Eur J Obstet Gynecol Reprod Biol 2014, 180:148-152.
77. Jamilian H, Jamilian M, Samimi M, Afshar Ebrahimi F, Rahimi M, Bahmani F, Aghababayan S, Kouhi M, Shahabbaspour S, Asemi Z. Oral carnitine supplementation influences mental health parameters and biomarkers of oxidative stress in women with polycystic ovary syndrome: a randomized, double-blind, placebo-controlled trial. Gynecol Endocrinol 2017, 33:442-447.
78. Homa ST, Carroll J, Swann K. The role of calcium in mammalian oocyte maturation and egg activation. Hum Reprod 1993, 8:1274-1281.
79. Ullah G, Jung P, Machaca K. Modeling Ca2+ signaling differentiation during oocyte maturation. Cell Calcium 2007, 42:556-564.
80. Defelici M, Dolci S, Siracusa G. An Increase of Intracellular Free Ca2+ Is Essential for Spontaneous Meiotic Resumption by Mouse Oocytes. Journal of Experimental Zoology 1991, 260:401-405.
81. Gedik O, Zileli MS. Effects of hypocalcemia and theophylline on glucose tolerance and insulin release in human beings. Diabetes 1977, 26:813-819.
82. Mazloomi S, Sharifi F, Hajihosseini R, Kalantari S, Mazloomzadeh S. Association between Hypoadiponectinemia and Low Serum Concentrations of Calcium and Vitamin D in Women with Polycystic Ovary Syndrome. ISRN Endocrinol 2012, 2012:949427.

83. Ranjzad F, Mahban A, Shemirani AI, Mahmoudi T, Vahedi M, Nikzamir A, Zali MR. Influence of gene variants related to calcium homeostasis on biochemical parameters of women with polycystic ovary syndrome. J Assist Reprod Genet 2011, 28:225-232.
84. Firouzabadi R, Aflatoonian A, Modarresi S, Sekhavat L, MohammadTaheri S. Therapeutic effects of calcium & vitamin D supplementation in women with PCOS. Complement Ther Clin Pract 2012, 18:85-88.
85. Anderson RA. Chromium in the prevention and control of diabetes. Diabetes Metab 2000, 26:22-27.
86. Chakraborty P, Ghosh S, Goswami SK, Kabir SN, Chakravarty B, Jana K. Altered Trace Mineral Milieu Might Play An Aetiological Role in the Pathogenesis of Polycystic Ovary Syndrome. Biological Trace Element Research 2013, 152:9-15.
87. Lucidi RS, Thyer AC, Easton CA, Holden AE, Schenken RS, Brzyski RG. Effect of chromium supplementation on insulin resistance and ovarian and menstrual cyclicity in women with polycystic ovary syndrome. Fertil Steril 2005, 84:1755-1757.
88. Jamilian M, Asemi Z. Chromium Supplementation and the Effects on Metabolic Status in Women with Polycystic Ovary Syndrome: A Randomized, Double-Blind, Placebo-Controlled Trial. Ann Nutr Metab 2015, 67:42-48.
89. Amooee S, Parsanezhad ME, Ravanbod Shirazi M, Alborzi S, Samsami A. Metformin versus chromium picolinate in clomiphene citrate-resistant patients with PCOs: A double-blind randomized clinical trial. Iran J Reprod Med 2013, 11:611-618.
90. Amr N, Abdel-Rahim HE. The effect of chromium supplementation on polycystic ovary syndrome in adolescents. J Pediatr Adolesc Gynecol 2015, 28:114-118.
91. Touyz RM. Magnesium in clinical medicine. Front Biosci 2004, 9:1278-1293.
92. Saris NE, Mervaala E, Karppanen H, Khawaja JA, Lewenstam A. Magnesium. An update on physiological, clinical and analytical aspects. Clin Chim Acta 2000, 294:1-26.
93. Bresink I, Danysz W, Parsons CG, Mutschler E. Different Binding Affinities of Nmda Receptor-Channel Blockers in Various Brain-Regions - Indication of Nmda Receptor Heterogeneity. Neuropharmacology 1995, 34:533-540.
94. Iseri LT, French JH. Magnesium: nature's physiologic calcium blocker. Am Heart J 1984, 108:188-193.
95. Eby GA, Eby KL. Rapid recovery from major depression using magnesium treatment. Medical Hypotheses 2006, 67:362-370.
96. Song YQ, Manson JE, Cook NR, Albert CM, Buring JE, Liu SM. Dietary magnesium intake and risk of cardiovascular disease among women. American Journal of Cardiology 2005, 96:1135-1141.
97. Young LT, Robb JC, Levitt AJ, Cooke RG, Joffe RT. Serum Mg2+ and Ca2+/Mg2+ ratio in major depressive disorder. Neuropsychobiology 1996, 34:26-28.
98. Mirone M, Giannetta E, Isidori AM. Selenium and reproductive function. A systematic review. Journal of Endocrinological Investigation 2013, 36:28-36.
99. Coskun A, Arikan T, Kilinc M, Arikan DC, Ekerbicer HC. Plasma selenium levels in Turkish women with polycystic ovary syndrome. European Journal of Obstetrics & Gynecology and Reproductive Biology 2013, 168:183-186.
100. Rezvanfar MA, Rezvanfar MA, Ahmadi A, Shojaei-Saadi HA, Baeeri M, Abdollahi M. Molecular mechanisms of a novel selenium-based complementary medicine which confers protection against hyperandrogenism-induced polycystic ovary. Theriogenology 2012, 78:620-631.
101. Stapleton SR. Selenium: an insulin-mimetic. Cellular and Molecular Life Sciences 2000, 57:1874-1879.
102. Zadeh Modarres S, Heidar Z, Foroozanfard F, Rahmati Z, Aghadavod E, Asemi Z. The Effects of Selenium Supplementation on Gene Expression Related to Insulin and Lipid in Infertile Polycystic Ovary Syndrome Women Candidate for In Vitro Fertilization: a Randomized, Double-Blind, Placebo-Controlled Trial. Biol Trace Elem Res 2017.
103. Jamilian M, Razavi M, Kashan ZF, Ghandi Y, Bagherian T, Asemi Z. Metabolic response to selenium supplementation in women with polycystic ovary syndrome: a randomized, double-blind, placebo-controlled trial. Clinical Endocrinology 2015, 82:885-891.
104. Tubek S. Zinc supplementation or regulation of its homeostasis: advantages and threats. Biol Trace Elem Res 2007, 119:1-9.
105. Chausmer AB. Zinc, insulin and diabetes. J Am Coll Nutr 1998, 17:109-115.
106. Dimartino G, Matera MG, Demartino B, Vacca C, Dimartino S, Rossi F. Relationship between Zinc and Obesity. Journal of Medicine 1993, 24:177-183.
107. Bolkent S, Yanardag R, Bolkent S, Mutlu O, Yildirim S, Kangawa K, Minegishi Y, Suzuki H. The effect of zinc supplementation on ghrelin-immunoreactive cells and lipid parameters in gastrointestinal tissue of streptozotocin-induced female diabetic rats. Mol Cell Biochem 2006, 286:77-85.
108. Guler I, Himmetoglu O, Turp A, Erdem A, Erdem M, Onan MA, Taskiran C, Taslipinar MY, Guner H. Zinc and Homocysteine Levels in Polycystic Ovarian Syndrome Patients with Insulin Resistance. Biological Trace Element Research 2014, 158:297-304.

109. Ciaraldi TP, el-Roeiy A, Madar Z, Reichart D, Olefsky JM, Yen SS. Cellular mechanisms of insulin resistance in polycystic ovarian syndrome. J Clin Endocrinol Metab 1992, 75:577-583.
110. Dunaif A. Insulin resistance and the polycystic ovary syndrome: mechanism and implications for pathogenesis. Endocr Rev 1997, 18:774-800.
111. Beletate V, El Dib RP, Atallah AN. Zinc supplementation for the prevention of type 2 diabetes mellitus. Cochrane Database Syst Rev 2007:CD005525.
112. Legro RS, Kunselman AR, Dunaif A. Prevalence and predictors of dyslipidemia in women with polycystic ovary syndrome. Am J Med 2001, 111:607-613.
113. Ahmed Z, Tabrizi S, Li A, Houlden H, Sailer A, Lees AJ, Revesz T, Holton JL. A case of Huntington's disease phenocopy characterised by pallido-nigro-luysian degeneration with brain-iron accumulation and p62-positive glial inclusions. European Journal of Neurology 2010, 17:379-379.
114. Adriaens I, Jacquet P, Cortvrindt R, Janssen K, Smitz J. Melatonin has dose-dependent effects on folliculogenesis, oocyte maturation capacity and steroidogenesis. Toxicology 2006, 228:333-343.
115. Tamura H, Nakamura Y, Korkmaz A, Manchester LC, Tan DX, Sugino N, Reiter RJ. Melatonin and the ovary: physiological and pathophysiological implications. Fertil Steril 2009, 92:328-343.
116. Carlomagno G, Nordio M, Chiu TT, Unfer V. Contribution of myo-inositol and melatonin to human reproduction. Eur J Obstet Gynecol Reprod Biol 2011, 159:267-272.
117. Wei D, Zhang C, Xie J, Song X, Yin B, Liu Q, Hu L, Hao H, Geng J, Wang P. Supplementation with low concentrations of melatonin improves nuclear maturation of human oocytes in vitro. J Assist Reprod Genet 2013, 30:933-938.
118. Tamura H, Nakamura Y, Terron MP, Flores LJ, Manchester LC, Tan DX, Sugino N, Reiter RJ. Melatonin and pregnancy in the human. Reprod Toxicol 2008, 25:291-303.
119. Kim MK, Park EA, Kim HJ, Choi WY, Cho JH, Lee WS, Cha KY, Kim YS, Lee DR, Yoon TK. Does supplementation of in-vitro culture medium with melatonin improve IVF outcome in PCOS? Reproductive Biomedicine Online 2013, 26:22-29.
120. Dickinson DA, Moellering DR, Iles KE, Patel RP, Levonen AL, Wigley A, Darley-Usmar VM, Forman HJ. Cytoprotection against oxidative stress and the regulation of glutathione synthesis. Biol Chem 2003, 384:527-537.
121. Liu J, Liu M, Ye X, Liu K, Huang J, Wang L, Ji G, Liu N, Tang X, Baltz JM, et al. Delay in oocyte aging in mice by the antioxidant N-acetyl-L-cysteine (NAC). Hum Reprod 2012, 27:1411-1420.
122. Santini MT, Cametti C, Indovina PL, Peterson SW. Membrane electrical properties associated with insulin receptor downregulation in human erythrocytes. Exp Hematol 1994, 22:40-44.
123. Fulghesu AM, Ciampelli M, Muzj G, Belosi C, Selvaggi L, Ayala GF, Lanzone A. N-acetyl-cysteine treatment improves insulin sensitivity in women with polycystic ovary syndrome. Fertil Steril 2002, 77:1128-1135.
124. Elnashar A, Fahmy M, Mansour A, Ibrahim K. N-acetyl cysteine vs. metformin in treatment of clomiphene citrate-resistant polycystic ovary syndrome: a prospective randomized controlled study. Fertil Steril 2007, 88:406-409.
125. Oner G, Muderris, II. Clinical, endocrine and metabolic effects of metformin vs N-acetyl-cysteine in women with polycystic ovary syndrome. Eur J Obstet Gynecol Reprod Biol 2011, 159:127-131.
126. Vargas ML, Almario RU, Buchan W, Kim K, Karakas SE. Metabolic and endocrine effects of long-chain versus essential omega-3 polyunsaturated fatty acids in polycystic ovary syndrome. Metabolism 2011, 60:1711-1718.
127. Calder PC. n-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. Am J Clin Nutr 2006, 83:1505S-1519S.
128. Poudyal H, Panchal SK, Diwan V, Brown L. Omega-3 fatty acids and metabolic syndrome: effects and emerging mechanisms of action. Prog Lipid Res 2011, 50:372-387.
129. Shahnazi V, Zaree M, Nouri M, Mehrzad-Sadaghiani M, Fayezi S, Darabi M, Khani S, Darabi M. Influence of omega-3 fatty acid eicosapentaenoic acid on IGF-1 and COX-2 gene expression in granulosa cells of PCOS women. Iran J Reprod Med 2015, 13:71-78.
130. Akinkuolie AO, Ngwa JS, Meigs JB, Djousse L. Omega-3 polyunsaturated fatty acid and insulin sensitivity: a meta-analysis of randomized controlled trials. Clin Nutr 2011, 30:702-707.
131. Kasim-Karakas SE, Almario RU, Gregory L, Wong R, Todd H, Lasley BL. Metabolic and endocrine effects of a polyunsaturated fatty acid-rich diet in polycystic ovary syndrome. J Clin Endocrinol Metab 2004, 89:615-620.
132. Phelan N, O'Connor A, Kyaw Tun T, Correia N, Boran G, Roche HM, Gibney J. Hormonal and metabolic effects of polyunsaturated fatty acids in young women with polycystic ovary syndrome: results from a cross-sectional analysis and a randomized, placebo-controlled, crossover trial. Am J Clin Nutr 2011, 93:652-662.

133. Kalgaonkar S, Almario RU, Gurusinghe D, Garamendi EM, Buchan W, Kim K, Karakas SE. Differential effects of walnuts vs almonds on improving metabolic and endocrine parameters in PCOS. Eur J Clin Nutr 2011, 65:386-393.
134. Mohammadi E, Rafraf M. Benefits of omega-3 Fatty acids supplementation on serum paraoxonase 1 activity and lipids ratios in polycystic ovary syndrome. Health Promot Perspect 2012, 2:197-204.
135. Rafraf M, Mohammadi E, Asghari-Jafarabadi M, Farzadi L. Omega-3 Fatty Acids Improve Glucose Metabolism without Effects on Obesity Values and Serum Visfatin Levels in Women with Polycystic Ovary Syndrome. Journal of the American College of Nutrition 2012, 31:361-368.
136. Oner G, Muderris, II. Efficacy of omega-3 in the treatment of polycystic ovary syndrome. J Obstet Gynaecol 2013, 33:289-291.
137. Roberfroid MB. Prebiotics and probiotics: are they functional foods? Am J Clin Nutr 2000, 71:1682S-1687S; discussion 1688S-1690S.
138. Fuller R. Probiotics in Human Medicine. Gut 1991, 32:439-442.
139. Allen SJ, Jordan S, Storey M, Thornton CA, Gravenor M, Garaiova I, Plummer SF, Wang D, Morgan G. Dietary supplementation with lactobacilli and bifidobacteria is well tolerated and not associated with adverse events during late pregnancy and early infancy. J Nutr 2010, 140:483-488.
140. Ejtahed HS, Mohtadi-Nia J, Homayouni-Rad A, Niafar M, Asghari-Jafarabadi M, Mofid V. Probiotic yogurt improves antioxidant status in type 2 diabetic patients. Nutrition 2012, 28:539-543.
141. Yadav H, Jain S, Sinha PR. Antidiabetic effect of probiotic dahi containing Lactobacillus acidophilus and Lactobacillus casei in high fructose fed rats. Nutrition 2007, 23:62-68.
142. Shoaei T, Heidari-Beni M, Tehrani HG, Feizi A, Esmaillzadeh A, Askari G. Effects of Probiotic Supplementation on Pancreatic beta-cell Function and C-reactive Protein in Women with Polycystic Ovary Syndrome: A Randomized Double-blind Placebo-controlled Clinical Trial. Int J Prev Med 2015, 6:27.
143. Goodarzi MO, Dumesic DA, Chazenbalk G, Azziz R. Polycystic ovary syndrome: etiology, pathogenesis and diagnosis. Nature Reviews Endocrinology 2011, 7:219-231.
144. Ahmadi S, Jamilian M, Karamali M, Tajabadi-Ebrahimi M, Jafari P, Taghizadeh M, Memarzadeh MR, Asemi Z. Probiotic supplementation and the effects on weight loss, glycaemia and lipid profiles in women with polycystic ovary syndrome: a randomized, double-blind, placebo-controlled trial. Hum Fertil (Camb) 2017, 20:254-261.
145. Tremellen K, Pearce K. Dysbiosis of Gut Microbiota (DOGMA) - A novel theory for the development of Polycystic Ovarian Syndrome. Medical Hypotheses 2012, 79:104-112.
146. Guo Y, Qi Y, Yang X, Zhao L, Wen S, Liu Y, Tang L. Association between polycystic ovary syndrome and gut microbiota. Plos one 2016. 1-15.
147. Arentz S, Smith CA, Abbott J, Bensoussan A. Nutritional supplements and herbal medicines for women with polycystic ovary syndrome; a systematic review and meta-analysis. BMC Complementary and Alternative Medicine 2017, 17:500.

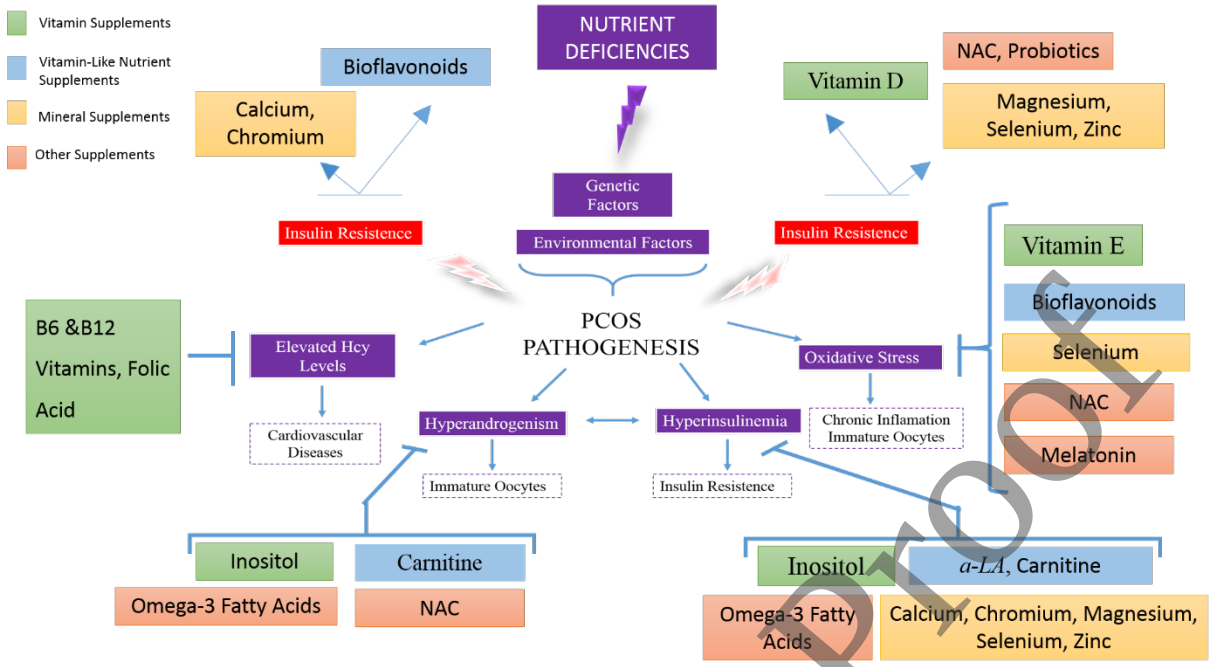

Figure 1. The effects of vitamins, minerals, vitamin-like substances and other supplements at PCOS pathophysiology.

Table 1. Effects of myo-inositol compounds within women with PCOS.

References	Patients	Treatment	Outcomes
Nestler et al, 1999	44 obese women with PCOS (Placebo group n=22, Inositol group n=22)	Oral administration of 1200 mg of D-chiro inositol per day during 7-8 weeks	• Plasma triglyceride ↓ • Diastolic and systolid pressure ↓ • DHEA-S ↓ • SHBG ↑
Baillargeon et al, 2004	19 obese women with PCOS	For 4-8 week, oral administration of metformin therapy (n=10) (500 mg orally thrice daily) and placebo group (n=9)	• Improvement of insulin mediated release of DCI-IPG
Gerli et al, 2007	92 women with oligomenorrhea and polycystic ovaries	During 12-16 weeks, 400 mcg folic acid intake in placebo group (n=47) and 400 mcg folic acid +4 gr inositol intake in treatment group (n=45)	• Higher ovulation rate • Weight loss • Folicular maturation • Circulating HDL ↑
Papaleo et al, 2007	25 women with PCOS who have oligo or amenorrhea since childbearing age	Myo-inositol + folic acid (inofolic) [2 gr twice a day] for 6 months	• Improvement in menstrual cyclicity • Replacement of healthy ovarian activity • Serum free testesterone ↓
Genazzani et al, 2008	20 overweight women with PCOS	Group A (n=10); 2 gr myo-inositol +200 μg folic acid per day Group B (n=10); 200 μg folic acid per day for 12 weeks	• Circulating LH, T, PRL and insulin level ↓ • Ratio of LH/FSH ↓ • Restroration of menstrual cyclicity
Costantino et al, 2001	42 women with PCOS from reproductive age (18-40)	Placebo group (n=19): 400 mcg folic acid alone; experiment group (n=23): 4 gr myo-inositol + 400 mcg folic acid during 12-16 weeks	• Insulin and androgen level ↓ • Improved glucose tolerance
Minozzi et al, 2011	155 women with PCOS	During 12 weeks, placebo group (n=75) oral contraceptive pills (OCP) intake while treatment group OCP + myo-inositol (4 gr/day) intake	• Insulin sensitivity ↑ • Recovery of hirsutism • Androgen synthesis ↓ • HDL cholesterol level ↑ • LDL cholesterol level ↓
Mongante et al, 2014	Insulin resistant women with PCOS (n=15)	Low dose step-down gonadotropin regimen + Redestop (1500 mg inositol, 100 mg lactoferrin)	• Improved clinical outcomes ↑ • Pregnancy rate • Number of follicles >15 mm in diameter ↓ • Cancellation rate ↓

Abbreviations: DHEA-S: Dehydroepiandestrone Sulfate, SHBG: Sex Hormone Binding Globulin, HDL: High Density Lipoprotein, LDL: Low Density Lipoprotein, T: Testesterone, LH: Luteinizing Hormone, PRL: Prolactin, FSH: Follicle Stimulating Hormone, ↓ decreasing, ↑ increasing

Table 2. According to the clinical studies, the role of omega-3 in the treatment of PCOS.

References	Subjects	Treatment	Outcomes
Kasım-Karakas et al, 2004	17 women with PCOS	Habitual diet process were followed during 3 months and then dietary fats were exchanged with PUFAs for another 3 months	Improvement of metabolic and endocrine parameters: • Fasting glucose and free fatty acid ↑ • Ketone bodies ↑
Phelan et al, 2011	126 women with PCOS	104 women with PCOS for asssessment of fatty acid metabolism in PCOS: (n=22) n-3 PUFA supplemented group which were used for evaluation of metabolic and endocrine parameters	• Atherogenic lipid profile ↓ • Bioavaliability of plasma testesterone concentration ↓
Kalgaonkar et al, 2011	32 women with PCOS	Subjects received 31 gr fats such as MUFA-riched almond (n=16) or PUFA-riched walnut (n=16) for 6 weeks	• Walnut group: increased adiponectin and leptin; decreased LDL cholesterol and ApoB • Almond group: increased adiponectin; decreased FAI
Mohammadi et al, 2012	64 PCOS women with 20-35 years old	Treatment group (n=32) received 4 gr/day omega-3 fatty acids and another 32 women were found in placebo group	Decreased cardiovascular risk: • Serum paraoxonase I activity ↑ • Improvement of lipid profile
Rafraf et al, 2012	61 overweight and obese PCOS patients with 20-35 years old	(n=30) treated group 1 gr/day n-3 capsules four times and another (n=31) women with PCOS were given placebo during 8 weeks	Improvement of insulin resistence associated parameters
Oner et al, 2013	45 non-obese patients with PCOS	Subjects were revealed 1,500 mg/d of omega-3 supplements during 6 months	• HOMA-IR and insulin level ↓ • LH and testesterone ↓ • SHBG and TNF-α ↑

Abbreviations: MUFA: Monounsaturated Fatty Acid, PUFA: Polyunsaturated Fatty Acid, SHBG: Sex Hormone Binding Globulin, TNF: Tumor Necrosis Factor, FAI: Fatty Acid Index, HDL: High Density Lipoprotein, LDL: Low Density Lipoprotein, T: Testesterone, LH: Luteinizing Hormone, FSH: Folicule Stimulating Hormone, ↓ decreasing, ↑ increasing