The Effects of Calcium, Vitamins D and K co-Supplementation on Markers of Insulin Metabolism and Lipid Profiles in Vitamin D-Deficient Women with Polycystic Ovary Syndrome

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ABSTRACT

Background Data on the effects of calcium, vitamins D and K co-supplementation on markers of insulin metabolism and lipid profiles among vitamin D-deficient women with polycystic ovary syndrome (PCOS) are scarce.

Objective This study was done to determine the effects of calcium, vitamins D and K co-supplementation on markers of insulin metabolism and lipid profiles in vitamin D-deficient women with PCOS.

Methods This randomized double-blind, placebo-controlled trial was conducted among 55 vitamin D-deficient women diagnosed with PCOS aged 18–40 years old. Subjects were randomly assigned into 2 groups to intake either 500 mg calcium, 200 IU vitamin D and 90 µg vitamin K supplements (n = 28) or placebo (n = 27) twice a day for 8 weeks.

Results After the 8-week intervention, compared with the placebo, joint calcium, vitamins D and K supplementation resulted in significant decreases in serum insulin concentrations (−1.9 ± 3.5 vs. +1.8 ± 6.6 µIU/mL, P = 0.01), homeostasis model of assessment-estimated insulin resistance (−0.4 ± 0.7 vs. +0.4 ± 1.4, P = 0.01), homeostasis model of assessment-estimated b cell function (−7.9 ± 14.7 vs. +7.0 ± 30.3, P = 0.02) and a significant increase in quantitative insulin sensitivity check index (+0.01 ± 0.01 vs. −0.008 ± 0.03, P = 0.01). In addition, significant decreases in serum triglycerides (−23.4 ± 71.3 vs. +9.9 ± 39.5 mg/dL, P = 0.03) and VLDL-cholesterol levels (−4.7 ± 14.3 vs. +2.0 ± 7.9 mg/dL, P = 0.03) was observed following supplementation with combined calcium, vitamins D and K compared with the placebo.

Conclusion Overall, calcium, vitamins D and K co-supplementation for 8 weeks among vitamin D-deficient women with PCOS had beneficial effects on markers of insulin metabolism, serum triglycerides and VLDL-cholesterol levels.

Introduction

Polycystic ovary syndrome (PCOS) is the commonest hyperandrogenic and dysmetabolic disorder which affects 6–10 % of women of child-bearing age [1, 2]. Subjects with PCOS have significantly higher rates of insulin resistance, impaired glucose tolerance, dyslipidemia, and metabolic syndrome than those subjects without the disease [3]. Insulin resistance and elevated lipid profiles occur in 50–75 % and 70 % of women with PCOS, respectively [4, 5]. Insulin resistance and dyslipidemia are central components of metabolic syndrome, and a significant risk factor for type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) [6].

Recently, the role of vitamins such as vitamin D and minerals including calcium, selenium and zinc in the developing of many diseases including PCOS has been evaluated [7, 8]. We have previously shown that supplementation with 1 000 mg/day calcium plus 50 000 IU/week vitamin D for 8 weeks among vitamin D-deficient subjects with PCOS had beneficial effects on markers of insulin metabolism, serum triglycerides and VLDL-cholesterol levels, but did

not influence fasting plasma glucose and other lipid profiles [9]. In addition, vitamins D, K and calcium co-supplementation for 12 weeks among diabetic patients with coronary heart disease (CHD) improved markers of insulin metabolism and HDL-cholesterol levels, but unchanged other lipid profiles [10]. However, vitamin D and calcium co-supplementation for 6 months did not affect insulin sensitivity, insulin secretion and β-cell function among multiethnic adults with low vitamin D status at risk of T2DM [11]. Moreover, vitamin K administration for 36 months at a dosage of 500 µg/day decreased insulin resistance among older men [12].

Vitamins D, K and calcium supplementation may improve metabolic status through their effects on up-regulation of the insulin receptor genes [13], the regulation of insulin secretion from the pancreatic beta-cell [14], and the enhancement of β-cell proliferation and adiponectin expression [15]. However, beneficial effects of combined vitamin D and calcium on markers of insulin metabolism and lipid profiles in PCOS subjects [9] and patients with T2DM [16] have previously reported, data on the effect of calcium, vitamins D and K co-supplementation on markers of insulin metabolism and lipid profiles among vitamin D-deficient subjects with PCOS are scarce. This study aimed to determine the effects of calcium, vitamins D and K co-supplementation on markers of insulin metabolism and lipid profiles in vitamin D-deficient subjects with PCOS.

Subjects and Methods

Trial design

This was an 8-week randomized, double-blind, placebo-controlled clinical trial.

Participants

Participants of this study were 55 PCOS women which were selected from the endocrinology and gynecology services of Iran University of Medical sciences (IUMS) from July 2016 to August 2016 at summer season. Diagnosis of PCOS was performed according to the Rotterdam criteria [17]: those with the 2 of the following criteria were considered as having PCOS: oligo- and/or anovulation (defined as delayed menses > 35 days or < 8 spontaneous hemorrhagic episodes/year), clinical (hirsutism using modified Ferriman-Gallwey score of ≥ 8) and/or biochemical signs of hyperandrogenism and polycystic ovaries (12 or more follicles in each ovary measuring 2–9 mm in diameter, and/or increased ovarian volume > 10 ml^3).

Ethics statements

This research was done in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines, and written informed consent was obtained from all subjects prior to the intervention. The current study was approved by the ethics committee of IUMS and was registered in the Iranian website for registration of clinical trials (http://www.irct.ir: IRCT201608115623N87).

Inclusion and exclusion criteria

Vitamin D-deficient (< 20 ng/mL) subjects with PCOS aged 18–40 years with phenotypes A (oligo-anovulation + hyperandrogenism + polycystic ovary morphology) and D (oligo-anovulation + polycystic ovary morphology) were included in the study. Exclusion criteria were pregnancy, adrenal gland disorders and/or other endocrine diseases and hormonal treatments in the previous 6 months.

Study design

At first, persons were randomly divided into 2 groups by random permuted blocks to intake either 500 mg calcium, 200 IU vitamin D plus 90 µg vitamin K supplements (n = 28) or placebo (n = 27) twice a day for 8 weeks. The randomized allocation sequence, enrolling subjects and assigning patients to the groups were conducted as blindness by a trained midwife. Calcium, vitamins D and K capsules and its placebos (cellulose) were provided by Arian Salamt Sina (Tehran, Iran) and Barij Essence Pharmaceutical Company (Kashan, Iran), respectively. At baseline, subjects were asked not to alter their routine physical activity or usual dietary intakes throughout the study and not to take any antioxidants supplements, anti-inflammatory medications and other medications that might affect their reproductive physiology during the 8-wk intervention [18]. All persons were recorded 3-day dietary records and 3 physical activity records at baseline, weeks 2, 4, 6 and 8 of intervention. To compute daily macro- and micro-nutrient intakes of persons according to 3-day food diaries, we used Nutritionist IV software (First Databank, San Bruno, CA) adjusted for Iranian foods.

Treatment adherence

Every 2 weeks, subjects were taken enough supplements and placebos and were instructed to return all unused supplements and placebos at each visit. Compliance to the take of calcium, vitamins D and K supplements and placebos was checked through asking participants to bring the medication containers and receiving short messages on their cell phones each day.

Assessment of anthropometric measures

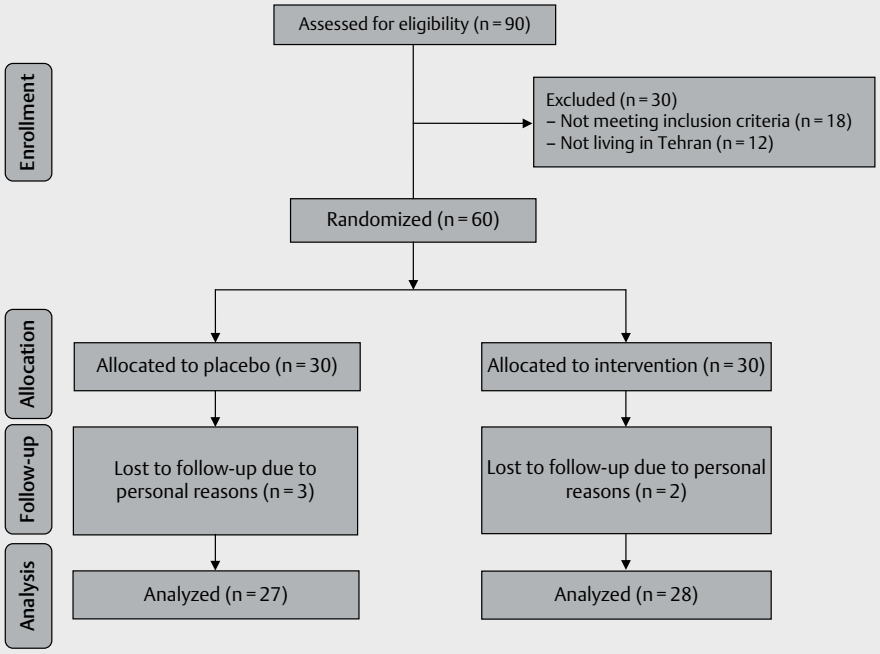
All patients were evaluated at the study start on the third day of a spontaneous or progesterone-induced menstrual cycle [18]. Anthropometric measures including height, weight (Seca, Hamburg, Germany) and body mass index (BMI) were quantified in the onset and the end of the study.

Assessment of outcomes

The primary outcome measurements were HOMA-IR in the current study. The secondary outcome measurements were lipid profiles.

Biochemical assessment

12-h fasting blood samples were taken by venipuncture at weeks 0 and 8 at the IUMS reference laboratory. Blood samples were taken based on a standard protocol and immediately centrifuged (Hettich D-78532, Tuttlingen, Germany). Then, the samples were stored at −80 °C until analysis at the IUMS reference laboratory. To evaluate fasting plasma glucose (FPG), serum triglycerides, VLDL-, total-, LDL- and HDL-cholesterol concentrations, we used available kits (Pars Azmun, Tehran, Iran) with enzymatic method. All inter- and intra-assay CVs for FPG and lipid fractions were lower than 5 %. Circulating concentrations of serum insulin were assessed using ELISA kit (Monobind, California, USA) with the intra- and inter-assay CVs 3.0 and 4.8 %, respectively. HOMA-IR, homeostatic model assessment for B-cell function (HOMA-B) and the quantitative insulin sensitivity check index (QUICKI) were calculated according to suggested formulas [19].



▶ **Fig. 1** Summary of patient flow diagram.

Sample size

To determine the sample size, we used a randomized clinical trial sample size formula where type one (α) and type 2 errors (β) were 0.05 and 0.20 (power = 80 %), respectively. Based on a previous study [10], we used 1.2 as SD and 1.0 as the difference in mean (d) of HOMA-IR as primary variable. Based on this, we needed 25 subjects in per group. Assuming a dropout of 5 persons in each group, we calculated to have 30 persons per group.

Statistical methods

To assess normal distribution of variables, we conducted the Kolmogrov-Smirnov test. To detect differences in the general characteristics, and daily macro- and micro-nutrient intakes between the 2 groups, we used independent samples Student's t test. To demonstrate the effects of calcium, vitamins D and K co-supplementation on glucose metabolism and lipid fractions, one-way repeated-measures ANOVA was used. To control the effect of confounders including baseline values of biochemical parameters, age and BMI at baseline, we applied ANCOVA. A P-value < 0.05 was considered statistically significant. All statistical analyses were conducted using the Statistical Package for Social Science version 18 (SPSS Inc., Chicago, Illinois, USA).

Results

At the screening visit, 850 participants were screened for PCOS. 760 participants of the 850 screened participants were excluded from the first visit due to not having PCOS. Thus, at baseline, we invited 90 women with PCOS according to Rotterdam criteria; however, 30 participants were excluded from the study because of not living in Tehran (n = 12) and not having inclusion criteria (n = 18) (▶ **Fig. 1**). Among participants in the calcium, vitamins D and K group, 2 participants (withdrawn due to personal reasons in the second month) and in the placebo group, 3 participants [withdrawn due to personal reasons (2 participants in the second month and 1 participant in the third month)] did not complete the trial. Finally, 55 participants [vitamins D and K co-supplements (n = 28) and placebo (n = 27)] completed the trial. On average, the rate of compliance in this trial was high, such that more than 90 % of tablets were taken throughout the study in both groups. No adverse events were reported following consumption of calcium, vitamins D and K co-supplements in participants with PCOS throughout the study.

Mean age, baseline weight and BMI, and end-of-trial weight and BMI were not significantly different between the 2 groups (▶ **Table 1**).

Based on the 3-day dietary records taken throughout the intervention, no significant change was observed between the 2 groups in terms of macro- and micro-nutrient intakes (Data not shown).

After the 8-week intervention, compared with the placebo, joint calcium, vitamins D and K supplementation resulted in significant decreases in serum insulin concentrations (− 1.9 ± 3.5 vs. + 1.8 ± 6.6 μIU/mL, P = 0.01), HOMA-IR (− 0.4 ± 0.7 vs. + 0.4 ± 1.4, P = 0.01), HOMA-B (− 7.9 ± 14.7 vs. + 7.0 ± 30.3, P = 0.02) and a significant increase in QUICKI (+ 0.01 ± 0.01 vs. − 0.008 ± 0.03, P = 0.01) (▶ **Table 2**). In addition, significant decreases in serum triglycerides (− 23.4 ± 71.3 vs. + 9.9 ± 39.5 mg/dL, P = 0.03) and VLDL-cholesterol levels (− 4.7 ± 14.3 vs. + 2.0 ± 7.9 mg/dL, P = 0.03) was observed following supplementation with combined calcium, vitamins D and K compared with the placebo. We did not see any significant change in FPG and other lipid profiles.

When we adjusted the analysis for baseline values of biochemical values, age and baseline BMI, QUICKI (P = 0.06) became non-significant, and other findings did not alter (▶ **Table 3**).

Discussion

We found that calcium, vitamins D and K co-supplementation among vitamin D-deficient subjects with PCOS for 8 weeks improved markers of insulin metabolism, serum triglycerides and VLDL-cholesterol levels compared with the placebo, but did not affect FPG and other lipid profiles.

There have been many studies indicating a significant relation between vitamin D concentration and beta cell function [20] and plasma glucose levels [21]. In addition, there is few evidence that low 25-hydroxyvitamin D concentrations are related to hyperinsulinemia in women with PCOS [22]; however, the findings are inconsistent. In line with our study, Tabesh et al. [16] found that supplementation with 1 000 mg/day calcium plus 50 000 IU/week vitamin D among patients with T2DM after 12 weeks improved glucose metabolism. Vitamin D supplementation (20 000 IU weekly for 24 weeks) also improved glucose metabolism and menstrual frequency in PCOS women [23]. Vitamin D and calcium co-supplementation for 4 months among women with PCOS resulted in a better outcome in a variety of PCOS symptoms including menstrual regularity and ovulation [24]. Furthermore, Pittas et al. [25] observed that co-supplementation with vitamin D and calcium for 3 years improved glucose homeostasis parameters in adults with normal glucose tolerance. In another study, vitamin K administration for 36 months at a dosage of 500 µg/day decreased HOMA-IR among older men, but it could not affect older women [12]. However, Pal et al. [26] observed no significant effect on glucose metabolism following supplementation with vitamin D and calcium in overweight vitamin D-deficient women with PCOS after 3 months. Previous studies have shown that impaired insulin metabolism are frequent findings in PCOS patients, and these traits have cause-consequence relationships with increased risk of CVD [27], T2DM [28] and dyslipidemia [29]. Therefore, based on our findings, we concluded that joint vitamins D-K-calcium supplementation due to improvement in insulin metabolism might help women with PCOS to control their insulin resistance. Vitamins D, K and calcium intake may improve markers of insulin metabolism through their effects on up-regulation of the insulin receptor genes [13], the regulation of insulin secretion from the pancreatic beta-cell [14], the enhancement of β-cell proliferation, adiponectin expression [15] and suppression of inflammation [30].

So far, several intervention studies, with divergent findings, have examined the effects of vitamin D or calcium administration on lipid concentrations [31, 32]. Major et al. [33] found that co-supplementation with 1 200 mg/day calcium and 400 IU vitamin D in overweight or obese women for 15 weeks improved lipid profiles. Unlike, other researchers [13, 14] did not find any significant effect of calcium-vitamin D supplementation on lipid profiles among postmenopausal women for 5 years [34] and 7 years [35]. Dyslipidemia is the most common metabolic abnormality in subjects with PCOS, although the type and extent of the derangement has been variable [36]. Elevated lipid profiles in subjects with PCOS would result

▶ **Table 1** General characteristics of study participants.

	Placebo group (n = 27)	Calcium, vitamins D and K group (n = 28)	P[1]
Age (y)	23.3 ± 3.4	23.5 ± 4.2	0.88
Height (cm)	163.0 ± 6.0	162.2 ± 4.2	0.57
Weight at study baseline (kg)	64.3 ± 8.5	63.8 ± 12.5	0.85
Weight at end-of-trial (kg)	64.1 ± 8.1	63.7 ± 12.3	0.89
Weight change (kg)	− 0.2 ± 1.6	− 0.1 ± 1.0	0.63
BMI at study baseline (kg/m^2)	24.3 ± 3.9	24.2 ± 4.8	0.95
BMI at end-of-trial (kg/m^2)	24.2 ± 3.7	24.2 ± 4.7	0.99
BMI change (kg/m^2)	− 0.1 ± 0.6	− 0.03 ± 0.4	0.58

Data are means ± SDs; [1] Obtained from independent t test

▶ **Table 2** The effect of calcium, vitamins D and K and calcium co-supplementation on markers of insulin metabolism and lipid profiles in PCOS patients[1].

	Placebo group (n = 27)			Calcium, vitamins D and K group (n = 28)			P[2]
	Baseline	End-of-trial	Change	Baseline	End-of-trial	Change	
Vitamin D (ng/mL)	14.8 ± 3.9	14.5 ± 5.0	− 0.3 ± 4.3	14.7 ± 2.5	20.0 ± 3.0	5.3 ± 1.9	< 0.001
Calcium (mg/dL)	9.4 ± 0.6	9.3 ± 0.7	− 0.1 ± 0.3	9.3 ± 0.5	9.5 ± 0.5	0.2 ± 0.5	0.02
FPG (mg/dL)	83.6 ± 13.0	88.6 ± 20.5	5.0 ± 19.7	84.2 ± 6.9	84.5 ± 6.7	2.1 ± 7.5	0.46
Insulin (µIU/mL)	10.4 ± 5.3	12.2 ± 6.1	1.8 ± 6.6	11.8 ± 4.7	9.9 ± 3.7	− 1.9 ± 3.5	0.01
HOMA-IR	2.1 ± 1.0	2.5 ± 1.3	0.4 ± 1.4	2.4 ± 1.0	2.0 ± 0.8	− 0.4 ± 0.7	0.01
HOMA-B	44.7 ± 30.9	51.7 ± 30.0	7.0 ± 30.3	47.9 ± 19.9	40.0 ± 15.9	− 7.9 ± 14.7	0.02
QUICKI	0.34 ± 0.02	0.34 ± 0.02	− 0.008 ± 0.03	0.33 ± 0.02	0.34 ± 0.01	0.007 ± 0.01	0.01
Triglycerides (mg/dL)	108.5 ± 49.6	118.4 ± 43.1	9.9 ± 39.5	107.1 ± 90.7	83.7 ± 41.1	− 23.4 ± 71.3	0.03
VLDL-cholesterol (mg/dL)	21.7 ± 9.9	23.7 ± 8.6	2.0 ± 7.9	21.4 ± 18.1	16.7 ± 8.2	− 4.7 ± 14.3	0.03
Total cholesterol (mg/dL)	159.0 ± 36.9	159.1 ± 30.5	0.1 ± 31.2	151.6 ± 30.9	144.7 ± 27.2	− 6.9 ± 25.8	0.36
LDL-cholesterol (mg/dL)	82.4 ± 29.4	77.2 ± 28.3	− 5.2 ± 32.9	74.4 ± 24.1	70.6 ± 21.2	− 3.8 ± 20.9	0.85
HDL-cholesterol (mg/dL)	55.0 ± 11.9	58.3 ± 18.4	3.3 ± 14.3	55.7 ± 9.8	57.4 ± 8.5	1.7 ± 5.1	0.55

[1] All values are means ± SDs; [2] Obtained from repeated measures ANOVA test; FPG, fasting plasma glucose; HOMA-IR, homeostasis model of assessment-estimated insulin resistance; HOMA-B, homeostasis model of assessment-estimated b cell function; QUICKI, quantitative insulin sensitivity check index

▶ **Table 3** Adjusted changes in markers of insulin metabolism and lipid profiles in PCOS patients[1].

	Placebo group (n = 27)	Calcium, vitamins D and K group (n = 28)	P[2]
Vitamin D (ng/mL)	−0.3 ± 0.6	5.3 ± 0.6	< 0.001
Calcium (mg/dL)	−0.1 ± 0.1	0.2 ± 0.1	0.03
FPG (mg/dL)	5.4 ± 2.8	1.8 ± 2.8	0.35
Insulin (μIU/mL)	1.5 ± 0.9	−1.5 ± 0.9	0.01
HOMA-IR	0.3 ± 0.2	−0.3 ± 0.2	0.01
HOMA-B	6.3 ± 3.9	−7.3 ± 3.8	0.01
QUICKI	−0.006 ± 0.004	0.005 ± 0.004	0.06
Triglycerides (mg/dL)	10.3 ± 6.5	−23.8 ± 6.4	< 0.001
VLDL-cholesterol (mg/dL)	2.1 ± 1.3	−4.7 ± 1.3	< 0.001
Total cholesterol (mg/dL)	2.0 ± 4.6	−8.8 ± 4.5	0.10
LDL-cholesterol (mg/dL)	−2.9 ± 4.4	−6.1 ± 4.3	0.59
HDL-cholesterol (mg/dL)	3.3 ± 2.1	1.7 ± 2.0	0.57

[1] All values are means ± SEs; [2] Obtained from ANCOVA test adjusted for baseline values, age and BMI at baseline; FPG, fasting plasma glucose; HOMA-IR, homeostasis model of assessment-estimated insulin resistance; HOMA-B, homeostasis model of assessment-estimated b cell function; QUICKI, quantitative insulin sensitivity check index

in increased risk of CVD [37]. Several mechanisms can explain the beneficial effects of combined calcium-vitamin D supplementation on serum triglycerides and VLDL-cholesterol levels. Calcium intake might result in reduced absorption of fatty acids and increased fecal fatty acid content through formation of insoluble calcium-fatty soaps in the gut [38], which in turn decrease serum triglycerides and VLDL-cholesterol levels. In addition, increased intracellular calcium in liver results in stimulating microsomal triglycerides transfer protein (MTP), and then causes decreased serum triglycerides and VLDL-cholesterol levels [39]. Moreover, vitamin D suppresses apo A1 gene expression at the transcriptional levels [40], which may result in decreased levels of triglycerides and VLDL-cholesterol.

This trial had some limitations. Due to limited funding, we did not evaluate the effects of calcium, vitamins D and K co-supplementation on serum vitamin K concentrations. Another limitation was that we did not assess gene expression related to insulin and lipid. It must be kept in mind that in the present study, we used the dosages of 400 IU vitamin D, 1 000 mg calcium plus 180 μg vitamin K per day based on observed beneficial effects of combined vitamin D, K and calcium supplementation on metabolic profiles in a previous study in overweight diabetic patients with CHD [10]. However, several studies have used higher doses of vitamin D in PCOS women [41, 42], we agree that future studies are needed to confirm our findings. In addition, our study was relatively of short duration of intervention for sustainable changes in the predefined outcome parameters. Long-term interventions might result in greater changes in circulating levels of metabolic profiles. It must be considered that we had not data about the time of sunlight exposure at baseline, end-of-trial and throughout the intervention. Although we believe that this status can affect our findings, this should be taken into account in the interpretation of our findings.

Overall, calcium, vitamins D and K co-supplementation for 8 weeks among vitamin D-deficient women with PCOS had beneficial effects on markers of insulin metabolism, serum triglycerides and VLDL-cholesterol levels, but it did not affect fasting plasma glucose and other lipid profiles.

Authors' Contributions

ZA contributed in conception, design, statistical analysis and drafting of the manuscript. MK, MA, M-SR, MJ, MK and MA contributed in data collection and manuscript drafting. ZA supervised the study.

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Clinical trial registration number

www.irct.ir: IRCT201608115623N87.

Conflicts of Interest

No conflicted.

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