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DIETARY CADMIUM EXPOSURE AND THE RISK OF HORMONE-RELATED CANCERS

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ABSTRACT

The toxic metal cadmium has been widely dispersed into the environment mainly through anthropogenic activities. Even in industrially non-polluted areas, farmland and consequently foods are, to a varying degree, contaminated. Food is the main source of exposure besides tobacco smoking. Cadmium accumulates in the body, particularly in the kidney where it may cause renal tubular damage. Recently, cadmium was discovered to possess endocrine disrupting properties, mainly mimicking the *in vivo*-effects of estrogen. The metal is classified as a human carcinogen by the International Agency for Research on Cancer based on lung cancer studies of occupational inhalation. It is, however, not clear whether cadmium exposure *via* the diet may cause cancer. Possible health consequences related to estrogenic effects such as increased risk of hormone-related cancers are virtually unexplored.

The aims of this thesis were to: 1) estimate the dietary exposure to cadmium, 2) estimate cadmium's toxicokinetic variability using a population model and to establish the link between urinary cadmium concentrations (a biomarker of accumulated kidney cadmium) and the corresponding long-term dietary exposure to cadmium in the population, 3) evaluate the comparability between food frequency questionnaire (FFQ)-based estimates of dietary cadmium exposure and urinary cadmium concentrations and 4) prospectively assess the association between dietary cadmium exposure and incidence of hormone-related cancers (endometrial, breast, ovarian and prostate cancers) in two population-based cohorts consisting of around 60 000 Swedish women and 40 000 men.

The main sources of dietary cadmium exposure (~80%) in both women and men were bread and other cereals, potatoes, root vegetables, and other vegetables. A one-compartment toxicokinetic model provided similar predictions of individual urinary cadmium concentrations as a more complex toxicokinetic model. We estimated the cadmium half-life to be about 11.6 years with 25% between-person variability in the population. The Pearson correlation between FFQ-based estimates of dietary cadmium exposure and urinary cadmium concentration was 0.2 and the observed sensitivity and specificity was 58% and 51%, respectively. Estimated dietary cadmium exposure was associated with a statistically significant increased risk of cancer of the endometrium, breast, and prostate (39%, 21% and 13% respectively) – but not with ovarian cancer – comparing the highest tertile of cadmium with the lowest. The risk estimates were higher in lean and normal weight women and men: we observed statistically significant increased risks of 52%, 27% and 49% for endometrial cancer, overall breast cancer and localized prostate cancer, respectively. Never-smoking women with lower endogenous (normal body mass index) and exogenous estrogens (no postmenopausal hormone use) and with a consistently high dietary exposure to cadmium assessed twice, 10 years apart, had a 2.9-fold increased risk of endometrial cancer, which may indicate an estrogenic effect. The highest risk of breast cancer (60% increase) was observed for diets high in cadmium and low in whole grain and vegetables, as

compared to diets low in cadmium and high in whole grain and vegetables. Taken together these results indicate that dietary cadmium exposure may play a role in the development of hormone-related cancers.

LIST OF PUBLICATIONS

I. Amzal B, **Julin B**, Vahter M, Wolk A, Johanson G, Åkesson A. Population toxicokinetic modeling of cadmium for health risk assessment. *Environ Health Perspect*. 2009 Aug;117(8):1293-301.

II. Åkesson A, **Julin B**, Wolk A. Long-term dietary cadmium intake and postmenopausal endometrial cancer incidence: a population-based prospective cohort study. *Cancer Res*. 2008 Aug 1;68(15):6435-41.

III. **Julin B**, Wolk A, Bergkvist L, Bottai M, Åkesson A. Dietary cadmium exposure and risk of postmenopausal breast cancer: A population-based prospective cohort study. *Cancer Res*, 2012 Mar 15;72(6):1459-66.

IV. **Julin B**, Wolk A, Åkesson A. Dietary cadmium exposure and risk of epithelial ovarian cancer in a prospective cohort of Swedish women. *Br J Cancer*. 2011 Jul 26;105(3):441-4.

V. **Julin B**, Wolk A, Johansson J-E, Andersson S-O, Andrén O, Åkesson A. Dietary cadmium exposure and risk prostate cancer: A population-based prospective cohort study. *Submitted*.

RELATED PUBLICATIONS

Engström A, Michaëlsson K, Vahter M, **Julin B**, Wolk A, Åkesson A.
Associations between dietary cadmium exposure and bone mineral density and risk of osteoporosis and fractures among women. *Bone.* 2012.
DOI 10.1016/j.bone.2012.03.18.

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Relation between dietary cadmium intake and biomarkers of cadmium exposure in premenopausal women accounting for body iron stores. *Environ Health*. 2011 Dec 16;10(1):105.

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Dietary cadmium exposure and fracture incidence among men: a population-based prospective cohort study. *J Bone Miner Res*. 2011 Jul;26(7):1601-8.

CONTENTS

LIST OF ABBREVIATIONS

AR	Androgen receptor
BMI	Body mass index
CI	Confidence interval
COSM	Cohort of Swedish men
CV	Coefficient of variation
EFSA	European Food Safety Authority
FAO	Food and Agriculture Organization
FFQ	Food frequency questionnaire
ER	Estrogen receptor
IARC	International Agency for Research on Cancer
ICPMS	Inductively coupled plasma mass spectrometry
IGF-1	Insulin growth factor-1
JECFA	Joint FAO/WHO expert committee on food additives
NFA	National Food Agency
MT	Metallothionein
PBTK	Physiologically-based toxicokinetic model
PR	Progesterone receptor
PSA	Prostate-specific antigen
PTWI	Provisional tolerable weekly intake
RR	Rate ratio
SMC	Swedish mammography cohort
TK	Toxicokinetic
TWI	Tolerable weekly intake
WHO	World Health Organization

1 INTRODUCTION

This thesis focuses on the associations between dietary exposure to cadmium and risk of hormone-related cancers, which includes cancer of the endometrium, breast, ovaries and the prostate.[1-3] Estrogen is an established or proposed risk factor in the etiology of cancers in the endometrium, breast and ovary[3, 4] and may play a role in prostate cancer development.[5] The incidence of these cancers displays a large geographical variation and is much higher in high-income countries, which raises the hypothesis that environmental factors may play a role in the etiology. Cadmium, a toxic metal, widely dispersed into the environment is known to exert a wide range of adverse health effects in humans, mainly on the kidney and bone.[6] Food is the main source to cadmium exposure in the general population, while smokers are additionally exposed. Although cadmium has shown estrogen-mimicking effects, there are almost no studies exploring the relationship between dietary cadmium and hormone-related cancers.[7]

2 BACKGROUND

2.1 CADMIUM

Cadmium is a toxic, heavy metal occurring naturally at low levels; however human activities have greatly increased these levels. Naturally cadmium occurs mainly associated with zinc, but also lead and copper, making it an unavoidable by-product when refining these metals.[8] Over the last century, human activities including the application of phosphate fertilizers, emissions from various industries, combustion of fossil fuels, waste from municipal refuse and the use sewage sludge on farmland have led to a considerable increase of cadmium in the environment.[8, 9]

More than 90% of cadmium occurring in the surface environment comes from anthropogenic sources (i.e. caused by humans).[8] After atmospheric emission, cadmium particles can travel over long distances depending on particle size, the height of emission outlets and meteorology. The metal is, however, mainly transported over local, national or regional distances,[10] where it can contaminate agricultural soil. The main anthropogenic sources of cadmium to agricultural soil in Europe are phosphate fertilizers (56%), atmospheric deposition (40%) and sewage sludge (4%).[8] In Sweden, based on emission and meteorological data, the deposition of cadmium was 4 tons in 2008 and atmospheric deposition is presently the main source of cadmium to agricultural soil. Due to regulations in Sweden during the early 1990-ies which restricted the cadmium content allowed in phosphate fertilizers, the contribution of cadmium to soil from phosphate fertilizers has decreased with 90 percent since the mid-1990-ies.[11]

Cadmium from soil accumulates in crops. The rate of uptake is influenced by soil pH, salinity, humus content, crop species and varieties and the presence of other elements (e.g., zinc). Plants grown in a greenhouse or a container take up more cadmium than plants grown in soil. The deposition of cadmium still exceeds removal as assessed in studies of cadmium balance in the upper most layers of arable soil. Thus, cadmium may still accumulate in certain soils, increasing the probability of future exposure through the diet.[10]

2.1.1 Exposure

The diet is the most important source of cadmium exposure in the general non-smoking population in Sweden, as in most other countries.[6, 12] Smokers are additionally exposed due to high cadmium content in tobacco leaves.[13, 14] The dietary exposure to cadmium is influenced from both the cadmium levels in foods and the consumption pattern. Even if the highest concentrations are found in certain seafood, edible offal and its products, and oil seeds, these foods are in general less important for the average overall dietary exposure in a population.[13] Instead, fiber-rich foods are the main sources of exposure[15, 16] due to their high consumption. Thus, all people are exposed; the exposure is life-long

and occurs mainly through foods generally considered healthy. Because mineral fertilizers are restricted within organic farming, one could expect that plants and animals from organic farms would contain less cadmium. In Sweden, plant foods from organic farming did not have lower levels of cadmium,[17] and organically grown pigs actually had higher cadmium levels in kidney,[18] whereas cows from organic farms had significantly lower levels of cadmium in kidney and liver, but not in muscle.[19] A limited number of mainly small Swedish and European studies suggest an average daily intake in the range 8-15 µg.[12, 20-25] The dietary exposure estimates reported in these studies were mostly generated by market basket studies, duplicate diet studies or included rather small samples of specific population subgroups. Thus, it is of interest to estimate the dietary cadmium exposure by a food frequency questionnaire (FFQs) in large populations and assess the main contributing food sources.

2.1.2 Uptake and distribution

Gastrointestinal absorption of cadmium in adults is usually less than 5% and thought to be mediated by the divalent metal transporter (DMT1), primarily responsible for the uptake of iron, but with high affinity for cadmium.[26] Absorbed cadmium is mainly bound to albumin in the blood and transported to the liver where the complex is split and cadmium is bound to metallothionein (MT) and redistributed to various tissues and organs.[14] Due to its small size, the cadmium-MT-complex can be filtered through the glomeruli and reabsorbed in the kidney tubules which lead to accumulation in the cortex. Excretion of cadmium goes mainly via urine, but due to the slow elimination rate (0.005-0.1% of the total body burden), the half-life is long (10-30 years).[14]

The rate of intestinal absorption of cadmium, organ retention and body burden is affected by numerous factors, such as calcium, zinc, iron and manganese.[23, 27-31] High concentrations of these essential metals reduce the rate of cadmium absorption and conversely trace element deficiency enhances cadmium absorption. Especially iron deficiency seems to be an important determinant of intestinal cadmium uptake.[23, 27] Animal experiments have indicated that the absorption of cadmium is inhibited by fiber and phytate,[32-34] which means that diets with a high content of natural fibers may result in a lower rate of intestinal absorption of cadmium even if fiber rich foods contain higher levels of cadmium compared to low-fiber foods.[33] The generally higher concentrations of cadmium in blood, urine and kidneys in women compared to men is most likely explained by the increased absorption of cadmium at low iron stores, which is common among women at fertile age.[23, 27]

2.1.3 Biomarkers of exposure

Cadmium in blood generally reflects recent exposure, but is also affected by the body burden of cadmium. In non-smoking, non-occupationally exposed persons, blood cadmium levels are usually between 0.1 and 0.8 µg/L in areas with no major contamination. In smokers the levels can be up to ten times higher. Urinary cadmium is

often used as a biomarker of long-term exposure as it is influenced by the body burden and proportional to the concentration in the kidneys.[35] Ideally, urinary cadmium should be measured in 24-h urine. Due to feasibility and the difficulties in sampling urine completely, urine spot samples are often used. In the case of spot samples, the dilution of the urine can vary considerably with regard to water solutes, both within and between persons.[14] These differences can be accounted for by adjusting the urine samples for either urinary creatinine or specific gravity.[36] Urinary cadmium concentrations of non-smokers are in general 0.02-0.7 μg/g creatinine, with increasing levels with age up to 50-60 years; thereafter the levels decrease. Smokers and people living in contaminated areas can have several-fold higher levels.[14]

The dietary exposure to cadmium has never been linked to the internal dose (e.g. urinary cadmium) in a population based on paired individual data on dietary intake and urinary excretion. Because cadmium continuously accumulates in human tissues, this relationship may change over the age span and is likely to be affected by several factors such as gastro-intestinal cadmium absorption, etc. (see further under 2.1.5). Nevertheless, this link is crucial for health risk assessment of adequate quality, i.e. to derive the distribution of the daily cadmium intake required to reach a given level of cadmium in urine after lifetime exposure.

2.1.4 Health effects

The critical organ for chronic cadmium exposure has long been considered to be the kidney and the main site of cadmium accumulation is in the proximal tubular cells of the renal cortex. The so called critical effect, the first adverse effect that occur as the dose increases, is renal tubular dysfunction detected as increased urinary excretion of low-molecular-weight proteins and intracellular tubular enzymes.[37, 38] However, associations between tubular damage and cadmium exposure can even occur at levels found in the general population,[38-41] but whether theses associations represent a causal relationship has recently been questioned.[42]

Long-term exceptionally high exposure to cadmium may give rise to bone disease, first discovered with the outbreak of the Itai-itai (meaning ouch-ouch in Japanese) disease in Japan more than 50 years ago, where severe renal and skeletal damage in women was associated with consumption of heavily cadmium polluted rice.[6] Although high exposure to cadmium is clearly detrimental to the kidney it is not clear whether cadmium exposure could be a risk factor for chronic renal failure and end stage renal disease at low level exposure via food as present in most countries. Recent studies, however, clearly support that cadmium may be a risk factor for osteoporosis and fractures even at the exposure present in the general population in Sweden.[15, 43, 44] The mechanisms behind cadmium induced bone effects are not fully clear but a direct effect on bone resorption and an uncoupling between bone resorption and formation has been suggested.[39] However, an indirect effect on bone mediated through kidney damage cannot be excluded.[45]

The International Agency for Research on Cancer (IARC) has classified cadmium as a human carcinogen because of the large incidence of lung cancers in occupationally exposed populations,[46] but the European Commission concluded in a report in 2007 that there were currently no evidence that cadmium is carcinogenic following oral exposure, but that cadmium oxide can be considered a suspected human inhalation carcinogen.[47] When comparing districts in Belgium (with a long tradition of cadmium-emitting zinc plants) with high and low environmental cadmium exposure, overall cancer was increased in the high-exposure group, but a clear excess was only seen regarding lung cancer[13, 48] thus, the relevance to dietary cadmium exposure is not clear. Recent studies also support a role of cadmium in the development of renal cancer, testicular cancer, bladder cancer, pancreatic cancer and cancer of the gall bladder,[49-51] but data are sparse and more studies are needed to clarify the relationship between cadmium and cancer risk. Recently, cadmium was proposed as a potent metalloestrogen.[52-55]

2.1.5 Population modeling

Toxicokinetic (TK) models are useful when linking external exposures to internal dose-measures. The main two classes of TK models found in the literature are the classical compartmental and the physiologically based models. An example of a classical TK model is the one-compartment model where the body is considered to behave as a single well-mixed container. In physiologically based models, the body is instead subdivided into a series of anatomical or physiological compartments that can either correspond to specific organs such as the kidney or liver or consist of groups that share a specific property such as fat content or blood flow.[56]

For cadmium, a one-compartment model has previous been used to predict urinary cadmium from food-cadmium exposure[57] and a physiologically based toxicokinetic model has been developed.[58] Neither model has however, been tested or validated in humans based on individual paired dietary and urinary cadmium data. Key model parameters of these models are, however, likely to vary between persons in a population and will thus most likely affect the target dose of a compound, for example cadmium. Therefore, quantification of the inter-individual variability in a population is crucial in risk assessment in order not to use uncertain safety factors to protect a given proportion of the population. Such quantification has never been fully integrated in the models from the literature. A tool for introducing variability into these models is the population (hierarchical) model, traditionally used in pharmacokinetic assessments. The population model aim at quantitatively describe the variability of the kinetics of a compound within a large population, based on individual level data.[59] The basic idea is that individual parameters are considered samples from a population of which the mean and standard deviation are the main parameters of interest. A powerful approach to calibrate such population models is to use Bayesian inference methods. Briefly, with Bayesian inference, information on all model parameters prior to the data analysis is summarized as prior distributions and subsequently updated based on

information in experimental toxicokinetic data.[56] In Bayesian inference, prior distributions need to be set on every statistical parameter to be estimated. Those distributions reflect the prior knowledge on the parameters, before accounting for the data. In case no prior knowledge is to be incorporated, the prior distribution is set as "non-informative". For mean parameters, a robust "non-informative" distribution is the uniform distribution, which ranges between two equally likely values. The posterior distributions received from such an analysis can then, by the use of Monte Carlo simulation, be used in risk assessment to protect a certain proportion of a population.

2.1.6 Risk assessment

A tolerable intake is "the estimated maximum amount of an agent, expressed on a body mass basis, to which individuals in a (sub)population may be exposed over a specific period without appreciable risk".[60] The Joint FAO/WHO Expert committee on Food Additives (JECFA) established already in 1972, a provisional tolerable weekly intake (PTWI) for cadmium of 7 µg/kg body weight per week.[61] For a person weighting 70 kg, this corresponds to a daily intake of 70 µg. In a risk assessment performed in 2009 by the European Food Safety Authority (EFSA) a new tolerable weekly intake (TWI) of 2.5 µg/ kg body weight was established.[13] This new TWI was set to keep 95% of the population below the "reference point" of 1 µg cadmium/g creatinine in urine at age 50. The intake for a person weighting 70 kg corresponds to 25 µg/day. Following this, in 2010, JECFA reviewed its previous evaluation and established a provisional tolerable monthly intake (PTMI) of 25 µg/kg body weight which corresponds to a weekly intake of 5.8 µg/kg body weight,[62] thus, leaving their old PTWI from 1972 virtually unchanged. The JECFA-value was based on the reference point of 5.4 µg/g creatinine as compared to the EFSA-value of 1 µg/g creatinine. Regardless of the discrepancies between these two risk assessments, EFSA concluded that the TWI established in 2009 (2.5 µg/ kg body weight) should be maintained in order to ensure a high level of protection of consumers.[63] Also, taking non-dietary exposure into account, some subgroups of the population such as children, vegetarians or people living in highly contaminated areas, could possible exceed both the EFSA TWI and the JECFA PTMI.

2.2 ENDOCRINE DISRUPTION

All steroid hormones are derived from cholesterol.[64] The sex steroid hormones, grouped into androgens, estrogens and progestins, are important for growth, differentiation and function of various tissues in both men and women. Testosterone, produced by the testis, is the main circulating androgen in men.[65] Estradiol, is the primary estrogen produced by the ovaries where it is derived from testosterone by aromatase activity. Estrogens are also produced locally, for example in adipose tissue.[66]

It acts on the breast to stimulate ductal growth, on the endometrium to stimulate and regulate its growth and cyclic changes, and on the ovarian follicles to promote granulosa cell differentiation. Progesterone is the major progestine, mainly made in the ovaries, but also locally in tissues. The biological effects of steroid hormones are believed to be mediated in part by specific receptor proteins with high affinity for their respective steroid ligands.[66] Estrogens, for example, are mediated via the estrogen receptors (ER) α and β, testosterone via the androgen receptor (AR) and progesterone via progesterone receptors (PRs) which regulate transcription through direct interaction with specific DNA binding sites of target genes.[67]

Compounds, either natural or synthetic, that alter the hormonal and homeostatic systems in the body are called endocrine disruptors.[68] Even though endocrine disruptors can disturb several hormone systems, the interference with sex steroid hormones has been of greatest interest. Originally, these compounds were thought to act mainly through nuclear hormone receptors, such as ERs or ARs. Today it is known that endocrine disruptors can mimic e.g. estrogenic effects in several ways, not only via nuclear receptors, but also through non-nuclear steroid hormone receptors (i.e. membrane ERs) or enzymatic pathways,[68] thereby disrupting the normal activity of estrogen ligands. Endocrine disruptors can exert negative effects through different mechanisms, for example by direct action to mimic the biological activity of a hormone (i.e. agonistic effect) or they can bind to, but not activate receptor (i.e. antagonistic effect). These compounds can also interfere by indirect mechanisms such as binding to transport proteins in blood, thus altering amounts of natural hormones or interfere with the metabolic processes of hormones.[69]

2.2.1 Cadmium and hormone-disruption

The research on the endocrine-disruptive effects of cadmium was discovered about a decade ago.[54, 55] In 2003, Johnson et al. reported that a single intraperitoneally non-toxic dose of cadmium (5 µg/ kg body weight) induced several well-characterized estrogenic responses.[53] These included increased uterine weight, hyperplasia and hypertrophy of the endometrial lining, induction of uterine progesterone receptor and complement C3 gene expression, increased mammary epithelial density and induction of milk protein synthesis in the mammary gland. Both cadmium and 17β-estradiol gave comparable responses, and an antiestrogen inhibited the cadmium-induced activities. In addition, to assess the estrogenic effects of cadmium after in utero exposure, pregnant rats were given two injections of cadmium intraperitoneally. Among other effects, cadmium affected mammary gland development and onset of puberty in female offspring – both prototypical endocrine disruptor-like responses.[53] Nevertheless, since then, 100-fold higher concentrations of cadmium has been required to produce similar effects[70, 71] and some studies could not find uterotrophic or proliferative effects of cadmium.[70, 72, 73]

Mechanistically, cadmium has been shown to bind the nuclear ERα with high affinity and appears to interact with its hormone binding domain[54, 55] and blocks the binding of

17β-estradiol in a non-competitive manner, increases ER-mediated proliferation and induces the expression of estrogen responsive genes.[74] Recently, cadmium was shown to also activate non-genomic signaling through membrane bound estrogen receptors such as GPR30[75, 76] and mER; thus cadmium would be able to exert effects through different pathways, even in the absence of classical ERs. Recent studies indicate that cadmium does not seem to activate classical estrogen signaling *in vivo*, but that the apparent estrogen-like activity of cadmium possibly stem from a mechanism different from that of steroidal estrogens.[72, 77] In addition, cadmium has been shown to mimic the effects of androgens on the prostate gland and induce the expression of an androgen-regulated gene through binding to the androgen receptor.[78]

Possible health consequences of the proposed estrogen-mimicking effects of cadmium have never been explored.

2.3 HORMONE-RELATED CANCERS

Cancers of the endometrium, breast, ovaries and the prostate, all considered hormone-related,[1-3] are proposed to share a distinct mechanism of carcinogenesis. The general idea is that hormones such as estrogen, progesterone or androgens, affect cell proliferation, and thus the risk for the accumulation of random genetic errors. Estrogen is an established key risk factor in the etiology of cancers in the endometrium, breast and ovary,[3, 4] and may play a role in prostate cancer development.[5] The incidence of hormone-related cancers are higher in high-income countries, supporting the hypothesis that environmental contaminants that mimic the effects of estrogen could play a role in the etiology[52] (Figure 1 and 2).

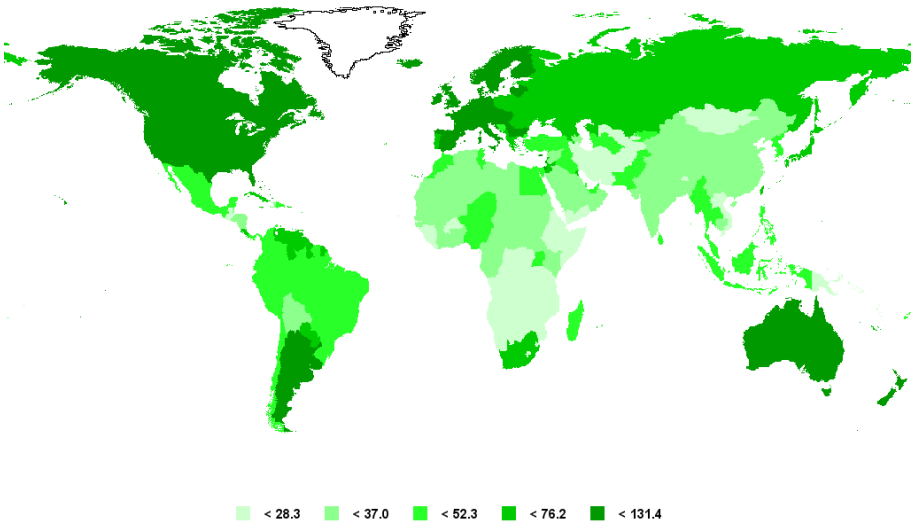


Figure 1. Age-standardized incidence rate per 100 000 in the world. Endometrial, ovary and breast cancer, all ages. Rates are age-standardized to the world population. Data from GLOBOCAN 2008 (IARC) 9.3.2012.

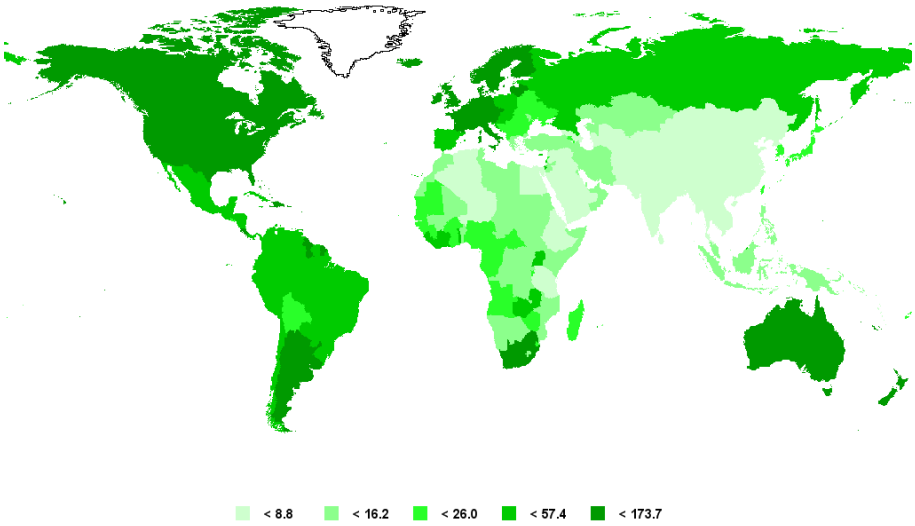


Figure 2. Age-standardized incidence rate per 100 000 in the world. Prostate cancer, all ages. Rates are age-standardized to the world population. Data from GLOBOCAN 2008 (IARC) 9.3.2012.

2.3.1 Endometrial cancer

Endometrial cancer is cancer that starts in the endometrium, the lining of the uterus. During a fertile woman's menstruation cycle, the endometrium is exposed to a process of cyclical change, with phases of high cell proliferation (follicular phase) followed by low proliferation phases (luteal phase).[79] Most of the cancers that occur in the body of the womb (corpus uteri) are endometrial cancers and the majority (80%) are endometrioid adenocarcinomas, which are estrogen driven.[80]

Incidence and trends

Endometrial cancer is the fifth most common cancer among Swedish women, with around 1400 Swedish women receiving this diagnose yearly and an age-standardized incidence of 25.5 per 100 000 individuals in 2009.[81] The incidence in Sweden has steadily increased, but flattened out since the mid-nineties. Worldwide, it is the sixth most common cancer, with around 288 000 cases recorded in 2008. The rates in high-income countries are nearly five times higher as compared to middle- to low-income countries.[80] The majority of women diagnosed with endometrial cancer are over 55 years of age. Since the symptoms (e.g. abnormal bleedings) of endometrial cancer often occur at early stages, the prognosis is good and survival is high; the 5-year survival rate in Sweden is over 85%.

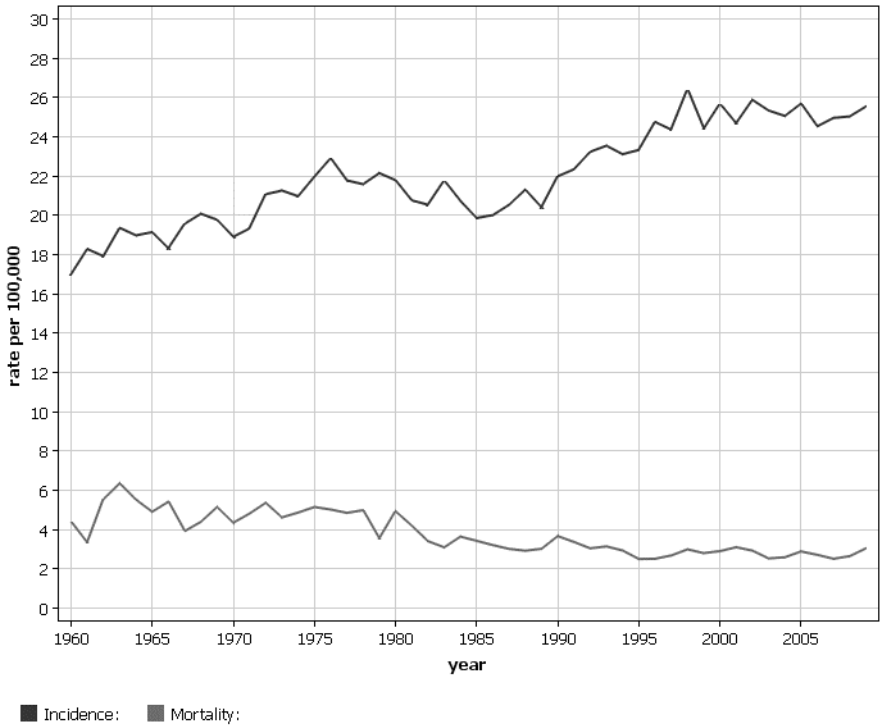


Figure 3. Trends in endometrial cancer incidence (upper line) and mortality (lower line) rates per 100 000 individuals in Sweden, 1960-2009. Rates are age-standardized to the Nordic population in year 2000. Data from NORDCAN © Association of the Nordic Cancer Registries (11.3.2012).

Risk factors

The risk for endometrial cancer is to a large extent influenced by the exposure of the endometrium to estrogens in the absence of progestagen and high levels of estrogens are associated with an increase in risk.[82] Compared to the other hormone-related cancers, endometrial cancer appears to be the cancer form most sensitive to sex hormones. Consequently, life events that protect against endometrial cancer include bearing children and having early menopause, which have the effect of reducing the number of menstrual cycles and therefore lifetime exposure to estrogens[80] and vice versa.

The use of postmenopausal hormones containing estrogens only, causes a large increase in risk, whereas hormones also containing a progestagen is associated with a smaller increase in risk.[82] The expert report on Food, Nutrition, Physical Activity and the Prevention of Cancer from 2007 concluded that there are convincing evidence that obesity is a cause of endometrial cancer and that abdominal fatness is a probable cause.[80] Physical activity probably protects against this cancer.[80, 83] The mechanisms behind the protective effect relates to lower levels of endogenous estrogen among physically active women.[84, 85] When it comes to dietary factors that could increase or decrease the risk, conclusions are more uncertain and there is a lack of prospective studies. Meta-analyses of case-control data states that non-starchy vegetables may well protect against endometrial cancer, suggestively through fiber and/or antioxidants and it

is likely that any protective effect may result from a combination of constituents of these foods.[80] Results from meta-analysis reports a positive association between diabetes and endometrial cancer[86] and such an association has also been found in the cohort of women that is the basis for this thesis.[87]

2.3.2 Breast cancer

Breast cancer develops as a result of abnormal proliferation of cells in the mammary gland and are almost all carcinomas of the epithelial cells lining the ducts.[88] An invasive breast cancer is characterized by the cancer cells occurring outside the basement membrane of the ducts and lobules of the breast, in the surrounding normal tissue.[88] Estrogens and progestagens are needed in normal growth and development of the mammary gland and also as growth factors for the majority of breast cancers.[89] Breast cancer can be divided into ER positive or negative tumors, based on the expression of the receptor in the tumor. Expression of ER (i.e. ER positive tumor) in a tumor is both a prognostic marker and predicts the response to therapies that target estrogen synthesis.[89] Increasing data indicate that risk factors may vary by hormone receptor status.[90]

Incidence and trends

Breast cancer is the most common cancer among women, both in Sweden and worldwide, with around 6400 diagnosed yearly in Sweden and 1.38 million worldwide. The age-standardized incidence per 100 000 women in Sweden was 122 in 2009[81] and breast cancer represents around 30% of total cancer cases in Swedish women.[91] Seen over the 20 last years, breast cancer incidence has increased with 1.3 per cent annually, but during the recent 10-year period this increase has become weaker. Incidence rates are much more common on high-income countries, but increase rapidly in middle- and low-income countries. Breast cancers can often be detected at a relatively early stage thanks to mammography screening[80] and the 5-year survival rate in Sweden is 86%.[91]

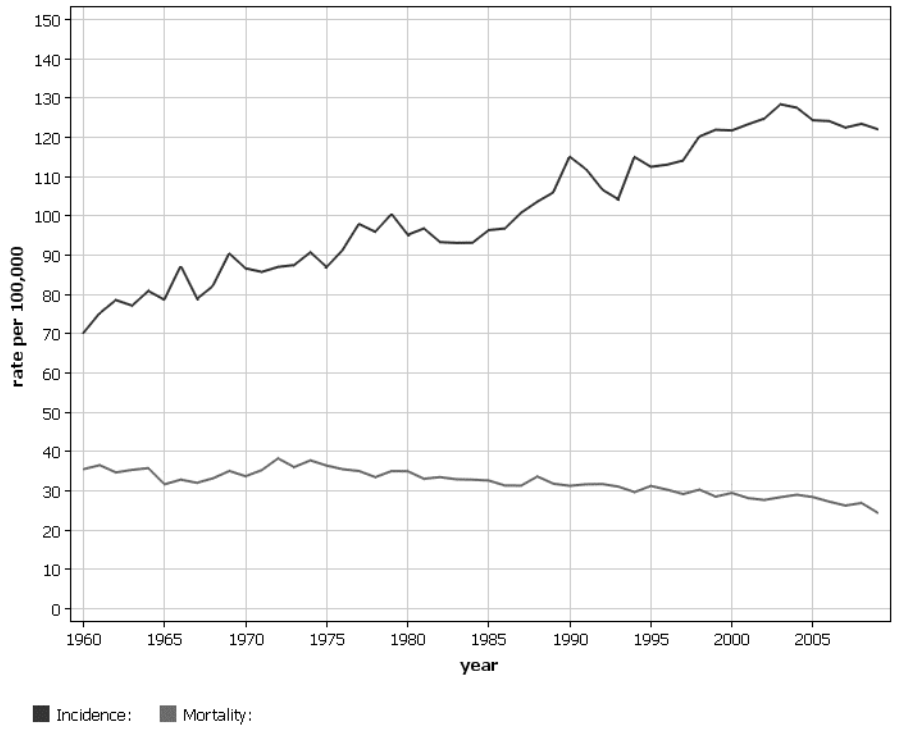


Figure 4. Trends in breast cancer incidence (upper line) and mortality (lower line) rates per 100 000 individuals in Sweden, 1960-2009. Rates are age-standardized to the Nordic population in year 2000. Data from NORDCAN © Association of the Nordic Cancer Registries (11.3.2012).

Risk factors

Since breast cancer is hormone related, the factors that modify the risk of this cancer when diagnosed premenopausally and postmenopausally differ. The majority of cases occur after menopause[91] and in this thesis the focus is on postmenopausal breast cancer (**paper III**). In postmenopausal women, increased exposure to estrogens increases the risk of breast cancer.[82] Thus, early age at menarche and late menopause, not bearing children, and late (> 30 years) first pregnancy increases the risk for this cancer.[80] Further, the use of postmenopausal hormones is also associated with a moderate increase in risk. The expert report on Food, Nutrition, Physical Activity and the Prevention of Cancer from 2007 concluded that there is convincing evidence that body fatness is a cause for postmenopausal breast cancer, and adult weight gain is a probable cause.[80] This suggests that the increase in risk may be mediated by elevated levels of endogenous estrogens found in heavier women.[90] Physical activity probably protects against postmenopausal breast cancer; proposed mechanisms are the beneficial effect that physical activity has on body weight,[80, 90] the effect on endogenous steroid hormone metabolism, such as reducing levels of circulating estrogens, or through a strengthening the immune system.[80] There is convincing evidence that consuming alcoholic drinks are a cause of breast cancer at all ages, and that the factors that lead to greater attained adult height are a cause of postmenopausal breast cancer is convincing.[80]

Whole grain and vegetables which are major dietary sources of fiber, phytoestrogens and antioxidants, proposed to have anticarcinogenic properties[80] have been inversely associated with breast cancer in some studies,[92-95] but not all.[80, 96, 97] Epidemiological findings have also shown a positive association between diabetes type 2 and breast cancer.[98]

2.3.3 Ovarian cancer

There are three types of ovarian tissue that can produce cancers: epithelial cells, which cover the ovary; stromal cells, which produce hormones; and germ cells, which become eggs.[80] The epithelial ovarian tumors account for approximately 90 % of malignant ovarian tumors.[99] They can be further classified into serous, endometrioid, mucinous, clear-cell, and transitional cell (or Brenner type), where serous tumors are the most common, constituting approximately 50% of all ovarian malignancies.[99] In this thesis, the group of epithelial ovarian cancer is referred to as "ovarian cancer".

Incidence and trend

Ovarian cancer is the ninth most common cancer in Swedish women[91] with around 700 women diagnosed yearly[100] and an age-standardized incidence of 14.5 per 100 000 individuals in 2009.[81] In Sweden, the incidence rate has decreased steadily since the 1970-ies; at the same time the survival rate has increased. Worldwide, ovarian cancer is the seventh most common cancer in women and it is most frequent in high-income countries.[80] It is possible to get the disease at all ages, but 85-90 % of the cases occur after the menopause.[80] Often this cancer type is symptomless at early stages, resulting in a generally advanced disease at time of diagnosis.[80] The 5-year survival rate ranges from approximately 30 to 50 % in Sweden.[91]

Figure 5. Trends in ovarian cancer incidence (upper line) and mortality (lower line) rates per 100 000 individuals in Sweden, 1960-2009. Rates are age-standardized to the Nordic population in year 2000. Data from NORDCAN © Association of the Nordic Cancer Registries (11.3.2012).

Risk factors

The etiology of ovarian cancer is not fully understood and the role of hormonal factors is much less clear than it is for endometrial and breast cancer.[82] Results from experimental studies support a potential role of estrogens to stimulate proliferation of ovarian surface epithelium cells,[101, 102] but epidemiological evidence is more inconclusive. Whereas long-term use of any type of hormone replacement therapy seems to increase the risk of ovarian cancer,[103] some studies observed a weaker association in users taking hormones containing estrogen combined with progestin.[104] The observed favorable effect of progestins could explain these findings. Nevertheless, taken together, the results support the hypothesis of long-term elevated estrogen concentrations as etiologic important for this disease. Also in favor of the estrogen hypothesis is the protective effect of oral contraceptive use, since it decreases ovarian estrogen production.[103] The risk of ovarian cancer decreases with increasing number of birth and early menarche and late menopause increases the risk.[82] The evidence regarding a possible relationship between body mass index (BMI) and ovarian cancer risk is inconclusive, but there is some suggestion that a high BMI may have a protective effect.[82] Further, it is suggested that several ovarian cancer risk factors, including age, duration of breastfeeding, age at natural menopause and duration of estrogen and smoking, may vary by subtype.[105]

2.3.4 Prostate cancer

The prostate is a walnut-sized gland surrounding the top of the urethra which produces the seminal fluid.[80] Androgens, especially dihydrotestosterone, influence the growth and development of the prostate gland[106] and acts to stimulate cell proliferation and inhibit apoptosis in the prostate.[82] Almost all prostate cancers are adenocarcinomas. Prostate cancer is usually a slow developing cancer and abnormal development of cells (i.e. dysplastic lesions) may occur many years or even decades before cancer development. Autopsy studies suggest that most men would have prostate cancer if they lived to be more than 100 years of age and small, localized cancers can be present for many years without any symptoms.[80] This means that men are more likely to die with, rather than from, prostate cancer.

Incidence and trend

Prostate cancer is the most common cancer in Swedish men, representing 34 % (around 8900) of the male cases in 2010.[91]The age-standardized incidence was 210 per 100 000 individuals for Sweden in 2009. Over the last 20 years, the incidence has, on average, increased except for the past five years where a slightly decrease has been observed. The incidence of prostate cancer is related to the use of Prostate-specific antigen (PSA) testing in health care and therefore it is uncertain how the incidence trend will develop over the coming years.[91] Worldwide, prostate cancer is the second most common cancer among men, with 899 000 new cases diagnosed in 2008[80] and the disease is more often diagnosed in high-income countries where screening is common. Often this cancer type is symptomless at early stages, resulting in a generally advanced disease at time of diagnosis.[80] The 5-year survival rate is 87 % in Sweden.[91]

Risk factors

The causes of prostate cancer are still largely unknown,[106] but may differ depending on subtype of disease (i.e. localized or advanced).[107] Elevated concentrations of androgens over long time may increase prostate cancer risk, but results have been inconclusive.[108] Studies have demonstrated an inverse association between diabetes and prostate cancer risk.[109] Diabetic men have lower serum concentrations of insulin and testosterone, insulin-like growth factor I (IGF-1) as well as higher concentrations of estrogens[107] which could be a reason behind the lower prostate cancer risk among diabetic men.[109] Since body fat is associated with both sex-hormones and IGF-1,[110, 111] BMI has been studied as a factor for prostate cancer risk, but results are inconclusive.[82] In prostate cancer subtypes, associations may differ between lean and obese men.[112, 113]

The expert report on Food, Nutrition, Physical Activity and the Prevention of Cancer from 2007 judged that diets high in calcium probably increase the risk of prostate cancer and consuming foods containing lycopene or selenium probably protect against this cancer.[80] There is limited evidence suggesting that pulses (legumes) including soya and soya products, foods containing vitamin E, and alpha-tocopherol supplements are protective, and that processed meat, milk and dairy products are a cause of this cancer.[80]

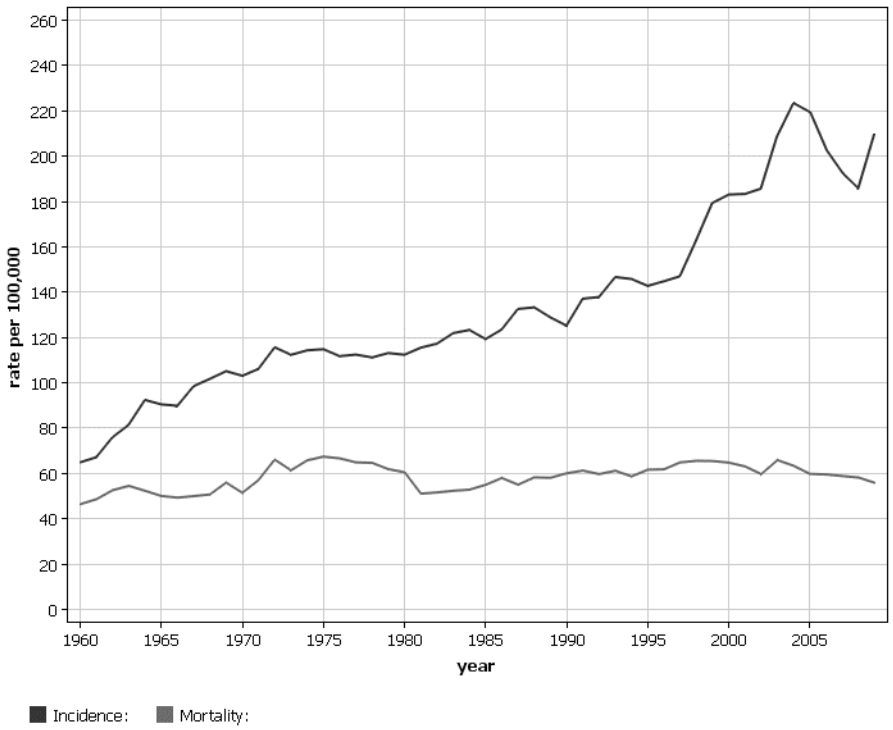


Figure 6. Trends in prostate cancer incidence (upper line) and mortality (lower line) rates per 100 000 individuals in Sweden, 1960-2009. Rates are age-standardized to the Nordic population in year 2000. Data from NORDCAN © Association of the Nordic Cancer Registries (11.3.2012).

2.4 CADMIUM AND HORMONE-RELATED CANCER

Given the fact that cancer of the endometrium, breast, ovaries and prostate are hormone-dependent, it is pertinent to hypothesize that cadmium, mimicking the effect of estrogens, may play a role in the development of these cancers. Few previous studies have explored the associations between cadmium exposure and risk of hormone-related cancers. No studies, except of the two presented in the current thesis, have explored the association between cadmium exposure and risk of endometrial or ovarian cancer. Regarding breast cancer, two previous reports of case-control studies have assessed the

risk of breast cancer among pre- and postmenopausal women in relation to cadmium exposure, assessed as the cadmium concentration in urine.[114, 115] McElroy and colleagues observed in the study of 246 cases, a multivariable-adjusted odds ratio of 2.29 (95% CI: 1.3–4.2) comparing the highest quartile of urinary cadmium versus the lowest. The second study examined the association between cadmium and breast cancer in two case-control samples from the United States, consisting of 100 and 98 cases, respectively. In both samples, increased odds ratios were observed (2.69; 95% CI: 1.07-6.78 and 2.5; 95% CI: 1.11-5.63, respectively) comparing the highest quartile of urinary cadmium with the lowest. Recently, a cross-sectional study lent further support to a relationship between cadmium exposure and breast cancer risk by observing an association between urinary cadmium and mammographic density.[116]

Four studies[117-120] have assessed the association between cadmium exposure present in the general non-occupationally exposed population and prostate cancer risk, with inconsistent results. Only one of them assessed the cadmium exposure through diet.[117] In a population-based case-control study in Utah, comparing extreme quartiles, an increase in prostate cancer risk for all tumors (OR 1.8; 95% CI: 1.1-3.1) was observed in elderly men (aged 68-74 years), but not in younger (OR 1.1; 95% CI: 0.7-1.9). In an Italian hospital-based case-control study, a 4.7-fold increased risk (95% CI: 1.3-17.5) of prostate cancer was observed comparing the highest quartile of toenail cadmium with the lowest.[119] However, no difference was observed in blood cadmium between Taiwanese hospital-based cases and controls, while cases had lower urinary cadmium (median 0.94 versus 1.40 μg/g creatinine).[120] Among cases with a Gleason score ≥8, however, the odds ratio was 2.89 (95% CI: 1.25-6.70) compared to cases with a score <6. Since blood cadmium primarily reflects recent exposure and urinary cadmium concentrations are acknowledged to mirror the accumulated body burden,[14] urinary cadmium seems a better marker than blood cadmium for assessing the association between cadmium exposure and prostate cancer risk. In an American nested case-control study, no association was observed between toenail cadmium and the risk of prostate cancer.[118] The validity of toenail cadmium concentration as a marker of cadmium body burden is uncertain as the factors influencing the deposition of cadmium in toenails and the time-course of deposition is unknown.[118]

3 AIM

The overall aim of this thesis was to assess the association between cadmium exposure *via* food and risk of hormone-related cancers.

The specific objectives were:

- To estimate the dietary cadmium exposure and major contributing food sources in two large populations consisting of 60 000 women and 40 000 men.
- To estimate cadmium's toxicokinetic variability using a population model and to establish the link between urinary cadmium concentrations and the corresponding long-term dietary exposure in the population (paper I).
- To evaluate the comparability between the food frequency questionnaire-based estimates of dietary cadmium exposure with measured urinary cadmium concentrations using different approaches (paper III, supplementary data).
- To prospectively assess the association between dietary cadmium exposure and the incidence of endometrial cancer (paper II), overall and hormone-receptor defined invasive breast cancer (paper III), ovarian cancer (paper IV) and prostate cancer and its subtypes (paper V) in two large population-based cohorts of women and men.

4 SUBJECTS AND METHODS

The following is a summary of the study populations and methods used in the studies included in this thesis. A detailed description can be found in each respective paper (**I-V**).

4.1 STUDY POPULATION

This thesis is based on data from two population-based prospective cohort studies, the Swedish Mammography Cohort (SMC) and the Cohort of Swedish Men (COSM). The Regional Ethical Review Board in Stockholm approved the studies. Obtained written information about the study and a returned completed questionnaire were considered to imply informed consent.

4.1.1 The Swedish Mammography Cohort

The SMC was established in 1987-1990 when all women born between 1914 and 1948 and living in the two counties of Uppsala and Västmanland in central Sweden were invited to a mammography screening (Figure 7). A mailed, self-administered questionnaire concerning diet, lifestyle, and reproductive factors was completed by the participants; response rate 74%. Information on history of oral contraceptive use, postmenopausal hormone use, and age at menarche and menopause was obtained from a supplemental questionnaire from the women in Uppsala County (54% of the cohort).

In 1997, a second questionnaire, expanded to include about 350 items concerning diet and other lifestyle factors (including smoking habits), was sent to all participants who were still alive and living in the study area; 39 277 women (70%) completed this questionnaire.

In 2003, a subcohort was initiated. Women living in the town of Uppsala were successively invited to complete a questionnaire on diet and lifestyle factors, and to undergo a health examination including bone mineral density, weight and height measurements as well as sampling of fat and blood. From 2004, sampling of morning spot urine was included. The recruitment continued until September 2009; at that time 8311 women had been invited (response rate 65%).

For details on exclusions the reader is referred to Figure 7. In short, for **paper I**, women of the subcohort that participated between 2004 and 2007, left a urine sample and were between 56-70 years of age were included; in total 1519 women. The age limit of 70 years was chosen in order to avoid an inverse effect of old age on kidney cadmium accumulation.[121] Because smoking is an additional source to cadmium exposure, we excluded women who had ever smoked (n = 813), thus 680 women were left for modeling analysis. In the **supplementary data** of **paper III**, we utilized data from the same 680 women to evaluate the comparability of dietary cadmium exposure with

urinary cadmium concentration. The reproducibility of the questionnaire-based dietary cadmium exposure was assessed in a random sample of women from the SMC, who completed two identical FFQs one year apart (during 2004-2005); 300 completed both questionnaires, response rate 50%.

For **papers II-IV**, those with a previous cancer diagnosis before baseline were excluded. Since a potential estrogenic effect of cadmium could be masked by the effect of endogenously produced estrogens from the ovaries, we restricted the analyses to postmenopausal women in **papers II** and **III**. We defined menopausal status and age at menopause based on reported age at cessation of menstruation. In case of missing data, women were considered to be postmenopausal if they had bilateral oophorectomy, obtained by computerized linkage of the study population with the National Hospital Discharge Registry, or were 55 years of age or older (because approximately 90% of women in the cohort stopped menstruating before the age of 55 years). Because of the fact that diabetes may increase the risk of endometrial cancer[87] and breast cancer[98] and that the dietary advice for diabetics involves high consumption of foods high in cadmium, we excluded women with diabetes mellitus from the cohort (**papers II and III**). For endometrial cancer, women that pre-baseline underwent hysterectomy (n = 202) and for ovarian cancer women who underwent oophorectomy (n = 544) were excluded.

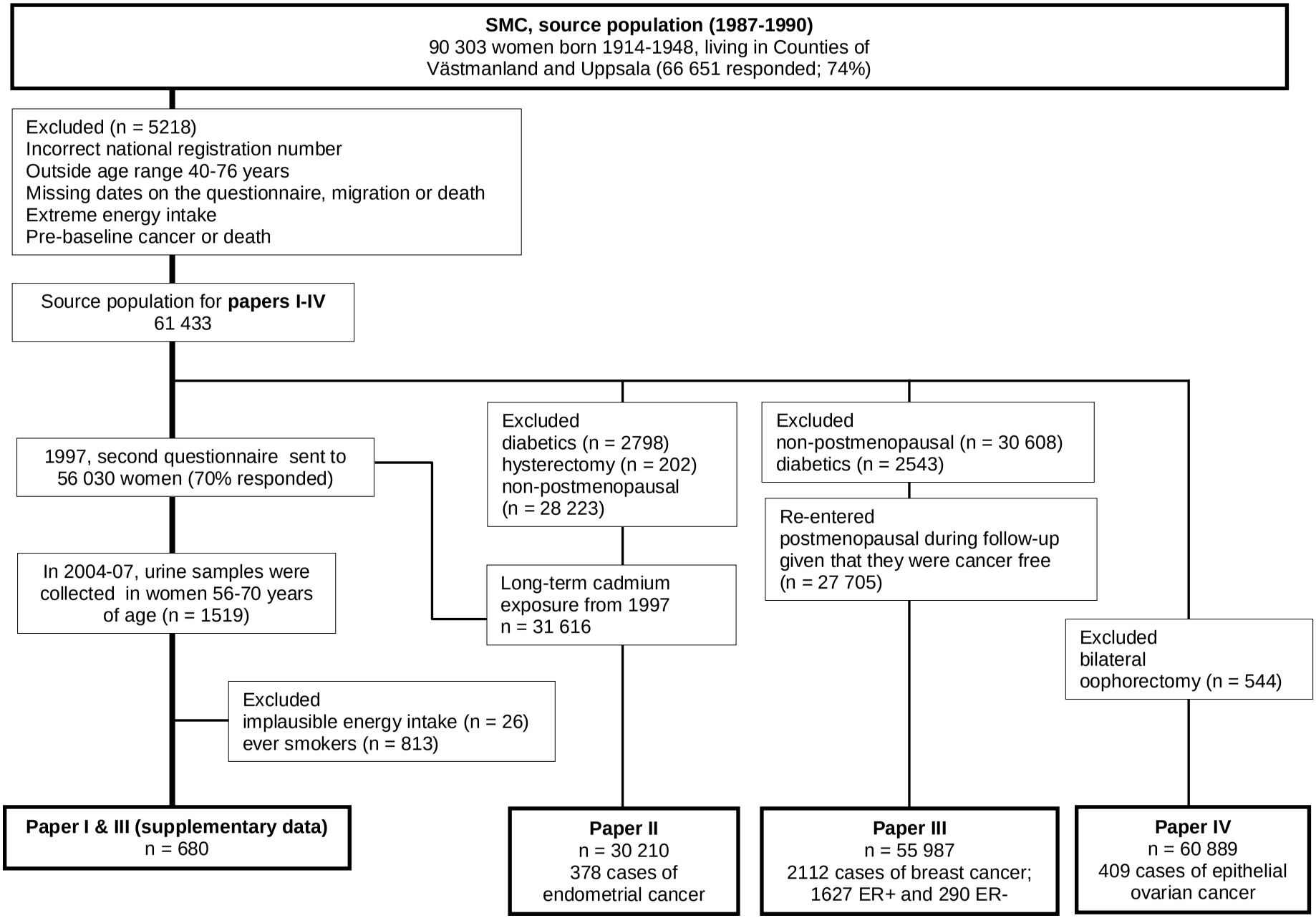


Figure 7. The Swedish Mammography Cohort (SMC) source and study populations for paper I-IV.

4.1.2 The Cohort of Swedish Men

The COSM was established in 1997-1998, when all eligible men born between 1918 and 1952 and residing in Västmanland and Örebro Counties in central Sweden received an invitation to participate in the study along with a self-administrated questionnaire, including almost 350 items on diet and other lifestyle factors (the same questionnaire as the women received in 1997); response rate 49%. This large population-based cohort is representative of Swedish males aged 45–79 years, in terms of age distribution, educational level, and prevalence of overweight.[122]

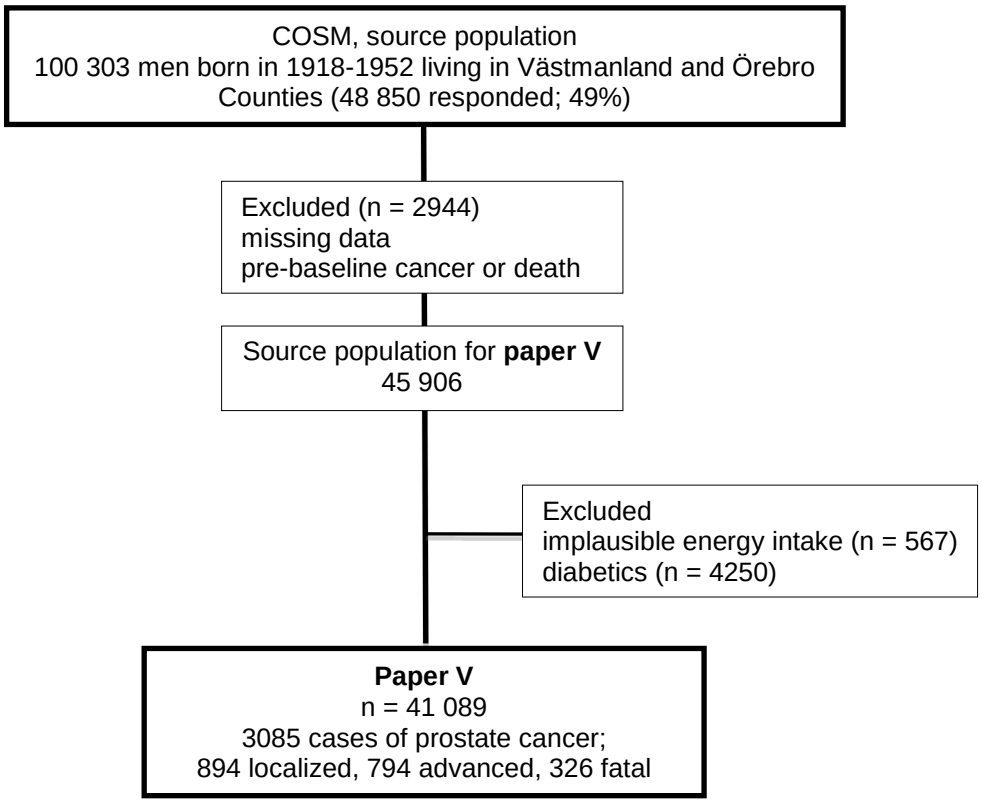


Figure 8. The Cohort of Swedish Men (COSM) source and study population for **paper V**.

For details on exclusions the reader is referred to Figure 8. In short, men with a previous cancer diagnosis were excluded. We also excluded those who reported an implausible energy intake and those diagnosed with diabetes before baseline as diabetes is associated with decreased risk of prostate cancer and the dietary advice given to diabetics is likely to lead to an increased exposure to of cadmium.[123] Thus, the analytical cohort for the primary analysis consisted of 41 089 men.

4.2 EXPOSURE ASSESSMENT

4.2.1 Assessment of diet and lifestyle factors

Diet was assessed in 1987-90 (baseline) and in 1997 among the women and in 1997 (baseline) among the men. In these FFQs, participants reported their usual average frequency of consumption of specific food item using eight predefined frequency consumption categories, ranging from never/seldom to four times a day. The consumption of bread and dairy products was assessed by open-ended questions.

The baseline FFQ in women has been validated in a sub-sample of 129 women randomly chosen from the study population. The Pearson correlation coefficients (r) between the FFQ and the mean of four 1-week weighted diet records were between 0.5 and 0.8 for the main cadmium-containing foods (0.5 for whole grain bread, 0.6 for breakfast cereals, 0.5 for potatoes, 0.5 for root vegetables, 0.8 for cabbage and 0.6 for spinach). Habitual physical activity was assessed by five questions concerning activity/inactivity in the 1997-questionnaire; these were work/occupation, walking/bicycling, home/household work, leisure time watching TV/reading and exercise. The time spent per day at specific activities was multiplied by its typical energy expenditure requirements (expressed in metabolic equivalents (METs) and summarized in MET-hours per day based on a physical activity compendium.[124] For women, we assessed leisure time activity using a validated questionnaire (r = 0.52)[125] with five predefined categories for time spent per day watching TV/sitting (**paper II**). Inactivity was defined as sitting ≥5 hours per day.[83]

The baseline FFQ in men has been validated in a random sample of 248 men aged 40-74 years living in the study area, which completed the FFQ and 14 repeated 24-hour recall interviews during a one year period. For macronutrients the mean Spearman's rank correlation coefficient between the FFQ and 14 repeated 24h recalls was 0.65.[126] For men, we obtained questionnaire data on family history of prostate cancer, education, height (at age 20), weight, waist circumference, smoking habits and physical activity. The Spearman correlation coefficient between total daily activity score estimated from the questionnaire and physical activity records was 0.56.[127]

Based on the reported weight and height, we calculated the BMI as weight (kg) divided by height^2 (m^2). The validity of BMI based on self-reported weight and height in the Swedish population is high in both men and women compared to measurements (r = 0.9).[128]

4.2.2 Assessment of dietary cadmium exposure

We created a comprehensive database on the cadmium content in food items. Data of cadmium concentration in different foods were obtained from the Swedish National Food Agency (NFA; Uppsala, Sweden). In order not to comprise the quality of the data, we excluded analyses performed before 1980, with the exception of butter, margarine and oils (analyzed 1976-78), were later results were lacking. Results below

the detection limit were assigned the half of the detection limit. In case of lacking analysis (e.g. pepper, spinach, leek, and citrus fruits) we used Finnish[129, 130] and Danish[22] data. Cadmium content in prepared dishes was calculated based on a recipe database from the NFA and on the cadmium concentration in each ingredient. Altogether, assessments of cadmium content were made for 422 food-items and 81 dishes.

The dietary cadmium exposure, as well as the intake of energy and specific nutrients, was estimated by multiplying the frequency of consumption of different foods by the average daily consumption (g/day) calculated from the mean values of age-specific (<53, 53-65, >65 years) portion sizes of scale-weighted foods recorded during 4 1-week periods 3-4 months apart, by 213 women randomly selected from the cohort (Wolk, A: unpublished data). Exposure from air contributes to less than 1%[131] and community-provided tap water and water from private wells contributes on average with 0.2%[12] of the total cadmium exposure and was thus ignored.

Because dietary cadmium exposure was positively correlated with energy intake (r = 0.76 among women and r = 0.79 among men), we energy-adjusted cadmium using the residual-regression method.[132] The adjustments were made to the mean energy intakes in the cohorts (1700 kcal/day for women and 2600 kcal/day for men). Energy-adjustment is based on the concept that the composition of the diet, independent of total caloric intake, is of primary interest - also in relation to the cadmium accumulation in the body. Thus, we create a kind of isocaloric diet. The adjustment also limits misclassification of exposure by reducing an artificial between-person variation in cadmium intake estimates due to some over- or underreporting of food intake in the questionnaires. The estimated dietary cadmium exposure was then categorized into tertiles (**papers II-V**).

4.2.3 Assessment of urinary cadmium

The concentration of cadmium was determined in the first voided morning urine, collected in urine cups, tested free from contamination, and transferred to acid washed (nitric acid; HNO_3) polypropylene tubes. To minimize the risk of cadmium contamination of urine, the women received detailed sampling instructions. Cadmium concentrations were measured at the Institute of Environmental Medicine, Karolinska Institutet, using inductively coupled plasma mass spectrometry with a collision/reaction cell operated in helium-mode (ICPMS; Agilent 7500ce, Agilent Technologies, Waldbronn, Germany), measuring cadmium isotope 111. The helium mode minimized polyatomic interferences. The urine samples were thawed in room temperature and then diluted 10 times with 1% HNO_3 (suprapur; Merck, Darmstadt, Germany). For quality control purposes, commercial reference materials (SeronormTM Trace Elements Urine, REF 201205, LOT NO2525 and SeronormTM Trace Elements Urine Blank, REF 201305, LOT OK4636; SERO AS, Billingstad, Norway) were analyzed. Mean cadmium concentrations in the Seronorm samples were 4.80 ± 0.13 µg/L (n = 68; recommended value 5.06 ± 0.22) and 0.24±0.03 µg/L (n = 121; recommended value

0.31 ± 0.05), respectively. The coefficient of variation (CV) was 2.6% and 13.0%, respectively. The limit of detection, calculated as three times the standard deviation of the blank values, was 0.003µg/L. In addition to the reference materials, we included a "in house" control urine sample, which showed good repeatability (mean concentration 0.79 µg/L, CV 5%). Because toxicokinetic models were only available for creatinine-adjusted data, all urinary cadmium concentrations were expressed in µg per g urinary creatinine, range 0.16-2.4 g/L, (Clinical Chemistry, Västerås Hospital, Västerås, Sweden) to account for variation in urine dilution.

4.3 TOXICOKINETIC MODELING (PAPER I)

In addition to the level of cadmium exposure, factors such as the intestinal absorption of cadmium and the half-life of cadmium in the kidney, which may vary between individuals, affect how much cadmium that is accumulated in the body. The quantification of the between-person variability of these factors in a population is important in risk assessment in order to build a robust statistical link between cadmium intake and urine concentrations. The quantification of the variability requires complex statistical modeling and could be obtained more easily with a simpler model. Therefore, in a first step, we fitted two models with different complexity without any population variability and compared their mean predictions. In this step all parameters are the same for everyone. If the two models were fairly similar the simpler model would be used to estimate inter-individual variability by adding a population layer. This means, a parameter for a specific individual is drawn from a predefined population distribution, thus adding variability. Finally, the estimated parameters were used for health risk assessment to derive the population distribution of daily cadmium intake required to reach a given level of cadmium in urine after lifetime exposure (50 years of age).

4.3.1 Model comparison

Two existing toxicokinetic models developed for cadmium were investigated and compared: 1) an eight-compartment physiologically-based toxicokinetic (PBTK) model developed by Kjellström and Nordberg[58, 133, 134] and 2) a simplified one-compartment model[57] with standard first-order elimination (i.e. elimination of a constant fraction per time unit) and bolus administration (i.e. a single, oral dose).[135] The simplified one-compartment model focuses on the kidney accumulation and urinary excretion, making rough assumptions on the other pathways. For both models, we calculated for each individual the mean yearly cadmium intake over the three measurement periods (1987, 1997, 2004-07) as a proxy for the average long-term cadmium intake and used the individual urinary cadmium concentrations. For inference, we used a Bayesian approach. This setup requires definition of prior distributions for each model parameter to be estimated. We defined priors based on available literature.

For the eight-compartment model, the parameters to be estimated were the absorption fraction and the fraction transferred from plasma to extravascular fluid. For both these parameters, non-informative priors restricted to positive values were used; parameter values were taken from Diamond et al., 2003[134], except for the absorption fraction where we used the value 0.1 (i.e. 10%).

For the one-compartment model, the parameters to be estimated were the half-life and a factor consisting of the gastrointestinal absorption coefficient as well as factors determining the amount of cadmium that end up in the urine (see Table 1 for separate factors). Since information on half-life is sparse, a non-informative (uniform) prior, restricted between 5 and 35 years was set for individual half-life. The time range was chosen to constrain the estimation to biologically plausible values. For the second factor, an informative prior was chosen in order to integrate the prior knowledge on cadmium-related and physiological parameters. This prior was set to a normal distribution centered on the central value given by the literature (Table 1) and with a CV of 30% covering the range of possible values.

Monte Carlo Markov chains were used for the fitting using an ad hoc Metropolis-Hastings algorithm coded in Matlab release 14 for the eight-compartment model and WinBUGS version 1.4 for the one-compartment model. The mean predictions of urinary cadmium using the two models were very close (Figure 9), thus we used the one-compartment model in the following calculations.

Table 1. Values taken from the literature and used as prior information for the one-compartment model.

Parameter	Value	References
Gastrointestinal absorption coefficient	1-10%	Flanagan 1978[136], Nordberg 2007[137]
Fraction of cadmium transported to kidney	1/3	Kjellström 1978[58], Nordberg 1985[138]
Coefficient between whole kidney cadmium and kidney cortex	1.25	Kjellström 1978[58], Nordberg 1985[138], Svartengren 1986[139]
Kidney weight	0.43% of total body weight	Kjellström 1978[58], Nordberg 1985[138]
Ratio between cadmium in urine and kidney cortex	a cadmium concentration of 50 mg/kg in kidney cortex corresponds to 1.7-2.5 µg cadmium/g creatinine in urine	Kjellström 1978[58], Nordberg 1985[138], Orlowski 1998[35]

4.3.2 Population modeling

Regarding the individual parameters to be estimated, the setting was as described before for the one-compartment model. A population layer was added to describe the inter-individual variability of half-life. A lognormal random distribution was chosen to describe the distribution of the half-life in the population, since this parameter tends to have a skewed distribution. The population mean of the half-life was allowed to vary between 10-30 years. From this distribution the individual half-lives were randomly sampled. A 25% variability of intra-individual daily intake was assumed and integrated into the model. The intra-individual variability in urinary cadmium could not directly be estimated from the data, but was assessed in sensitivity analysis. We ran three parallel Markov chains (> 300 000 simulations) in order to obtain the posterior distributions and their estimates. Thinning was set to 10 and the 150 000 first simulations were viewed as burning time (i.e discarded).[140, 141]

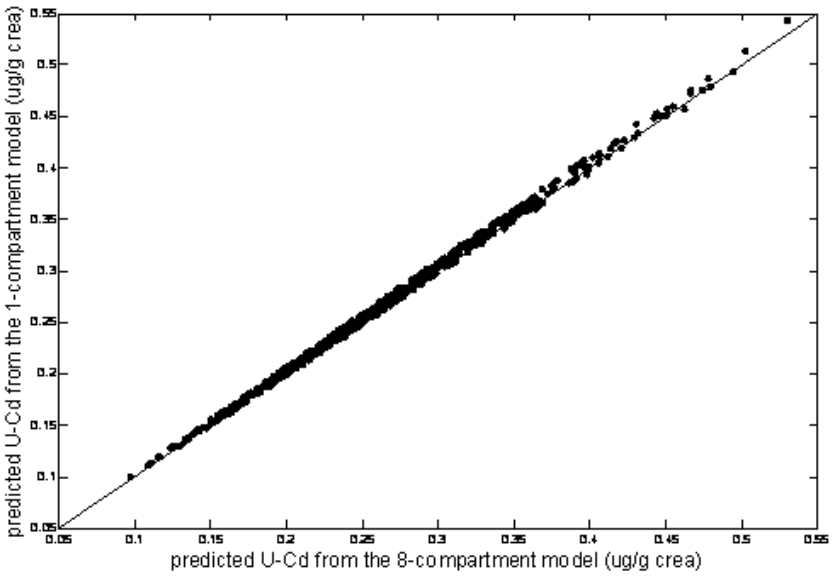


Figure 9. Comparison of individual urinary cadmium predictions using the one-compartment model (y-axis) and the eight compartment model (x-axis).

4.3.3 Application of model estimates on risk assessment

At last, based on the estimated parameters, we ran Monte Carlo simulations in order to predict the population distribution of daily cadmium intake linked to a given level of urinary cadmium after lifetime exposure.

4.4 CASE ASCERTAINMENT (PAPERS II-V)

Incident cases of endometrial (**paper II**), breast (**paper III**), ovarian (**paper IV**) or prostate cancer (**paper V**) were identified by computerized linkage of the study populations to the Swedish National Cancer Registry. The Swedish Cancer Registry have been estimated to provide close to 100% coverage.[142] Ascertainment of hysterectomies (**paper II**) and oophorectomies (**paper IV**) were obtained from the National Hospital Discharge Registry. By linkage to the Swedish Death and Population Registries we obtained dates of death and migration from the study area.

Data on estrogen receptor status of breast tumors was obtained from pathology logs at the Uppsala University Hospital (1987–1994) and from the Quality Registry at the Regional Oncology Centre in Uppsala (1994–2008); a proportion of positive cells over 10% were defined as ER+.

With regard to prostate cancer, information on Tumor-Node-Metastasis stage (T-N-M), Gleason grade and prostate-specific antigen (PSA) were ascertained through medical records and the Swedish Prostate Cancer Quality Registry. Incident cases were then classified by subtype as localized (T<3, and PSA<10 and Gleason grade ≤6) or advanced (T=4, or N=1, or M=1 or PSA>100 or Gleason grade >7). Because of this exclusive classification, 55% of the prostate cancer cases were classified as either localized or advanced. Information on prostate cancer death was ascertained through linkage to the Swedish Register of Death Causes at the National Board of Health and Welfare. Classification of deaths was based on International Classification of Diseases (ICD-10, code C61 for prostate cancer).

4.5 STATISTICAL ANALYSIS (PAPERS II-V)

4.5.1 Dietary cadmium versus urinary cadmium concentrations (paper III, supplementary data)

Sensitivity was defined as the probability of being classified as high in the FFQ-estimated dietary cadmium if also urinary cadmium was high and specificity as the probability of being classified as low in FFQ-cadmium if also urinary cadmium was low. We calculated the sensitivity and specificity by cross-classify extreme tertiles (high and low) of FFQ-based dietary cadmium estimates with extreme tertiles (high and low) of urinary cadmium concentrations. This approach was chosen because we wanted to assess the ability of the questionnaire to correctly classify individuals in the same groups as later used in the exposure-disease association studies. The reproducibility of the dietary cadmium intake was assessed by calculating the intraclass correlation between the two identical FFQs answered by 300 women. We compared long-term

dietary cadmium exposure with urinary cadmium using the partial Pearson correlation coefficient for continuous data. Because physiological factors, such as gastrointestinal absorption, age and body weight, affect the concentration of cadmium in urine and thus the link between dietary and urinary cadmium, we adjusted the correlation for dietary iron and fiber intake (as approximations for gastrointestinal absorption), age and weight. To account for the exponential shape of the elimination rate of cadmium, we also compared predicted urinary cadmium concentrations using the one-compartment model with the actual measured urinary cadmium concentrations. The prediction of urinary concentration by the model was based on individual data for dietary cadmium intake, age and weight, while unknown factors such as the gastrointestinal cadmium absorption was set to 3% and the cadmium half-life to 11.6 years as estimated by the population modeling.[143] In a final step, the correlation was adjusted for the within-person variation of the FFQ-based dietary cadmium estimates (deattenuated correlation).[144]

4.5.2 Dietary cadmium exposure and the risk of hormone-related cancers (Paper II-V)

For an overview of time of exposure assessment and follow-up in each respective paper, please see Figure 10. Each participant contributed to follow-up time from start of follow-up until date of diagnosis of the cancer type under study (endometrial, breast, ovarian or prostate cancer), date of death, or end of follow-up, whichever occurred first. Women were censored at date of hysterectomy (**paper II**) or oophorectomy (**paper IV**).

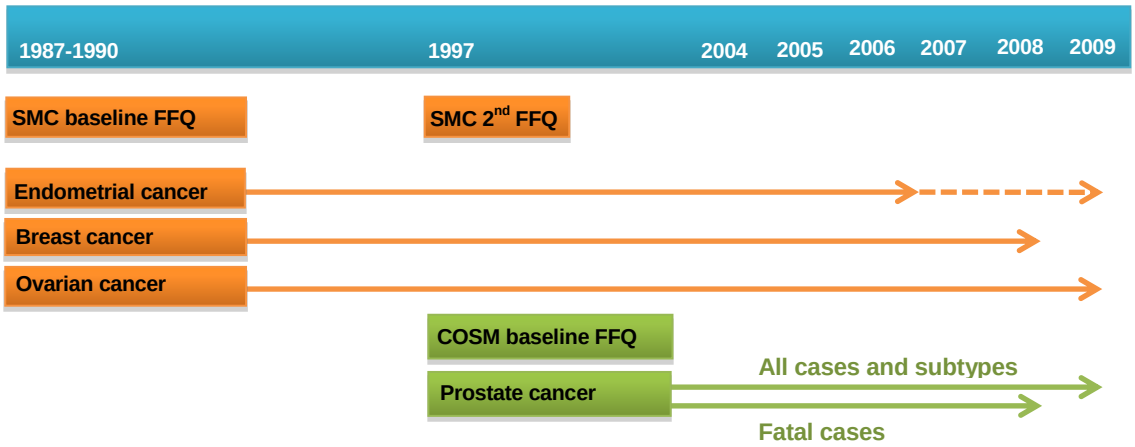


Figure 10. Overview of time of exposure assessment and follow-up in the Swedish Mammography Cohort (SMC) (orange) and the Cohort of Swedish Men (COSM) (green).

Start of follow-up was counted from date of entry into the SMC (**papers II** and **IV**). For **paper III**, start of follow-up differed between the two Counties (1987–90 in Uppsala County and Jan 1, 1998 in Västmanland County) because routine assessment of breast tumor subtypes was introduced in Västmanland County first during 1997 and we wanted to have the same start of follow-up for the analysis with and without receptor status. Furthermore, we re-entered women that became postmenopausal during follow-up; i.e. for women not being postmenopausal at enrollment, date of becoming menopausal was counted as start of follow-up. This was done in order to increase the power of the study. For members of the COSM, start of follow-up was 1st of January 1998 (**paper V**). The end of follow-up was June 30st, 2006 (**paper II**), December 31st, 2008 (**paper III** and fatal cases of prostate cancer; **paper V**) or December 31st, 2009 (**papers IV** and **V**). In additional analyses only performed for this thesis summary and thus not presented in the original paper II, the end of follow-up was extended to December 31, 2009.

Rate ratios (RR) and 95% confidence intervals (CIs) were estimated with Cox proportional hazards regression models with attained age (1-y units) as the underlying timescale. The Schoenfeld's residual test was used to ensure that the proportional hazard assumption was not violated. Linear trends across categories were tested using the median cadmium values within categories as a continuous variable in order to avoid too strong influence of possible outliers. If appearing, missing values were treated as a separate category in the models. In **paper III** and **V,** we performed multiple imputation analysis using chained equations[145] with 30 imputed data sets to evaluate a potential effect of missing values on the observed results. In multivariable models, we adjusted for established risk factors for the cancer type under study and for covariates that changed the beta-estimates with more than 10%. In additional analyses, we adjusted for consumption of vegetables and whole grain (**papers II** and **III**) and potatoes (**paper II**) to examine whether potentially beneficial substances in these foods are attenuating the observed association between cadmium intake and cancer risk.

Analyses were stratified by categories of BMI and use of postmenopausal hormones (**papers II-IV**) as sources of endogenous and exogenous estrogens and additionally by smoking status (**paper II**), because smoking is associated with decreased risk of endometrial cancer. In analyses stratified by BMI, we included BMI in the models as a continuous variable to adjust for a possible effect of body fat within strata. Among men (**paper V**), stratifications were made by waist circumference (<95 cm or ≥ 95 cm) since abdominal fat may be a better predictor of body fatness compared to BMI in elderly.[112] We also examined the association between cadmium and breast cancer by strata of total whole grain and vegetable consumption as well as the association between tertiles of dietary cadmium, total whole grain and vegetable consumption and breast cancer risk (**paper III**).

Long-term cadmium intake (**paper II**), i.e. longitudinal, repeated exposure assessment, was estimated based on cadmium intake at baseline 1987 and in 1997 and categorized into three categories (long-time low, inconsistent, long-time high).

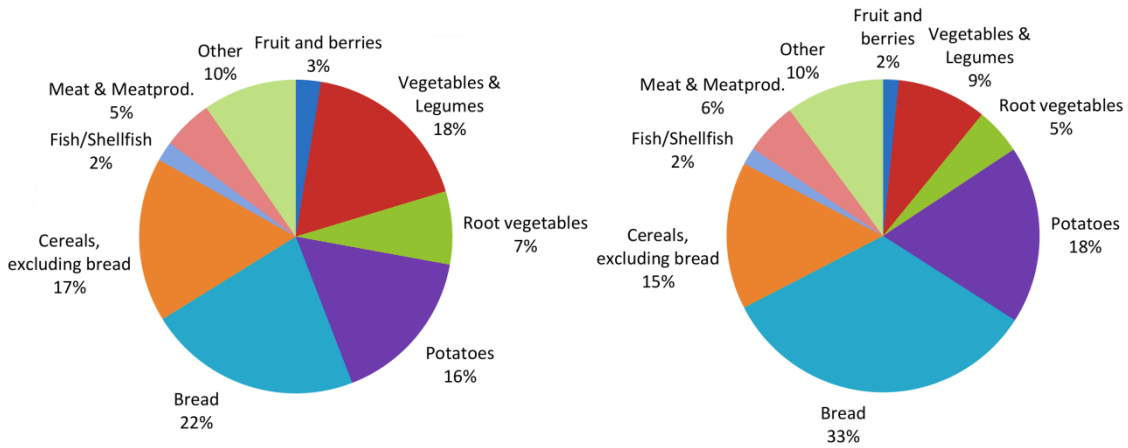
To assess the impact of exposure misclassification on the observed RRs, we performed a probabilistic sensitivity analysis[146, 147] (**paper III**). The sensitivity and specificity of the exposure ranged from 50% to 60% (see section on relationship between FFQ-based dietary cadmium estimates and urinary cadmium concentrations). Therefore, based on 10 000 simulations, we specified uniform distributions with equally probable values between 50% to 60% for both sensitivity and specificity (assuming non-differential exposure misclassification).

In an additional analysis, not included in the paper (**III**), we used restricted cubic-spline analysis (four knot positions and the median dietary cadmium exposure as the reference) to flexibly model the association between dietary cadmium exposure and breast cancer risk. We also estimated the difference in time to diagnosis between highest and lowest tertiles of cadmium exposure using Laplace regression. Laplace regression is a method that can be applied to censored data to enable inference on time to diagnosis and may provide an alternative, intuitive description of diagnose-free survival.[148] We explored the association between tertiles of dietary cadmium and time (in years) from enrollment to breast cancer diagnosis (i.e. disease-free time). Because overall, 3.8% of the women in the cohort were diagnosed with breast cancer, we assessed the second percentile of time to breast cancer diagnosis since the information at higher percentiles was limited. Standard errors were estimated with 100 bootstrap samples.

We used the Wald test to evaluate the heterogeneity of the multivariable-adjusted associations across prostate cancer subtypes (localized, advanced and fatal)[149] (**paper V**). All statistical analyses were performed using the software Stata (StataCorp, College Station, TX, USA) or SAS (SAS Institute, Cary, NC). All reported P-values were two-sided; P-values below 0.05 were considered statistically significant.

5 RESULTS

The mean estimated energy-adjusted dietary cadmium exposure at baseline was 15 µg/day (standard deviation 3.2) in all women and 19 µg/day for men (standard deviation 3.7). This corresponds to 1.6 µg/kg body weight per week for a woman of 67 kg (mean weight in whole SMC) and 1.7 µg/kg body weight per week for a man of 80 kg (mean weight in whole COSM). Among women, 3 % exceeded the tolerable weekly intake (2.5µg/kg of body weight) set by the EFSA[13]; among men the corresponding number was 4%. The main foods contributing to the dietary cadmium exposure for women and men respectively are illustrated in Figure 11 a) and b). Plant foods including bread and other cereals, potatoes, root vegetables and other vegetables constituted ~80% of the total exposure in both women and men.



a) b)

Figure 11. Major sources to dietary cadmium exposure within a) the SMC, and b) the COSM; age range 49-79.

5.1 POPULATION TOXICOKINETIC MODELING (PAPER I)

The cadmium half-life was estimated to about 11.6 years (95% confidence interval (CI): 10.1-14.7) with 25% population variability (3 years; 95% CI: 2.5-4.0). Figure 12 shows the distribution of the half-life in the population (assumed to be lognormal, truncated to a range of 5-35 years). The population distribution of daily dietary cadmium exposure corresponding to a pre-defined urinary cadmium concentration could be derived in a robust manner (Figure 13). In order to remain below e.g. 1 µg cadmium/g creatinine in urine in 50% of the population by the age of 50, the average daily dietary cadmium exposure should not exceed about 0.8 µg cadmium/kg body weight. The corresponding dietary exposure for the 95th percentile of the population is about 0.4 µg cadmium/kg body weight and day.

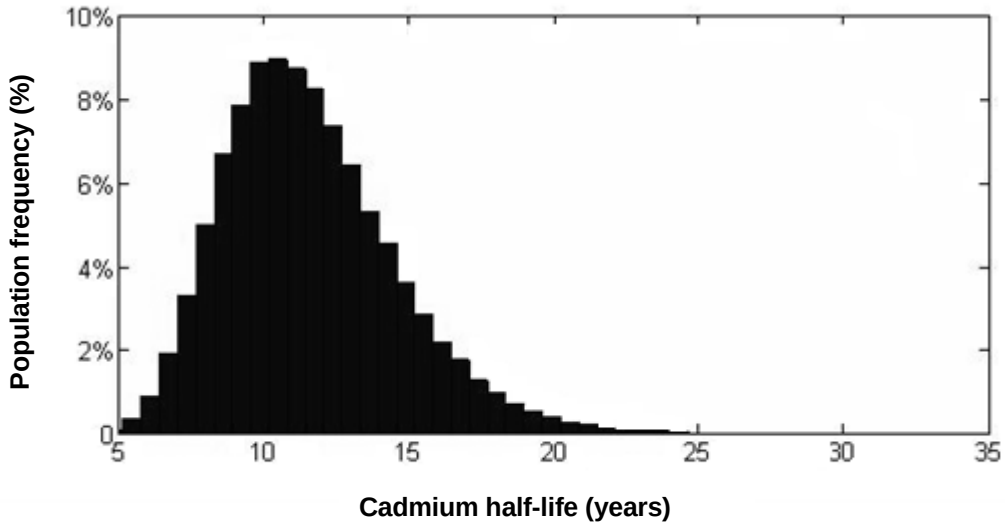


Figure 12. Estimated distribution of apparent half-life of kidney cadmium in the study population based on the one-compartment model assuming lognormality.[143]

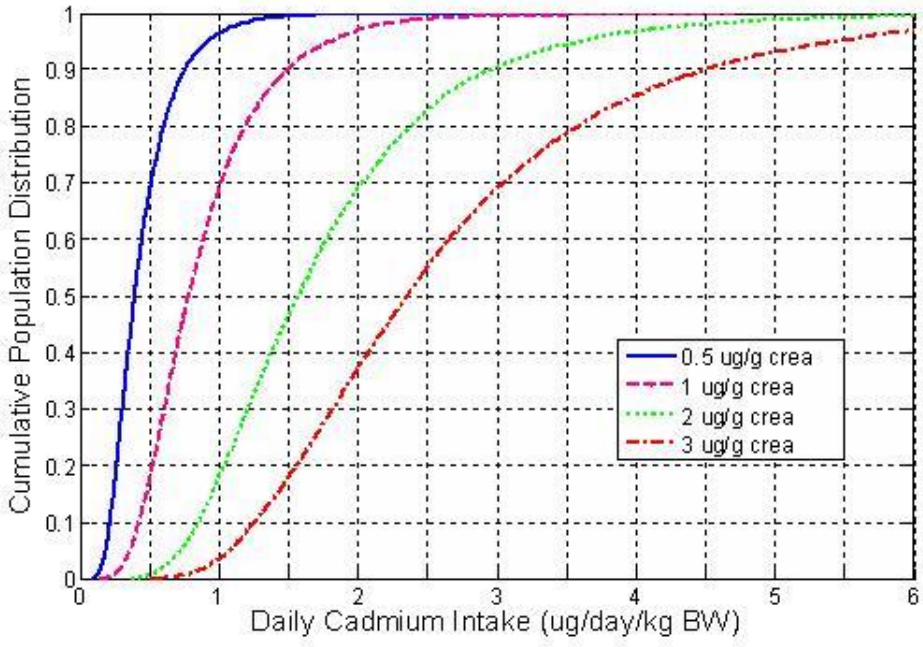


Figure 13. Cumulative population distribution of daily dietary cadmium exposure corresponding to urinary cadmium concentrations of 0.5, 1, 2 and 3 µg/g creatinine, at age 50. The curves show for each population percentile (Y-axis), the maximum dietary cadmium intakes (X-axis) allowed in order not to exceed the predefined urinary cadmium concentrations.[143]

Thus, the link between the long-term daily dietary exposure to cadmium and the corresponding urinary cadmium concentration at age 50 among never-smoking women could be derived (Figure 14). The mean dietary cadmium exposure in these Swedish women was 0.20 µg/day and kg body weight (mean cadmium exposure was 14 µg/day and mean body weight was 69 kg in the 680 women studied here).

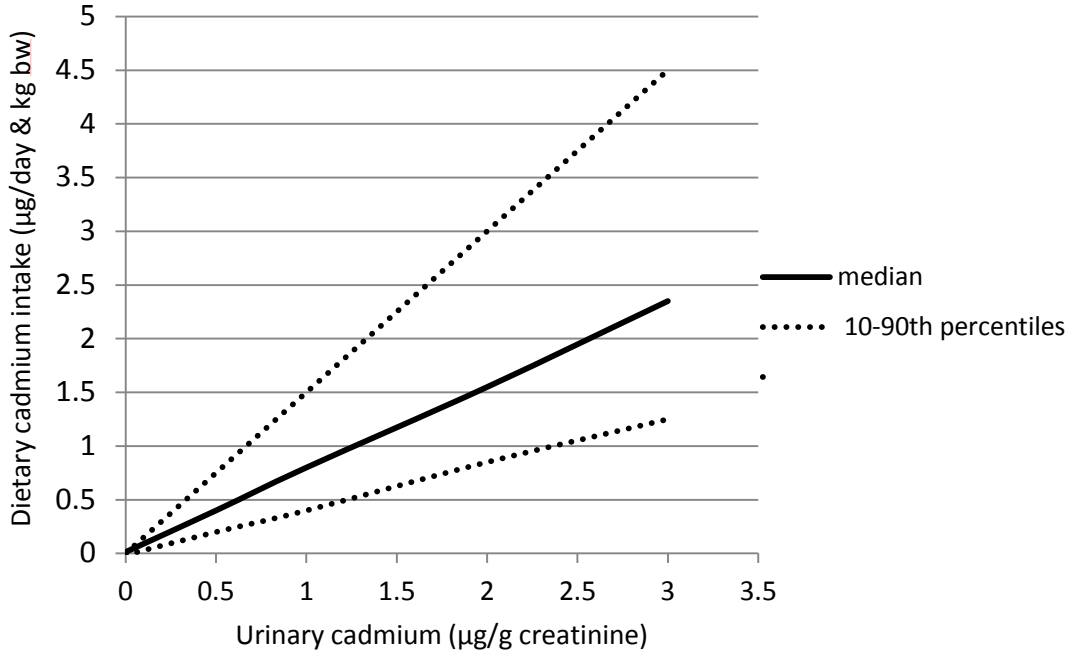


Figure 14. Modeled relationship between long-term daily dietary cadmium exposure and urinary cadmium concentrations at age 50 in never-smoking women.

5.2 FFQ-BASED ESTIMATES OF DIETARY CADMIUM VERSUS URINARY CADMIUM CONCENTRATIONS (PAPER III, SUPPLEMENTARY DATA)

The sensitivity of the FFQ-based dietary cadmium was 58% and the specificity was 51% (see Table S1, **paper III**, **supplementary data**). The reproducibility of the FFQ-based dietary cadmium estimates, based on two FFQs completed one year apart was 0.58 (P <0.001). The partial Pearson correlation was 0.1 (95% CI: 0.01-0.21) between questionnaire-based estimates of dietary cadmium and measured urinary cadmium concentration when accounting for within-person variation in the FFQ-based estimates. When accounting for the exponential shape of the elimination rate (i.e. using the one-compartment model) the corresponding correlation was 0.2 (95% CI: 0.1-0.3).

5.3 CADMIUM AND ENDOMETRIAL CANCER (PAPER II)

With the exception of smoking status, education and consumption of whole grain and vegetables, there was no considerable difference in characteristics of women with respect to dietary cadmium exposure (see Table 1, **paper II**). During a mean of 16.0 years (484 274 person-years) of follow-up, we ascertained 378 incident cases of endometrioid adenocarcinoma among the 30 210 postmenopausal women.

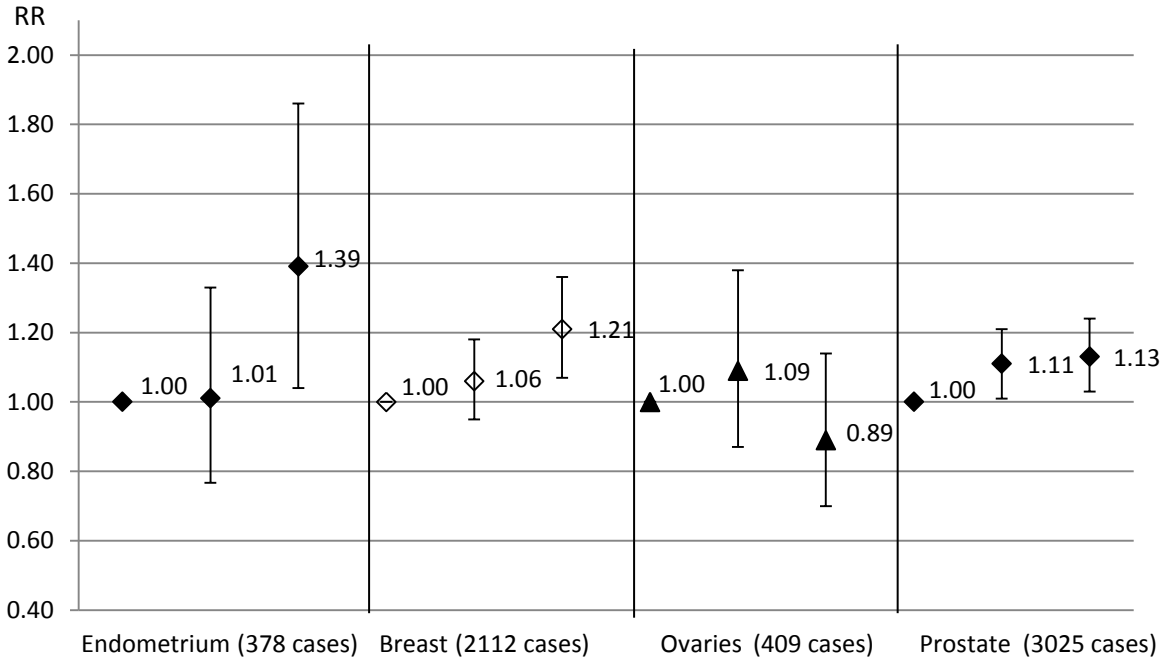


Figure 15. Multivariable-adjusted rate ratios (RR) and 95% confidence intervals of hormone-related cancer according to tertiles of dietary cadmium exposure.

The highest tertile of dietary cadmium exposure was associated with a statistically significant 39% increased risk (95% CI: 4-86%) of endometrial cancer (Figure 15) in the multivariable-adjusted model, additionally adjusted for whole grain, vegetables and potatoes; foods that are major contributors to the dietary cadmium exposure (for more details see Table 2, **paper II**). Whole grain and vegetables also contain potentially anticarcinogenic compounds such as antioxidants, fiber and phytoestrogens. In additional analyses, not included in **paper II**, we explored the effect of excluding potatoes from the model to resemble the adjustments made in **paper III**. In this analysis, the end of follow-up was extended to December 31st 2009, thus increasing the number of cases to 434. Excluding potatoes from the model resulted in a slightly decreased risk estimate; RR 32% (95% CI: 1-72%) comparing the highest with the lowest tertile of dietary cadmium.

Adipose tissue is a major source of estrogen in postmenopausal women. In women with BMI <27 kg/m^2, representing a group with lower adipose tissue-derived estrogen exposure, we observed a multivariate-adjusted dose-dependent 52% increased risk (P for trend 0.039) of endometrial cancer, comparing the highest tertile of dietary cadmium exposure with the lowest (Figure 16). There was no association between cadmium intake and endometrial cancer risk among overweight and obese women. Because obesity is such a strong risk factor for endometrial cancer, the disease is not very common among lean women. Thus, to avoid power problems, the cut-off of 27 kg/m^2 for BMI was chosen in the original analyses, which is higher than the cut-off for overweight (25 kg/m^2). However, with the extended follow-up (to 2009) it was possible to use BMI 25 kg/m^2 as the cut-off, leading to an increase of the RR to 1.61 among lean and normal weight women (Figure 16).

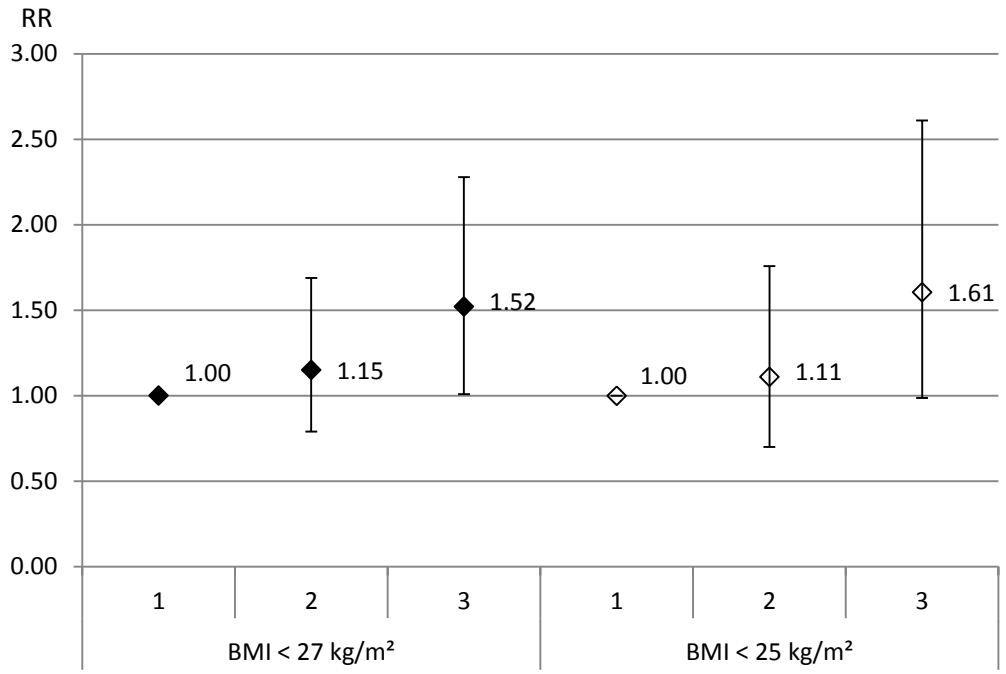


Figure 16. Multivariable-adjusted rate ratios (RR), including whole grains, vegetables and potatoes, and 95% confidence intervals of endometrial cancer according to tertiles of dietary cadmium exposure, among women with BMI <27 or BMI <25 kg/m^2. Note that follow-up was extended to 2009 for BMI <25 kg/m^2.

To minimize the effect of exposure to both endogenous (adipose tissue) and exogenous (postmenopausal hormones) sources of estrogen as well as smoking-derived cadmium, we performed analyses among women with BMI <27 kg/m^2, who were never-smokers and did not use postmenopausal hormones. In order to also examine the association between long-term cadmium intake and endometrial cancer, these analyses were made in women with available information on cadmium intake at baseline (1987) and in 1997, with follow-up from 1997 to mid-2006. We observed that never-smoking women, with body-mass index <27 kg/m^2 and with long-term consistently higher cadmium intake (corresponding to above the median in the cohort at both baseline 1987

and ten years later in 1997) had an multivariate-adjusted RR of 2.55 (95% CI, 1.33-4.89) compared to those with cadmium intake below the median at both occasions (Figure 17). The corresponding RR for never-smoking women with BMI <27 kg/m^2 and who were non-users of postmenopausal hormones was 2.84 (95% CI, 1.04-7.73).

Energy intake was not adjusted for in **paper II** as was done in **papers III**, and **V**. Thus, in additional analyses we adjusted the models for total energy intake also in **paper II**. This adjustment only marginally changed the point estimates (full multivariable-adjusted RR 1.40; 95% CI: 1.07-1.83 without adjustment for energy and RR 1.42; 95%CI: 1.07-1.88 with adjustment for total energy intake, based on follow-up prolonged until 2009).

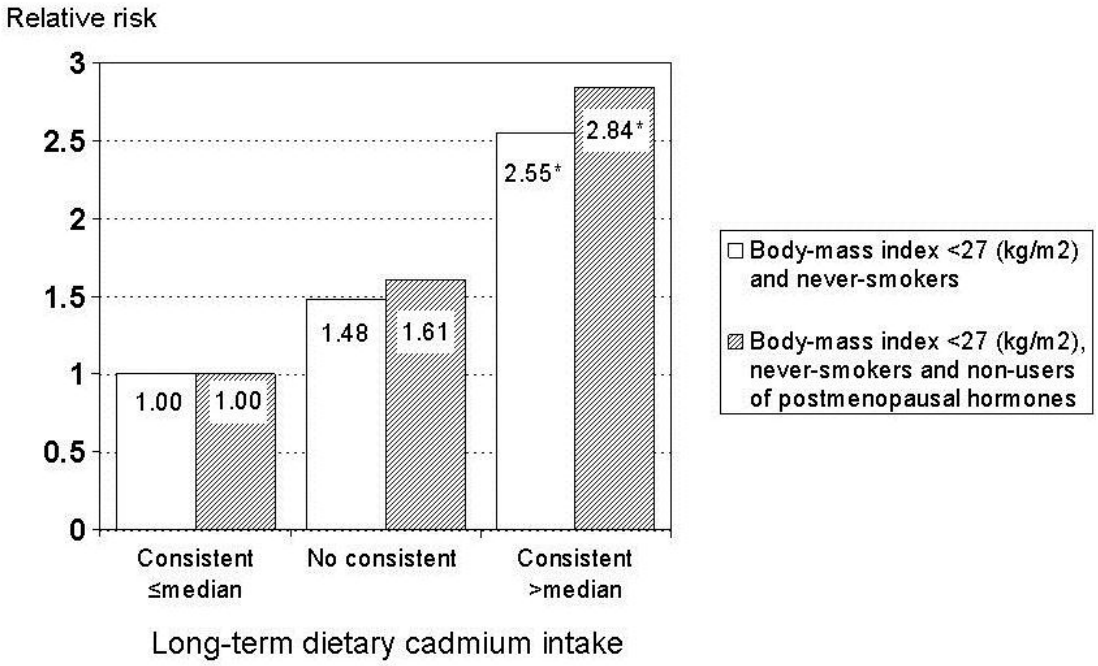


Figure 17. Multivariate adjusted relative risk of endometrial cancer in relation to long-term cadmium intake (assessed as consistent below the median, no consistent and consistent above the median cadmium intake in 1987 (baseline) and in 1997) in women with body-mass index <27 kg/m^2 who were never-smokers (empty bars) and in those with body-mass index <27 kg/m^2 who were never-smokers and did not use postmenopausal hormones (shaded bars). Numbers in each bar denote multivariate-adjusted relative risks and (*) statistically significant compared with the reference group.[150]

5.4 CADMIUM AND BREAST CANCER (PAPER III)

With the exception of smoking status, postsecondary education and consumption of whole grain and vegetables, there was no considerable difference in characteristics of women with respect to dietary cadmium exposure (see Table 1, **paper III**). During an average of 12.2 years of follow-up (712 075 person-years), we ascertained 2112 incident cases of breast cancer among the 55 987 postmenopausal women. Information on ER status was available for 1916 cases (1626 ER+ and 290 ER-).

The highest tertile of dietary cadmium exposure was associated with a statistically significant 21% increased risk (95% CI: 7-36%; $P_{trend} = 0.02$) of overall breast cancer in the multivariable-adjusted model, additionally adjusted for whole grain and vegetables; foods that are major contributors to cadmium and contain potentially anticarcinogenic compounds (Figure 15). The corresponding results for ER+ and ER- tumors were 19% (95% CI, 3-36%) and 33% (not statistically significant), respectively (for more details please see **paper III**, table 2.

To estimate the likely impact of exposure misclassification of estimated dietary cadmium exposure, we utilized information from the SMC subcohort, see **paper III, supplementary data**. In the probabilistic sensitivity analysis performed to quantify the likely impact of the estimated exposure misclassification of dietary cadmium, we observed a median RR of 2.88 for all breast cancer cases comparing the highest tertile with lowest.

For the same reason as in **paper II** (i.e. adipose-tissue is the major source of endogenously produced estrogen in postmenopausal women), we performed analyses stratified by BMI to restrict the influence of endogenous estrogen on the observed association. In lean and normal weight women (BMI 18.5-25 kg/m^2), we observed, in the fully-adjusted model, a statistically significant RR of 1.27 (95% CI: 1.07-1.50) of overall invasive breast cancer, comparing the highest tertile of cadmium with the lowest (Figure 18). Likewise, we observed a RR of 1.25 (95% CI: 1.03-1.52) for the ER+ subtype, and a similar, but not statistically significant, association for ER- subtype (RR 1.22; 95% CI: 0.76-1.93). Among overweight and obese women (BMI $\geq$ 25 kg/m^2), point estimates were in general lower and not statistically significant except for ER- tumors where the point estimate was higher (full multivariable-adjusted RR and 95% CI for the highest tertile of cadmium: 1.10; 0.91- 1.33 for overall tumors, 1.09; 0.88-1.36 for ER+, and 1.50; 0.89- 2.53 for ER-).

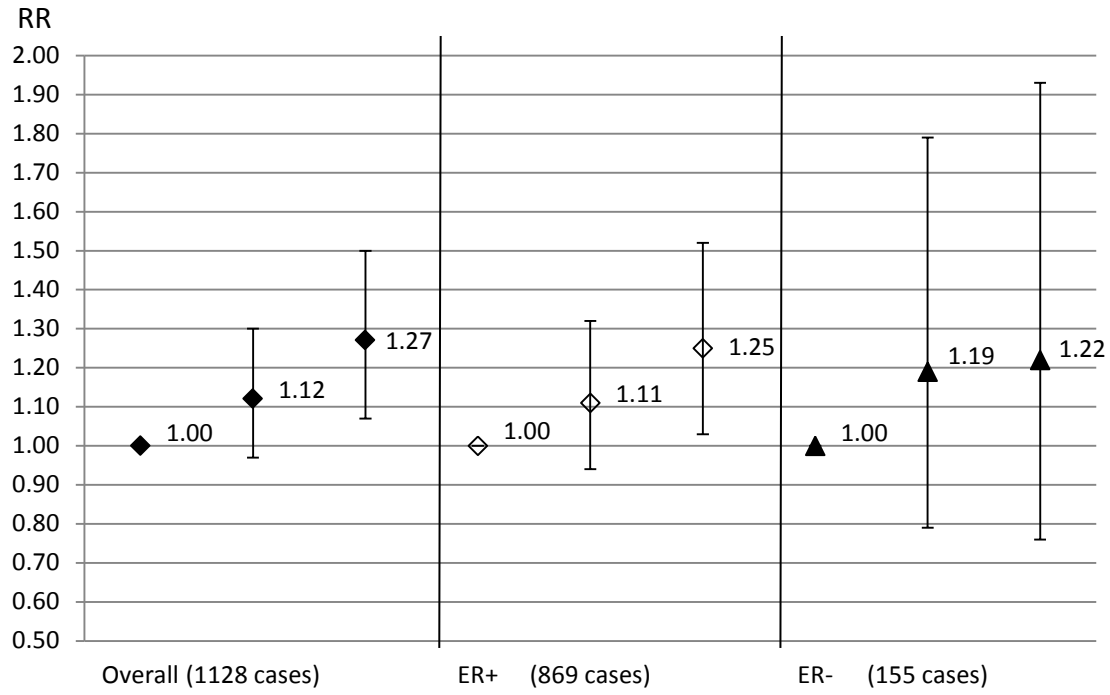


Figure 18. Multivariable-adjusted rate ratios (RR) and 95% confidence intervals of overall, ER+, and ER- breast cancer according to tertiles of dietary cadmium exposure among normal weight women (BMI 18.5-25 kg/m^2).

Because dietary cadmium was correlated with the consumption of whole grain and vegetables (r = 0.41 with whole grain, r = 0.49 with vegetables and r = 0.59 with total whole grain and vegetable consumption), we also examined the joint relationship between cadmium exposure, whole grain and vegetable consumption, and breast cancer risk. The risk of postmenopausal breast cancer increased with increasing cadmium intake within each tertile of total whole grain and vegetable consumption (Figure 19).

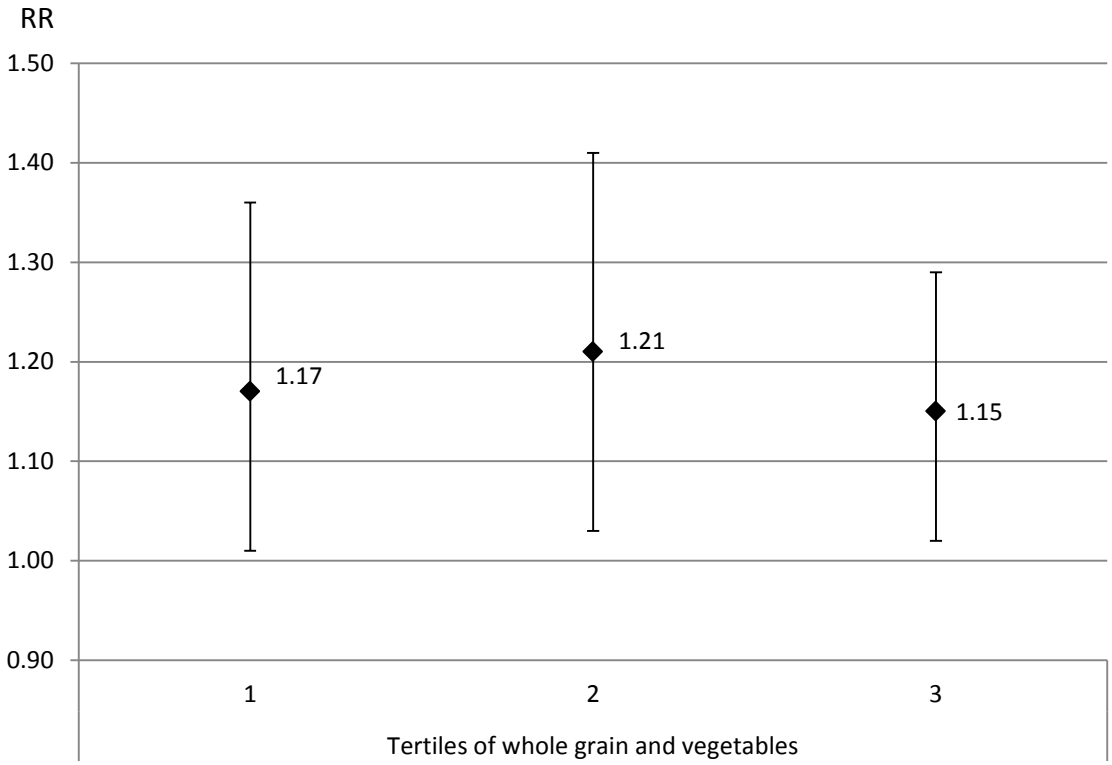


Figure 19. Multivariable-adjusted rate ratios (RR) and 95% confidence intervals of overall breast cancer per 5 µg increment in dietary cadmium exposure according to tertiles of whole grain and vegetable intake.

In further analyses based on tertiles of both cadmium exposure and whole grain and vegetable consumption (Figure 20), we observed an increased risk of breast cancer with increasing dietary cadmium exposure and with decreasing consumption of whole grain and vegetables. The RR for overall breast cancer was 1.60 (95% CI: 1.28–2.00) among those within both the highest tertile of cadmium intake and lowest tertile of whole grain and vegetable consumption compared to the reference (low intake of cadmium and high consumption of whole grain and vegetables). There was no interaction on a multiplicative scale (P for interaction = 0.73). Similar results were observed for ER+ tumors (Figure 21), while the interpretation of the results for ER- tumors was limited by the small number of cases.

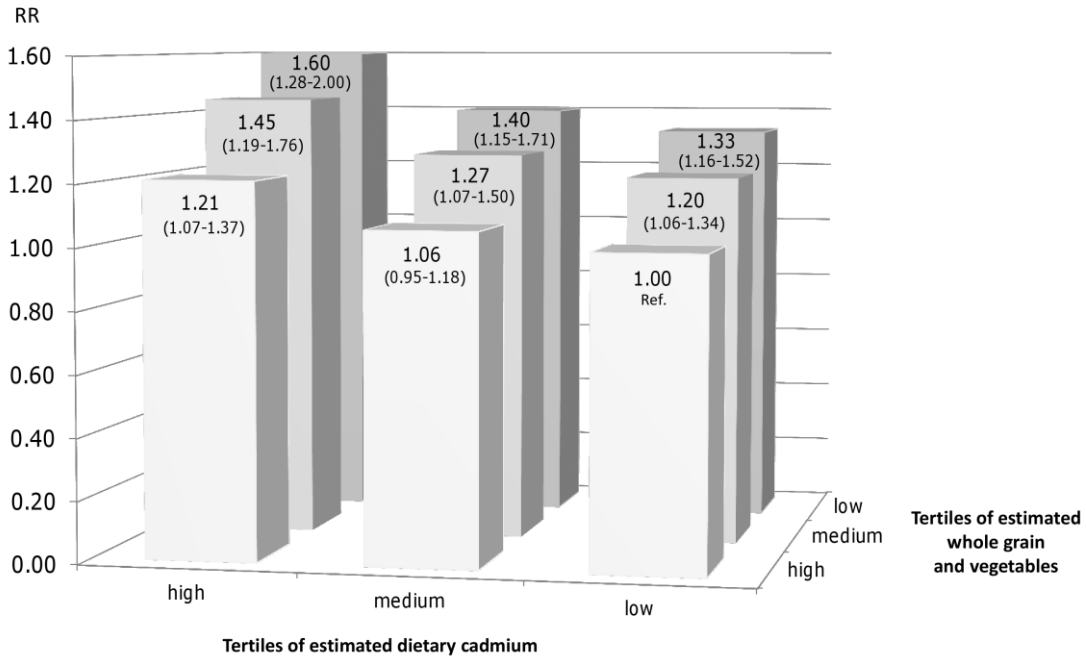


Figure 20. Overall breast cancer according to tertiles of estimated dietary cadmium exposure and whole grain and vegetable consumption jointly.[151]

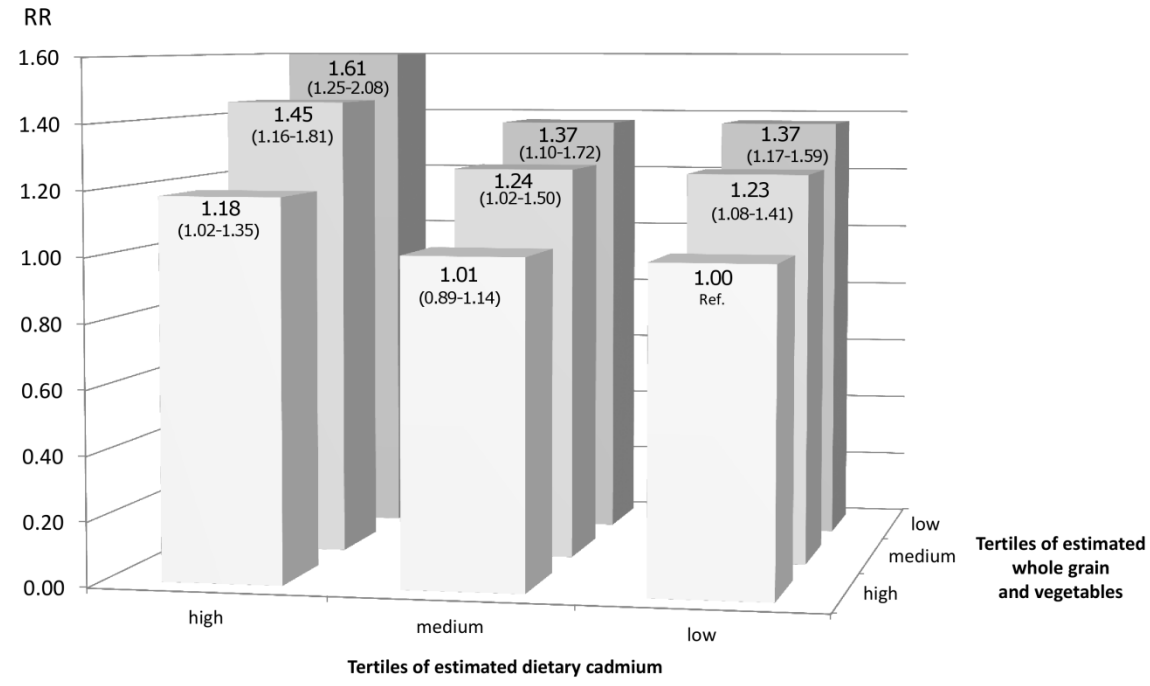


Figure 21. Estrogen receptor positive breast cancer according to tertiles of estimated dietary cadmium exposure and whole grain and vegetable consumption jointly.

To explore the possible effects of the categorization of cadmium into tertiles, the joint analysis of dietary cadmium, whole grain and vegetable consumption and breast cancer risk (Figure 20) was also performed with continuous data. Dietary cadmium was, per continuous 5 µg/d increment, associated with a RR of 1.18 (95% CI, 1.08-1.29) for overall breast cancer and the RR per 100 g/day increment in whole grain and vegetables was 0.91 (95% CI, 0.86-0.96), supporting the results observed for tertiles.

The association was also tested for non-linearity using a restricted cubic spline model with four knot positions (additional analysis not included in paper III), but no support of a non-linear relationship was indicated (Figure 22).

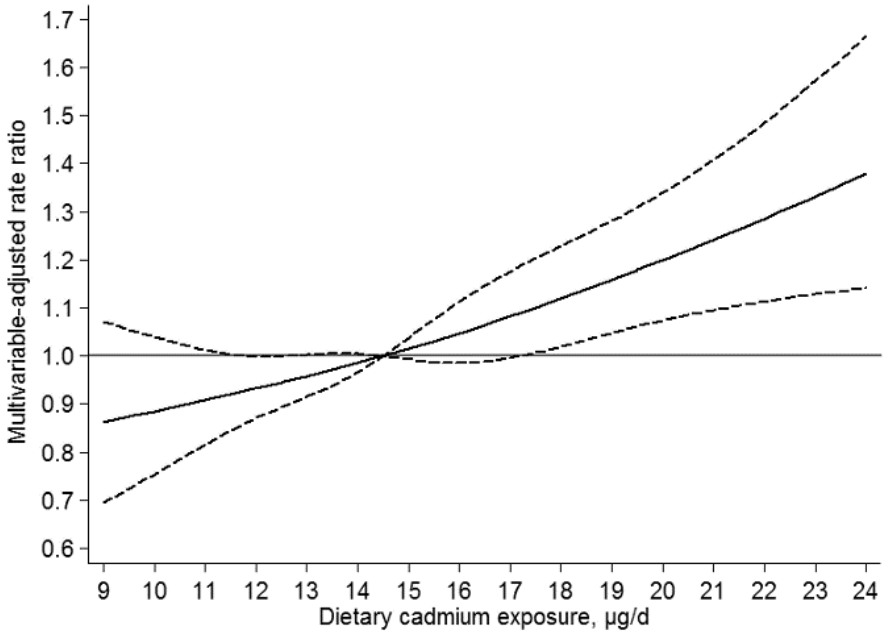


Figure 22. Full multivariable-adjusted rate ratios and 95% confidence intervals (CIs) of dietary cadmium exposure in relation to risk of overall breast cancer using restricted cubic splines. Plot covers the central 98% of the dietary cadmium exposure values. Splines (solid line), 95% CIs (dashed lines), and reference line (grey line).

Laplace regression may provide an alternative, more intuitive description of diagnose-free survival compared to relative risks. By applying Laplace regression, we explored the association between cadmium exposure and disease-free time (additional analysis not presented in paper III). As shown in Table 2, the second percentile of time to diagnosis of breast cancer in an age-adjusted model was 0.25 years (0.25 *12 = 3.0 months) shorter for each tertile increment in dietary cadmium (P = 0.22). Thus, comparing the highest with the lowest tertile, the first 2% of the cohort were diagnosed with breast cancer 6 months earlier (3.0 months per tertile*2 tertiles), equivalent to a 7% reduction of disease-free time. The corresponding result for multivariable-adjusted cadmium, was 0.59 years (7.1 months) earlier per tertile increment of dietary cadmium intake (P = 0.02). This reduction of disease-free time corresponds to 14.2 months (7.1 months per tertile*2 tertiles), i.e. 13%, comparing time to diagnosis in the highest tertile of cadmium intake with the lowest.

Table 2. Estimates in years per tertile of dietary cadmium exposure for the second percentile of time from enrollment to breast cancer diagnosis (95% confidence intervals; CI) from Laplace regression.

	Estimate (y)	95% CI	P-value
Age-adjusted[a]			
Years to diagnosis in the lowest tertile of cadmium intake (intercept)	7.35	6.98, 7.73	<0.001
Decrease in years to diagnosis per tertile increment of cadmium intake	-0.25[b]	-0.66, 0.15	0.22
Multivariable-adjusted[a, c]			
Years to diagnosis in the lowest tertile of cadmium intake (intercept)	9.15	8.72, 9.56	<0.001
Decrease in years to diagnosis per tertile increment of cadmium intake	-0.59[d]	-1.10, -0.08	0.02

[a] age was centered around the median of 52 years.
[b] 0.25 years = 0.25*12 = 3.0 months; comparing the lowest with the highest tertile: 0.25*12*2 = 6.0 months.
[c] Adjusted for age (y), adult height (140-159, 160-163, 164-168, ≥168cm), BMI (18.5-24, 25-30, ≥30 kg/m^2), >12 y of education (yes, no), use of oral contraceptives (yes, no), use of postmenopausal hormones (yes, no), age at menarche (<13, 13, >13 y), age at menopause (≤51, >51 y), parity (nulliparous, 1-2, >2 children), age at first birth (nulliparous, <26, 26-31, ≥ 31 y), alcohol consumption (non-drinker, <3.4g/day; 3.4-10 g/day, ≥ 10 g/day), glycemic load, total energy intake and intake (g/day) of whole grain and vegetables in tertiles.
[d] 0.59 years = 0.59*12 = 7.1 months; comparing the lowest with the highest tertile: 0.59*12*2 = 14.2 months.

5.5 CADMIUM AND OVARIAN CANCER (PAPER IV)

With the exception of smoking status and postsecondary education, there was no considerable variation in characteristics of women with respect to dietary cadmium exposure (see Table 1, **paper IV**). During a mean follow-up of 18.9 years (1 149 470 person-years) of 60 889 women, we identified 409 incident cases of epithelial ovarian cancer, including 215 serous, 27 mucinous, 62 endometrioid and 12 clear cell tumors. We found no association between dietary cadmium and risk of total epithelial ovarian cancer (RR 0.89; 95% CI: 0.70-1.40), comparing the highest tertile of dietary cadmium with the lowest (Figure 15). Likewise, no association was observed for any subtype of ovarian cancer when modeled with continuous dietary cadmium exposure; multivariable RR for each 1 µg/day increment of cadmium: 0.97 (95% CI: 0.93-1.02) for serous tumors, 0.94 (95% CI: 0.82-1.07) for mucinous tumors, and 1.00 (95% CI: 0.92-1.08) for endometrioid and clear cell tumors.

5.6 CADMIUM AND PROSTATE CANCER (PAPER V)

With the exception of smoking status, consumption of alcohol and intake of calcium and lycopene, there was no considerable difference in characteristics of men with respect to dietary cadmium exposure (see Table 1, **paper V**). During an average of 10.8 years (443 599 person-years), we ascertained 3085 incident cases of prostate cancer, whereof 894 localized and 794 advanced cases. Based on prostate cancer as primary cause of death, 326 of the cases were classified as fatal.

Dietary cadmium exposure was, after multivariable-adjustment, associated with a statistically significant 13% increased risk (95% CI: 3-24%; P for trend = 0.01) of overall prostate cancer, comparing the highest tertile with the lowest (Figure 15). In subtypes of prostate cancer tumors, the RR was 1.29 (95% CI: 1.08-1.53; P for trend <0.01) for localized cases, 1.05 (95% CI: 0.87-1.25; P for trend = 0.70) for advanced cases, and 1.14 (95% CI: 0.86-1.51; P for trend = 0.34) for fatal cases, although the associations were not statistically significant for the latter two categories. No statistically significant difference was observed in the multivariable-adjusted risk estimates between localized, advanced and fatal tumors (P for heterogeneity = 0.27).

In men with a waist circumference below 95 cm, the associations between dietary cadmium exposure and prostate cancer risk was pronounced for all subtypes of prostate cancer as compared to those equal to or above 95 cm, but did only reach significance for localized (1.49; 95% CI: 1.13-1.97) prostate cancer (Figure 23). Stratification by BMI (cut-off 25 kg/m^2) resulted in slightly less pronounced risk estimated.

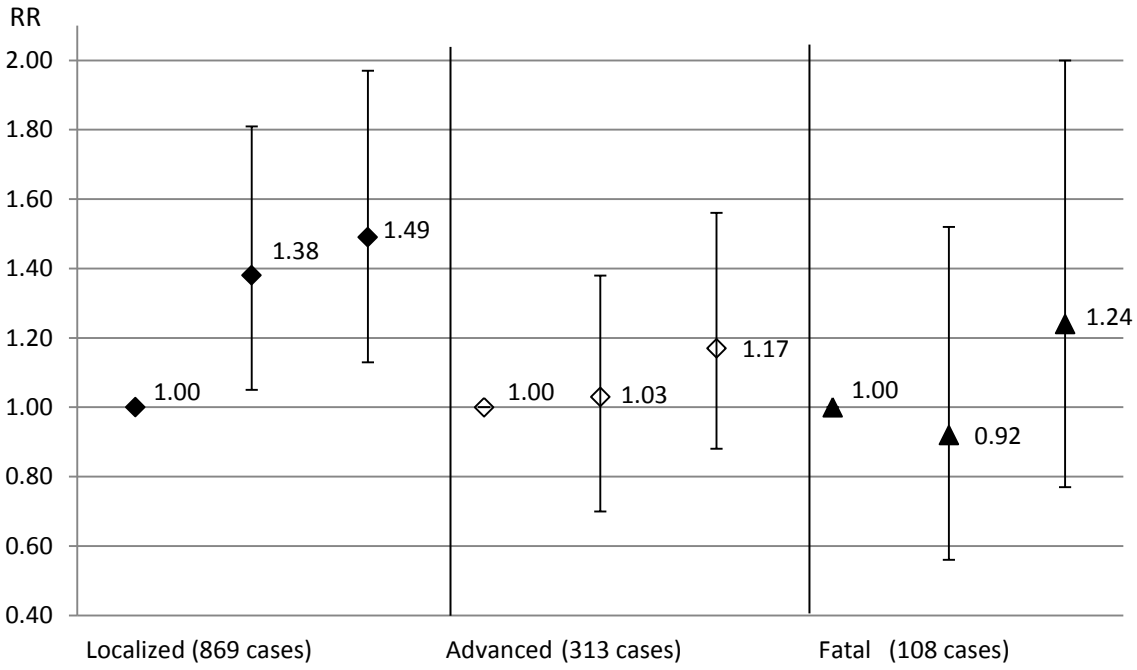


Figure 23. Multivariable-adjusted rate ratios (RR) and 95% confidence intervals for localized, advanced and fatal prostate cancer according to tertiles of dietary cadmium exposure among lean men (waist circumference < 95 cm).

6 DISCUSSION

In the following section, considerations of the epidemiological methodology are followed by a general discussion of the main findings.

6.1 METHODOLOGICAL CONSIDERATIONS

Different sources of errors, both of random and systematic nature can be present in observational studies to different degrees and it is important to consider the impact of these biases as alternative explanations for the observed associations (**paper II-V**). Below follows a discussion of the types of biases that may have influenced the results.

6.1.1 Precision

Precision is defined as the absence of random errors in measurements and can be improved by increasing the size of the study.[152] In the present studies, we used the confidence interval to estimate precision. A large number of cases narrow the range of the confidence interval, thus increasing the precision of the point estimate. Both cohorts studied in this thesis had generated, in general, a large number of cases that made risk estimates precise. In some of the subgroup analyses, however, precision was lower due to a smaller number of cases in each exposure category.

6.1.2 Validity

Validity is defined as the absence of systematic errors and can be classified into three major components; these are information bias, selection bias and confounding. A low validity tells us that we are not measuring what we intended to measure.

Information bias

Information bias occurs when measurement or classification of exposure or outcome are not valid.[152] Misclassification can be divided into two types depending on their dependency to the exposure or disease under study; namely differential and nondifferential misclassification. Differential misclassification refers to the situation where error in measurement of the exposure is related to disease, or where errors in the classification of disease are related to exposure. Consequently, in nondifferential misclassification, the error in measurement of the exposure is unrelated to the disease under study and vice versa. In the studies presented in this thesis, all participants were free of disease when they filled in the FFQ. Thus differential misclassification is most unlikely and thus not discussed in the following section.

Misclassification of exposure

The most important issue to consider based on the studies presented in this thesis is whether the FFQ-estimated exposure to cadmium provides a valid measure of exposure. Dietary assessments are always subject to misclassification due to the difficulty of reporting diet correctly; both because of errors in self-report and due to normal within-person variation.[144] In the case of cadmium, although the used mean concentrations in specific foods in some cases were based on several hundred measurements, we may not account for all the variability in cadmium content in the reported food (see further section 6.2.4).

We categorized the cohort participants into tertiles of exposure. Misclassification is almost certainly present and are as already mentioned most likely nondifferential. When the exposure is measured on a dichotomous scale (i.e. non-exposed versus exposed), nondifferential misclassification attenuates the true relationship between exposure and disease.[144] A dichotome categorization is, however, uncommon within the field of nutritional epidemiology since individuals to some extent almost always are exposed to a certain food or food component. In the case of a polychotomous categorization, under certain circumstances, the risk estimates for intermediate levels can also be biased away from the null.[153] To explore this, I applied different scenarios of misclassification to the results in **paper III**. In contrast to the findings reported by Dosemeci et al, our risk estimates seemed to be biased towards the null (Table 3). In addition, the sensitivity analysis performed on the results in **paper III**, taking the misclassification of exposure into account, indeed indicated that the observed associations were likely biased towards the null, so that the true associations were even stronger.

Table 3. Examples of the effect of non-differential misclassification on the risk estimates presented in paper III. a) the unadjusted RR observed in paper III, b) unadjusted RR after two different scenarios of non-differential misclassification

Breast cancer	Dietary cadmium exposure					
	Low	Medium	High			
a. Reference distribution						
Cases	677	691	744			
Person-years	233 546	228 121	230 981			
RR	1.00	1.04	1.11			
	Example I 40% from high to low and 40% from medium to high			**Example II** 60% from low to high and 60% from high to low		
	Dietary cadmium exposure					
	Low	Medium	High	Low	Medium	High
b. Misclassified distribution						
Cases	975	415	723	717	691	704
Person-years	325 938	136 873	229 837	232 007	228 121	232 520
RR	1.00	1.01	1.05	1.00	0.98	0.98

The associations observed between diet and cancer risk may also be dependent on how the exposure categories were chosen. In **paper III**, we explored the possible effects of the categorization into tertiles. The joint analysis of dietary cadmium exposure, whole grain and vegetable consumption and breast cancer risk (Figure 20) was therefore performed with continuous data (dietary cadmium as well as whole grain and vegetables). In this analysis, a possible impact of any extreme observations (outliers) was also considered. This had no impact on the results.

Misclassification of outcome

In both the SMC and the COSM, we identified cases of the hormone-related cancers by linkage with the national and regional cancer registries, and when applicable with the quality registries, all of which provided close to 100% complete case ascertainment in Sweden.[142] Thanks to this practically complete case ascertainment, any potential bias due to under ascertainment would be minimal. The accuracy of the estrogen receptor status measurement may however be lower.[154]

Selection bias

Selection bias can be defined as "distortions that result from procedures used to select subjects and from factors that influence study participation".[152] In other words, the relation between exposure and disease differs between those who participate and all who could have participated in a study but did not. Selection bias is not a big problem in prospective studies since exposed and unexposed individuals are both free from disease when the study is initiated. A factor that could influence selection bias, however, is the completeness of follow-up between exposed and unexposed participants. The nearly complete follow-up of the study population through linkage with various population-based registers[142] minimized the possibility that our results could be affected by differential loss to follow-up.

Confounding and effect modification

Confounding can be explained as a mixing of effects and may result in both an overestimation and underestimation of the true risk. A confounder should fulfill three characteristics: it should be an independent risk factor for the disease under study; it must be associated with the exposure under study; and must not be an intermediate step in the causal path between exposure and disease.[152]

In order to control for confounding in the analyses between dietary cadmium exposure and hormone-related cancers (**papers II-IV**), we adjusted for factors, either seen as established or potential risk factors for these cancers or factors that changed the beta-coefficients in the model with more than 10%. The fact that we were able to control for a large number of potential confounders is considered an advantage, but a large number of covariates may potentially overload of the models. This seemed not to be the case in our analyses as the confidence intervals were very stable.

Important to mention is that also the confounding factors are measured with error. This would lead to some confounding still present after adjustment, i.e. residual confounding. The possibility that unmeasured confounding may have contributed to the observed findings cannot be totally ruled out. For example, we cannot be absolute certain that our findings were not explained by some other aspects of the diet. However, in light of the fact that the exposure occurred mainly through foods proposed to protect against cancer and that adjustments for proposed risk factors such as glycemic load and energy intake did not affect the results, this alternative explanation is highly unlikely.

Within the context of cadmium exposure, smoking is an important factor. Women with the highest cadmium exposure from food tended to a larger extent to be never smokers. For endometrial cancer, smoking is considered a protective factor,[155] for breast cancer[156] and ovarian cancer[157] the relationship to smoking appear more complex. In the SMC, smoking was only assessed in 1997 and not at baseline, which must be considered a major limitation in the studies on women. Nevertheless, we do not consider misclassification of smoking status of women at baseline very likely, because women who reported to be never-smokers in 1997 were most likely also never-smokers in 1987. Yet, the about one-third of our cohort with no information on smoking status at baseline (i.e. those who did not participate in 1997) might result in a selective follow-up. The results from our sensitivity analysis among never-smoking women with complete information on smoking (i.e. follow-up starting in 1997) in **paper II** did, however, support the results obtained from baseline follow-up.

An effect modifier is defined as a factor that changes (increases or decreases) the effect of the exposure on the outcome and thus give rise to interaction.[152] In **paper III**, examining the association between dietary cadmium exposure and breast cancer, we tested if whole grain and vegetable consumption could modify the observed association between dietary cadmium and breast cancer, but we found no statistical evidence for an interaction here (see Figure 20).

6.1.3 Generalizability

Both the SMC and the COSM are population-based studies. This means they were recruited from the general population and thus the results from these studies should be most direct generalizable to middle-aged and elderly Swedish women and men. Since the study population of the SMC and the COSM are primarily Caucasians, our findings may not directly apply to other ethnic groups. The response rate in women was fairly high (74%), but in men only 49%. Nevertheless, the incidence rate of prostate cancer in the COSM was almost the same as in the whole male population of Sweden and the cohort participants were considered representative in terms of age distribution, educational level and prevalence of overweight.[122]

6.2 MAIN FINDINGS AND GENERAL DISCUSSION

The papers based on urinary concentrations of cadmium (**paper I** and **paper III, supplementary data**) are discussed separately, followed by a common discussion of the associations between dietary cadmium exposure and the risk of hormone-related cancers (**papers II-V**).

6.2.1 Dietary cadmium exposure

The estimated average daily dietary cadmium exposure was 15 µg in women and 19 µg in men, which are in the same range as that observed in other areas of Europe and the US with no particular industrial cadmium contamination.[12, 22, 23, 158-162] This supports the accuracy of the created food cadmium database. The intake was, however, slightly higher than that recently reported by the NFA,[163] which may be explained by differences in study designs such as the included age range, the dietary assessment method used (7-day dietary record versus FFQ) or the sample size.

The detailed food-cadmium database was based on the cadmium content in practically all foods available on the Swedish market. The use of the average cadmium concentration for each food item in the questionnaire was substantiated by the fact that there is no known industrial cadmium contamination of agricultural soil in the study area and no known geographical variation in cadmium content of consumed foods across Sweden (Personal communication L. Jorhem, NFA). In addition, most foods are distributed throughout the country by wholesale companies, giving a fair representation of what the women in our study would encounter. The NFA has monitored cadmium content in Swedish food regularly and very frequently since the late 1970-ties, thus the average cadmium content was calculated based on analyses ranging from a few to several hundred per single food item. To make the food-cadmium database as detailed as possible, we also calculated the cadmium content in the dishes that occurred in the FFQ by combining recipes with the cadmium content in each ingredient, taking possible changes in content during preparation into account. Although the FFQ was not especially designed for measuring cadmium exposure and the average for some foods was based on a few samples only, thus maybe not capturing all variation, the database was in general very thorough, resulting in a good accuracy.

The major sources in both men and women were bread and other cereals, potatoes, root vegetables and vegetables, indicating that the main sources to cadmium via food are from foods mainly considered healthy. This is in agreement with a previous study in Sweden.[12]

6.2.2 Urinary cadmium

In **paper I** and **paper III, supplementary data**, a subgroup of the SMC donated urine samples between the years 2004 and 2007 and 65% responded. Here we used first voided morning urine spot samples. The concentrations of markers in spot urine samples are highly influenced by variation in the dilution caused by variation in the intake of for example fluids, physical activity, or temperature.[36] Cadmium excretion is lower in the night than during daytime and spot urinary samples may therefore

underestimate the true cadmium excretion.[164] On the other hand the correlation between cadmium concentration in spot urine and 24-h urine is high, both for samples adjusted for creatinine and specific gravity.

We cannot exclude that women who did participate in the subcohort were healthier than those that did not. Nevertheless, there were no major difference in estimated dietary cadmium exposure or BMI between women included in **paper I** and the whole SMC in 1987 (mean dietary cadmium: 14.9 µg/d and 14.7 g/day; mean BMI: 23.3 kg/m^2 and 23.9 kg/m^2, respectively).

The mean urinary cadmium concentration in the SMC is lower than those observed in Belgium[41] and USA[165] and much lower than those in Japan.[14]

6.2.3 Population toxicological modeling

The major advantage of **paper I** is the unique availability of both long-term intake and biomarker data at the individual level. This allowed, for the first time, the assessment of the inter-individual variability in cadmium toxicokinetics at the population level, hence allowing the derivation of a robust model to be used in health risk assessment. Accordingly, the results have been used both by EFSA and by FAO/WHO (JECFA) to derive new tolerably intakes of cadmium.[13, 62] Based on Figure 13, these authorities estimated the tolerable weekly/monthly intake in order to keep 95% of the population below a certain cadmium concentration in urine by the age of 50 years.

The estimated average daily dietary cadmium exposure of 15 µg (as assessed in 1987) in the 680 women was the same as observed in the SMC cohort indicating that the subgroup of women was representative of the whole cohort with respect to the cadmium exposure.

Since the results of the modeling were based on several assumptions, such as the use of *a priori* information (see Table 1), a constant cadmium intake per kg body weight over the life course and the nature of intra-individual variability of daily cadmium exposure, sensitivity analyses were performed to test the robustness. The uncertainty (CV) of the prior distribution of the factor housing, among others the gastrointestinal absorption coefficient, was originally set to 30%. Increasing the CV to 100% resulted in parameter estimates close to the original ones. The assumption of a constant dietary cadmium exposure per kg body weight over a lifetime may of course be challenged, especially at younger ages. Worth to mention is, however, that even large variations in intake before the age of 20 years, are expected to have limited impact on the cadmium burden at older ages (half-life was estimated to around 12 years and our study participants were > 50 years of age). The inter-day variability in dietary cadmium exposure was set to 25%. A wider range of variability (15-50%) showed <5% difference in all parameters estimated.

To evaluate the intra-individual variability of urinary cadmium, an additional 25% variability within individuals was added. Although this lead to a constant underestimation of urinary cadmium predicted by the model (especially for concentrations >0.4 µg/g creatinine; see Figure 8 **paper I**), it did not have a great impact with respect to health risk assessment purposes. In conclusion, the sensitivity analyses showed that the model was very robust and the use of it within health risk assessment is considered reliable.

The population variability estimates may be affected by the fact that they are derived from upper middle-aged women only. However, as women generally have higher concentrations of cadmium in blood, urine and kidney than men, due to a higher gastrointestinal cadmium absorption,[166] only including women is appropriate for risk assessment purposes. Thus, women are considered more at risk of adverse effects of cadmium and by protecting them it can be anticipated that the whole population is protected.

6.2.4 The agreement between dietary and urinary cadmium

The use of a FFQ to estimate the dietary exposure to cadmium opens up the possibility to perform large scale epidemiological studies for assessing the relation between exposure and risk of disease, thereby overcoming disadvantages like feasibility and high costs of sample collection and chemical analyses of biomarkers. Important, however, is that the FFQ show reasonable validity.

We observed a partial Pearson correlation of 0.1 (95% CI: 0.01-0.21) between questionnaire-based estimates of dietary cadmium and measured urinary cadmium concentration when accounting for within-person variation in the FFQ-based estimates. Because the excretion of cadmium is non-linear we also compared model-predicted urinary cadmium concentration with measured urinary cadmium concentration; Pearson correlation 0.2 (95% CI: 0.1-0.3). The sensitivity (58%) and specificity (51%) were also modest. In contrast, the validation based on the consumption of major cadmium–contributing foods in a subgroup of the women was satisfactory (r = 0.5- 0.8).

There could be several reasons for the rather modest agreement. First, measurement error is inevitable when estimating dietary cadmium exposure, both because of the self-reporting and the use of the mean cadmium concentration in each food item reported. Because cadmium is primarily due to contamination rather than being an intrinsic component of a food, the levels in a food may vary widely. By excluding women with implausible energy intake and by adjusting dietary cadmium exposure to the mean total caloric intake, we compensated for under- or over reporting of habitual food intake.[144]

Second, the bioavailability of cadmium in food may also vary depending on both dietary factors and nutritional status and may lead to exposure misclassification in relation to the internal dose. An individual's iron status seems to be the most important factor of cadmium bioavailability in the diet and low iron status is associated with

higher concentrations of cadmium in blood and urine.[12, 23, 30] Low iron status is common among fertile women due to menstrual losses and to pregnancy. Unfortunately, we did not have any information on the iron stores at younger ages in these women. In a recent publication we showed that measured dietary cadmium intake (duplicate portions) could reasonably well predict biomarkers of both long-term kidney accumulation (urine) and short-term exposure (blood) and that the predictions were substantially improved when taking data on the iron status into account.[167]

Third, all biomarkers are imprecise and include analytical as well as pre-analytical sources of variation. Although urinary cadmium concentration is generally considered to be a good biomarker of the amount of cadmium accumulated in the kidney,[35] the association at low exposure levels present among the women studied here, has not been investigated. Further it is not known how well it reflects the whole body-accumulated internal dose or more importantly it is not known if it is the accumulated dose in the kidney that is important for the development of cancer in others sites. In a recent study (*Bone*, in press), we concluded that the adverse effect of cadmium on bone (e.g. fractures) in the same women was underestimated using either one of urinary or dietary cadmium exposure alone, while these two exposure estimates combined reduced the level of misclassification substantially.[168] These new results question how well urinary cadmium, at least in the present concentration range, reflects the internal dose. They also suggest that the obtained correlation of 0.2 underestimate the relation between the diet and the internal dose responsible for adverse effects.

6.2.5 Dietary cadmium exposure and the risk of hormone-related cancers

In these two large, population-based cohorts of middle-aged and elderly women and men, we observed positive associations between dietary exposure to the food contaminant cadmium and risk of hormone-related cancers of the endometrium, breast and prostate, but not the ovaries. Our studies were the first to examine the association between dietary cadmium exposure in specific and risk of endometrial, breast and ovarian cancer. Our study on the association between dietary cadmium exposure and the risk of prostate cancer was the first to prospectively assess this relationship.

The associations were stronger for endometrial cancer (**paper II**). This is not surprising, considering the estrogen-mimicking properties of cadmium and that estrogen unopposed by progesterone is the main risk factor of this cancer.[169] Also the fact that the risk estimates showed a clear graded increase with less available estrogen exposure in **paper II**, support the hypothesis of cadmium as a potential metalloestrogen. There are however, several other potential mechanisms of cadmium-induced carcinogenesis. These include indirect genotoxic mechanisms such as oxidative stress, inhibition of DNA repair, stimulation of cell proliferation, blockage of apoptosis or epigenetic mechanisms[170] and experimental studies are needed to further elucidate possible mechanisms. For the other cancer forms there were less support of an estrogenic effect, although this could not be totally ruled out.

The associations between dietary cadmium exposure and endometrial and breast cancer were more pronounced when adjusting for the consumption of whole grain, vegetables and, in the case of endometrial cancer, consumption of potatoes, foods that at the same time are among the main sources of dietary cadmium exposure. This confounding was especially pronounced for breast cancer (**paper III**). Consumption of specific vegetables and whole grain foods are more accurately estimated when using a FFQ compared to estimates of any separate putative anti-carcinogenic compounds e.g. lignans. To obtain a more comprehensive effect of the adjustment we therefore chose to adjust for these food groups rather than specific compounds. Ideally, the models should be adjusted for the specific dietary components *per se*. However, both the wealth of such substances and the limited knowledge available make such an approach intricate. Also, multivariable-adjustment by a large number of compounds may overload the models. Nevertheless, in **papers II and III** (not presented in the published papers), we did adjust for some of the compounds such as specific vitamins (C and E), cereal fiber, specific carotenoids, specific lignans etc. but much less pronounced confounding effect as that for whole grains and vegetables was observed.

For **paper V** on prostate cancer, we adjusted the multivariable models for selenium, lycopene and calcium; because these nutrients have been linked with a probable decreased (selenium and lycopene) or increased (calcium) risk for prostate cancer [80] and was measured with high validity with our FFQ (r = 0.72 for selenium, r = 0.77 for calcium, lycopene not assessed).[126]

Stronger association among lean individuals

The associations between dietary cadmium and hormone-related cancers were more pronounced in lean and normal weight women and men (**papers II, III** and **V**). Regarding the female cancer types, as adipose tissue is the main determinant of estrogens in postmenopausal women,[79] the effect of cadmium could be masked by high BMI. Our results suggest that, in the absence of increased levels of available estrogens from adipose tissue in women with BMI <25 kg/m^2, an estrogenic effect of cadmium may be of importance for the risk of endometrial and breast cancer. Among overweight and obese women, already at increased risk of these cancers because of higher levels of endogenously produced estrogen,[79] cadmium seems not to pose an additionally increased risk. When it comes to men we can only speculate that differences in endogenous sex hormone profile, depending on amount of body fat,[112] may affect the potential effect of dietary cadmium on prostate cancer risk. Finally, we cannot rule out that the risk estimates in lean and normal weight individuals may be higher due to a lower validity in reporting food intake among overweight and obese people.

Estrogen receptor status of breast cancer tumors

In **paper III**, we observed similar risk estimates regardless of estrogen receptor status of the tumor. Although, the classical mode of estrogen action is mediated by the nuclear estrogen receptors, ERα and ERβ, ERs have also been found in the plasma membrane.[171] A G-protein coupled receptor (GPR30) was shown to have all the binding and signaling characteristics of the membrane ER in human breast cancer cells,[172, 173] and ER-negative tumors have shown to express GPR30, suggesting a possibility for a ER-negative tumor to have some estrogen responsive signaling.[174] Recent studies show that cadmium can also activate the membrane bound ER pathways through ERK1/2 (MAPK pathway) and Akt (PI3K pathway).[72, 175, 176] Further, Yu and colleges demonstrated that cadmium stimulated breast cancer cell proliferation in cells that contain only GPR30 and no ERα or ERβ, in a manner similar to that of estrogens.[76]

Differences in associations between localized and advanced prostate tumors

We assessed the association in subtypes of prostate cancer based on their aggressiveness. The stronger association observed for localized tumors compared to advanced and fatal ones (although not statistically different), might suggest that cadmium plays a role in the initiation rather than the progression of already established tumors.

Lack of association between dietary cadmium exposure and ovarian cancer

We did not observe an association between dietary cadmium exposure and ovarian cancer. A possible reason for the lack of association, in contrary to that observed between dietary cadmium and endometrial cancer, breast cancer and prostate cancer, is that estrogen may not be the most important etiologic factor for ovarian cancer.[177] Indeed, a strong direct association was observed between circulating levels of estrogen and endometrial cancer,[178] but not ovarian cancer.[179] Except for a true non-existing association, there may be several reasons explaining the lack of an observed association. Due to a suggested ovarian cancer latency of 25-30 years,[177] we may not have captured the relevant exposure time for ovarian carcinogenesis. Further, in this study we had the power (>80%) to detect increased relative risks of 1.4, which means that we would have missed a small association.

Concluding remarks

Our results are in line with all previous studies on cadmium exposure and breast cancer[114, 115] and some studies on cadmium and prostate cancer risk.[117, 119] Very recently, a Japanese prospective cohort study[180] found no associations between dietary cadmium estimated with a FFQ and overall cancer. Although a tendency towards an increased risk with endometrial cancer was observed, cases were in general too few to look at specific cancer types, not to mention specific cancer subtypes or subgroup analyses.

The results from the study of breast cancer indicated that wholegrain products and vegetables provide some degree of protection against cancer, despite them also being among the major sources to cadmium exposure.

Furthermore, our results indicate that a possible impact of cadmium on cancer risk is present at dietary exposure levels clearly below the TWI set by EFSA to protect 95 % of the population by the age of 50 years from renal tubular damage and far below that set by FAO/WHO. As such the findings of this thesis are important given the current debate on what levels of cadmium we are to accept in our environment, bearing in mind the health hazards it poses.

7 CONCLUSIONS

- The major dietary sources of cadmium are whole grains, vegetables and root vegetables, which at the same time are important sources of fiber, antioxidants, and phytochemicals with proposed anticarcinogenic properties.
- The estimated cadmium half-life was 11.6 years with 25% between-person variability in the population. The established link between urinary cadmium concentrations and the corresponding long-term dietary cadmium exposure for different population strata could be further used in international health risk assessment.
- Food frequency questionnaire-based estimates of dietary cadmium exposure were considered to reflect the exposure dose reasonably well, allowing these estimates to be used in large scale epidemiological studies.
- Cadmium exposure *via* food was associated with an increased risk of endometrial cancer, breast cancer but not ovarian cancer in women and with an increased risk of prostate cancer in men. Taken together these results indicate that dietary cadmium exposure may play a role in the development of hormone-related cancers.

From a public health perspective, the results presented in this thesis indicate that cadmium exposure *via* food may play a role in the development of two major cancers – breast cancer and prostate cancer – both with a high incidence in high income countries and an increasing trend in low- and middle-income countries. Thus, it is of great importance for the public health to decrease the level of cadmium contamination in food.

8 FUTURE RESEARCH

The results presented in this thesis contribute to the knowledge about the association between dietary cadmium exposure and hormone-related cancers. Future research should include:

- Experimental studies for increasing the understanding on by which mechanisms cadmium may affect the risk of hormone-related cancers.

- Large, prospective population-based cohort studies with urine and blood collected to measure cadmium exposure using nested-case-control design.

- Studies linking urinary cadmium concentrations to estimated dietary cadmium exposure in men.

- Studies that explore the association of early life cadmium exposure. Of interest in the context of cadmium as an endocrine disruptor, developmental effects may be of particular importance.

- Studies to elucidate the benefit-risk of consumption of foods/diets that contain healthy components, but are contaminated with cadmium.

Together such studies will be very valuable as scientific evidence-based knowledge in the discussion on what levels of cadmium contamination are acceptable in our environment.

9 POPULÄRVETENSKAPLIG SAMMANFATTNING

Kadmium är en giftig metall naturligt förekommande i jordskorpan, men halterna i miljön har under det senaste århundratet ökat till följd av den omfattande industrialiseringen. Kadmium tillförs till åkermark via nedfall från luften (till exempel från renframställning av zink, förbränning av fossila bränslen och sopor) och genom användningen av handelsgödsel och rötslam. Kadmium tas sedan i varierande grad upp av växter vilket leder till att kosten är den i särklass största exponeringsvägen för människan. Med andra ord – alla exponeras och exponeringen är livslång. Rökare får även i sig kadmium via tobaksrök. Inälvsmat, vissa skaldjur och fröer kan innehålla höga halter kadmium, men den huvudsakliga exponeringen sker genom våra viktigaste baslivsmedel såsom bröd, spannmål, rotfrukter inklusive potatis och grönsaker. Kadmium utsöndras långsamt och ansamlas därför i kroppen, främst i njuren och det är sedan länge känt att metallen kan orsaka njurskador. I studier på råttor fann man nyligen att kadmium även kan ha hormonstörande effekter genom att härma östrogenets funktion i kroppen. Det har föreslagits att hormonstörande kemikalier har bidragit till den, över tid, ökade förekomsten av hormonberoende cancer. Kadmium har klassats som cancerframkallande under vissa omständigheter. Det är dock inte klarlagt om även kadmium från kosten kan påverka risken för cancer. Hur kadmiums eventuellt östrogenlika effekter yttrar sig i förekomst av hormonberoende cancerformer i befolkningen har i princip inte studerats.

Artiklarna som ligger till grund för denna avhandling bygger på två stora befolkningsbaserade studier som består av cirka 60 000 kvinnor i Uppsala och Västmanland län och 40 000 män i Västmanland och Örebro län. Studiedeltagarna svarade på en enkät om matvanor; kvinnorna tillfrågades 1987 och männen 1997. Vi räknade sedan ut hur mycket kadmium deltagarna fick i sig via maten med utgångspunkt från kadmiuminnehållet i varje enskilt livsmedel som studiedeltagarna åt. Utifrån mängden kadmium deltagarna fick i sig genom kosten delades de in i tre lika stora grupper.

Syftet med denna avhandling var dels att uppskatta exponeringen av kadmium via kosten, dels att undersöka sambandet mellan långsiktig, låg kadmiumexponering från kosten och förekomsten av hormonberoende cancer (livmoder-, bröst-, äggstocks- och prostatacancer). Med hjälp av en matematisk modell som beskriver upptag, ansamling och utsöndring av kadmium, ville vi dessutom koppla halten av kadmium i urin (en markör för livslång kadmiumexponering) till kadmium från kosten, vilket sedan har kunnat användas i hälsoriskbedömningar för kadmium. Vi testade även hur väl denna kostenkät kunde spegla individuell exponering av kadmium från kosten. Sammantaget talar resultaten för att vi kan uppskatta exponeringen via kostenkäten på ett tillfredställande sätt. Alltså kan vi använda den uppskattade kostexponeringen i stora befolkningsbaserade studier för att uppskatta risken för hormonberoende cancer.

De huvudsakliga källorna till kadmiumexponering via kosten (~80%) hos både kvinnor och män var bröd och andra spannmål, potatis, rotfrukter och grönsaker; livsmedel som samtidigt är viktiga källor till fiber och antioxidanter som skulle kunna motverka cancer. Sambanden mellan kadmium från maten och livmodercancer, bröstcancer och prostatacancer visade alla på en statistiskt säkerställd ökad risk. Kvinnor i gruppen med den högsta exponeringen av kadmium via maten hade 39 procents förhöjd risk att drabbas av cancer i livmodern och 21 procents ökad risk för bröstcancer, jämfört med gruppen som hade lägst kadmiumexponering. Vi kunde däremot inte se något samband mellan kadmium från kosten och äggstockscancer. Vad gäller prostatacancer hade män i gruppen med den högsta exponeringen av kadmium via maten 13 procents förhöjd risk jämfört med män i den lägsta gruppen. Vi fann även indikationer på hormonstörande effekter från kadmium, men detta måste utredas vidare. Ett viktigt fynd var att de kvinnor som åt mest fullkornsprodukter (exempelvis fullkornsbröd) och grönsaker hade lägre risk att drabbas av bröstcancer jämfört med kvinnor som exponerats för kadmium via annan sorts mat.

Sammanfattningsvis pekar resultaten från denna avhandling på att exponering för kadmium via kosten kan spela en roll för utvecklingen av bland andra två av våra absolut vanligaste cancerformer – bröst- och prostatacancer. Sett utifrån ett folkhälsoperspektiv är det här viktig kunskap med tanke på den pågående diskussion om vilka nivåer av kadmium vi ska acceptera i vår miljö ställt emot de eventuella hälsorisker som det för med sig.

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