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ORIGINAL ARTICLE

The effect of vitamin K1 supplementation on sensitivity and insulin resistance via osteocalcin in prediabetic women: a double-blind randomized controlled clinical trial

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BACKGROUND/OBJECTIVES: A relationship between osteocalcin (OC) levels and factors associated with energy metabolism and insulin resistance has been reported recently. The aim of this study was to investigate whether modulation of ostecalcin isoforms via vitamin K1 supplementation would affect glucose metabolism or insulin sensitivity in prediabetic and premenopause women.
SUBJECTS/METHODS: Eighty-two prediabetic women were randomized to consume vitamin K1 supplement ($n = 39$) or placebo ($n = 43$) for 4 weeks. Participants in the vitamin K1 supplement group received one pearl softgel capsule containing 1000 μm of phylloquinone, and the placebo group received one placebo capsule daily for 4 weeks. Blood samples were collected at baseline and after the 4-week intervention period to quantify carboxylated OC (cOC), undercarboxylated OC (ucOC) and relevant variables.
RESULTS: Phylloquinone supplementation increased the serum levels of cOC and decreased ucOC, compared with placebo (12.53 ± 5.95 compared with 7.43 ± 4.85 ng/ml and 2.47 ± 1.91 compared with 4.79 ± 2.43 ng/ml, respectively; $P < 0.001$). Furthermore, intake of phylloquinone supplement led to significant decreases in %ucOC (17.97 ± 12.24 compared with 43.80 ± 19.86) and 2-h post-oral glucose tolerance test (OGTT) glucose (7.32 ± 1.50 compared with 8.62 ± 1.45 mmol/l), and 2- h post-OGTT insulin level (80.34 ± 42.24 compared with 112.43 ± 53.19 μIU/ml) and increased insulin sensitivity index (2.46 ± 0.71 compared with 1.75 ± 0.61) compared with placebo. Overall, a significant association was found between changes in %ucOC and changes in 2-h post-OGTT glucose ($r = 0.308$, $P = 0.028$).
CONCLUSIONS: The results of this study demonstrated that vitamin K1 supplementation for 4 weeks did not affect insulin resistance in premenopausal and prediabetic women but had beneficial effects on glycemic status and insulin sensitivity.

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INTRODUCTION

With regard to the hypothesis that the skeleton may act as an endocrine organ to regulate energy metabolism and the key concept of this hypothesis is that the regulation is mediated by osteocalcin (OC), the possible roles of OC in glucose metabolism were investigated in this study.[1] OC is a vitamin K-dependent calcium binder that carries three γ-carboxylated glutamic acid residues (Gla), which are known to mediate strong binding of OC to hydroxyapatite. Vitamin K stimulates the γ-carboxylation of Gla residues. Total OC (tOC) includes both carboxylated OC (cOC) and undercarboxylated OC (ucOC) forms. Many nutritional studies have established that circulating ucOC is a measure of the vitamin K status. Serum percentage of ucOC increases in response to vitamin K depletion and decreases in response to vitamin K supplementation.[2] Studies suggested that OC could increase β-cell proliferation in islets and increase adiponectin expression in adipocytes, indicating that OC is involved in glucose metabolism by improving both β-cell function and insulin sensitivity.[1] Moreover, several epidemiological studies have shown that serum OC levels are negatively associated with body mass index (BMI), fat mass, blood glucose, insulin concentrations and insulin resistance.[3–5] However, there is a controversy over the kind of OC that is involved in glucose metabolism. According to the results of Lee *et al.*'s[1] study on genetically modified mice, only the undercarboxylated isoform of OC functions as a hormone that regulates glucose metabolism and fat mass. Kanazawa *et al.*[6] reported that OC is positively associated with both insulin sensitivity and insulin secretion in Japanese patients with type 2 diabetes mellitus. Hwang *et al.*[7] reported that ucOC is related to enhanced β-cell functions, whereas cOC leads to improved insulin sensitivity in Korean middle-aged male subjects. In contrast, some studies did not find any association between tOC or ucOC and glucose levels in subjects with normal glucose tolerance.[8–11] With regard to the fact that the majority of previous studies have focused on healthy people or elderly diabetic patients, this study investigated whether modulation of OC isoforms via vitamin K1 supplementation (with no medication) would affect glucose metabolism or insulin sensitivity in premenopausal and prediabetic women.

MATERIALS AND METHODS

Subjects

A randomized, double-blinded, placebo-controlled clinical trial was designed and conducted over a total period of 4 weeks. This clinical trial

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was approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences. Informed consent was obtained from all study participants. Premenopausal women with diagnosed prediabetes aged 22–45 years and with a BMI of 18.5–30 kg/m^2 were eligible for the study. Subjects were recruited on the basis of the results of a standard 75-g oral glucose tolerance test (OGTT) at screening (blood drawn in the 0 and 120 min), according to the American Diabetes Association criteria:[12] Prediabetes was diagnosed according to the criteria established by the American Diabetes Association[12]—that is, impaired fasting glucose (5.6 mmol/l < fasting blood glucose (FBG) < 7 mmol/l) or impaired glucose test (7.8 mmol/l < glucose120 min < 11.1 mmol/l). A total of 82 prediabetic women met the inclusion criteria. None of the subjects suffered from rheumatic, thyroid, parathyroid, kidney or liver diseases, pregnancy, lactation, menopause and taking drugs known to influence glucose, vitamin K and bone metabolism, lipid-lowering drugs, warfarin, corticosteroids, vitamin and mineral supplements within the 6 months before the study. Overall, 82 women were randomized to consume vitamin K1 supplement ($n = 39$) or placebo ($n = 43$) for 4 weeks. Participants in the vitamin K1 supplement group received one pearl softgel capsule containing 1000 μg of phylloquinone (DSM Nutritional Products, Inc., Basel, Switzerland), whereas the placebo group received one placebo capsule (Barij Essence co., Kashan, Iran) daily for 4 weeks. Placebo capsules were similar in color, shape, size appearance and packaging and were indistinguishable for participants and investigators. Soy oil was used in both supplement and placebo capsules. The participants were asked to maintain their habitual food consumption and physical activity pattern throughout the study and not to consume any supplements other than the item provided to them by the investigator. Dietary intake was assessed using a 3-day food record consisting of three nonconsecutive days, including two working days and one weekend. The dietary records were based on estimated values in household measures. To obtain the mean of nutrient intakes of participants on the basis of these 3-day food diaries, Nutritionist IV software (First Databank Division, The Hearst Corporation, San Bruno, CA, USA) modified for Iranian foods was used.

Assessment of variables

Body weight was measured to the nearest 0.1 kg after overnight fasting, without shoes and wearing minimal clothing, by the use of a digital scale (Seca). Height was measured to the nearest 0.1 cm by using a nonstretched tape measure (Seca). BMI was calculated as weight in kilograms divided by height in meters squared (kg/m^2). Body fat was measured by OMRON BF 306 (OMRON HEALTHCARE (DALIAN) CO., LTD., Dalian, China) body fat monitor using bioelectric impedance method. Blood samples (10 ml) were collected at baseline and after the 4-week intervention period. After an overnight fast, 75 g of glucose was given to each subject between 0800 and 1000 hours. Blood glucose was determined at times 0 and 120 min after OGTT on the same day. Then, the remaining separated serum was stored at −70 °C before analysis in the laboratory of Ahvaz Jundishapur University of Medical Sciences.

Serum cOC and ucOC were quantitatively determined using a commercially available enzyme immunoassay (EIA) kit (Gla-type Osteocalcin EIA kit, cat. no. MK111 and Undercarboxylated osteocalcin EIA kit, cat. no. MK118 Takara Bio Inc., Japan, respectively). The intra- and inter-assay coefficient of variations (CVs) for serum cOC and ucOC were 3.3% and 1% and 4.58% and 5.67%, respectively. Total OC was estimated as the sum of cOC and ucOC. Serum insulin was assayed by using an enzyme-linked immunosorbent assay kit (Diaplus, San Francisco, CA, USA), and intra- and inter-assay CVs for insulin were 4.9% and 4.9% respectively. FBG and 2-h post-OGTT glucose were measured using auto-analyser (Hitachi, Holliston, MA, USA). Insulin resistance was calculated with homeostasis model assessment-estimated insulin resistance (HOMA-IR), which was defined as follows: HOMA-IR = (FBG (mg/dl) × fasting insulin (FINS (μU/ml))/405. Basal insulin secretion was calculated by using the formula HOMA-%B:360 × FINS(μU/ml)/(FBG-63)[13] and insulin sensitivity index was calculated using the formula (ISI)(composite):10 000/$\sqrt{}$(FBS × FINS × 2-h post-OGTT glucose × 2-h post-OGTT insulin).[14,15]

Statistical analyses

Data are expressed as means ± s.d. The normality of data distribution was assessed by using the Kolmogorov–Smirnov goodness-of-fit test. Analysis of covariance was used to assess differences between the treatment groups at the 4-week postintervention period, adjusted for baseline values. Between-group comparison of baseline and postintervention period was done by independent-samples Student's *t*-test. The within-groups comparison of values was performed by the paired-samples *t*-test. Correlations between variables were evaluated by using Pearson's (*r*) correlation. All statistical analyses were done by using the Statistical Package for Social Sciences (SPSS version 16; SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered as the statistical significance level.

RESULTS

The means and s.d. of age, weight and BMI were 40.17 ± 4.9 years, 71 ± 6.5 kg and 28.08 ± 1.65 kg/m^2, respectively. Baseline and end-of-trial means of weight, BMI and body fat were not significantly different between vitamin K1 and placebo groups (Table 1). Table 2 shows dietary intakes of energy and relevant nutrients. No significant difference was observed between two groups in baseline and end-of-trial dietary variables (Table 2). Baseline values of cOC, ucOC, %ucOC, tOC, FBS, 2-h post-OGTT glucose, fasting and 2-h post-OGTT insulin, HOMA-IR, homeostasis model assessment β-cell function (HOMA-B) and ISI were not different between two groups. Vitamin K1 supplementation had a significant effect on cOC, ucOC, %ucOC, 2-h post-OGTT glucose and ISI ($P < 0.001$, for all variables). As expected, phylloquinone supplementation resulted in increased cOC and decreased ucOC serum levels, compared with placebo (12.53 ± 5.95 compared with 3.60 ± 3.49 ng/ml and 2.47 ± 1.91 compared with 4.79 ± 2.43 ng/ml, respectively; $P < 0.001$ (Table 3)). Furthermore, intake of phylloquinone supplement led to a significant decrease in %ucOC (17.97 ± 12.24 compared with 43.80 ± 19.86) and 2-h post-OGTT glucose (7.32 ± 1.50 compared with 8.62 ± 1.45 mmol/L), and 2 h post-OGTT insulin level (80.34 ± 42.24 compared with 112.43 ± 53.19 μIU/ml) and increased ISI (2.46 ± 0.71 compared with 1.75 ± 0.61) compared with placebo. No significant effect on serum tOC, FBS, fasting insulin, HOMA-IR and HOMA-B was observed from phylloquinone supplementation (Table 3). To evaluate the relationship between changes in different forms of osteocalcin and changes of glycemic and insulin status, Pearson's correlation analyses were done between cOC, ucOC, tOC and % ucOC with glucose and insulin homeostasis variables (Table 4). Overall, a significant association was found between changes in % ucOC and changes in 2-h post-OGTT glucose ($r = 0.308$, $P = 0.028$).

Table 1. General characteristics of prediabetic premenopausal women who received either vitamin K1 supplement or placebo

	Placebo group (n = *43)*	*Phylooquinone group* (n = *39)*	P-*value*[a]
Age (years)	40.09 ± 4.65	40.25 ± 5.32	0.88
BMI (kg/m^2) at study baseline	27.93 ± 1.53	28.34 ± 1.72	0.25
BMI (kg/m^2) at end of trial	27.91 ± 1.61	28.19 ± 1.80	0.46
Weight (kg) at study baseline	71.09 ± 6.59	71.21 ± 6.47	0.93
Weight (kg) at end of trial	71 ± 6.76	70.72 ± 6.39	0.84
Fat mass (%) at study baseline	38.55 ± 3.99	38.77 ± 3.86	0.79
Fat mass (%) at end of trial	38.57 ± 4.10	38.46 ± 4.05	0.90

Abbreviation: BMI, body mass index. All values are means ± s.d. [a]Obtained from independent-samples *t*-test.

Table 2. Dietary intakes of prediabetic premenopausal women who received either vitamin K1 supplements or placebo before and after the intervention

	Week 0			*Week 4*		
	Placebo group (n = *43*)	*Phyloquinone group* (n = *39*)	P[a]	*Placebo group* (n = *43*)	*Phyloquinone group* (n = *39*)	P[a]
Energy (kJ/day)	7444.1 ± 1197.4	7582.29 ± 1142.9	0.59	7452.5 ± 996.4	7607.4 ± 916.9	0.47
Carbohydrate (g/day)	246.64 ± 44.94	252.13 ± 4418	0.57	245.03 ± 38.73	249.96 ± 40.29	0.56
Protein (g/day)	73.81 ± 12.31	76.48 ± 11.39	0.31	73.50 ± 10.63	76.21 ± 9.02	0.21
Fat (g/day)	64.71 ± 9.18	66.17 ± 9.36	0.48	65.37 ± 10.19	66.91 ± 11.16	0.51
Vitamin K (μg/day)	55.55 ± 17.73	60.94 ± 14.13	0.13	55.23 ± 18.12	61.22 ± 13.71	0.10
Vitamin D (mg/day)	3.71 ± 1.54	4.14 ± 1.64	0.22	3.79 ± 1.35	4.17 ± 1.47	0.22
Calcium (mg/day)	710.53 ± 238.73	692.73 ± 220.81	0.72	696.12 ± 219.12	686.71 ± 236.87	0.85

All values are means ± s.d. [a]Obtained from independent-samples *t*-test.

Table 3. Metabolic variables, biomarkers of insulin resistance and comparison of variables within and between placebo and phyloquinone groups in prediabetic premenopausal women

	Placebo group (n = *43*)		*Phyloquinone group* (n = *39*)		P-*value*[a]
	Week 0	*Week 4*	*Week 0*	*Week 4*	
cOC (ng/ml)	7.17 ± 5.07	7.43 ± 4.85	8.93 ± 4.88	12.53 ± 5.95[b]	0.00
ucOC (ng/ml)	4.77 ± 2.49	4.79 ± 2.43	5.57 ± 2.34	2.47 ± 1.91[b]	0.00
tOC	11.95 ± 12.08	12.08 ± 5.65	14.50 ± 15.01	15.01 ± 6.51	0.387
%ucOC	44.58 ± 21.11	43.80 ± 19.86	42.33 ± 20.67	17.97 ± 12.24[b]	0.00
FBG (mmol/l)	5.58 ± 0.751	5.79 ± 0.68	5.93 ± 0.59	5.73 ± 0.62	0.51
2-h post-OGTT glucose (mmol/l)	8.55 ± 0.88	8.62 ± 1.45	7.92 ± 1.94	7.32 ± 1.50	0.00
FINS (μIU/ml)	23.83 ± 12.08	23.80 ± 8.28	20.74 ± 9.90	20.56 ± 8.04	0.208
2-h post-OGTT INS (μIU/ml)	106.55 ± 46.96	112.43 ± 53.19	97.80 ± 54.5	80.34 ± 42.24[b]	0.001
HOMA-IR	6.27 ± 3.62	6.22 ± 2.40	5.50 ± 2.65	5.28 ± 2.08	0.14
HOMA-B	226.09 ± 167.53	220.66 ± 105.76	178.18 ± 104.80	193.14 ± 98.47	0.906
ISI	1.89 ± 0.78	1.75 ± 0.61	2.31 ± 1.17	2.46 ± 0.71	0.00

Abbreviations: cOC, carboxylated OC; FBG, fasting blood glucose; FINS, fasting insulin; HOMA-B, homeostatic model assessment β-cell function; HOMA-IR, homeostasis model assessment insulin resistance index; INS, insulin; ISI, insulin sensitivity index; OC, osteocalcin; OGTT, oral glucose tolerance test; tOC, total osteocalcin; ucOC, uncarboxylated OCN. All values are means ± s.d. [a]Obtained from an analysis of covariance adjusted for baseline values of the variable analyzed to determine the effects of the treatment on the metabolic variables. [b]Significantly different from values in week 0, $P < 0.05$ obtained from paired-samples *t*-test.

Table 4. Pearson's correlations between changes of different form of osteocalcin and metabolic variables changes, in 82 prediabetic premenopausal women

	Δ*FBG (mmol/l)*	Δ*2-h post-OGTT glucose (mmol/l)*	Δ*FINS (μIU/ml)*	Δ*2-h post-OGTT INS (μIU/ml)*	Δ*HOMA-IR*	Δ*HOMA-B*	Δ*ISI*
ΔcOC (ng/ml)	− 0.042	− 0.032	0.037	− 0.199	0.011	0.153	0.107
ΔucOC (ng/ml)	0.053	0.172	0.024	0.124	0.040	− 0.054	− 0.057
ΔtOC	− 0.001	0.172	0.057	− 0.108	0.043	0.116	0.066
Δ%ucOC	0.049	0.308*	0.024	0.175	0.036	− 0.069	−0.137

Abbreviations: FBG, fasting blood glucose; FINS, fasting insulin; HOMA-B, homeostatic model assessment β-cell function; HOMA-IR, homeostasis model assessment insulin resistance index; INS, insulin; ISI, insulin sensitivity index; OC, osteocalcin; cOC, carboxylated OC; tOC, total osteocalcin; ucOC, uncarboxylated OCN; OGTT, oral glucose tolerance test. *$P < 0.05$.

DISCUSSION

Findings of this study showed that vitamin K1 supplementation in premenopausal women with prediabetes resulted in significant changes in cOC, ucOC, %ucOC, 2-h post-OGTT glucose, insulin concentrations and ISI but did not affect other aspects related to glycemic and insulin status. To the best of our knowledge, the present study is the first one that investigated the effects of vitamin K1 supplementation on the glycemic status and insulin sensitivity via different forms of OC in prediabetic women. As expected, phylloquinone supplementation for 4 weeks significantly increased serum cOC and consequently decreased ucOC and %ucOC levels. Moreover, the supplementation decreased 2-h post-OGTT glucose and insulin concentrations, and it increased ISI. However, it did not alter tOC, HOMA-IR, HOMA-B, FINS and FBS compared with placebo.

Lee *et al.*[1] suggested that only the undercarboxylated, but not the carboxylated, OC might improve glucose tolerance in mice. In addition, Ferron *et al.*[16] showed that administration of ucOC may

improve glucose tolerance by stimulating the expression of insulin and adiponectin. Several clinical studies have shown that glucose metabolism was not only related to ucOC[7,17–20] but also to carboxylated and total ones.[7,17,21–24] Kanazawa *et al.*[17] reported that both forms of osteocalcin were associated with a blood glucose level in type 2 diabetes. In contrast, Abseyi *et al.*,[10] Lu *et al.*,[11] Kumar *et al.*[8] and Yoshida *et al.*[9] did not find any association between tOC or ucOC and glucose. To evaluate the relationship between different forms of osteocalcin with glycemic and insulin status variables, Pearson's correlation analyses were done. No statistically significant association was observed between OC forms and surrogate markers of insulin resistance. However, the results of this study revealed that the changes in %ucOC were correlated with changes of 2-h post-OGTT. Hence, the %ucOC reduction that occurs usually following vitamin K1 supplementation is associated with improvement in 2-h post-OGTT. On the other hand, a relationship between %ucOC as an index of vitamin K status and 2-h post-OGTT was observed in this study. Although this relationship was observed while analyzing pooled data, it did not remain when the study groups were analyzed separately. Reduced sample size might be the reason for this observation.

Most previous studies have applied a cross-sectional method to explore the relationships between undercarboxylated and glycemic status among prediabetic and diabetic patients; this study is the only investigation that uses an interventional method to study the effects of vitamin K1 on glycemic status and insulin resistance in prediabetic and premenopausal women. Pollock *et al.*[25] suggested that lower ucOC concentration in children with prediabetes may be associated with β-cell dysfunction. Kanazawa *et al.*[17] demonstrated that ucOC was negatively associated with fasting glucose and hemoglobin A1C in adults with type 2 diabetes. In a study of Korean men with prediabetic complications and type 2 diabetes, Hwang *et al.*[7] found that higher undercarboxylated osteocalcin levels resulted in better glucose tolerance. These studies attest to the fact that the effects of undercarboxylated osteocalcin on β-cell function have been observed only among individuals with prediabetes and diabetes or among individuals with an abnormal glucose metabolism.

High vitamin K intakes are associated with a low percentage of undercarboxylated osteocalcin[2,26] and are also associated with reduced insulin resistance.[3,5,9] The results of this study showed that phylloquinone supplementation does not affect insulin resistance, but at the same time it could increase insulin sensitivity. In this study, simple methods were used to measure insulin resistance under the fasting state and fasting plus the postabsorptive state. HOMA-IR is the surrogate measure of insulin resistance at fasting state, and it tends to represent hepatic insulin resistance,[27] whereas ISI-based whole-body measures capture both hepatic and skeletal muscle insulin resistance, glucose disposal and is a direct measurement of the β-cell response to energy stress.[14] This finding suggests that any potential effect of phylloquinone supplementation on insulin sensitivity may affect peripheral insulin action. Although limited data are available assessing the effects of phylloquinone supplementation on glycemic status in prediabetic patients, the reports of the effect of vitamin K1 supplementation on insulin resistance in various subjects are conflicting. Consistently, Kumar *et al.*[8] reported that in elderly women phylloquinone administration is not associated with changes in insulin secretion and action, despite reductions in ucOC concentrations. Along the same lines, Yoshida *et al.* showed that 3 years of phylloquinone supplementation among nondiabetic men and women reduced HOMA-IR only among men but not among women. On the basis of these studies, it seems safe to argue that sex can also have a role in the relationships between OC and insulin resistance. In another study by Choi *et al.*,[28] in which 42 healthy young men were studied, it was demonstrated that 30 mg of vitamin K2 supplementation for 4 weeks increased insulin sensitivity. Hence, it is suggested to compare the effects on male and female individuals in a study in order to shed more light on the relationship between osteocalcin and insulin resistance in the future studies.

It seems that the vitamin K dosage and intervention duration may affect insulin resistance. It is possible that changes in HOMA-IR may have occured over a longer period, whereas our study continued for 4 weeks. On the other hand, we applied an indirect method of measuring insulin resistance, that is, HOMA-IR. It is possible that using different methods of measuring insulin resistance would have yielded different results.

The manipulative effect of vitamin K supplementation on osteocalcin in human had been confirmed. In this study, phylloquinone supplementation increased serum levels of cOC and decreased ucOC, but did not alter tOC. Because of simultaneous effects of vitamin K supplementation and antiresorptive therapies in decreasing ucOC[29] and in order to investigate their effects on glucose and insulin metabolism separately, it is suggested not to limit to osteocalcin and measure bone formation and resorption markers such as N-terminal propeptide of type I collagen and C-terminal telopeptide in the future studies. With regard to the simultaneous effects of vitamin K supplementation and antiresorptive therapy on bone turnover, such studies will show whether the improvement of glycemia status would be contributed to the vitamin supplementation (via independent role of osteocalcin) or other mechanisms, which may affect glycemia status and insulin sensitivity. Moreover, measurement of serum phylloquinone, which was not conducted in the present study, could reveal the relationships between serum vitamin K1 and glucose homeostasis.

In summary, the results of this study demonstrated for the first time that vitamin K1 supplementation for 4 weeks did not affect insulin resistance in prediabetic and premenopause women but had beneficial effects on glycemic status and insulin sensitivity.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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