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Dietary intake of vitamin K, serum undercarboxylated osteocalcin, genetic variation in the vitamin K epoxide reductase gene, and risk of prostate cancer

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Prostate cancer is the third leading cause of cancer death and the most frequent cancer diagnosis in German men. Geographic variations in incidence rates and observations from migration studies suggest that environmental factors such as diet may play an important role in the etiology of prostate cancer. Vitamin K is a fat-soluble vitamin that physiologically acts as a cofactor during the post-translational gamma-carboxylation of vitamin K-dependent proteins including blood coagulation factors and the bone protein osteocalcin. Vitamin K from food includes phylloquinone (vitamin K_1), which is abundant in green leafy vegetables, and the group of menaquinones (vitamin K_2), which mainly occur in fermented dairy products such as cheese as well as in meat. Serum undercarboxylated ostoecalcin (ucOC) has been suggested as a sensitive biomarker of vitamin K status. Anticancer activities of vitamin K have been observed in several cancer cell lines, including prostate cancer cells. The mechanism by which phylloquinone and menaquinones exert growth inhibitory effects is mediated by modulation of proto-oncogenes that foster cell cycle arrest and apoptosis. Epidemiological studies examining the association between dietary vitamin K and risk of prostate cancer have not been conducted so far. The primary aim of this thesis was to investigate the association between habitual dietary intake of vitamin K and the risk of prostate cancer, using dietary intake data (phylloquinone and menaquinones) and serum ucOC as a biomarker of vitamin K status. In addition, potential effect modification by a genetic variant in the vitamin K epoxide reductase (*VKORC1*) gene that determines the vitamin K turnover, should be tested. Prior to addressing the main study question in the prospective cohort study EPIC-Heidelberg, the Bavarian Food Consumption Survey II (BVS II) was used to investigate dietary intake of vitamin K, determinants of serum ucOC and its association with dietary vitamin K intake in the general population.
The BVS II is designed as a representative, cross-sectional study of the Bavarian population to investigate dietary habits that were assessed by means of three 24-hour dietary recalls. Analyses were based on data from 231 male and 320 female participants (aged 18-81 years). The EPIC-Heidelberg cohort contributes to the multi-centre prospective cohort study "European Prospective Investigation into Cancer and Nutrition" (EPIC). At baseline, habitual dietary intake was assessed by means of a validated food frequency questionnaire. During a mean follow-up time of 8.6 years, 268 incident cases of prostate cancer, including 113 advanced cases, were identified among 11,319 male participants. A nested case-control study including 250 prostate cancer cases (106 advanced cases) and 492 controls matched by age and time of recruitment was conducted within EPIC-Heidelberg to allow for biomarker measurements and genotyping. Dietary intake of phylloquinone and menaquinones was calculated using previously published food content data analysed by high performance liquid chromatography (HPLC). UcOC and total intact osteocalcin (iOC) were determined by

specific ELISA (enzyme-linked immunosorbent assay) tests in serum samples of the BVS II and the nested case-control study. UcOC was expressed as absolute concentration (ng/ml) or relative to total intact osteocalcin (ucOC/iOC ratio). The +2255 allelic variant in the *VKORC1* gene was genotyped by means of restriction fragment length polymorphism in both studies. The statistical analysis of determinants of serum ucOC in the BVS II was performed by analysis of variance. The association between dietary intake of vitamin K and serum ucOC/iOC ratio was analysed using linear regression analysis. The effect of dietary intake of phylloquinone and menaquinones on the risk of prostate cancer in the EPIC-Heidelberg cohort study was examined using Cox proportional hazards regression models calculating hazard ratios (HR) with corresponding 95% confidence intervals (95% CI). Conditional logistic regression analysis was used to investigate the association between serum ucOC/iOC ratio and prostate cancer in the case-control study nested in EPIC-Heidelberg.

In male and female BVS II participants, median intakes of phylloquinone were 83.4 and 79.6 µg/day, those of menaquinones were 37.6 and 29.8 µg/day, respectively. Mean serum concentration of ucOC was 2.46 ng/ml in men and 2.34 ng/ml in women; corresponding mean ucOC/iOC ratios were 0.28 and 0.29, respectively. Identified determinants of serum ucOC included age, smoking status, sports activity and the season when blood was collected. Dietary intake of vitamin K (phylloquinone and menaquinones) was significantly inversely associated with the ucOC/iOC ratio. This inverse association was modified by the +2255 polymorphism of the *VKORC1* gene, showing that only carriers of the GG genotype (39% of the population) responded to higher vitamin K intake by a decrease in the ucOC/iOC ratio.

Dietary intake of menaquinones was non-significantly inversely associated with total prostate cancer in EPIC-Heidelberg. The multivariate adjusted HR (95% CI) comparing the highest with the lowest quartile was 0.65 (0.39-1.06). The association was stronger for advanced prostate cancer with a HR of 0.37 (0.16-0.88). Phylloquinone intake was not associated with total [HR (95% CI) highest versus lowest quartile 1.02 (0.70-1.48)] or advanced prostate cancer [HR (95% CI) 0.84 (0.46-1.56)].

The ucOC/iOC ratio was not associated with total [odds ratio, OR (95% CI) per 0.1 increment 1.01 (0.90, 1.13)] and non-significantly positively associated with advanced prostate cancer [OR (95%CI) 1.20 (0.98-1.47)] in the case-control study nested in EPIC-Heidelberg. The association with advanced prostate cancer tended to be modified by the +2255 polymorphism of the *VKORC1* gene, i.e., the strongest effects were observed in carriers of the AA genotype, who are characterised by the highest activity of the enzyme vitamin K epoxide reductase ($p_{interaction}$=0.17). However, the statistical power for this analysis was limited.

The results from the BVS II provide reliable data for phylloquinone and menaquinonone intake in the general German population. The observed inverse association between dietary intake of vitamin K and serum ucOC/iOC ratio confirms prior suggestions of this measure as a sensitive biomarker of vitamin K supply. However, the magnitude by which the ucOC/iOC ratio can be influenced by increased vitamin K intake depended on the genotype of the +2255 *VKORC1* polymorphism, suggesting that subjects differ with respect to vitamin K-sensitivity. Thus, this thesis provides important new information on vitamin K intake in Germany that may also be relevant for future intake recommendations. The findings from EPIC-Heidelberg suggest an inverse association between dietary intake of menaquinones but not phylloquinone and the risk of prostate cancer. This is consistent with experimental studies showing substantially higher anticancer activities for menaquinones as compared to phylloquinone. The increased, although not statistically significant, risks of advanced prostate cancer in subjects with high ucOC/iOC ratio, which is indicative of a poor vitamin K supply, strengthens the hypothesis that vitamin K status plays a role in the etiology and progression of prostate cancer. This is the first investigation of the association between dietary intake of vitamin K and risk of prostate cancer. Therefore, further epidemiological studies on this topic are warranted.