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Lower diastolic blood pressure in healthy subjects with vitamin K deficiency: a preliminary cross-sectional study

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ABSTRACT

Introduction. There is a growing body of evidence for the role of vitamin K in cardiovascular health. As a cofactor of carboxylation of the matrix Gla protein it prevents arterial calcification. However, the data on the relationship between vitamin K status and the blood pressure are scarce, and particularly so in persons without the burden of cardiovascular risk factors.

Material and Methods. We performed a pilot cross-sectional study, in which we hypothesized that vitamin K deficiency is associated with a higher blood pressure in young, healthy people. The concentration of protein induced by vitamin K absence-II (PIVKA-II) larger than 2 ng/mL was chosen as a proxy for vitamin K deficiency; it was assessed in serum using ELISA. Blood pressure was measured using a validated, automated oscillometric monitor in triplicate.

Results. Twenty-three healthy subjects were enrolled (16 female; mean age 21.3 ± 1.6 years; body mass index 20.6 ± 2.4 kg/m^2). The diastolic blood pressure (DBP) was lower in vitamin K-deficient subjects (58 ± 9 vs. 67 ± 5 mmHg, $p = 0.01$). The mean arterial blood pressure also differed (75 ± 9 vs. 83 ± 6, $p = 0.02$). PIVKA-II levels correlated with DBP only (Pearson's $R = -0.41$, $p < 0.05$; Spearman's ρ ns.). Stepwise regression identified PIVKA-II concentrations as the only independent parameter associated with DBP (adjusted $R^2 = 13.1\%$; PIVKA-II: $\beta = -0.41$; 95%CI -1.87-(-0.00098), $t = -2.08$, $p < 0.05$).

Conclusions. The relationship between vitamin K deficiency and low DBP in young adults should be investigated further.

Keywords: menaquinone, hypertension, K2, arterial stiffness, osteocalcin.

Introduction

Vitamin K is a group of enzyme cofactors that enable carboxylation of proteins containing gamma-carboxyglutamate (Gla) domains. Apart from the constituents of the coagulation cascade other proteins belong to this group, including the matrix Gla protein (MGP, present in the vasculature). Importantly, MGP carboxylation is affected by vitamin K insufficiency in the first line, long before clotting processes become disturbed. The resulting larger fraction of uncarboxylated MGP (ucMGP) favorizes arterial calcification.

This link between vitamin K and the cardiovascular health has been confirmed by a number of studies in humans and animal models. For instance, in 1001 participants the levels of ucMGP were shown to correlate

with vascular stiffness measured by pulse wave velocity calculated on the basis of applanation tonometry and this effect sustained after adjustment for potential confounders [1]. On a populational level, the insufficient supply of vitamin K2 in the diet was recently predicted to be responsible for 6.9% of cardiovascular mortality before the age of 65 years – more than the male gender (6.1%) [2].

However, the data on the relationship between vitamin K status and the blood pressure are scarce, and particularly so in persons without the burden of cardiovascular risk factors. We performed a pilot study, in which we hypothesized that vitamin K deficiency is associated with a higher blood pressure in young, healthy people.

Material and Methods

Healthy subjects were recruited in the year 2014 in the city of Poznan, Poland, as a control group for a study concerning atherosclerosis in cystic fibrosis [3]. The inclusion criterion was no acute or chronic disease. Exclusion criteria were: hypercholesterolemia or hypertriglyceridemia and early coronary disease or vascular brain diseases in the family (< 65 years in women and < 55 years in men).

As a proxy of vitamin K status we employed the level of protein induced by vitamin K absence-II (PIVKA-II). PIVKA-II concentrations above 2 ng/mL were considered to indicate moderate vitamin K deficiency; they were determined in serum using enzyme-linked immunosorbent assay (MyBioSource, San Diego, USA).

Non-high density lipoprotein (non-HDL) cholesterol concentration was chosen and calculated as the most relevant supplementary cardiovascular risk factor. Total cholesterol was assessed with the use of an enzymatic method with esterase, cholesterol oxidase, and Trinder reaction. HDL cholesterol concentrations were measured employing glycerophosphate oxidase and Trinder reaction (Advia 1800 Chemistry, Siemens Healthcare, Erlangen, Germany).

Blood pressure was measured using a validated, automated oscillometric monitor Omron M5-I (Omron, Kyoto, Japan) [4, 5] in the Department of Cardiology-Intensive Therapy of Poznan University of Medical Sciences, Poznan, Poland. An average of three measurements was obtained for systolic (SBP) and diastolic (DBP) pressures and subsequently the mean arterial pressure (MAP) was calculated.

Statistical analyses were performed using Statistica 12 (Statsoft Inc., Tulsa, USA). The Shapiro-Wilk test was used to check for the normality of distribution in subgroups. The F-test was used to verify that there were no statistically significant differences in variances between the compared subgroups. The hypothesis that mean values of parameters in subgroups are equal was tested using the Student's t-test. Correlations were checked by computing both Pearson's r and Spearman's ρ. Forward stepwise multivariable regression models were built to compensate for confounding, which included the following parameters: age, sex, BMI, non-HDL cholesterol, and PIVKA-II concentration. In additional explorative analyses the Mann-Whitney U-test was applied.

The study respected the rules and values set forth in the Declaration of Helsinki and was approved by the bioethical committee at Poznan University of Medical Sciences (decision no. 250/10). All the volunteers provided written, informed consent for their participation in the research.

Results

Twenty-three subjects were recruited for the study, of whom 16 were female (70%). The mean age was 21.3 ± 1.6 years. The average mass, height and body mass index (BMI) were: 60.2 ± 9.2 kg, 171 ± 9 cm, 20.6 ± 2.4 kg/m^2. The PIVKA concentration in the group was varied: mean 3.85 ± 3.58 ng/mL (minimum 0.22, maximum 10.13 ng/mL). The average pressures were: SBP 113 ± 9 mmHg, DBP 63 ± 8 mmHg, and MAP 79 ± 8 mmHg. Non-HDL cholesterol concentration was 104 ± 32 mg/dL.

The comparison of the above parameters in vitamin K-deficient and vitamin K-sufficient healthy persons is presented in **Table 1**. A lower average diastolic and mean blood pressure was found in vitamin K-deficient subjects (**Figure 1**).

PIVKA-II levels correlated with DBP only (Pearson's R = -0.41, $p < 0.05$; Spearman's ρ ns.; **Figure 2**). Of the three regression models built to predict blood pressure values, only the one explaining DBP was valid. PIVKA-II alone was found to independently associate with DBP (adjusted R^2 = 13.1%, model $p < 0.05$; PIVKA-II: β = -0.41; 95%CI -1.87-(-0.00098), t = -2.08, $p < 0.05$).

We performed explorative analyses to characterize the five vitamin K-deficient participants in whom DBP < 55 mHg was found (Figure 2). The five subjects (4 female, 1 male) did not differ from the rest of the group nor from the other vitamin K-deficient persons in age, weight, height, BMI, or non-HDL cholesterol concentration (all p values > 0.40).

Table 1. Comparison of blood pressure and other characteristics of vitamin K-deficient and vitamin K-sufficient healthy persons

Parameter	Vitamin K-deficient	Vitamin K-sufficient	P
Number of persons	11	12	
Age, years	20.9 ± 1.7	21.7 ± 1.5	0.21
Sex	7 female (64%)	9 female (75%)	0.67[a]
Weight, kg	61.2 ± 6.6	59 ± 11	0.62
Height, cm	170 ± 9	171 ± 10	0.81
BMI, kg/m^2	21.1 ± 2.0	20.1 ± 2.7	0.30
Non-HDL cholesterol, mg/dL	108 ± 31	100 ± 34	0.55
SBP, mmHg	110 ± 9	115 ± 8	0.23
DBP, mmHg	58 ± 9	67 ± 5	0.01
MAP, mmHg	75 ± 9	83 ± 6	0.02
PIVKA-II, ng/mL	7.01 ± 2.62	0.95 ± 0.47	< 10^{-6}

[a] Fisher's exact test, two-tailed p-value.
BMI – body mass index, DBP – diastolic blood pressure, MAP – mean arterial pressure, non-HDL – non-high density lipoprotein, SBP – systolic blood pressure

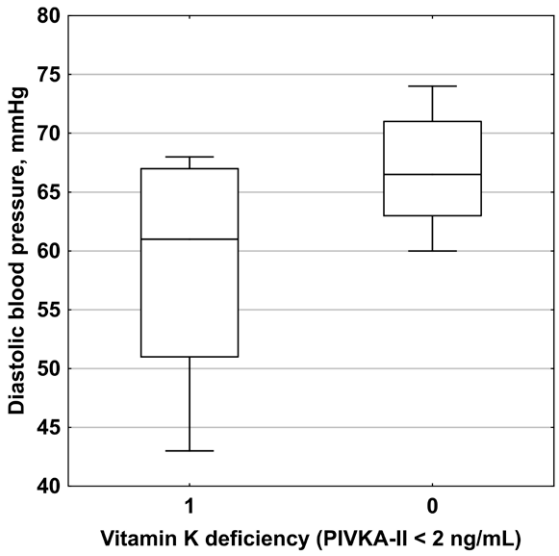

Figure 1. Medians, 1st–3rd quartiles and 5th–95th percentiles of diastolic blood pressure depending on vitamin K status assessed using protein induced by vitamin K absence-II

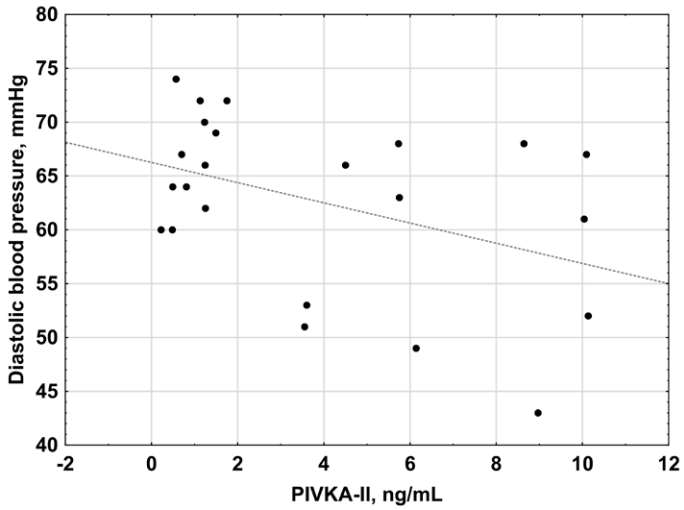

Figure 2. Scatterplot illustrating the relationship between the diastolic blood pressure and protein induced by vitamin K absence-II in 23 healthy subjects. A regression line is shown

Discussion

This pilot study reveals an unexpected association between vitamin K deficiency and a lower DBP in healthy subjects. This goes against the common knowledge, which focuses on the role of MGP and arterial calcification. However, some indirect support for our findings is provided by the study of Streit et al., who analyzed data regarding 4412 patients with hypertension, of whom 569 received phenprocoumon, a vitamin K antagonist. Even after the adjustment for potential confounding, the patients on phenprocoumon had lower mean SBP (-8.4 mmHg) and DBP (-1.5 mmHg). The authors proposed that this could be explained by better compliance to antihypertensive pharmacotherapy – our study adds that there might indeed be a mechanistic explanation to their observation.

Other research regarding vitamin antagonists also yielded important information on the role of Gla carboxylation in cardiovascular health. The Stroke Prevention in Nonrheumatic Atrial Fibrillation (SPINAF) trial, which investigated the impact of warfarin and placebo in 284 patients with atrial fibrillation, did not find any changes in DBP after a mean follow-up of 24 months. A cohort study by Lim et al. followed 116 diabetes patients, half of whom received warfarin, over a period of 36 months. No increases in SBP in the warfarin group were found [6]. Unfortunately, blood pressure data are missing from many other vitamin K antagonists trials [7].

Treatment with warfarin was proposed as an animal model for isolated systolic hypertension [8] and vitamin K was shown to rescue warfarin-induced increase in SBP [9]. Furthermore, a striking warfarin-induced vascular calcification was found in rats with adenine-induced chronic kidney disease. In this case the effect was also mitigated by vitamin K [10]. In humans, total arterial calcium associated with a high osteocalcin ratio, which reflects vitamin K insufficiency [11]. A randomized study in postmenopausal women (n = 244) showed that supplementation of vitamin K2 (180 µg of menaquinone 7 – MK7) during 3 years led to a decrease in arterial stiffness [12]. A large trial investigating high-dose vitamin K2 (360 µg of MK7) in coronary calcification is ongoing in the Netherlands (VitaK-CAC) [13].

In a randomized controlled study by Fulton et al. 80 older people were given either 100 mg of vitamin K2 (MK7) or placebo daily over 6 months. The blood pressure did not change [14]. It should be added that the dose used by Fulton et al. was moderate [15]. In a small randomized study comparing vitamin K2 (1.5 mg daily of menaquinone 4 – MK4) and placebo a reduction in both SBP and DBP was observed, but the results remained inconclusive in this respect since a decrease in blood pressure was observed in the control group as well [16]. Menaquinones of various length are naturally produced by the intestinal microbiome and may have different biological functions. This is an area of ongoing research; one of its fruits is the latterly introduced concept of menaquinotype [17].

In a cross-sectional analysis of the National Health and Nutrition Examination Survey (NHANES) study vitamin K1 (phylloquinone) intake inversely correlated with elevated blood pressure [18]. There are also other strong data indicating that a simultaneous supplementation with vitamins K and D could lower blood pressure [19]. Interestingly, in a group of 1035 patients ucMGP positively associated with the renal resitive index [20].

A major limitation of this preliminary observational, cross-sectional study is the small sample size, which both reduces the chance of finding a true effect and increases the probability of a false positive finding [21]. In order to address this problem, we used various approaches to the data, one of which (Spearman's rank-sum correlation) did not replicate the main finding. On the one hand, the forward stepwise regression, which is a useful tool for identifying correlations, is known to inflate the significance of findings. On the other hand, the t test, which is robust, indicated a highly significant finding and a linear correlation between the raw (non-categorized) PIVKA-II levels and DBP was also confirmed. As a consequence, the thought-provoking finding that we present herein should be interpreted with due caution.

Conclusion

The relationship between vitamin K deficiency and low DBP in young adults should be investigated further.

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Conflict of interest statement
The authors declare no conflict of interest.

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References

1. Pivin E, Ponte B, Pruijm M, Ackermann D, Guessous I, Ehret G et al. Inactive Matrix Gla-Protein Is Associated With Arterial Stiffness in an Adult Population-Based Study. Hypertens Dallas Tex. 2015;66:85–92.
2. Cundiff DK, Agutter PS. Cardiovascular Disease Death Before Age 65 in 168 Countries Correlated Statistically with Biometrics, Socioeconomic Status, Tobacco, Gender, Exercise, Macronutrients, and Vitamin K. Cureus. 2016;8:e748.
3. Madry E, Nowak J, Wykretowicz A, Wenska-Chyzy E, Miskiewicz-Chotnicka A, Walkowiak J. Predicting the risk of atherosclerosis in patients with cystic fibrosis – rationale and design of a prospective cohort study. J Med Sci. 2015;84:126–128.
4. El Assaad MA, Topouchian JA, Asmar RG. Evaluation of two devices for self-measurement of blood pressure according to the international protocol: the Omron M5-I and the Omron 705IT. Blood Press Monit. 2003;8:127–133.
5. Omboni S, Riva I, Giglio A, Caldara G, Groppelli A, Parati G. Validation of the Omron M5-I, R5-I and HEM-907 automated blood pressure monitors in elderly individuals according to the International Protocol of the European Society of Hypertension. Blood Press Monit. 2007;12:233–242.
6. Lim MA, Shafique S, See SY, Khan FN, Parikh CR, Peixoto AJ. Effects of warfarin on blood pressure in men with diabetes and hypertension--a longitudinal study. J Clin Hypertens Greenwich Conn. 2007;9:256–258.
7. Kallistratos MS, Manolis AJ, Mancia G. Blood pressure. The forgotten factor in previous and recent studies regarding anticoagulation in atrial fibrillation. Int J Cardiol. 2013;168:4434–4435.
8. Essalihi R, Dao HH, Yamaguchi N, Moreau P. A new model of isolated systolic hypertension induced by chronic warfarin and vitamin K1 treatment. Am J Hypertens. 2003;16:103–110.
9. Ware KM, Vance JC, Muni N, Hebert LA, Satoskar AA, Nadasdy G et al. Oral warfarin and the thrombin inhibitor dabigatran increase blood pressure in rats: hidden danger of anticoagulants? Am J Hypertens. 2015;28:182–189.
10. McCabe KM, Booth SL, Fu X, Shobeiri N, Pang JJ, Adams MA et al. Dietary vitamin K and therapeutic warfarin alter the susceptibility to vascular calcification in experimental chronic kidney disease. Kidney Int. 2013;83:835–844.
11. Rennenberg RJMW, de Leeuw PW, Kessels AGH, Schurgers LJ, Vermeer C, van Engelshoven JMA et al. Calcium scores and matrix Gla protein levels: association with vitamin K status. Eur J Clin Invest. 2010;40:344–349.
12. Knapen MHJ, Braam LAJLM, Drummen NE, Bekers O, Hoeks APG, Vermeer C. Menaquinone-7 supplementation improves arterial stiffness in healthy postmenopausal women. A double-blind randomised clinical trial. Thromb Haemost. 2015;113:1135–1144.
13. Vossen LM, Schurgers LJ, van Varik BJ, Kietselaer BLJH, Vermeer C, Meeder JG et al. Menaquinone-7 Supplementation to Reduce Vascular Calcification in Patients with Coronary Artery Disease: Rationale and Study Protocol (VitaK-CAC Trial). Nutrients. 2015;7:8905–8915.
14. Fulton RL, McMurdo MET, Hill A, Abboud RJ, Arnold GP, Struthers AD et al. Effect of Vitamin K on Vascular Health and Physical Function in Older People with Vascular Disease--A Randomised Controlled Trial. J Nutr Health Aging. 2016;20:325–333.
15. Dalmeijer GW, van der Schouw YT, Magdeleyns E, Ahmed N, Vermeer C, Beulens JWJ. The effect of menaquinone-7 supplementation on circulating species of matrix Gla protein. Atherosclerosis. 2012;225:397–402.
16. Koitaya N, Ezaki J, Nishimuta M, Yamauchi J, Hashizume E, Morishita K et al. Effect of low dose vitamin K2 (MK-4) supplementation on bio-indices in postmenopausal Japanese women. J Nutr Sci Vitaminol (Tokyo). 2009;55:15–21.
17. Karl JP, Fu X, Wang X, Zhao Y, Shen J, Zhang C et al. Fecal menaquinone profiles of overweight adults are associated with gut microbiota composition during a gut microbiota-targeted dietary intervention. Am J Clin Nutr. 2015;102:84–93.
18. Pan Y, Jackson RT. Dietary phylloquinone intakes and metabolic syndrome in US young adults. J Am Coll Nutr. 2009;28:369–379.
19. Van Ballegooijen A, Van Schoor N, Brouwer I, Visser M, Beulens J. OS 06–09 the synergistic association between vitamin D and vitamin K with incident hypertension. J Hypertens. 2016;34(Suppl 1):e64.
20. Pivin E, Pruijm M, Ackermann D, Guessous I, Ehret G, Pechère-Bertschi A et al. 1D.03: Inactive matrix GLA protein is associated with renal resistive index in a population-based study. J Hypertens. 2015;33(Suppl 1):e15.
21. Button KS, Ioannidis JPA, Mokrysz C, Nosek BA, Flint J, Robinson ESJ et al. Power failure: why small sample size undermines the reliability of neuroscience. Nat Rev Neurosci. 2013;14:365–376.

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