Effects of Vitamin K on Glucose Homeostasis in Women with Rheumatoid Arthritis

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Abstract

Background: Given the high prevalence of insulin resistance in inflammatory diseases and due to increasing rate of some chronic inflammatory disease like rheumatoid arthritis (RA) during past decades, it seems that an agent which controls glucose homeostasis might be an adjuvant therapy. Among dietary factors, vitamin K is considered as a nutrient which controls inflammation and glucose homeostasis, may improve insulin resistance in patients with rheumatoid arthritis.

Objective: The aim of this study was to examine the effects of vitamin K_1 (phylloquinone) on fasting glucose, insulin and HOMA-IR in RA patients.

Materials and Methods: The study was a before-after clinical trial in which 32 women with rheumatoid arthritis who fulfilled the eligibility criteria were participated. Vitamin K_1 (10 mg/day) was given to the participants for eight weeks. Baseline dietary records, anthropometric measures, and characteristics were recorded. Fasting glucose, insulin and HOMA-IR were assessed before and after the intervention.

Results: There was no significant change in subjects regarding anthropometric measurements and intake of energy and nutrients compared to baseline values. However, a significant reduction was observed in the levels of fasting blood glucose (P=0.002) after the intervention period. Vitamin K did not alter the levels of insulin and HOMA-IR in comparison to baseline.

Conclusions: In this trial fasting glucose level in women with rheumatoid arthritis was reduced after vitamin K1 supplementation (10 mg/day) for 8 weeks while insulin levels remained unchanged. It seems that vitamin K may improve glucose tolerance through insulin independent pathway; or perhaps the significant change in insulin secretion would appear after a load of glucose following a course of vitamin K treatment.

Keywords: Vitamin K, Phylloquinone, Insulin resistance, Glucose homeostasis, Rheumatoid Arthritis

Introduction

Insulin resistance (IR), a dominant feature of metabolic syndrome, accompanies most of inflammatory diseases like rheumatoid arthritis (RA) which is the most common inflammatory arthritis [1]. It is postulated that a state of chronic inflammation and inflammatory mediators are involved in insulin resistance [2]. In support of this correlation, several lines of evidence found an improvement in insulin resistance following anti-TNFα therapies in such inflammatory disorders [1,3-7]. Therefore, anti-inflammatory agents may mitigate inflammation and consequently insulin resistance in these diseases. Meanwhile, there is a great attention to anti-inflammatory effects of vitamin K recently [8,9], in addition, the increasing body of reports underlined its direct beneficial role in glucose homeostasis [10,11].

Vitamin K is an important cofactor in gamma-carboxylation of certain proteins which are involved in different actions including coagulation, bone formation and also insulin secretion and insulin sensitivity [10]. Among these proteins which are associated with glucose homeostasis are vitamin K-dependent growth arrest-specific protein 6 (Gas6) [12] and osteocalcin [10].

Findings of recent clinical researches investigating effects of vitamin K on insulin sensitivity support its protective effects [11,13]. One experimental showed that plasma glucose was significantly lower in control rats compared with those fed with low

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vitamin k diet [13] and in another study an association between pharmacological doses of vitamin K and insulin response was also observed [11]. However, several studies have not met significance to demonstrate this association [14-16]. Kumar et al. reported that phylloquinone supplementation for 12 months did not change glucose metabolism in postmenopausal women [14]. In another randomized clinical trial insulin sensitivity was not improved in women following vitamin K intake [15]. Sakamato et al. investigated the association between vitamin K intake (using one-week food check list) with serum glucose concentration after an oral load of 75 g glucose. No significant differences in fasting serum glucose were observed between groups with high and low intake of vitamin K [16].

Therefore, due to confined evidence with contradictory findings about the role of vitamin K and since far too little attention has been paid to the function of vitamin K in glucose homeostasis in inflammatory diseases to date; we purposed to survey the effects of vitamin K supplementation on insulin resistance in rheumatoid arthritis.

Materials and Methods

Study design and subjects

This study was performed on 32 women with rheumatoid arthritis. The patients were recruited from the rheumatology clinic of Imam Reza Hospital of Tabriz University of Medical Sciences, Tabriz, Iran. The trial was approved by the Regional Medical Ethics Committee of Tabriz University of Medical Science, and registered in Iranian Registry of Clinical Trials (http://www.irct.ir; Code number: IRCT 201205203140N4).

The exclusion criteria were pregnancy, lactation, smoking, receiving contraceptive drugs, coumarins, antibiotics, or antiepileptic drugs, being under hormonal therapy, postmenopausal status, being in severe stage of rheumatoid arthritis (DAS28>5.1), using antioxidants, anti-inflammatory or other supplements during the past 3 months, liver and kidney disorders, history of chronic diseases, i.e., diabetes, cardiovascular diseases, malabsorption, coagulation or thyroid disorders, hyper-prolactinemia, super obesity (body mass index over than 40), cancer, Cushing' syndrome, changing lifestyle and treatment protocol during the study and unwillingness to continue the study. Thirty two eligible women with the age group of 20-50 yrs who had definitive RA according to rheumatology specialist diagnosis on the basis of the American College of Rheumatology classification for RA (ACR, 2010) [17], were informed about the study purpose and protocol and fulfilled consent paper prior to inclusion into the study.

The sample size was determined by considering a 15% change in primary outcome of glucose level. It was based on primary information from a pilot study (n = 5) and using the Pocock formula and was estimated 24 to achieve power of 80% and a 95% confidence interval. Finally 32 subjects, given 30% rate of loss, were assigned in the intervention.

At baseline, information on age, disease history, medication, etc. was collected using a demographic questionnaire through a face-to-face interview. Patients were requested not to change their usual lifestyle including physical activity, medication, and dietary intake during the intervention period. Height and weight were measured before and after the trial using a non-stretchable tape and a balance scale (Seca, Germany) with the precision of 0.5 cm and 0.1 kg, respectively. Then, the body mass index (BMI) was calculated by dividing the weight in Kg to height in squared meters. The three-day dietary record for 2 week days and 1 weekend was used to dietary intakes assessment before and after the period. After entering amounts (grams) of all consumed food and beverage, Nutritionist IV software (First Databank, San Bruno, CA, USA) modified for Iranian foods were used for analyzing the data. Disease Activity Scores of 28 joints (DAS-28) were calculated on the basis of the swollen and tender joint count and the serum CRP level at baseline. The following formula was used [18]: DAS28= 0.56 (tender joint count)2 + 0.28 (swollen joint count)2+ 0.36* Ln (CRP+1) * 1.10+1.15

The participants were requested to take one pill daily after lunch. A chewable pill of Vit. K1, containing 10 mg phylloquinone (manufactured by Minoo Company, Iran), was used for this intervention.

The vitamin K doses, used in other clinical trials, were different and were in the range from microgram to milligram. We didn't find enough evidence for optimal doses of vitamin K. Considering safety of vitamin K [19] and different high doses of it in previous studies [20-22], we decided to use 10 mg/day pills of vitamin K_1. To control any probable adverse event during the trial period, all subjects were monitored by phone call every week.

Blood sampling and biochemical assays

Blood samples were taken in, after 12 hour fasting, at the beginning and end of the study. Then they were centrifuged for 15 minutes at 3000 rpm (Round Per Minute), and separated serum samples were kept at -70ºC until biochemical analysis.

Blood glucose was assayed by enzymatic method using commercial kits (Pars Azmun Co., Iran) and the results stated as milligram per deciliter (mg/dl). The blood concentration of insulin was detected by ELISA (Enzyme Linked Immuno-sorbent Assay) method using the kit (Diametra Co., Italy) as well; the results were expressed as micro international unit per milliliter (μIU/mL).

HOMA-IR index (Homeostasis Model of Assessment - Insulin Resistance) was used to calculate the insulin resistance via this formula:

HOMA-IR = Fasting glucose (mg/dl) × Fasting insulin (μIU/ml) / 405

Statistical Analysis

Statistical analyses were carried out using SPSS for Windows (version 11.5; SPSS Inc., Chicago, IL, USA). Normality of data distribution was determined using the Kolmogorov-Smirnov test. For expressing of normally distributed, non-normally distributed, and qualitative data, we used mean ± standard deviation (SD), median ± interquartile range, and frequency (percentage), respectively. Paired t-test was used for comparing the mean values before and after the study period for normally distributed data and Wilcoxon test was used for non-normally ones. P-value < 0.05 were considered statistically significant.

Results

Of 32 patients initially recruited, 2 persons were excluded

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Page 3 of 5

during the study. One of whom needed to change the medication during the intervention and the other one experienced slight heartburn following vitamin K consumption. However, no serious adverse side effects were reported except that case.

Table 1 displays the data on baseline characteristics including age, BMI, disease activity and conventional therapy which remained unchanged regarding the dose and type of consumed drugs throughout the supplementation period. No significant differences in dietary intakes were observed during the study period (data not shown).

Table 2 represents weight changes and biochemical markers including the levels of fasting blood glucose, insulin and homeostasis model assessment before and after the intervention.

This table shows that the fasting glucose levels of these overweight and obese women (mean BMI=29) with inflammatory disease are surprisingly low. According to table 2, vitamin K supplementation significantly reduced the levels of FBS (P=0.002). However, fasting insulin level and HOMA-IR was not significantly affected by vitamin K.

Discussion

On the basis of present study's findings there was a significant reduction in fasting glucose level in women with rheumatoid arthritis after 8 weeks of vitamin K administration. Considering the inflammatory nature of the disease and close relation between inflammation and developing insulin resistance, it seems that RA patients are more susceptible to develop glucose intolerance as a part of metabolic syndrome [1]. Therefore, control of glucose homeostasis might open a new window to reduce the severity and consequently mortality rate in these patients [1,2].

Table 1: Baseline characteristics of the studied participants

Variables	
Age (y) *	37.97 ± 8.22
BMI*	29.43 ± 5.27
Disease duration (y) # DAS-28 #	3 (1.5,8.5) 1.74 (1.15,2.59)
Conventional therapy Prednisolone Methotrexate Chloroquine Sulfasalazine Folic acid Ca-Vitamin D	 30 (100%) 26 (86.7%) 23 (76.7%) 3 (10%) 25 (83.3%) 22 (73.3%)

DAS-28: Disease activity score in 28 joints

Values are the number (%) of patients (except where indicated otherwise)

* Refers to values which are presented as mean ± SD.

Refers to values which are presented as median (percentile 25, 75).

Table 2: Weight and Biochemical markers at baseline and after 8 weeks intervention

Variables	Baseline	After 8 weeks	P$_{value}$
FBS(mg/dL)	81.41 ± 8.24	77.35 ± 7.17	0.002*
Insulin level(μIU/mL)	9.30(5.05, 10.50)	10.20(5.45,15.25)	0.492
HOMA-IR(mIU/L)	1.72(0.92,2.30)	1.89(1.05,2.89)	0.740
Weight (Kg)	71.63 ± 13.79	72 ± 13.59	0.197

Data are presented as mean ± SD and median (percentile 25, 75).

P to compare values before and after eight weeks using paired t-test and Wilcoxon rank t-test

* Significant differences between before and after intervention *(P < 0.05)*

Unfortunately, most of studies in this regard have been conducted only in men population and are in support of the evidence representing potential protective function of vitamin K in men [13,16]. Thus, it is worth investigating more on the role of vitamin K in women population who are more susceptible to chronic inflammatory diseases.

In support of our findings, the clinical trial was conducted by Yoshida et al. revealed that phylloquinone (500 μg) supplementation for 36 months on healthy older nondiabetic subjects (age > 60) improved HOMA-IR only in men not women [15]. Yoshida et al. attributed this null finding in women to the high percentage of adipose tissue in women compared with men. It is plausible that adipose tissue reduces the availability of vitamin K in peripheral organs by storing it as a fat- soluble nutrient [15].

A cross-sectional study showed this association between levels of vitamin K intake and plasma glucose in healthy men. They showed that plasma glucose at 30 minutes after a load of 75 g glucose was significantly lower in subjects with high intake of vitamin K compared to the subjects with low vitamin K intake. However, there was not any difference in fasting plasma glucose between two groups [16]. Sakamoto et al. also underlined that healthy men had an improvement in insulin response after a load of glucose following one week vitamin K administration (90 mg/d of menaquinone-4) [11].

Framingham Off-spring cohort study underlined a significant association between high dietary intake of phylloquinone and insulin sensitivity as measured by (2-h post-OGTT insulin and glucose) in healthy men and women. However, there was no significant correlation between phylloquinone intake and HOMA-IR, fasting insulin and glucose concentrations [10].

The findings of the current study are consistent with those of Kumar et al. who conducted a clinical trial on 21 postmenopausal healthy women to assess the effects of 1 mg vitamin K1 for 12 months on insulin secretion and undercarbocylated form of osteocalcin. They did not find any significant change in insulin secretion despite reductions in ucOC concentrations [14]. Insulin secretion by beta cells and hepatic clearance of insulin are the most important contributors in serum insulin concentrations. So, the authors related this unchanged insulin concentration to increase in hepatic insulin clearance despite insulin secretion [14].

A direct significant association between total serum osteocalcin and carboxylated osteocalcin with insulin sensitivity was observed in one cross-sectional study conducted by Shea et al.

Carboxylation of osteocalcin which is dependent on vitamin K reduces in result of vitamin K deficiency and undercarboxylated form of osteocalcin (ucOC) concentrations increases consequently. However, precise contribution of undercarboxylated or carboxylated form of osteocalcin to glucose metabolism remains to be clarified [23].

Surprisingly, animal studies demonstrated that high level of undercarboxylated osteocalcin (that presents in vitamin K deficiency) can improve insulin secretion and sensitivity through increasing of beta energy expenditure, cell proliferation, and adiponectin expression [24,25]. This association has not

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Page 4 of 5

been found in human studies. In contrast, subjects who had an improvement in glucose homoeostasis following vitamin K intake, had a lower level of undercarboxylated osteocalcin compared with control group and it is suggested that ucOC is not related to glucose homeostasis in human [15].

One cross-sectional study indicated that other vitamin K dependent proteins such as Gas-6 might have a pivotal role in glucose homeostasis. On the basis of their findings, Gas-6 is negatively correlated with HOMA-IR and interleukin 6 in women [12].

The potential mechanisms relating vitamin K to glucose homeostasis are not known in depth up to now. Not only are Vitamin K1 and vitamin K2 found in pancreas, but also, some certain vitamin K dependant proteins like osteocalcin is found in pancreas which might have a potent role in insulin sensitivity (10). The possible mechanism for vitamin K involvement in glucose homeostasis is its function in control of oxidative stress and inhibition of aldose reductase in pancreas which increases at high glucose concentration in diabetes [26]. Meanwhile, vitamin K suppresses inflammation through some pathways in addition to its direct role in glucose homeostasis [8,9,27].

Suppression of NF-κB (Nuclear Factor Kappa-lightchain enhancer of activated B-cells) activation and iNOS (Inducible Nitric Oxide Synthase) expression in the cells exposed to streptozotocin (STZ) by vitamin K may explain its contribution in inflammatory cascade and glucose metabolism [1,26].

Thus, our findings represent that vitamin K may improve glucose tolerance through the alternative pathways beyond its effect on insulin secretion. Furthermore, delayed insulin secretion by beta-cells might account for its inefficacy on fasting insulin secretion. So, it is the other explanation for the null findings in several investigations which revealed protective effects of vitamin K only at 2-hpost-OGTT (2-h post-oral glucose tolerance test) glucose and insulin concentrations [10,16].

Limitations and Strengths

The interpretation of present study's findings is limited by several factors. First, due to financial problems, this study was conducted as a before-after research. The outcomes will be more reliable if the study repeats as a clinical-control trial to compare the results with the control group. Confining participants to specific group in present study is the second limitation. It seems that trials on different and big population like considering postmenopausal women, women with severe range of the disease, also men, etc. deserve more attention. In addition, we acknowledge that measurement of 2-hpost-OGTT insulin and glucose concentrations and also osteocalcin (undercarboxylated and carboxylated form) levels would have provided more information regarding precise function of vitamin K in glucose homoestasis. Different dosages, long-term intervention or larger sample size might result in the different findings.

Besides these limitations, to the best of our knowledge, this is the first study to investigate the effects of vitamin K on glucose tolerance in patients with inflammatory diseases such as rheumatoid arthritis. High compliance of drug, uniformity of target group regarding hormonal status, disease activity, dietary intake and physical activity let us interpret outcomes of the study without any effects of residual confounding.

In conclusion, in this trial fasting glucose level in women with rheumatoid arthritis was reduced after vitamin K1 supplementation (10 mg/day) for 8 weeks.

Acknowledgement

The authors declare that there is no conflict of interest and they are responsible for this paper's content.

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Citation: Neda Ghamarzad Shishavan, Bahram Pourghassem Gargari, Mehran Mesgari Abbasi, Sahar Masoudi (2017). Effects of Vitamin K on Glucose Homeostasis in Women with Rheumatoid Ar-thritis

Page 5 of 5

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