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Dietary glucosinolate intake and selenium and risk of prostate cancer in EPIC-Heidelberg

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Prostate cancer is one of the most frequent cancers diagnosed among men worldwide; however, the few established risk factors to date are all non-modifiable. Nevertheless migration studies strongly support a role of environmental factors in prostate cancer etiology. Two currently discussed protective factors are a high glucosinolate intake and a good selenium status. Both substances have been show to exert anticarcinogenic properties in cell-based studies. Glucosinolates are secondary plant metabolites occurring mainly in cruciferous vegetables. They induce the expression of biotransformation enzymes such as glutathione S-transferases (GST) or NADPH quinone oxidoreductase 1 (NQO1), which play a role in the detoxification and elimination of carcinogens. Selenium is a trace element essential for the human body, since it is specifically incorporated into so-called selenoproteins, many of which catalyze redox reactions, like glutathione peroxidases which reduce cellular oxidative stress and subsequent DNA damage.
The aim of this thesis was to evaluate the association between glucosinolate intake and selenium status and prostate cancer risk. Furthermore, effect modification of these associations by genetic variation in genes induced by glucosinolates or in selenoproteins was investigated.
These study questions were addressed in the frame of the EPIC-Heidelberg cohort, an ongoing prospective study with information on habitual diet, lifestyle, socio-demography, and anthropometry and with blood samples collected at recruitment available. Glucosinolate intake was calculated based on participant's food intake data and a newly established database on glucosinolate content of foods commonly consumed and hazard ratios (HR) and 95 % confidence intervals (CI) for prostate cancer were calculated with Cox proportional hazard models based on the male cohort. A case-control study of 248 cases and 492 controls matched by age and time of recruitment was nested in this EPIC cohort to allow for analyzing biological specimen. Serum samples were used to measure GST-alpha (enzyme immunoassay), selenium (DRC ICP-MS), and selenoprotein P (immunoluminometric assay) concentration and GPx3 activity (UV method). Genotyping was performed with real-time PCR or MALDI-TOF mass spectrometry. Additionally, plasma samples of 115 participants were analyzed for glucoraphanin metabolites (LC-ESI-MS/MS) and total isothiocyanates (cyclocondensation assay). Analysis of the case-control data was done by conditional logistic regression estimating odds ratios (OR) and 95 % CI.
Based on 24-hour dietary recalls of 1 034 men, mean intake of total glucosinolates was 14.2 ± 1.1 mg/d with glucobrassicin and sinigrin being the major individual

glucosinolates ingested. Major food sources contributing to total glucosinolate intake were broccoli, Brussels sprouts, cauliflower and radish. During a mean follow-up time of 9.4 years, 328 incident and verified prostate cancer cases occurred among the 11 405 male participants. The intake of glucosinolates was significantly inversely associated with prostate cancer risk (adjusted HR 4^{th} versus 1^{st} intake quartile = 0.68, 95 % CI = 0.48-0.94, p_{trend} = 0.03) in analyses of the full male EPIC-Heidelberg cohort. Within the case-control study nested within the EPIC-cohort, the inverse association was predominantly found in subjects with 3-4 deleted alleles of the *GSTM1* and *GSTT1* gene (adjusted OR for 10 mg/d increment of glucosinolate intake = 0.56, 95 % CI = 0.35-0.87, $p_{interaction}$ = 0.009) and in those with the wild type of the *NQO1* C609T polymorphism (adjusted OR for 10 mg/d increment of glucosinolates = 0.64, 95 % CI = 0.44-0.91, $p_{interaction}$ = 0.10). There was no effect modification by *GSTP1* A313G or *GSTA1* G-52A polymorphisms. Serum concentration of GST-α, an enzyme induced by administration of glucosinolates, was not positively associated with long-term glucosinolate intake as estimated by FFQ data, but showed a strong inverse association with prostate cancer (adjusted OR for the 3^{rd} versus 1^{st} serum GST-α tertile = 0.53, 95 % CI = 0.35-0.80). Additionally, plasma glucoraphanin metabolites or total isothiocyanates measured in a small pilot sample of participants did not prove useful as biomarkers for long-term glucosinolate intake.

Among healthy participants of the nested case-control study, mean serum selenium concentration was 87.7 µg/l and correlated well with serum SepP concentration and GPx3 activity. No lifestyle factors determined selenium status, but the G>A SNP in the 3'UTR of the *SEPP* gene led to higher mean serum SepP concentrations in carriers of at least one mutant allele. SepP is responsible for the transport of selenium and, thus, plays a critical role in the distribution of selenium to the different body tissues. There was a borderline significantly inverse association between serum selenium concentration and prostate cancer risk (adjusted OR per 10 µg/l increment of serum selenium = 0.89, 95 % CI = 0.79-1.01), which was more pronounced in high-grade cancer cases (adjusted OR = 0.84, 95 % CI = 0.69-1.02). This association was modified by the C>T SNP in *GPX1*, a gene encoding for an enzyme that reduces hydrogen peroxides and, thus, protects cells from oxidative damage ($p_{interaction}$ = 0.03). Carriers of at least one T allele had a significantly reduced risk of prostate cancer for increasing selenium concentration (per 10 µg/l) as compared to those with CC genotype (adjusted OR = 0.87, 95 % CI = 0.76-0.99). Again effects were more pronounced when analyses were repeated in the subgroup of high-grade prostate cancer (adjusted OR = 0.64, 95 % CI = 0.49-0.83, $p_{interaction} < 0.001$). There was no effect modification by polymorphisms in *GPX4* (C718T), *SEP15* (G1125A and C>T) and *SEPP* (G>A coding and G>A in 3'UTR).

The results of the present thesis add further evidence to the protective role of glucosinolate intake and selenium status in prostate cancer etiology. Considering genetic variation and potential intermediate effect biomarkers are important steps in elucidating the biological mechanisms of action of potentially protective substances in vivo and might further help to explain inconsistent results of prior studies. However, the findings of this explorative study need to be confirmed in future epidemiologic research.