Prostate cancer risk and recurrence

the role of nutrition and clinical aspects

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Abstract

Background Prostate cancer is the most common cancer among men in Western countries. Knowledge on prostate cancer aetiology is required for identification of high-risk groups, optimization of treatment strategies, and development of prevention programs. The aim of this thesis was to obtain insight into nutritional and clinical factors relevant to different stages of prostate cancer.

Methods and results First, an inventory of potential risk factors for prostate cancer was made by asking 956 patients with prostate cancer about perceived causes of their disease. Among the 143 patients who provided self-reported causes, heredity, specific environmental factors, nutrition or lifestyle, and stress were most frequently reported.

Second, two potential risk factors, i.e. blood lipid levels and a previous cancer diagnosis, for incident prostate cancer were evaluated. Higher levels of total and LDL cholesterol were significantly associated with an increased risk of (aggressive) prostate cancer after 6.5 years of follow-up in a population-based cohort of 2,118 men (43 cases). Analyses from another population-based cohort among 551,553 men (9,243 cases) showed that cancer survivors diagnosed with a first cancer (other than prostate cancer) between 1989 and 2008 had an overall increased risk of prostate cancer in the first year after their first cancer diagnosis. This increased prostate cancer risk is most likely the result of active screening or incidental detection, because the effects disappeared after one year of follow-up for most of the specific first cancer sites.

Third, the effect of body mass index (BMI) on risk of biochemical recurrence was studied in two cohorts of 493 patients (142 cases) and 1,302 patients (297 cases) treated with radical prostatectomy for prostate cancer. BMI was not associated with risk of biochemical recurrence in these patients.

Finally, the effects of selenium, a suggested candidate for prostate cancer chemoprevention, on gene expression profiles in the prostate were examined in a randomized and placebo-controlled intervention trial with 15 participants (n=8 selenium, n=7 placebo). Selenium (300 µg/day as selenized yeast) affected the expression of genes towards an anti-inflammatory gene expression profile. Furthermore, we were able to detect expression changes in genes implicated in the epithelial-to-mesenchymal transition.

Conclusion The results of this thesis show that specific nutritional and clinical factors might influence risk of prostate cancer or have an effect on gene expression in the prostate. A future challenge is the confirmation and 'translation' of these findings into the development and implementation of effective treatment and prevention strategies for prostate cancer.

Contents

1 Introduction

The overall aim of this thesis is to obtain insight into nutritional and clinical aspects relevant to different stages of prostate cancer. In the following paragraphs, an introduction to prostate cancer will be provided. Basic aspects of the epidemiology, pathology, carcinogenesis, diagnosis, and treatment of prostate cancer will be discussed in light of the topics addressed in this thesis **(paragraph 1.1)**. Furthermore, an overview of potential risk factors and targets for prevention of incident **(paragraph 1.2)**, progressive **(paragraph 1.3)** and recurrent **(paragraph 1.4)** prostate cancer will be presented. As this thesis covers a relatively broad and extensive research area, some of the aspects discussed in this chapter will not be specifically addressed in the following chapters. Instead, this information can be used as background information and might be useful for the interpretation or discussion of the study outcomes. This chapter ends with the specific research aims and the outline of this thesis.

1.1 General introduction to prostate cancer

The incidence of prostate cancer

Prostate cancer is the most common cancer among men in Western countries[1, 2]. It is estimated that almost one out of ten Dutch men will develop prostate cancer before the age of 80[3, 4]. With 21%, prostate cancer accounted for the majority of all newly diagnosed cancers in Dutch men in 2009[4]. In the Netherlands, 10,166 men were newly diagnosed with prostate cancer in 2009, while 2,492 men died from this disease in the same year[4]. Prostate cancer is predominantly diagnosed in elderly men. In 2009, 70% of all newly diagnosed Dutch prostate cancer patients were men aged 65 years and older[4].

Diagnosis

There are no characteristic symptoms for prostate cancer, because most of the reported local symptoms, such as lower urinary tract symptoms (LUTS), refer to benign prostatic disorders more specifically. The detection and diagnosis of prostate cancer is often based on serum levels of the prostate-specific antigen (PSA) and a digital rectal examination, followed by ultrasound-guided prostate biopsies. Several prostate cancer-specific biomarkers in urine, blood and tissue have been evaluated, but so far none of these biomarkers is widely used in clinical practice[5].

In contrast to few other cancers (i.e. cervical cancer and breast cancer in females), there is no national screening program for prostate cancer in the Netherlands. The controversy about such screening program refers to the lack of cancer specificity of PSA testing[6]. Furthermore, it has been determined that the benefits are not likely to balance the possible harms (i.e. complications of treatment and mental burden[7]) of early screening[8-10].

Pathology and staging

Normal prostate tissue consists of glandular tissue surrounded by non-glandular components (e.g. fibromuscular stroma)[11]. The prostate ducts are lined by two layers of epithelial cells, with neuroendocrine cells dispersed throughout these layers[12]. The secretory luminal cells directly line the lumen of the prostate ducts, while the basal cells form the second layer of epithelial cells[12]. The vast majority of the cancers in the prostate arises from the epithelial cells and are therefore defined as prostatic adenocarcinomas[13]. Within the prostate, three morphological zones can be distinguished; the peripheral zone, the transition zone, and the central zone[11, 14]. The majority of all prostatic adenocarcinomas (~70%) occur in the peripheral zone, which is also highly susceptible to inflammation, while 20-25% and 5-10% arise from the

transition zone and central zone, respectively[11, 15, 16]. Benign prostate enlargement (benign prostate hyperplasia or BPH) typically originates from the transition zone[17].

Histological grading is a tool for pathologists to assess the architecture of a tumour. The Gleason grading system for prostatic adenocarcinomas is grouped into five categories[18]. Gleason grade 1 refers to well-differentiated tumours (resemble normal prostate tissue), whereas poorly-differentiated tumours (abnormal architecture) are assigned as Gleason grade 5[19, 20]. The sum of the two most common Gleason grades provides clinical implications for treatment and prognosis[20].

Clinical or pathological staging is another approach to evaluate the prognostic characteristics of prostate cancer. Clinical staging is based on the findings during digital rectal examination or imaging techniques, while pathological staging is evaluated in surgically removed tissues. The TNM staging (**Table 1.1**) system refers to local tumour growth (T), spread to regional lymph nodes (N) and distant metastases (M)[21]. The tumour (T) classification for prostate cancer ranges from T1 (tumour present but not detected clinically or with imaging) to T4 (tumour invades into adjacent structures)[21].

Non-aggressive and aggressive prostate cancer

In clinical practice, both Gleason grade and TNM stage are used as predictors for prognosis. The prognosis of prostate cancer also depends on several other factors, because prostate cancer is a heterogeneous disease with different levels of aggressiveness. As observed in autopsy studies, prostate tumours were found in 33-65% of all men aged above 70 years[22-24]. In most cases, these tumours were slow-growing, indolent tumours without clinical significance. Various criteria have been suggested for the classification of clinically insignificant prostate cancer[25]. According to the Epstein criteria, insignificant prostate cancer is defined as an organ-confined cancer with no Gleason pattern 4 or 5, PSA density <0.15 ng/mL, dominant tumour volume <0.5 cm^3 in surgically removed specimens, or fewer than three positive biopsies with <50% cancer per biopsy specimen[26, 27]. The aim of these criteria is to identify low-risk patients who do not require active treatment[26].

Prostate cancer, however, can also be an aggressive disease which is resistant to therapy, shows distant metastases, and might have a fatal ending. In epidemiological studies, these aggressive, high-grade or advanced forms of prostate cancer usually refer to tumours with stage T3 or worse, Gleason scores ≥7, and involvement of regional lymph nodes (N1) or distant metastases (M1) (e.g. Platz et al.[28]). Many studies aimed to determine biomarkers that can distinguish between insignificant, indolent and aggressive types of cancer[29-31]. So far, none of the suggested biomarkers is

implemented in clinical practice, mostly because they were not able to provide more information than Gleason score alone[32].

Table 1.1 Grading and staging system for prostate cancer

System	Category	Description	
Gleason Grading[a]	Gleason 1	Well-differentiated, closely packed, uniform, medium-sized glands	
	Gleason 2	Glands somewhat loosely arranged, there may be minimal infiltration	
	Gleason 3	Smaller, discrete glands, marked variation in size and shape of the glands with some infiltration	
	Gleason 4	Fused glands with an irregular border	
	Gleason 5	Poorly-differentiated, no glandular differentiation	
TNM Staging[b]	Tumour (T)	T1	Clinically inapparent tumour (neither palpable nor visible by imaging)
		T1a	Tumour in ≤5% of resected tissue
		T1b	Tumour in >5% of resected tissue
		T1c	Tumour identified by needle biopsy
		T2	Tumour confined within prostate
		T2a	Unilateral, ≤one-half of one lobe
		T2b	Unilateral, >one-half of one lobe
		T2c	Bilateral
		T3	Tumour extends through the prostate capsule
		T3a	Extracapsular extension
		T3b	Invasion into seminal vesicle(s)
		T4	Tumour invades adjacent structures other than seminal vesicles
	Node (N)	N0	No regional lymph node metastases
		N1	Regional lymph node metastases
	Metastasis (M)	M0	No distant metastases
		M1	Distant metastases
		M1a	Nonregional lymph node(s)
		M1b	Bone(s)
		M1c	Other site(s)

[a] International Society of Urological Pathology Consensus Conference[19]; [b] American Joint Committee on Cancer Staging Manual, 7th edition[21]

Treatment

The treatment of prostate cancer depends on the stage of the disease, presence of co-morbidities, and the age, condition and preference of the patient. For patients with insignificant or low-risk prostate cancer, conservative management ("active surveillance") is often indicated[25, 33]. Active treatment regimens for prostate cancer include surgical removal of the prostate (radical prostatectomy), and radiation therapy[34]. Androgen-deprivation therapy and chemotherapy are mostly used to control and relieve the symptoms of advanced prostate cancer and to improve quality of life[35].

Prostate carcinogenesis

Prostate cancer is a complex disease, with a number of molecular events and pathways involved in its development and progression. The process of development and progression of cancer is defined as 'carcinogenesis'. Carcinogenesis is mostly driven by changes in the sequence, structure or stability of the genetic material. A brief overview of some of these changes is provided below (**Figure 1.1**).

Genetic changes

Genetic changes usually refer to changes or variants in the sequence of the DNA. These changes can either be inherited or acquired, the latter for instance as a consequence of prolonged exposure to harmful substances or oxidative stress. Mutations in relevant parts of the DNA (i.e. genes) can result in loss or aberrant function of these genes. In particular, mutations in genes that are involved in control of cell growth (e.g. proto-oncogenes, tumour suppressor genes) and DNA repair (DNA repair genes) might substantially contribute to the carcinogenic process. Family-based studies identified several variations (single nucleotide polymorphisms, SNP's) in genes that are associated with an increased risk of developing prostate cancer (e.g. the ribonuclease L gene, *RNASEL*)[37]. In the past years, the efforts of the so-called genome-wide association (GWAS) studies, which compare DNA sequences of prostate cancer patients with a control population, also revealed several prostate cancer susceptibility loci[38, 39].

More extensive alterations in the DNA sequence are associated with prostate carcinogenesis as well. Duplication or deletion of substantial parts of the DNA (defined as chromosomes) is referred to as chromosomal gain and loss, respectively[32]. With some exceptions, chromosomal loss is often observed during early stages of carcinogenesis, whereas chromosomal gain occurs mainly during later stages[40, 41]. Deletions in the 8p region are often accompanied by a reduced expression of the gene *NKX3.1*[42], which is located at 8p21[43]. As *NKX3.1* is considered a tumour suppressor gene with a critical function in response to DNA damage[44], the deletion of this gene might explain its relevance with respect to prostate cancer. In parallel with the effects for *NKX3.1*, deletions in the 10q region often result in a loss of *PTEN*[45], another gene implicated in prostate carcinogenesis. *PTEN* is also considered a tumour suppressor gene with a regulatory role in a survival (PI3K/AKT) signalling pathway[46].

Epigenetic changes

Epigenetic changes refer to changes in the genetic material that occur without altering the sequence of the DNA. Several epigenetic processes have been described[47-49]. It is beyond the scope of this thesis to review all of these phenomena, however, epigenetic

processes which might be of special relevance to prostate cancer will be shortly highlighted. DNA methylation primarily refers to the binding of methyl groups to cytosine nucleotides of the DNA. DNA methylation plays an essential role in the regulation of gene expression[49]. Excessive methylation or hypermethylation, which often occurs in specific regions rich of cytosine and guanine nucleotides (CpG islands), might result in a detrimental blocking or silencing of the genes and thereby contribute to the development of cancer[49]. For prostate cancer, over fifty genes have been identified that are frequently hypermethylated during carcinogenesis[50]. One of the best studied genes which is often hypermethylated in prostate tumours is involved in DNA repair and encodes for the glutathione S-transferase P1 (*GSTP1*)[51].

Other phenomena, which are more recently associated with prostate cancer development and progression, are the small, non-coding RNA fragments[52, 53]. These so-called microRNA's (miRNA) are able to regulate gene expression via binding to the coding RNA (messenger RNA or mRNA)[53, 54]. Alterations in the expression of several microRNA's might affect cell cycle control, cellular migration and invasion, apoptosis, and androgen signalling in prostate tissue[53], and thereby explain its possible relevance with respect to prostate cancer.

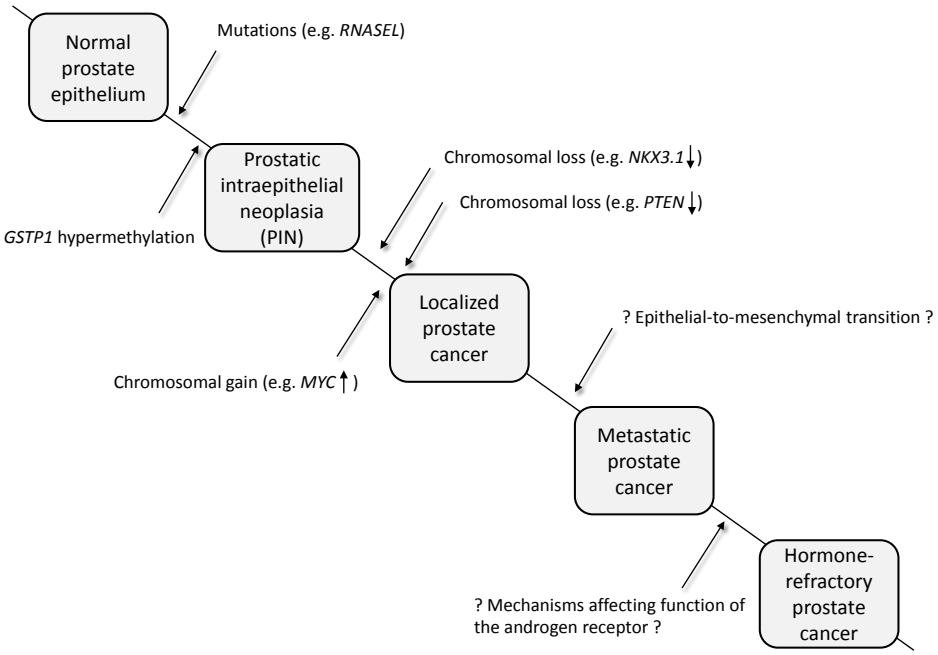

Figure 1.1 Schematic overview of suggested molecular processes occurring during prostate carcinogenesis (based on Nelson et al, 2003[36])

Androgen receptor

Most of the molecular processes implicated in prostate carcinogenesis directly result from the genetic or epigenetic changes described above. One process that deserves some special attention is the androgen signalling pathway. Androgens are, through their interaction with the androgen receptor (AR), involved in growth and development of both normal and cancerous prostate tissue[55]. After binding of androgens, the androgen receptor is subjected to conformational changes and is transported to the nucleus[55]. In the nucleus, the activated androgen receptor binds to specific regions (androgen-response elements) and thereby regulates the expression of target genes involved in cellular growth and survival[55, 56]. Initially, prostate tumours require androgens for their growth, which explains the efficiency of androgen-deprivation therapy for advanced prostate cancer[57]. Sooner or later, most tumour cells gain the ability to grow in the absence or at minimal levels of androgens, which result in the androgen-independent or hormone-refractory stage of the disease[55, 58]. Several mechanisms may lead to the development of hormone-refractory prostate cancer, including[58] mutations in the androgen receptor[59], overexpression of the androgen receptor[60], changes in expression of factors that collaborate with the androgen receptor[61], activation of the androgen receptor by alternative factors or pathways[62, 63], or the production of androgens by the tumour itself[64]. As reviewed by others, more recent studies suggest that alternative mechanisms, independent of the androgen receptor, might also contribute to the development of hormone-refractory prostate cancer[32, 58].

Epithelial-to-mesenchymal transition

Progression of prostate cancer is not only characterized by the development of hormone-refractory disease, but also by the formation of metastases. The molecular mechanisms underlying metastatic prostate cancer are poorly defined. Given its important role in cellular invasion and migration, it is suggested that epithelial-to-mesenchymal transition (EMT) might facilitate the spread of prostate cancer cells[65]. During the epithelial-to-mesenchymal transition, the unique characteristics of epithelial cells are replaced by mesenchymal properties[66]. Epithelial cells typically form structured layers of cells and have close contacts with their neighbouring cells, whereas cells with a mesenchymal phenotype do not form cell layers and might have the ability to migrate[66]. Important molecular events that come along with the epithelial-to-mesenchymal transition are loss of markers for epithelial cells (e.g. E-cadherin)[67] and aberrant expression of markers for mesenchymal cells (e.g. vimentin and N-cadherin)[66]. Although the exact mechanisms remain unclear, it has been clearly demonstrated that processes related to the epithelial-mesenchymal transition might play a role in development of metastatic prostate cancer[65].

1.2 Risk factors and prevention of prostate cancer

Relatively little is known about the aetiology of prostate cancer. Knowledge on aetiology and risk factors is required for the identification of high-risk groups, development of new treatment strategies, and optimization of effective prevention programs. In the following sections, an overview of established and some suggested risk factors for prostate cancer will be presented. Furthermore, examples of potential prevention strategies will be described.

Established risk factors for prostate cancer

So far, old age, black race, a positive family history, and a few dozen of low-penetrance genetic markers have been established as risk factors for prostate cancer[68, 69]. Previous studies, however, suggested that a considerable part (~60%) of all prostate cancer diagnoses is most likely attributable to clinical or environmental factors[70]. Apparently, these factors have not yet been identified or confirmed and further research is therefore needed to elucidate the role of these factors in the aetiology of prostate cancer.

Clinical factors

High-grade prostatic intraepithelial neoplasia (HGPIN) refers to the presence of atypical epithelial cells in the prostate[71]. In contrast to prostatic adenocarcinomas, the basal epithelial cell layer of HGPIN foci is disrupted, but not absent[71]. Currently, HGPIN is considered as a premalignant stage of prostate cancer[72]. If HGPIN is found in an initial diagnostic biopsy, the chance of detecting prostate cancer in subsequent biopsies ranges from 30-75% and seems to depend on the number of previous biopsies with HGPIN[73].

Inflammation is very common in prostate tissue[74-76], however, its role in prostate cancer aetiology remains controversial[77]. Based on the estimation that up to 15-20% of all cancers is caused by either infections or inflammation[78-80], it is suggested that inflammation might be involved in prostate carcinogenesis as well[16, 80]. Prostatitis and sexually-transmitted diseases have been associated with increased risks of prostate cancer[81, 82]. However, the role of detection bias should be considered, because men with prostate-related symptoms might have relatively intensive screening for prostate cancer[75].

It has been suggested that a previous cancer diagnosis might influence the subsequent risk of developing prostate cancer. The incidence of prostate cancer, for instance, is relatively high in bladder cancer patients[83, 84].

The co-occurrence of bladder cancer and prostate cancer can be explained by incidental detection in surgical specimens or active medical surveillance. Also previous cancer treatments might influence prostate cancer risk. It has been suggested that pelvic radiotherapy reduces the risk of developing prostate cancer[85, 86]. Finally, the association between prostate cancer and other malignancies might be explained by common aetiological factors, such as genetic susceptibility or shared exogenous factors as nutrition and lifestyle factors. In order to explore the aetiology of prostate cancer, more insight into the co-occurrence of prostate cancer and other malignancies is warranted.

Nutrition and lifestyle factors

There has been considerable interest in the role of nutrition and lifestyle factors in prostate cancer development and progression. In 2007, the World Cancer Research Fund (WCRF) and the American Institute for Cancer Research presented an extensive report on food, nutrition, and physical activity in relation to cancer[87]. The aim of this report was to evaluate and judge factors that can modify the risk of cancer. For prostate cancer, an extensive systematic literature review (558 publications) was performed and several factors were judged according to the strength of evidence[87]. For the majority of the nutritional and lifestyle factors, there was no or limited evidence that these factors modify the risk of prostate cancer[87]. Only diets high in calcium are reported as a probable cause of prostate cancer according to this report[87].

Overweight and obesity have also been suggested as risk factors for prostate cancer[88]. Body mass index (weight in kilograms divided by height in meters squared, kg/m^2) is often used to define overweight (BMI 25-30 kg/m^2) and obesity (BMI ≥30 kg/m^2). Although results are fairly inconsistent, several prospective studies demonstrated that a high body mass index (BMI) is associated with an increased risk of prostate cancer, particularly for advanced prostate cancer[89-91]. Most of these studies, however, were conducted in the United States, where a rapidly growing epidemic of overweight and obesity is reported with over 68% of adult Americans being overweight or obese[92]. So far, most European studies were not able to confirm these results, although some prospective studies did find an association between measures of overweight, obesity and fat distribution (e.g. BMI and waist circumference) and prostate cancer incidence[93-95].

According to the WCRF report, foods containing lycopene and selenium, and dietary supplements with selenium are probably protective against prostate cancer[87]. In theory, intervention strategies with these nutrients might provide relatively simple and straightforward possibilities for the prevention of prostate cancer.

At this moment, however, the long-term effects of these factors on cancer biology and other health outcomes need to be explored.

Prevention of prostate cancer

Prevention is a strategy to reduce the development and progression of cancer. It is suggested that at least 40% of all cancer cases is preventable[96, 97]. Avoidance or reduction of risk factors, and pharmacological, dietary or lifestyle interventions can be effective prevention strategies. Chemoprevention is defined as an intervention with synthetic or naturally occurring substances in order to prevent, reverse, or inhibit the development of cancer in any of its stages[98, 99].

Prostate cancer is considered as an important target for chemoprevention, because of the high prevalence, the long latency period, and the roles of hormones[100] and possible modifiable risk factors in prostate cancer development and progression. Currently, the most studied strategies for prostate cancer chemoprevention could be classified either as hormonal interventions or dietary interventions.

Hormonal interventions

An intervention targeting a hormonal pathway is a reasonable possibility for prostate cancer chemoprevention, because hormones play an important role in the prostate[100]. The enzyme 5α-reductase facilitates the conversion from testosterone to dihydrotestosterone (DHT), which is the main androgen responsible for prostate development and growth[101, 102]. The 5α-reductase inhibitors decrease the levels of DHT and are therefore used in the treatment of symptomatic benign prostate hyperplasia (BPH)[101, 103]. Finasteride and dutasteride are 5α-reductase inhibitors that are also evaluated as candidates for prostate cancer chemoprevention.

Finasteride specifically inhibits the activity of the 5α-reductase isoenzyme type 2. The Prostate Cancer Prevention Trial (PCPT) showed that finasteride reduced the risk of prostate cancer by 25%[104]. Stratified analyses, however, suggested that high-grade tumours (Gleason score ≥7) were more frequently detected among finasteride-users[104]. Dutasteride is also considered as a potential chemopreventive agent for prostate cancer, because it is a dual inhibitor of the 5α-reductase isoenzymes type 1 and 2[105]. The Reduction by Dutasteride of Prostate Cancer Events (REDUCE) trial was initiated to examine the effects of dutasteride on the risk of incident prostate cancer in high-risk men[106]. First results of the REDUCE trial showed that dutasteride reduced the risk of incident prostate cancer detected on biopsy by 23%[107]. Stratified analyses showed that the preventive effects were confined to the low-grade prostate cancers (Gleason grade 5 or 6)[107].

Dietary interventions

Lycopene, a carotenoid and major constituent of tomatoes and other red fruit and vegetables, is classified as 'probably protective against prostate cancer' by the WCRF report[87]. Small intervention studies with lycopene supplements or tomato-based products examined the effects of lycopene on PSA levels[108, 109], pathological outcomes[108] and DNA damage[109]. The overall evidence that lycopene influences the prostate in a beneficial way, however, is inconsistent[110, 111].

Based on their roles as antioxidants, several vitamins have also been suggested as candidates for prostate cancer chemoprevention. For vitamin A, D, and C, there is no conclusive evidence that these vitamins prevent the development of prostate cancer[87, 112]. Although vitamin E intake and status were associated with a significantly decreased risk of prostate cancer in some prospective cohort studies[113-115] and a randomized trial[116], two large chemoprevention trials with incident prostate cancer as primary outcome did not confirm these results[112, 117], or even suggest an increased risk of developing prostate cancer[118].

Selenium is an essential trace element and has been considered as a promising candidate for prostate cancer chemoprevention. This hypothesis is supported by several observations that selenium deficiencies increased the risk of prostate cancer, whereas high selenium status or selenium supplementation decreased the risk of prostate cancer [87, 119-122]. Recent observational and intervention studies, however, did not consistently confirm a preventive effect of selenium for prostate cancer[117, 123]. The Selenium and Vitamin E Cancer Prevention Trial (SELECT) is a randomized, controlled intervention trial that was initiated in order to determine the effects of selenium, vitamin E or a combination on prostate cancer incidence in a large sample of the general population[124]. The planned follow-up for the SELECT trial was seven to twelve years, however, the trial was discontinued after five and half years, because no preventive effects of selenium or vitamin E were found for prostate cancer, or were expected with additional exposure[117, 118]. The inconsistent findings of prospective studies and intervention trials highlight the need for extensive research on the possible mechanisms of selenium in the prostate, as well as the role different doses, formulations and baseline status of selenium.

Other interventions

Several other chemopreventive strategies for prostate cancer have been suggested. The use of cholesterol-lowering drugs is consistently associated with decreased risk of (advanced) prostate cancer[125]. This observation provides leads for research on the role of cholesterol in the aetiology of prostate cancer. Use of aspirin and non-steroidal anti-

inflammatory medications might lower prostate cancer risk through anti-inflammatory mechanisms[126]. Oestrogen receptor modulators have been implicated in the prevention of prostate cancer through mechanisms that have not been elucidated[127, 128]. Most of these agents, however, have not been validated in randomized, controlled intervention trials primarily designed for prostate cancer outcomes.

Implementation of chemopreventive strategies

So far, none of the chemopreventive strategies described above is recommended or implemented in clinical practice or in the general population. It is beyond the scope of this thesis to address all aspects involved in decision-making related to chemoprevention. However, few aspects will be addressed below. Overall, the cost-effectiveness of chemoprevention depends on the balance between costs, desired effects, and manifestation of adverse effects[129]. Depending on the aim and design of the chemopreventive strategy, the target population should be carefully defined. For chemoprevention aimed at the entire, lower-risk population (e.g. SELECT[117] and PCPT[104]), the expected effects should be relatively strong in order to achieve a cost-effective intervention[130]. Furthermore, the acute and long-term effects of the intervention are extremely important; every minor adverse effect should be taken into consideration. From this perspective, dietary and lifestyle interventions are frequently suggested as appropriate and safe candidates for widespread chemoprevention. However, also dietary and lifestyle interventions, especially at supranutritional or supraphysiological levels, might be accompanied by adverse health effects[118, 131, 132]. A high-risk population for prostate cancer (e.g. REDUCE[107]) is an effective target population for chemoprevention, however, this population might have more aggressive forms of cancer that are not susceptible to the suggested prevention strategies[133]. Extensive research on the biological mechanisms and the long-term effects on cancer biology and other health outcomes is therefore required before any prostate cancer prevention program should be implemented or recommended.

In conclusion

The aetiology of prostate cancer remains poorly understood. Only few non-modifiable risk factors for prostate cancer have been established. For other factors, evidence is missing or inconsistent. Identification of risk factors involved in prostate carcinogenesis is important in order to understand the aetiology, to identify high-risk groups for early detection and to develop effective prevention strategies. Several chemopreventive agents for prostate cancer have been suggested. Before any of these chemoprevention strategies can be implemented, the balance between desired effects, adverse effects and costs should be carefully considered. Extensive research on the biological mechanisms and the long-term effects on cancer biology and other health outcomes is therefore required.

1.3 Risk factors and prevention of prostate cancer progression

From non-aggressive to aggressive prostate cancer

Progression of prostate cancer refers to the transformation from HGPIN and insignificant or indolent tumours to more advanced, aggressive or fatal forms of prostate cancer. Although numerous studies revealed that different factors are involved in the development of non-aggressive and aggressive prostate cancer, there is relatively little known about what exactly drives prostate cancer towards an aggressive form.

Risk factors for prostate cancer progression

Several clinical, nutritional and lifestyle factors, such as calcium intake[134], body mass index[88], use of cholesterol-lowering drugs[135], and blood lipid profiles[28, 136] have been linked to aggressive prostate cancer in particular. These findings suggest that these factors might play a substantial role in prostate cancer progression. At present, few observational studies examined the effects of post-diagnostic variables on risk of prostate cancer progression (defined as prostate cancer death, metastases, rising PSA levels or start of secondary treatment) as primary outcome[137-139]. Intake of eggs and poultry with skin[137], as well as whole milk (but not total milk or dairy intake)[139], were significantly associated with higher risk of progression, whereas intake of fish and tomato sauce protected against progression in these studies[138]. Furthermore, it was demonstrated that men with moderate to high levels of physical activity after their diagnosis had lower risk of prostate cancer-specific mortality or progression in some prospective studies[140, 141]. Also, several genetic (e.g. single nucleotide polymorphisms) and epigenetic (e.g. DNA hypermethylation) variations have been associated with prostate cancer progression[142-145].

Prevention of prostate cancer progression

Stopping or delaying prostate cancer progression and recurrence, sometimes referred to as tertiary prevention, might result in a reduction of treatment-related complications and prostate cancer-related mortality[133]. Previous studies demonstrated that cancer patients are relatively motivated to change their diet and lifestyle[146, 147]. This starting point provides good opportunities for adherence to tertiary prevention programs. Several studies addressed the effects of dietary and lifestyle interventions in relation to prostate cancer progression[148-150]. The primary outcomes in most of these studies were indicators of disease progression (e.g. start of primary treatment, rising PSA levels) among men with HGPIN or low-risk prostate cancer undergoing active surveillance.

Prescription of an intensive lifestyle program, including a vegan diet (supplemented with selenium, vitamin E and C, soy, and fish oil), moderate aerobic exercise, stress management techniques, and participation in a support group seemed to reduce serum PSA levels[149], and delay the start of a conventional therapy for prostate cancer[148], suggesting that disease progression was attenuated in these patients with low-risk prostate cancer. Supplementation with selenium alone did not influence changes in PSA levels over time (PSA velocity) in men with low-risk prostate cancer[150].

As can be concluded from above, mixed results were found for the effects of dietary and lifestyle interventions on risk of prostate cancer progression. Before considering the development and implementation of prevention programs, further research is warranted. First, it is important to identify factors that are responsible for the potential protective effects. The advantage of comprehensive dietary and lifestyle interventions is that they mimic daily life situation, however, the disadvantage is that the role of individual factors or specific combinations of factors cannot be evaluated. Second, feasibility and adherence to such comprehensive intervention programs should be evaluated. Third, long-term effects on cancer outcome, other health outcomes, and prognosis should be carefully assessed. Of special importance is the clinical relevance of indicators for progression; preventive effects on indicators of progression (e.g. start of primary treatment or rising PSA levels) should also reflect protection against clinical or pathological progression and, in the end, improve survival. Finally, biological effects should be studied in order to understand the mechanisms of prevention.

At present, some studies examined the molecular effects of dietary and lifestyle interventions in prostate tissue[151-155]. Results of these studies showed that dietary and lifestyle interventions are able to induce pronounced effects on gene expression in either non-malignant or malignant prostate tissue. The relevance of the changes in gene expression with respect to prostate cancer progression, however, warrants further investigation.

In conclusion

The progression to aggressive or fatal prostate cancer reveals important implications for prognosis and survival. There is little known about what exactly drives prostate cancer towards an aggressive form. In order to obtain more insight into prostate cancer progression, observational studies should stratify their analyses for aggressive and non-aggressive prostate cancer whenever possible. Furthermore, extensive research on the molecular events occurring during progression, and the possible mechanisms underlying prevention of progression is warranted.

1.4 Risk factors and prevention of prostate cancer recurrence

Indicators for prostate cancer recurrence

Recurrence of prostate cancer is defined as the return of the disease after a curative treatment. Confirmation of recurrence is often based on PSA levels or imaging techniques[156]. Biochemical recurrence (BCR) is widely used as a measure of recurrence in patients treated with radical prostatectomy[157]. Ideally, PSA levels should become undetectable after a radical prostatectomy, because the entire prostate is removed. Persistently detectable, post-operative PSA levels usually indicate residual disease, while rising PSA levels after an undetectable PSA refer to biochemical recurrence[156, 157]. For other forms of therapy (e.g. radiotherapy) alternative definitions for biochemical recurrence have been suggested[157, 158], however, recognizing biochemical recurrence is often more complicated because PSA levels do not necessarily become undetectable[158]. Therefore, the majority of studies on prostate cancer recurrence focused on patients treated with radical prostatectomy[157]. This paragraph will highlight the main predictors and role of preventive strategies for (biochemical) recurrence after radical prostatectomy.

Risk factors for recurrence

Following radical prostatectomy, up to 35% of the patients develop biochemical recurrence[159-164]. Although some suggest that a rising PSA does not perfectly predict prognosis or prostate cancer-specific mortality[165, 166], it is an important 'tool' for the identification of recurrent prostate cancer. Many studies evaluated clinical or pathological factors that predict risk of biochemical recurrence after radical prostatectomy. Several prognostic factors for biochemical recurrence have been established; i.e. advanced stage, high Gleason grade, positive surgical margins, invasion into seminal vesicles, extracapsular extension, lymph node involvement, and pre-operative PSA levels[167].

Except from positive surgical margins, which may sometimes depend on the experience of the urologist[168], none of the prognostic factors described above can be easily controlled. Identification of modifiable risk factors, such as nutritional and lifestyle factors, allows development of prevention strategies for recurrent prostate cancer. So far, obesity is the main modifiable risk factor studied in relation to risk of prostate cancer recurrence. Several studies examined whether body mass index, as a measure of obesity, was associated with biochemical recurrence after radical prostatectomy[169]. Prospective studies from the USA almost consistently demonstrated that a higher body mass index was associated with an increased risk of biochemical

recurrence or mortality[169]. Also a recent weight gain is associated with an increased risk of biochemical recurrence after radical prostatectomy[170]. Only few European studies examined the effects of body mass index on risk of prostate cancer recurrence after radical prostatectomy and these studies demonstrated inconsistent results[171, 172]. Since the incidence of obesity increases substantially[173], additional research on the effects of obesity on recurrent prostate cancer in Europe is needed.

Prevention of prostate cancer recurrence

Adequate strategies for the prevention of recurrent prostate cancer may ultimately contribute to the reduction of prostate cancer-specific mortality. Also from the patients' perspective, an active preventive strategy may help to deal with the difficult and anxious period of frequent PSA testing and thereby improve quality of life[174]. In line with the results for progressive prostate cancer, relatively few studies evaluated preventive strategies for recurrent prostate cancer[133].

A number of dietary interventions have been evaluated in the light of the prevention of prostate cancer recurrence. A diet rich in plant-based foods and fish, together with a mindfulness program, increased the PSA doubling time in prostate cancer patients with rising PSA levels after primary treatment[175]. The PSA doubling time is the time needed for the PSA levels to double, and is considered as a predictor of recurrence and mortality after radical prostatectomy[176]. Also, different dietary supplements containing isoflavones, carotenoids, phytosterols, trace-elements and vitamins did influence PSA doubling time[177] or levels of specific subclasses of PSA (free PSA)[178], however, risk of biochemical recurrence itself was not examined as outcome in any of these studies because participants already had rising PSA levels[175, 177, 178].

In conclusion

Recurrence of prostate cancer after a radical therapy is often characterised by rising PSA levels. Several clinical and pathological factors can predict risk of biochemical recurrence after radical prostatectomy. A number of modifiable factors, such as high body mass index, have been suggested as risk factors for prostate cancer recurrence, however, current evidence is inconclusive.

1.5 Overview of this thesis

Gaps of knowledge

As can be concluded from the previous paragraphs, there is extensive knowledge on various aspects of prostate cancer; however, a number of other important aspects remain poorly understood. Prostate cancer is still the most common cancer among men, and an important cause of cancer death. As already suggested for years, prevention of prostate cancer is the ultimate strategy to stop this alarming development[179]. For the design of effective prevention programs, better understanding of the aetiology of prostate cancer is required. Moreover, insight into risk factors allows identification of high-risk groups for early detection. Focusing on high-risk groups reduces the detection of insignificant tumours and overtreatment in low-risk men.

Important issues that need to be addressed:

1. Understand the biology of prostate cancer
2. Identify risk factors for prostate cancer development, progression, or recurrence
3. Identify targets for effective prevention and/or treatment

Extended knowledge on these issues might subsequently shed a new light on other research areas, such as the finding of prostate cancer-specific biomarkers, identification of methods to distinguish between insignificant and aggressive tumours, and development of new treatment strategies.

Aims

The overall aim of this thesis is to obtain insight into nutritional and clinical factors relevant to different stages of prostate cancer. Insight into these factors allows identification and evaluation of risk factors for prostate cancer as well as potential candidates for chemoprevention.

For this purpose, randomized, controlled intervention trials usually provide the highest level of evidence and are therefore considered as the gold standard. However, these trials are often time-consuming, expensive and characterized by rather complicated logistics. Therefore, we sought to initiate one randomized, controlled intervention trial with molecular endpoints, while other studies and comprehensive datasets were added in order to address our main research aims.

The specific research aims addressed in this thesis are:

1. Identification of leads for research on potential risk factors **(Chapter 2)**
2. Evaluation of risk factors for incident prostate cancer **(Chapter 3 and 4)**
3. Evaluation of factors that might influence risk of recurrent disease **(Chapter 5)**
4. Studying the molecular mechanisms of chemoprevention **(Chapter 6)**

Outline of this thesis

Chapter 1 provides a general introduction to prostate cancer and highlights the need for research on potential risk factors. Identification of risk factors is often based on agnostic or biology-driven observational studies. Unexpected and useful leads for research on risk factors may also come from patients themselves. In **Chapter 2,** self-reported causes and patients' perceptions of prostate cancer are presented.

In chapter 3 and 4, potential risk factors for incident prostate cancer are examined. **Chapter 3** presents the results from a population-based cohort study on the association between serum levels of cholesterol and triglycerides and the risk of incident prostate cancer. **Chapter 4** evaluates whether a previous cancer diagnosis influences the risk of prostate cancer as a second primary cancer.

In **Chapter 5**, the focus shifts from incident to recurrent prostate cancer. In chapter 5a and chapter 5b, body mass index is examined as prognostic factor for biochemical recurrence after a radical prostatectomy. **Chapter 5a** is based on a cohort of patients identified through the population-based cancer registry, while C**hapter 5b** refers to a study with patients from two tertiary referral hospitals.

In **Chapter 6,** the results of a short-term, randomized, controlled intervention trial with selenium are presented. Selenium is considered a chemopreventive agent for prostate cancer, however, the possible mechanisms by which selenium might lower prostate cancer risk have not been elucidated. The aim of this study was to examine the molecular effects, as determined by gene expression profiles, in prostate tissue before and after a five-week intervention with selenium in patients suspected for prostate cancer.

In **Chapter 7**, the main findings of our studies are summarized and the methodology and relevance of each of the chapters are discussed. Finally, implications for clinical practice and areas of interest for future research are suggested.

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Chapter 2

Why I got prostate cancer: an explorative study on perceived causes of prostate cancer

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Purpose The aim of this study was to evaluate self-reported causes of prostate cancer among patients recently diagnosed with this disease in order to identify potential leads for aetiological research and to obtain insight into patients' perceptions of causes.

Subjects and methods A total of 956 patients, who were identified from a population-based cancer registry and provided written informed consent, completed a questionnaire on sociodemographic characteristics, lifestyle, medical history and family history of cancer. The final open-ended question: "You have been diagnosed with prostate cancer. Do you have any idea what may have been the cause of your cancer?" was evaluated for this study.

Results In total, 143 patients (15%) reported that they were aware of any factors that might have caused their prostate cancer. Reported causes reflect a wide range of different factors that are often highly specific. Patients reported factors related to heredity (37%), specific environmental factors (17%), nutrition and physical activity (13%), and stress (13%) as most likely causes. Interestingly, although a positive family history is established as a risk factor for prostate cancer, only 19% of the patients with an affected first-degree relative reported this as possible cause.

Conclusion Established risk factors for prostate cancer were not commonly perceived, not even among patients with these risk factors. This finding might be taken into account while developing future cancer education programs. Some suggestions given by the patients, such as psychological stress, infections, and a sedentary lifestyle may warrant further investigation, because current evidence is missing or inconclusive.

Introduction

Prostate cancer is the most common cancer among men in Western countries[1, 2]. As for many other types of cancer, little is known about causes of prostate cancer. So far, old age, black race, a positive family history, and a few dozen of low-penetrance genetic markers have been established as risk factors for prostate cancer[3, 4]. The identification of new risk factors for prostate cancer is important in order to understand the aetiology, to identify high-risk groups for early detection and to develop effective prevention strategies.

Searching for associations in agnostic (such as genome-wide association studies) or biology-driven observational studies might reveal new factors that modify prostate cancer risk. A limitation of this approach, however, is that unexpected associations may be missed. Leads for new risk factors may sometimes come from unexpected sources such as patients themselves. For example, maternal exposure to diethylstilbestrol (DES) was identified as a cause of vaginal clear-cell adenocarcinomas after the mother of one of the patients reported that she had taken DES during early pregnancy[5]. Although prostate cancer seems to be a multifactorial disease with a complex aetiology and different factors involved in non-aggressive and aggressive tumours, asking patients about perceived causes of their prostate cancer might give leads regarding potential risk factors.

Furthermore, understanding of what patients think and to what extent they are aware of potential risk factors might provide valuable information for education and prevention programs. Previous studies showed a low awareness of risk factors for prostate cancer among the general population[6-8]. Data on patients' perceptions of individual risk factors for prostate cancer, however, is scarce[9-11]. The aim of the present study was to evaluate self-reported causes of prostate cancer among patients diagnosed with this disease in order to identify potential leads for aetiological research and to obtain insight into patients' perceptions of causes.

Subjects and methods

Self-reported possible causes of prostate cancer were evaluated in this study among Dutch men with prostate cancer. Recruitment and characteristics of the study population have been described in detail previously[12, 13]. Briefly, patients diagnosed with prostate cancer between 2003 and 2006 (n=1668) were identified from the population-based cancer registry held by the Comprehensive Cancer Centre East (CCCE) in the Netherlands. Patients diagnosed with prostate cancer before the age of 76 years were invited between September 2006 and June 2007 to participate in a

European study named Polygene (www.polygene.eu) which aimed to identify common genetic variants that influence the risk of developing breast or prostate cancer[13].

All eligible patients with prostate cancer (n=1330) received an invitation letter and an information brochure. The information brochure explained the aim of the study and highlighted the need for aetiological research into risk factors for prostate cancer and breast cancer. Lifestyle factors ("nutrition and physical activity") and genetic factors were mentioned as established risk factors for prostate cancer in this information brochure. Overall, 956 men agreed to participate and filled out a baseline postal questionnaire on sociodemographic and lifestyle characteristics, physical activity, occupational history, sun exposure, medical history, use of medicines, and family history of cancer. The final open-ended question: "You have been diagnosed with prostate cancer. Do you have any idea what may have been the cause of your cancer?" (No / Yes, namely…) was evaluated for this study.

Categories of perceived causes were based on answers given by the patients and were presented as clusters of risk factors (environment / heredity / stress / nutrition and physical activity / clinical interventions / voiding problems / vasectomy / infections / other physical problems / cycling / smoking / screening / other). Patients were divided into subgroups in order to search for patterns among reported causes and shared patients' characteristics. For these subgroup analyses, patients were stratified based on their age, family history of prostate cancer, educational level, body mass index (BMI), and reasons for PSA testing. Strata of age were defined as below and equal or above 68 years of age, which was the median age of the study population. A positive family history of prostate cancer was defined as at least one reported first-degree family member (father, brother, son) with prostate cancer. Educational level was classified as low (primary school, secondary school, vocational education) or high (college and university), based on the seven response options in the questionnaire. BMI (kg/m^2) was calculated using self-reported weight (kg) and height (cm). Strata of BMI were below 25 kg/m^2 (normal weight) and equal or above 25 kg/m^2 (overweight and obesity). Initial reasons for PSA testing were either based on screening and routine check-ups or to complaints and symptoms.

The institutional review board approved the Polygene study and all participants provided written informed consent. The Statistical Package for Social Sciences (SPSS, version 16.0, Chicago, Illinois), was used for all analyses.

Results

Patients' characteristics, stratified for patients who did and did not report a causal explanation, are presented in **Table 2.1**. The median (interquartile range (IQR)) age at completion of the questionnaire was 68 (63-73) years. Median (IQR) time between diagnosis of prostate cancer and completion of the questionnaire was 26.9 (17.7-36.8) months. The majority of the participants indicated that they were not aware of any causal factor that might have contributed to the development of their prostate cancer (n=809; 85%) or did not answer this question (n=4; <1%).

Table 2.1 Sociodemographic and clinical characteristics of the study population

	All patients with prostate cancer	Patients with causal explanation for their prostate cancer	Patients without causal explanation for their prostate cancer
Number of patients	956	143 (15%)	813 (85%)
Age at completion of questionnaire (years)	68.2 (63.2-72.8)	66.1 (60.7-70.7)	68.8 (63.6-73.1)
Time between diagnosis and completion of questionnaire (months)	26.9 (17.7-36.8)	26.9 (18.7-34.9)	26.9 (17.4-37.0)
Body mass index (kg/m^2)	25.2 (23.8-26.9)	24.8 (23.6-26.1)	25.2 (23.9-27.0)
Marital status (%)			
Married / cohabiting	858 (90%)	126 (88%)	732 (90%)
Single	46 (5%)	8 (6%)	38 (5%)
Divorced / widowed	52 (5%)	9 (6%)	43 (5%)
Educational level (%)[a]			
Low	664 (70%)	93 (65%)	571 (70%)
High	286 (30%)	50 (35%)	236 (29%)
Currently employed (%)			
Yes	181 (19%)	40 (28%)	141 (17%)
No	775 (81%)	103 (72%)	672 (83%)
Positive family history of prostate cancer (%)	211 (22%)	55 (39%)	156 (19%)
Reason for PSA screening (%)			
Complaints	484 (51%)	71 (50%)	413 (51%)
Routine check-up	324 (34%)	50 (35%)	274 (34%)
Screening	27 (3%)	3 (2%)	24 (3%)
Other[b]	118 (12%)	19 (13%)	99 (12%)

Data are presented as median (interquartile range) or numbers (%). Percentages may not add up to 100% because of missing values. [a] Educational level is defined as low (primary school, secondary school, vocational education) or high (college and university). [b] The category 'Other' consisted of explanations given by the patients and includes for example: *"prostate cancer diagnosis among family or friends", "article in newspaper", "other physical complaints or diseases"*.

In total, 143 (15%) patients suggested a possible cause. These patients were younger, were more likely to have a positive family history of prostate cancer and were more frequently currently employed compared to patients who did not report any cause. Patients with causal explanations had the same reasons for initial PSA testing compared to patients without causal explanations (i.e. complaints and symptoms 51%, routine check-up 34%, screening 3%, and other 12%).

The perceived causes as mentioned by the prostate cancer patients are summarized in **Table 2.2**. Results were stratified by age, family history, educational level, BMI, and reasons for PSA testing.

Heredity is most commonly reported (n=53; 37%) by the 143 patients who mentioned at least one possible cause. Patients with a positive family history were more likely to mention heredity (n=41; 75%) compared to patients without prostate cancer in their family (n=12; 14%). However, of all patients with a positive family history in the first degree (n=211), only 41 patients (19%) reported this positive family history as one of the potential causes of their disease, while 14 patients (7%) only mentioned other causes and 156 patients (74%) indicated that they were not aware of any causes of their prostate cancer. By contrast, 12 out of 745 patients without a positive first-degree family history indicated that heredity might have caused their prostate cancer. Some of these patients reported second or third-degree affected family members ("*a grandfather with prostate cancer*", "*cousins from my mother*"), referred to other types of cancer in the first-degree ("*father had testis and lymphatic cancer*") or mentioned heredity in general ("m*y father also was a prostate-person, he got several surgeries since he was 55 years of age*").

Environmental factors like pesticides and other chemicals, electromagnetic radiation, and air pollution were frequently mentioned as well (n=25; 17%). Most environmental factors were linked to current or previous occupations ("*occupational-disease for painters*", "*chemicals in rubber industry*", "*cold or sun exposure during work*") although incidental or accidental exposures were also reported ("*defect fridge*", "*Chernobyl disaster*"). Most patients reported highly specific causes ("*DDT intoxication at young age*", "*mobile phone in right trouser pocket*", "*use of felt-tip pens*") rather than general environmental factors (e.g. "*air pollution*" and "*car exhaust*"). Environmental factors were more frequently reported among patients with lower education (n=22; 24%) compared to patients with a high educational level (n=3; 6%).

For factors related to nutrition and physical activity an opposite pattern was found; patients with a low educational level were less likely (n=7; 8%) to report these factors compared to patients with higher education (n=11; 22%). Nutrition and related factors comprise both abundant intake ("*abundant alcohol intake*", "*salt*", "*tomatoes*" "*dairy*") and deficient intake of foods ("*low fruit intake*"), as well as specific food patterns ("*irregular*", "*monotonous diet*", "*Mediterranean lifestyle with meat and wine*"). Also additives ("*chemicals in food*", "*hormones in meat industry*") and a sedentary lifestyle ("*sedentary occupation*", "*traveling by train*") belong to this category.

Table 2.2 Categories of perceived causes of prostate cancer among Dutch patients recently diagnosed with this disease

	All patients	Age		Family history	
	-	< 68 years	≥ 68 years	Negative	Positive
Number of patients	956	462[a]	492	745	211
Number of patients giving a causal explanation	143 (15%)	85 (18%)	58 (12%)	88 (12%)	55 (26%)
Causal explanations					
Heredity *"Heredity, DNA, mutations"*	53 (37%)	31 (36%)	22 (38%)	12 (14%)	41 (75%)
Environmental *"Pesticides, air pollution"*	25 (17%)	18 (21%)	7 (12%)	18 (20%)	7 (13%)
Nutrition / physical activity *"Food additives, meat, alcohol, sedentary lifestyle"*	18 (13%)	12 (14%)	6 (10%)	13 (15%)	5 (9%)
Stress / psychological *"Stress, burnout, workaholic, depression"*	18 (13%)	15 (18%)	3 (5%)	16 (18%)	2 (4%)
Voiding problems *"Urgent, frequent"*	8 (6%)	4 (5%)	4 (7%)	7 (8%)	1 (2%)
Vasectomy	7 (5%)	5 (6%)	2 (3%)	7 (8%)	0 (0%)
Infection *"Prostatitis, virus infection"*	7 (5%)	5 (6%)	2 (3%)	7 (8%)	0 (0%)
Other physical problems *"Immune system, thrombosis, digestion"*	7 (5%)	3 (4%)	4 (7%)	5 (6%)	2 (4%)
Cycling	7 (5%)	4 (5%)	3 (5%)	7 (8%)	0 (0%)
Smoking	6 (4%)	3 (4%)	3 (5%)	4 (5%)	2 (4%)
Clinical interventions *"X-ray, light therapy for eczema"*	6 (4%)	3 (4%)	3 (4%)	5 (6%)	1 (2%)
Age	5 (3%)	1 (1%)	4 (7%)	4 (5%)	1 (2%)
Other *"Use of drugs, high testosterone"*	4 (3%)	3 (4%)	1 (2%)	3 (3%)	1 (2%)
Screening	2 (1%)	0 (0%)	2 (3%)	2 (2%)	0 (0%)

Data are presented as numbers (%). Numbers might exceed the total number of patients in our study as some patients provided more than one answer to the question. [a] For two patients, age at completion of the questionnaire is missing.

Six patients mentioned drinking alcohol as the potential cause of their prostate cancer. One patient suggested overweight, together with other causes, as a possible cause of his prostate cancer; *"Somewhat abundant weight? Eaten too much fat? Deficient fruit intake during adolescence? Too little physical activity?".*

Patients with overweight or obesity (BMI ≥ 25kg/m^2) did not clearly report causes related to nutrition or physical activity more frequently (n=9; 13%) than patients with normal weight (n=9; 12%). Patients with overweight and obesity reported other physical problems relatively frequently in comparison to patients with normal weight. Their explanations seem not to be specifically related to overweight; *"hypertension", "thrombosis", "polyp", "cyst in prostate", "auto-immune system"* and *"deficient auto-immune system, neuropathy, thyroid".*

Table 2.2 Continued

	Educational level[b]		Body mass index		Detection[c]	
	Low	High	< 25 kg/m^2	≥ 25 kg/m^2	Screening	Complaint
Number of patients	664	286	449	499	351	484
Number of patients giving a causal explanation	93 (14%)	50 (17%)	74 (16%)	69 (14%)	53 (15%)	71 (15%)
Causal explanations						
Heredity *"Heredity, DNA, mutations"*	35 (38%)	18 (36%)	26 (35%)	27 (39%)	23 (43%)	19 (27%)
Environmental *"Pesticides, air pollution"*	22 (24%)	3 (6%)	13 (18%)	12 (17%)	8 (15%)	16 (23%)
Nutrition / physical activity *"Food additives, meat, alcohol, sedentary lifestyle"*	7 (8%)	11 (22%)	9 (12%)	9 (13%)	7 (13%)	7 (10%)
Stress / psychological *"Stress, burnout, workaholic, depression"*	10 (11%)	8 (16%)	11 (15%)	7 (10%)	3 (6%)	13 (18%)
Voiding problems *"Urgent, frequent"*	5 (5%)	3 (6%)	5 (7%)	3 (4%)	1 (2%)	5 (7%)
Vasectomy	3 (3%)	4 (8%)	6 (8%)	1 (1%)	2 (4%)	5 (7%)
Infection *"Prostatitis, virus infection"*	3 (3%)	4 (8%)	3 (4%)	4 (6%)	2 (4%)	4 (6%)
Other physical problems *"Immune system, thrombosis,digestion"*	2 (2%)	5 (10%)	0 (0%)	7 (10%)	2 (4%)	5 (7%)
Cycling	4 (4%)	3 (6%)	3 (4%)	4 (6%)	3 (6%)	4 (6%)
Smoking	6 (6%)	0 (0%)	4 (5%)	2 (3%)	3 (6%)	2 (3%)
Clinical interventions *"X-ray, light therapy for eczema"*	5 (5%)	1 (2%)	3 (4%)	3 (4%)	1 (2%)	4 (6%)
Age	2 (2%)	3 (6%)	2 (3%)	3 (4%)	2 (4%)	3 (4%)
Other *"Use of drugs, high testosterone"*	4 (4%)	0 (0%)	2 (3%)	2 (3%)	3 (6%)	1 (1%)
Screening	1 (1%)	1 (2%)	2 (3%)	0 (0%)	2 (4%)	0 (0%)

Data are presented as numbers (%). Numbers might exceed the total number of patients in our study as some patients provided more than one answer to the question. [b] Educational level is defined as low (primary school, secondary school, vocational education) or high (college and university). [c] Reasons for initial PSA testing are either related to complaints / symptoms (n=484) or to screening / routine check-up (n=351). Another, minor category in the questionnaire was 'other' (n=118) and includes for example *"prostate cancer diagnosis among family or friends", "article in newspaper", "other physical complaints or diseases".*

Stress was reported relatively frequently (n=18; 13%) as a possible cause of prostate cancer. Patients gave causal explanations such as "*Fatigue and stress caused prostate enlargement and subsequently cancer"," busy lifestyle", "high workload", "depression",* and *"burnouts".* Stress-related explanations were somewhat more common among young men with a high educational level, which may suggest that stress is mainly related to current occupation.

Patients (n=7; 5%) who mentioned infections as a possible cause of their prostate cancer either specified infections related to the prostate (n=3) or urinary tract (n=3). One patient reported a theory about an unknown retrovirus that is suggested to cause prostate cancer. All three patients who mentioned prostatitis, gave a clear cause of the infection *("prostatitis in adolescence because of cycling", "frequent episodes of*

prostatitis because of poorly performed vasectomy", and *"chronic prostatitis during 2 years because of catheterization after a serious accident"*). From the questionnaire, we identified 93 patients with self-reported prostatitis. Only four of them mentioned infections or prostatitis as a possible cause of their prostate cancer, suggesting that most patients do not consider prostatitis as a main risk factor.

Other causes that were mentioned are related to voiding problems (n=8; 6%), other physical problems (n=7; 5%), vasectomy (n=7; 5%), cycling (n=7; 5%), smoking (n=6; 4%), clinical interventions (n=6; 4%), age (n=5; 4%), screening (n=2; 1%), and others (n=4; 3%). The last category comprised a variety of causal explanations; *"it is a man-thing?", "use of prednisone and azathioprine", "an accident as testing engineer",* and *"high levels of testosterone".* These and other examples of reported causes are presented in **Table 2.3**.

Table 2.3 Selection of perceived causes of prostate cancer reported by Dutch patients diagnosed with this disease.

Perceived causes …	Examples of perceived causes given by the patients
…established as risk factors for prostate cancer	"Heredity", "Conform age incidence", "My father; he was diagnosed with prostate cancer when he was 70 years", "It is in my genes", "It is a man-thing?", "My father also was a prostate-person, he got several surgeries since he was 55 years of age", "Hereditary mutation (father) could not be excluded", "DNA", "Heredity, therefore I asked for a screening", "Because my father also had prostate cancer, I think that it is hereditary"
…with an unknown or unidentified effect on prostate cancer risk	"Sweeteners", "Abundant intake of tomatoes", "Little physical activity during the last 25 years", "Fluid from defect fridge", "Air pollution from the Ruhr district", "Television switched on for 6 h/day", "I assume because I was exposed to chemicals in rubber industry", "Traffic jam during rush hours", "DDT intoxication at young age", "Electromagnetic radiation", "Unknown retrovirus", "Chronic voiding problems", "Polyps in the rectum", "Stress" "Completely overtired because of family problems", "Work-related stress and traveling by car", "Sadness", "Depression", "Maybe stress and depression in the past", "Fatigue and stress for years caused prostate enlargement and subsequent cancer", "Many X-rays lower part of the body", "Disorder of the autoimmune system?", "Association with neuropathy and thyroid", "Outdoor profession (cold)", "Sun exposure during work", "Light therapy for eczema in 1999/2000", "Chronic prostatitis", "Frequent episodes of prostatitis because of poorly performed vasectomy", "Ignorance of voiding problems by general practitioner", "Air pollution in Nijmegen-West"
…unlikely to have an effect on prostate cancer risk	"Vasectomy", "Cycling", "Smoking", "Cyst in the prostate", "Is there any relationship between vasectomy and prostate cancer?", "Increased risk because of vasectomy, I have read that somewhere", "A lot of cycling 5 years before diagnosis, pressure on prostate because of problems with saddle", "Smoking and moderate alcohol intake", "Cycled 65.141 km between 1993-2003", "Smoking!!!"

This table is provided in order to give insight into the types of perceived causes reported by the patients. The classification of perceived causes into the different categories is based on a rather arbitrary selection. Future research and new insights might result in a shift of perceived causes among the different categories. Some of the perceived causes reported by the patients may be proxies for other underlying risk factors.

Many patients reported possible causal explanations to which they apparently have been exposed during a long period ("*Thirty-seven years of unhealthy and dirty work", "I have been a workaholic during my whole life", "Too little physical activity and sports during the last 25 years"*) or that has occurred during the past ("*Monotonous diet during the war", "Not much fruit during childhood", "Prostatitis in adolescence due to frequent cycling", "Frequent X-rays in the sixties and seventies"*). Only few patients reported more acute or recent events that might have contributed to the development of their prostate cancer *("A defect fridge" and "Serious infection in bladder and prostate during the past 9 months").*

If a causal explanation was given, this was frequently coupled with a measure of uncertainty. *"If I knew that! Perhaps a monotonous diet during the war in 1940-1945 or during my stay abroad in 1964-1995?"* Almost one third of the patients provided a causal explanation followed by a question mark, or mentioned words as "possibly" and "perhaps". These measures of uncertainty may suggest that many patients did not recognize one clear, outstanding responsible cause of their prostate cancer.

Discussion

In this study among patients diagnosed with prostate cancer, we evaluated self-reported potential causes of prostate cancer. Only 15% of the patients reported at least one causal explanation, whereas the majority of the patients (85%) indicated that they were not aware of any cause that might have contributed to the development of their prostate cancer.

A few studies examined perceived causes of prostate cancer among patients recently diagnosed with this disease[9-11]. It was demonstrated that patients with prostate cancer were least likely (41%) to report perceived causes, as compared to other cancer patients (47-74%), which was explained by the authors by the lack of scientific evidence available for factors involved in prostate carcinogenesis[11]. Fitzpatrick and colleagues reported on awareness of risk factors and perceived levels of risk among prostate cancer patients and the general population[9]. Participants were asked to identify risk factors for prostate cancer from a prompted list. In addition to this, the participants were requested to explain why they did perceive their risk of getting prostate cancer as low or high[9]. Similar studies with patients were performed for prostate cancer[10], breast cancer[14] and other chronic diseases, like heartburn[15], and gastro-oesophageal reflux[16]. Most of these studies[9, 10, 14, 15], however, used multiple-answer questions from a prompted list. The prompted question format is considered to reflect recognition and usually indicates higher levels of knowledge compared to open-ended questions[17].

Furthermore, the prompted question format does not allow unique, unexpected or new responses and is strongly directed by views of the health professionals. Because the aim of our study was to evaluate self-reported causes of prostate cancer in order to identify leads for aetiological research and to obtain insight into patients' thoughts about causes, we used the open-ended question format.

In our study, we expected to find three categories of answers; factors that have been established as risk factors for prostate cancer, factors that are unlikely to have an effect on prostate cancer risk, and factors with an inconsistent or unidentified effect that might provide potential leads for aetiological research.

Factors that have been established as risk factors for prostate cancer

Black race, old age, a positive family history, and several low-penetrance genetic markers have been established as risk factors for prostate cancer[3, 4]. Race was not mentioned by any of the patients, which is expected because 99% of the population in our study was Caucasian. Although age is established as a main risk factor for prostate cancer, this was mentioned by only few patients (n=5; 4%) in our study, which is consistent with previous studies[11]. Reasons for the small number of patients reporting age may be related to lack of knowledge, the open-ended question format, or to the perception of 'old age'. Median age of our population was 68 years and many of the patients may not perceive themselves as 'old'. The patients (n=5) who did report age were indeed somewhat older than 68 years (namely 74-75 years), except for one patient of 62 years who was working in a medical profession.

A positive family history is another main risk factor for prostate cancer[18]. Although causes related to heredity were the most frequently reported aetiological factors in our study (n=53, 37%), only 19% of the patients with a positive family history in the first degree reported this as a possible cause of their prostate cancer. This finding highlights the modest prostate cancer awareness. Patient education might theoretically stimulate adherence to screening programs and thus lead to early detection, improvement of prognosis and reduction of mortality. However, before such education programs can be implemented and optimized, it should be carefully considered whether these benefits balance the possible harms (i.e. complications of treatment, mental burden) of early detection and treatment, especially in the field of prostate cancer.

Factors that are unlikely to have an effect on prostate cancer risk

For several causes mentioned in our study there is no or only limited evidence that these factors increase prostate cancer risk[3]. Vasectomy, for example, was perceived as

a cause of prostate cancer among several patients. Although early studies suggested vasectomy as a risk factor for prostate cancer[19, 20], other studies refuted this[21, 22]. We assume that patients remember the 'old rumours' ("*Increased risk because of vasectomy, I have read that somewhere*") or that they associate prostate cancer with other disorders or procedures in the genitourinary system *("When I was 44 years of age, I had a vasectomy. Since then I had pain in that area during ejaculations").* From that perspective, it is reasonable that several patients also reported voiding problems (which might refer to benign prostate hyperplasia), prostatitis, or cycling as possible causes of their prostate cancer. However, for none of these factors there is consistent evidence that they increase the risk of prostate cancer[3]. Also smoking and alcohol intake were sometimes perceived as potential causes of prostate cancer. Since smoking and alcohol are common risk factors for other types of cancer and a variety of other diseases and conditions, it seems reasonable that patients link these factors to prostate cancer as well.

Factors with an unknown or unidentified effect on prostate cancer risk

Many of the suggested causes have not yet been extensively studied in relation to prostate cancer, or have shown inconsistent results in previous studies. In parallel with the study of Willcox *et al.*[11], stress and stress-related causes such as burnouts, depression, and fatigue were reported by relatively many patients in our study. From either an epidemiological or biological perspective, the role of psychological stress in prostate cancer development has not been uniformly confirmed. The common hypothesis states that stress impairs immune function, which in turn may increase susceptibility to malignancies[23]. *In-vitro* studies showed that glucocorticoids, such as cortisone and cortisol, might activate a mutated androgen receptor and thereby stimulate androgen-independent growth of prostate cancer cells[24]. Whether physiological levels of glucocorticoids can promote growth of prostate cancer cells *in-vivo,* and to what extent the mutated androgen receptors occur in prostate cancer patients need further investigation. Although psychological stress has been previously associated with risk of cervical cancer,[25] lung cancer,[26] and breast cancer,[27] only few studies examined the relationship between stress and prostate cancer risk[28, 29]. Perceived stress was not associated with risk of prostate cancer in a case-control study among older men (65-79 years) from the United States[29]. A major limitation of this study, however, was the timing of exposure, which reflected only a one-year period before the reference date. A prospective cohort study from Denmark, did not find an association between self-reported stress level and prostate cancer risk either[28]. Although the prospective design was a major strength of this study, the measure of exposure was only based on two questions concerning stress intensity and frequency, rather than the more detailed Global Perceived Stress (GPS) scale[30].

Concluding, psychological stress is perceived as a major cause of prostate cancer in patients recently diagnosed with this disease. We cannot rule out the possibility that patients perceived and reported high levels of stress as a consequence of their recent cancer diagnosis or treatment. Future prospective studies may include a psychological stress measurement in order to further evaluate the effects of stress on prostate cancer risk. Measures of stress should include both stressful life events and chronic stress as measured by an adequate method, wherever possible supported by reliable biomarkers, and reflecting a relevant timing of exposure.

Limitations and strengths

Prostate cancer is a multifactorial disease with a complex aetiology and different factors involved in development of non-aggressive and aggressive tumours. Identification of new risk factors for prostate cancer is therefore complicated. We realize that asking patients for perceived causes of their prostate cancer is not the most conventional strategy for aetiological research. However, the unique and individual responses of patients allow an explorative and creative way of thinking, which can provide directions for future research. The relatively small number of patients who provided a causal explanation should be considered in the interpretation of our findings. This small number, however, also indicates the current state of awareness among prostate cancer patients. Furthermore, causal explanations of patients might be somewhat biased by the design of the provided questionnaire. Since the open-ended question on the causes of the prostate cancer was the final question, previous questions on exposures to chemicals, smoking, physical activity, sun exposure, baldness, medical history or family history might have influenced thoughts of the patients. However, we assume that addressing these topics in the questionnaire did not discourage the patients from reporting new and unique causes. In addition, we did not observe a striking overrepresentation of the topics addressed in the questionnaire. The median time between diagnosis of prostate cancer and completion of the questionnaire was 26.9 months. We cannot rule out the possibility that patients changed their perception during this period of time. Strengths of our study are the population-based design and the open-ended question format, which gave the patients the opportunity to provide their own answers, without being led by suggested causal relationships from a prompted list.

In conclusion, the results of this study show that only few patients were aware of any causal factors that might have contributed to the development of their prostate cancer. Established risk factors for prostate cancer were not commonly perceived, not even among patients with these risk factors. This finding might reflect the current state of knowledge on risk factors for prostate cancer and stresses the need for

development of effective education and prevention programs. Few unexpected or unidentified causes that could provide leads for further research were reported by the patients in this study. Nevertheless, some of the suggestions given by the patients, such as psychological stress, infections, a sedentary lifestyle, and specific environmental factors may warrant special attention, as there is still insufficient scientific evidence available for the possible role of these and many other factors in prostate carcinogenesis. Or as stated by one of the patients: "*I can mention some things here, but science does not know the answer itself".*

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Conflict of interest

The authors declare no conflict of interest.

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Chapter 3

Blood lipid levels and prostate cancer risk: a cohort study

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Purpose It has been hypothesized that blood lipid levels might be associated with prostate cancer risk. The aim of the present study was to evaluate the association between serum total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides and prostate cancer risk in a cohort study among 2842 Dutch men.

Subjects and methods By the end of follow-up, 64 incident cases of prostate cancer were identified. Serum total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides were evaluated as potential risk factors for prostate cancer using multivariable Cox proportional hazards regression models. These analyses were restricted to men who never used cholesterol-lowering drugs (2118 men, 43 cases).

Results Higher total and higher LDL cholesterol were significantly associated with an increased risk of prostate cancer (hazards ratios (HR) and 95% confidence interval (CI) per mmol/L were 1.39 (95% CI 1.03–1.88) and 1.42 (95% CI 1.00-2.02), respectively). Similar results were observed for aggressive prostate cancer, whereas for non-aggressive prostate cancer a significant association with HDL cholesterol was found (HR 4.28, 95% CI 1.17-15.67).

Conclusion The results of this study suggest that blood lipid levels may influence risk of prostate cancer. However, the exact roles of different cholesterol fractions on prostate cancer aggressiveness should be further evaluated.

Introduction

Epidemiological studies suggest that lipid profiles in blood are associated with risk of prostate cancer. Although some studies indicated that high serum triglycerides[1, 2], low or high serum high-density lipoprotein (HDL) cholesterol[3, 4], and high total[3-8] or low-density lipoprotein (LDL) cholesterol[3, 4] might contribute to the development or progression of prostate cancer, overall results are relatively scarce and inconclusive. So far, few studies have assessed the relation between serum triglycerides and prostate cancer risk[2, 9]. A case-control study among 504 cases with prostate cancer and 565 controls with benign prostatic hyperplasia (BPH) found a positive association between serum triglycerides and prostate cancer risk (odds ratio (OR) 1.15, 95% CI 1.00-1.32)[2]. A recent, prospective study based on 29,364 Norwegian men (687 incident cases), however, did not confirm an association between serum triglycerides and risk of incident or fatal prostate cancer[9].

Cholesterol has also been regarded as a potential risk factor for prostate cancer. Although two case-control studies have suggested that hypercholesterolemia increases the risk of prostate cancer[3, 5], most prospective studies did not find any association[9-15] or suggested that risk of prostate cancer decreases with increasing cholesterol levels[16, 17]. Recent studies were able to examine risk of clinical subtypes of prostate cancer in relation to cholesterol levels[4, 6-8]. An association between total cholesterol levels and risk of high-grade prostate cancer was consistently reported, whereas for total or low-grade prostate cancer risk results were inconsistent[4, 6-8]. Supportive evidence for the potential role of cholesterol in prostate cancer development has been provided by observations that cholesterol-lowering drugs (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, commonly known as statins) might be inversely associated with risk of (advanced) prostate cancer[4, 18-21]. A meta-analysis by Bonovas et al.[22] confirmed that use of statins lowers the risk of advanced prostate cancer (relative risk (RR) 0.77, 95% CI 0.64-0.93); however, no effect for total prostate cancer risk was found (RR 0.95, 95% CI 0.73-1.23). Others proposed that the cholesterol-lowering effects of statins might not be the only reason why these drugs are associated with a reduced risk of advanced prostate cancer[22]. Direct pro-apoptotic and anti-inflammatory effects of statins are suggested to inhibit the development or progression of prostate cancer independent of cholesterol[23, 24]. Furthermore, differences in PSA screening patterns between statin users and non-users might be responsible for the observed associations[18, 22]. As results are conflicting and underlying mechanisms have to be elucidated, further research is needed to evaluate the effects of cholesterol-lowering drugs on prostate cancer prevention, whereas studies on serum cholesterol and other blood lipids should confirm whether these blood lipids itself are potential risk factors for prostate cancer.

As described above, the relation between blood lipid profiles and prostate cancer risk has been previously investigated, but only few recent, prospective and population-based studies have been reported[4, 6-9]. The aim of the present study was to address the association between serum cholesterol, triglycerides and prostate cancer risk in a prospective, population-based study from the Netherlands.

Subjects and methods

The Nijmegen Biomedical Study is a survey of the general population in which a random, age- and sex-stratified sample was recruited among adult inhabitants of Nijmegen, Lent and Oosterhout (eastern part of the Netherlands) between 2001 and 2003. The study was approved by the Institutional Review Board, and all participants provided written informed consent. In total, 21,756 inhabitants received an invitation to participate in this study. Of these, 9350 (43%) subjects agreed to participate, and filled out a postal questionnaire on lifestyle and medical history at baseline. Majority of the participants (90%) were Caucasian. Furthermore, 6468 (69%) participants donated two non-fasting blood samples, which were collected in tubes containing heparin (8.5 mL) or EDTA (8.5 mL). Blood samples were processed within two hours after withdrawal and aliquots of serum were stored at -40°C. All analyses of blood lipid profiles were performed between October 2004 and April 2005. Levels of serum total cholesterol, HDL cholesterol and triglycerides were analysed enzymatically using an Abbott Aeroset autoanalyser (Abbott Diagnostics, Hoofddorp, the Netherlands). Levels of LDL cholesterol were estimated using the Friedewald formula[25]. As the Friedewald formula appears to be accurate only up to triglyceride levels of 4.52 mmol/L[25], we did not calculate LDL levels for participants with triglyceride levels above 4.52 mmol/L (n=132).

All 3050 male participants of the Nijmegen Biomedical Study who provided blood samples were initially included in our analyses. Of these, 36 participants were excluded because they had a diagnosis of prostate cancer before blood withdrawal, 160 participants were excluded because no blood lipid measurements were available, and another 12 participants were excluded because of incomplete follow-up data, leaving 2842 cohort members for final analyses. Cases of incident prostate cancer (n=64) were identified through record linkage with the Dutch population-based cancer registry. Follow-up duration was defined as the day of blood withdrawal until date of death, emigration, prostate cancer diagnosis, or the end of follow-up (31 December, 2009), whichever came first.

Information on age, height, weight, smoking status (current, former, never), history of hypertension (yes, no), history of diabetes mellitus (yes, no), and use of cholesterol-

lowering drugs (current, former, never) were obtained from the self-reported questionnaires. For the prostate cancer cases, date of diagnosis, clinical and pathological stage (TNM based on the 2002 American Joint Committee on Cancer guidelines[26]), Gleason score (from biopsy or radical prostatectomy specimen) and PSA levels were obtained through the cancer registry, whenever available.

Cox proportional hazards regression analysis was used to calculate hazard ratios (HR) and 95% confidence intervals (CI) for prostate cancer risk. In order to attenuate any distorting effect of cholesterol-lowering drugs, these analyses were restricted to men who never used cholesterol-lowering drugs (cohort n=2118, cases n=43). Age (continuous), body mass index (BMI) (continuous), and self-reported history of diabetes mellitus were included in the multivariable Cox model, because these factors were previously shown to be associated with prostate cancer risk[27, 28] and substantially (>10%) affected the effect estimates in our analyses. Each blood lipid was evaluated individually as a continuous parameter (per mmol/L). Although the number of cases is relatively small, we aimed to repeat the analyses for non-aggressive and aggressive prostate cancer separately. Non-aggressive prostate cancer is defined as a clinical or pathological stage T1 or T2, no evidence of positive lymph nodes (N0, NX) or metastases (M0, MX), Gleason score <7 and prediagnostic PSA levels <20 ng/mL. Aggressive prostate cancer is defined as a clinical or pathological stage T3 or T4, N1, M1, Gleason score ≥7, or prediagnostic PSA levels ≥20 ng/mL. The Statistical Package of Social Sciences (SPSS, version 17.0, Chicago, Illinois) was used for all statistical analyses.

Results

Baseline characteristics of the participants are presented in **Table 3.1**. Among 2842 participants, 64 incident prostate cancer cases were identified during a median (interquartile range) follow-up of 79.5 (IQR: 74.0-83.1) months. The median time between blood withdrawal and diagnosis of prostate cancer was 43.7 (IQR: 20.0-66.8) months. Cases were slightly older compared with cohort members. Median serum levels of total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides appear to be fairly similar among the cohort members and the prostate cancer cases. After exclusion of participants who reported former or current use of cholesterol-lowering drugs (n=416) or with missing data on use of cholesterol-lowering drugs (n=308), 2118 cohort members and 43 cases were available for analyses. The participants who were excluded because of former or current use of cholesterol-lowering drugs (n=416) tended to be somewhat older, had a slightly higher BMI and lower total and LDL cholesterol levels and were more likely to have a history of smoking, diabetes or hypertension compared with the participants included in the analyses (data not

shown). Of the 43 cases included in the analyses, 15 had a tumour with Gleason score <7, 20 cases had a Gleason score ≥7 and 8 cases had no data available for Gleason score. Tumour stage T1 or T2 was diagnosed in 27 cases, while T3 or T4 was found in 12 cases, and 4 cases had an unknown tumour stage. Furthermore, 6 cases had positive lymph nodes (N1), 7 patients had distant metastasis (M1), and 15 patients had PSA levels ≥20 ng/mL (data not shown).

Table 3.1 Baseline characteristics of all cohort members and members with incident prostate cancer in a Dutch, population-based cohort study

Median (interquartile range) or numbers (%)	Total cohort	Prostate cancer cases
Number	2842	64
Follow-up (months)	79.5 (74.0-83.1)	43.7 (20.0-66.8)
Age (years)	62.0 (47.3-73.1)	70.8 (65.1-75.7)
Height (cm)	178 (173-183)	175 (170-178)
Weight (kg)	80 (73-88)	80 (71-84)
BMI (kg/m^2)	25.2 (23.4-27.5)	26.1 (24.0-27.7)
Smoking (ever, %[a])	2214 (78%)	54 (89%)
Diabetes (yes, %[a])	189 (8%)	6 (11%)
Hypertension (yes, %[a])	667 (26%)	20 (33%)
Use of cholesterol-lowering drugs (ever, %[a])	416 (16%)	14 (25%)
Serum total cholesterol (mmol/L)	5.6 (4.9-6.3)	5.7 (5.0-6.8)
Serum HDL cholesterol (mmol/L)	1.2 (1.0-1.4)	1.2 (1.0-1.4)
Serum LDL cholesterol (mmol/L)	3.5 (2.9-4.1)	3.6 (2.9-4.5)
Serum triglycerides (mmol/L)	2.0 (1.4-2.7)	1.8 (1.4-2.4)

Abbreviations: *BMI* body mass index, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein

[a] Percentages are based on the number of cohort members and cases without missing values for these variables. Cohort members with missing values for smoking n=8, history of diabetes mellitus n=309, history of hypertension n=245, use of cholesterol lowering drugs n=308.

As shown in **Table 3.2**, serum levels of total cholesterol (HR 1.39 per mmol/L, 95% CI 1.03-1.88) and LDL cholesterol (HR 1.42 per mmol/L, 95% CI 1.00-2.02) were associated with an increased risk of total prostate cancer in the model adjusted for age, BMI and history of diabetes mellitus. Similar results were found for aggressive prostate cancer, which was positively associated with total cholesterol (HR 1.65 per mmol/L, 95% CI 1.10-2.47) and LDL cholesterol (HR 1.83 per mmol/L, 95% CI 1.15-2.90). In contrast, non-aggressive prostate cancer risk seemed to be associated predominantly with HDL cholesterol (HR 4.28 per mmol/L, 95% CI 1.17-15.67) in this cohort. Levels of triglycerides did not statistically significantly influence prostate cancer risk. Inclusion of men who reported former, current or unknown use of cholesterol-lowering drugs resulted in lower effect estimates; none of the previously reported associations remained statistically significant. In order to exclude any possible reverse causal effect, we also excluded cohort members and cases with a follow-up less than 12 months.

Although HDL cholesterol was no longer statistically significantly associated with non-aggressive prostate cancer risk (HR 3.03, 95% CI 0.73-12.51), this restriction did not substantially influence results for total cholesterol (HR for aggressive prostate cancer 1.58, 95% CI 1.04-2.39) and LDL cholesterol (HR for aggressive prostate cancer 1.72, 95% CI 1.06-2.77).

Table 3.2 Cox proportional hazards regression models for prostate cancer incidence associated with blood lipid levels in a Dutch, population-based cohort study

Model[a]		Age-adjusted			Multiple-adjusted[c]		
		No. of cases	HR[b]	95% CI	No. of cases	HR[b]	95% CI
Prostate cancer	Cholesterol	43	1.34	1.00-1.80	41	1.39	1.03-1.88
	HDL cholesterol	43	1.82	0.79-4.23	41	2.16	0.92-5.05
	LDL cholesterol[d]	42	1.40	1.00-1.97	40	1.42	1.00-2.02
	Triglycerides	43	0.87	0.65-1.18	41	0.88	0.65-1.20
Aggressive prostate cancer[e]	Cholesterol	23	1.54	1.04-2.29	22	1.65	1.10-2.47
	HDL cholesterol	23	1.41	0.43-4.68	22	1.65	0.49-5.60
	LDL cholesterol[d]	22	1.72	1.09-2.71	21	1.83	1.15-2.90
	Triglycerides	23	0.89	0.59-1.33	22	0.90	0.59-1.36
Non-aggressive prostate cancer[f]	Cholesterol	16	1.32	0.82-2.13	15	1.34	0.82-2.20
	HDL cholesterol	16	3.37	0.94-12.16	15	4.28	1.17-15.67
	LDL cholesterol[d]	16	1.31	0.76-2.27	15	1.26	0.71-2.24
	Triglycerides	16	0.82	0.49-1.37	15	0.84	0.50-1.42

Abbreviations: *CI* confidence interval, *HDL* high-density lipoprotein, *HR* Hazard Ratio, *LDL* low-density lipoprotein
[a] Restricted to men who never used cholesterol-lowering drugs (n=2118); [b] Hazards ratios per mmol/L; [c] Adjusted for age (continuous), BMI (continuous), and history of diabetes mellitus; [d] Restricted to men with triglyceride levels ≤ 4.52 mmol/L, n=2033; [e] Aggressive prostate cancer is defined as: clinical or pathological stage T3, T4, or N1, M1, or Gleason ≥7 or PSA ≥20 ng/mL; [f] Non-aggressive prostate cancer is defined as: clinical or pathological stage T1, T2, N0, M0, Gleason<7, and PSA <20 ng/mL.

Discussion

In this prospective, population-based study, we evaluated the association between blood lipid profiles and prostate cancer risk. We found that high levels of both total cholesterol and LDL cholesterol were associated with an increased risk of total and aggressive prostate cancer. In contrast, high levels of HDL cholesterol were associated with an increased risk of non-aggressive prostate cancer, while triglycerides were not statistically significantly associated with prostate cancer risk.

It has previously been suggested that associations between blood lipid profiles and prostate cancer risk depend on the aggressiveness of the disease[4, 6-8]. Platz *et al.*[6] evaluated the association between plasma total cholesterol and prostate cancer risk in a case-control study nested in the Health Professionals Follow-Up Study. Low total cholesterol levels (in the bottom quartile) were not associated with total prostate cancer risk (OR 0.93, 95% CI 0.72-1.20), however, those participants with low total

cholesterol levels had a lower risk of high-grade prostate cancer (OR 0.61, 95% CI 0.39-0.98)[6]. Subsequent prospective studies confirmed an association between levels of cholesterol and high-grade prostate cancer[7, 8]. Consistent with these results, we found a positive association between total cholesterol and aggressive prostate cancer risk. Next to aggressive disease, however, we also observed a modest association for total prostate cancer risk. Farwell *et al.*[4] presented similar findings from a retrospective cohort study in which total cholesterol was associated with total and high-grade prostate cancer risk.

The findings from our study suggest that increased levels of serum LDL cholesterol might be positively associated with total or aggressive prostate cancer risk. Studies focussing on the association between levels of LDL cholesterol and prostate cancer risk are relatively scarce. A hospital-based, case-control study suggested that high levels of LDL cholesterol (OR 1.60, 95% CI 1.09-2.34), but also total cholesterol (OR 1.58, 95% CI 1.11-2.24) and low levels of HDL cholesterol (OR 1.57, 95% CI 1.04-2.36), increase the risk of prostate cancer[3]. In the cohort of Farwell *et al.*, LDL cholesterol in the highest quartiles was statistically significantly associated with total and high-grade prostate cancer[4]. Our results with respect to LDL cholesterol need to be interpreted with some caution, as non-fasting blood samples were used. The Friedewald formula for calculating levels of LDL cholesterol is based on the assumption that total cholesterol minus HDL and very-low-density lipoprotein (VLDL) cholesterol equals LDL cholesterol[25]. This method requires measurements of total cholesterol, HDL cholesterol and triglycerides (as a proxy for VLDL cholesterol) levels[25]. Using non-fasting samples might result in high levels of triglycerides, a subsequent overestimation of VLDL cholesterol and therefore might underestimate levels of LDL cholesterol[29]. In our cohort, 132 cohort members had non-fasting triglyceride levels above 4.52 mmol/L, which is suggested as the upper level for accurate Friedewald calculations[25]. For these 132 cohort members, we did not calculate levels of LDL cholesterol. Nevertheless, use of the Friedewald formula for non-fasting blood samples remains controversial[30, 31]. Although we assume that the non-fasting state is not different between men who later develop prostate cancer and men who do not, its possible role as source of bias should be considered when interpreting the results. Most likely, a non-differential underestimation of LDL cholesterol might result in a bias towards the null, i.e. an underestimation of the hazards ratios for LDL cholesterol.

In contrast to total cholesterol and LDL cholesterol, the effects of HDL cholesterol were most pronounced for non-aggressive prostate cancer, that is, the results suggest that men with high levels of HDL cholesterol are at increased risk of developing non-aggressive prostate cancer. Similar findings were presented by Farwell *et al.*, who

described a statistically significant association between HDL cholesterol (as continuous measure) and total and low-grade prostate cancer, whereas for high-grade prostate cancer the association did not reach statistical significance. However, detailed analyses with quartiles of HDL cholesterol did show statistically significant associations with high-grade prostate cancer risk[4]. Other studies on HDL cholesterol and prostate cancer risk are inconclusive. Hammersten *et al.*[1] evaluated features of the metabolic syndrome among 299 patients recently diagnosed with prostate cancer. Subjects with poorly differentiated prostate cancer had lower HDL cholesterol levels and higher triglyceride levels compared with those with well differentiated disease[1]. A hospital-based, case-control study found a statistically significant association between low levels of HDL cholesterol and total prostate cancer risk[3]. Large prospective studies, however, did not consistently confirm an association between HDL cholesterol and localized, advanced, or total prostate cancer risk[9, 15]. Our findings might be explained by several factors. First, high levels of HDL cholesterol may be associated with a healthy lifestyle[32]. In theory, participants with a healthy lifestyle, and consequently high HDL levels, may on average be more health conscious and may have consulted their physicians for periodic PSA testing. As a result, more early-stage prostate cancers might have been diagnosed among these men compared with other men in the cohort. Second, the positive association between HDL cholesterol and non-aggressive prostate cancer may be a chance finding, resulting from the relatively small number of cases.

The mechanisms underlying the possible association between blood lipid profiles and prostate cancer risk are poorly understood, but are possibly related to signalling functions of cholesterol. Cholesterol is incorporated into moving platforms in the fluid bilayer of cellular membranes, which are referred to as lipid rafts[33]. As reviewed by others[34-36], these lipid rafts might have an important role in cell signalling (such as the EGFR/Akt1[37] or IL6/STAT3[38] pathways) and could thereby act on growth and survival of prostate cancer cells[37, 38]. Future experiments focusing on lipid rafts should elucidate these and other signalling networks and their effects with respect to prostate cancer development or progression. Another hypothesis is based on the theory of steroidogenesis, which postulates that prostate cancer cells itself might be able to produce androgens which can bind to the androgen receptor and stimulate growth and survival[39]. It has recently been shown that prostate cancer cells in advanced stages can synthesize androgens directly from cholesterol[40]. These findings might explain the suggested association between serum cholesterol levels and risk of aggressive forms of prostate cancer. Future studies are needed to confirm the exact role of cholesterol and other blood lipids in the development and progression of prostate cancer.

Unfortunately, because of the relatively small number of cases in our cohort, we were not able to perform extensive analyses based on different tumour characteristics such as Gleason grade or tumour stage. Instead we used a rather arbitrary, combined definition of prostate cancer aggressiveness. It should be noted that only 69% of the participants of the Nijmegen Biomedical Study was willing to provide blood samples. Although we aimed at recruiting a random, population-based sample, we cannot exclude the possibility that only specific subgroups of participants agreed to provide blood samples. Although this selection is not an issue for the validity of the study, in theory, it may be so for the generalizability. Other potential limitations of the present study were the non-fasting blood samples as discussed previously, the relatively short follow-up (median 79.5 months) and the incapability to adjust for other potential confounders such as family history or socioeconomic status. Strengths of this study are the prospective design and the analyses of several blood lipid fractions in blood samples from a population-based cohort.

In conclusion, this study provides further evidence that blood lipid levels are associated with prostate cancer risk. Furthermore, our results suggest that different fractions of cholesterol are involved in aggressive and non-aggressive prostate cancer. The associations between the different fractions of cholesterol and clinical sub-types of prostate cancer warrant confirmation in larger, prospective studies.

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Conflict of interest

The authors declare no conflict of interest.

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Chapter 4

Risk of prostate cancer among cancer survivors in the Netherlands

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Purpose In parallel with increasing numbers of cancer patients and improving cancer survival, the occurrence of second primary cancers becomes a relevant issue. The aim of our study was to evaluate risk of prostate cancer as second primary cancer in a population-based setting.

Subjects and methods Data from the Netherlands Cancer Registry were used to estimate standardized incidence ratios (SIRs) and 95% confidence intervals (CIs) for prostate cancer as second primary cancer. The effect of time since first cancer diagnosis, specific first cancer sites, age, and pelvic radiotherapy was taken into account.

Results Out of 551,553 male patients diagnosed with a first primary cancer between 1989-2008, 9,243 patients were subsequently diagnosed with prostate cancer. Overall, cancer survivors showed an increased risk (SIR 1.3, 95% CI 1.2-1.3) of prostate cancer. The increased prostate cancer risk was limited to the first year of follow-up for the majority of the specific first cancer sites. More than ten years after the first cancer diagnosis, only melanoma patients were at increased risk (SIR 1.5, 95% CI 1.2-1.9), while patients with head or neck cancers were at decreased risk (SIR 0.7, 95% CI 0.5-0.9) of being diagnosed with prostate cancer. Patients treated with primary pelvic radiotherapy for their first cancer had a decreased risk of prostate cancer in the long term (SIR 0.5, 95% CI 0.4-0.6).

Conclusion Our data showed that cancer survivors have an increased prostate cancer risk in the first year following a first cancer diagnosis, which is most likely the result of active screening or incidental detection.

Introduction

The number of patients newly diagnosed with cancer increased substantially during the past decades and this trend is expected to continue in the coming years[1, 2]. At the same time, survival for most cancer sites improved by early detection and more effective treatment strategies[3, 4]. As a growing number of patients survive their first cancer, the occurrence of second primary cancers becomes a relevant issue[5]. Prostate cancer is the most common cancer among elderly men in Western countries[2, 6]. The incidence of prostate cancer as second primary cancer is likely to increase as a consequence of demographic aging and increased diagnostic activities, combined with the improved cancer survival[7]. Risk of prostate cancer among cancer survivors might also depend on various clinical as well as biological factors. It has been suggested that initial cancer treatment might influence subsequent cancer risk. As such, pelvic radiotherapy for a first cancer has been associated with a reduced prostate cancer risk as compared to non-irradiated patients or the general population[8-11]. Furthermore, incidental detection in surgical specimens or intensive screening after a previous cancer diagnosis might also influence prostate cancer risk. Likewise, the detection of prostate tumours in cystoprostatectomy specimens[12] is therefore a plausible explanation for the reported co-occurrence of bladder cancer and prostate cancer[13, 14]. Finally, common aetiological factors, such as genetic susceptibility or shared environmental factors, might explain an association between prostate cancer and other malignancies.

Insight into the occurrence of prostate cancer as second primary cancer may yield important implications for aetiological research. Several studies have addressed the relevance of prostate cancer as a second cancer. Most of these studies, however, were limited to specific first cancer sites[13, 15-17] or focussed on treatment effects[8, 9, 11] or family history of the first cancer[18] in particular. The aim of the present study was to evaluate the risk of prostate cancer as second primary cancer in a population-based setting while taking into account the first cancer sites and time since first cancer diagnosis. This approach allowed us to compare prostate cancer risk among different first cancer sites and to evaluate the possible effects of detection and treatment.

Subjects and methods

Male patients diagnosed with a first primary cancer between 1989 and 2008 were identified through the nationwide, population-based Netherlands Cancer Registry[19]. The analyses were restricted to primary cancers as defined by the International Agency for Research on Cancer (IARC)[20]. Non-invasive cancers, except from bladder cancer because of its common non-invasive character, were excluded from all analyses. Patients with a first and second primary cancer diagnosed on the same day, and

patients diagnosed with cancer found during autopsy were not included in the analyses. Furthermore, patients with a first primary prostate cancer were excluded, resulting in a study population of 551,553 male cancer patients.

Follow-up duration was defined as the time between date of first primary cancer diagnosis until date of death, emigration, diagnosis of prostate cancer as second primary cancer, diagnosis of any (other than prostate cancer) second primary cancer, or end of follow-up (1st of January 2009), whichever came first. Information on death and emigration were obtained from the municipal registries and since 1995 from the Dutch Municipal Personal Records Database which keeps information about vital status of all inhabitants in the Netherlands. Information on primary cancer treatment was recorded from the medical charts. Clinical tumour stages were grouped into six categories (0, I, II, III, IV and other/unknown) according to the fourth (tumours diagnosed before 1999), fifth (tumours diagnosed between 1999 and 2002) or sixth (tumours diagnosed after 2002) edition of the American Joint Committee on Cancer guidelines (AJCC).

Standardized incidence ratios (SIRs) were estimated to compare incidence rates of prostate cancer as a second cancer in the study population versus incidence rates of prostate cancer in the general Dutch population. The SIR was calculated as the number of observed patients with prostate cancer as a second cancer divided by the number of expected patients. The number of expected patients with prostate cancer was estimated by multiplying age- and calendar period-specific incidence rates (5-year age and 1-calendar year groups, respectively) in the general Dutch population by the number of person-years at risk. The 95% confidence intervals (CIs) were calculated assuming a Poisson distribution for the observed number of prostate cancers. Absolute excess risks (AERs) were calculated to estimate the excess burden of the prostate cancers occurring as second cancer. The AER (expressed per 10,000 person-years) was calculated by subtracting the number of expected patients from the number of observed patients in the study population and subsequently divided by the person-years at risk. Analyses were presented according to the time since first cancer diagnosis, the first cancer site or age of the patients at first cancer diagnosis. In addition to estimates for all cancer sites together, results were provided for all sites excluding bladder cancer in order to take into account possible distorting effects of early and incidental detection of prostate tumours in cystoprostatectomy specimens.

In order to assess the effect of radiotherapy on the subsequent prostate cancer risk, we computed SIRs for patients treated with or without pelvic radiotherapy. Pelvic radiotherapy was defined as primary radiotherapy for one of the following first

primary cancers: sigmoid colon, rectum, anus and anal canal, penis, testis, other male genital organs, renal pelvis, ureter, and other urinary tract. Patients with bladder cancer were not included in this analysis, because most patients who were not treated with pelvic radiotherapy were likely to undergo radical cystoprostatectomy and were therefore not at risk of developing prostate cancer in the long term. The subgroup analysis for patients treated without pelvic radiotherapy comprised all patients diagnosed with the aforementioned tumours located in the pelvic area (without bladder cancer), who were not treated with primary radiotherapy. SAS software (version 9.3, SAS Institute, Cary, North Carolina) was used for all analyses.

Results

The study population includes 551,553 male cancer patients diagnosed with a first primary cancer between 1989 and 2008. Of these, 9,243 patients subsequently developed prostate cancer after a median follow-up of 2.3 years (range: 1 day to 19 years). The median age (interquartile range (IQR)) of these patients was 70 years (64-76) at the time of first cancer diagnosis (**Table 4.1**). Clinical tumour stages of the prostate cancers occurring as second cancer were compared to clinical stages of first prostate cancers diagnosed in the general Dutch population in the same period (**Figure 4.1**). Overall, stage III and IV tumours were somewhat less common for subsequent prostate cancers (especially in the period 1989-1998) as compared to first prostate cancers, while the opposite was observed for unknown tumour stages. Also for subsequent prostate cancers diagnosed more recently (2004-2008), a larger percentage of unknown tumour stages was found, while especially stage II tumours tended to be less common in comparison to first prostate cancers.

The risk of prostate cancer as second primary cancer according to first cancer site and years since first cancer diagnosis is presented in **Table 4.2.** Overall, cancer survivors showed an increased risk (SIR 1.3, 95% CI 1.2-1.3) of being diagnosed with prostate cancer as compared to the general Dutch population. This effect was mainly observed shortly (0-1 year) after the first cancer diagnosis (SIR 2.1, 95% CI 2.0-2.2). The increased prostate cancer risk in the first year following the first cancer diagnosis was also shown for several specific cancer sites. The corresponding SIRs ranged from 1.3 (95% CI 1.1-1.5) for cancers in the digestive tract (without colorectal cancer) up to 9.2 (95% CI 8.7-9.8) for invasive bladder cancer. These effects disappeared after one year of follow-up for most of the specific cancer sites. Contrary, for patients with skin cancer as first primary cancer, an increased prostate cancer risk was mainly observed after one year since first cancer diagnosis. These effects were most pronounced for melanoma skin cancer. Prostate cancer risk was reduced in patients diagnosed with

head or neck cancer. This effect was observed ten years since first cancer diagnosis in particular (SIR 0.7, 95% CI 0.5-0.9).

Table 4.1 Characteristics of patients diagnosed with or without prostate cancer as second primary cancer after a first primary cancer diagnosis between 1989 and 2008 in the Netherlands

	Patients who were not diagnosed with prostate cancer as second primary cancer (n=542,310)	Patients who were diagnosed with prostate cancer as second primary cancer (n=9,243)
Age at first cancer diagnosis		
Median (interquartile range, in years)	68 (58-75)	70 (64-76)
< 50 years	71,116 (13%)	167 (2%)
50-74 years	327,929 (60%)	6338 (69%)
75+ years	143,265 (26%)	2738 (30%)
Period of first cancer diagnosis		
1989-1993	124,057 (23%)	2717 (29%)
1994-1998	128,434 (24%)	2694 (29%)
1999-2003	135,556 (25%)	2316 (25%)
2004-2008	154,263 (28%)	1516 (16%)
Time at risk[a]		
Median (interquartile range, in years)	1.3 (0.3-4.5)	2.3 (0.3-5.9)
< 1 year	241,149 (44%)	3386 (37%)
1-10 years	248,943 (46%)	4946 (54%)
10+ years	52,218 (10%)	911 (10%)
Pelvic radiotherapy for first primary cancer[b]		
Yes	18,218 (27%)	202 (16%)
No	49,823 (73%)	1096 (84%)

[a] Time at risk is defined as the time between date of first primary cancer diagnosis until date of death, emigration diagnosis of any (other than prostate cancer) second primary cancer, or end of follow-up (1st of January 2009), whichever came first. For patients with prostate cancer as a second primary cancer, time at risk is defined as the time between their first and second primary cancer diagnosis. [b] Restricted to patients with first primary cancers of the: sigmoid colon, rectum, anus and anal canal, penis, testis, other male genital organs, renal pelvis, ureter, and other urinary tract (without bladder cancer).

The analyses stratified by age at first cancer diagnosis further confirmed that cancer survivors had an increased prostate cancer risk mainly during the first year following first cancer diagnosis (**Table 4.3**). This finding applies to all age groups, although a more pronounced effect was found for patients who were diagnosed with a first cancer at a relatively young (<50 years) age (SIR 12, 95% CI 8.0-17).

As shown in **Table 4.3**, both patients treated with or without pelvic radiotherapy for their first primary cancer had an increased risk of prostate cancer during the first year following first cancer diagnosis. Patients treated with pelvic radiotherapy, however, showed a decreased prostate cancer risk in the long term (SIR 0.5, 95% CI 0.4-0.6 and SIR 0.6, 95% CI 0.3-0.98, for 1-10 and 10+ years after first cancer diagnosis, respectively).

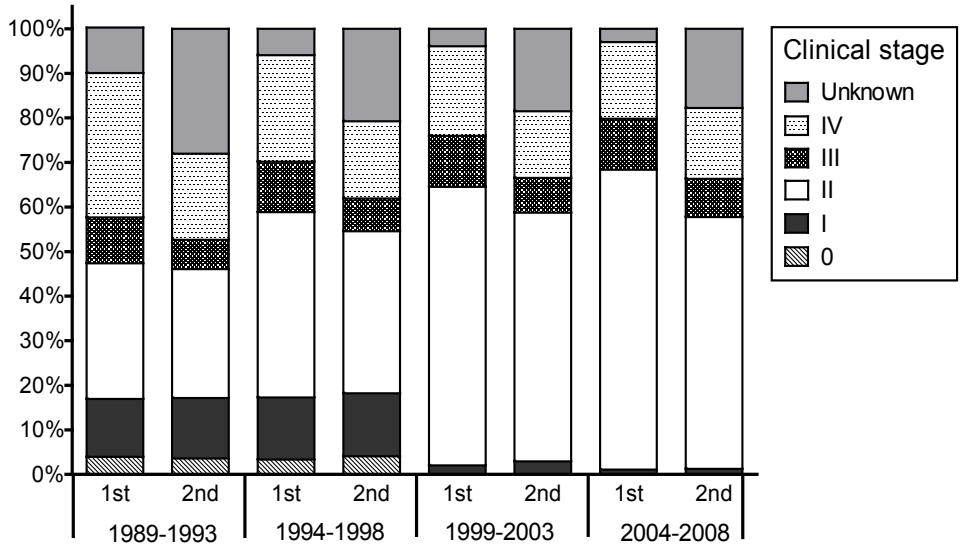

Figure 4.1 Clinical stage distribution of prostate cancer diagnosed as a first primary cancer (from the general population) and as a second primary cancer (among previously diagnosed cancer patients) in the Netherlands between 1989 and 2008 according to period of prostate cancer diagnosis.

Discussion

Overall, our study showed a 30% increased risk of prostate cancer among Dutch cancer survivors in the first year of follow-up, whereas in the long term prostate cancer risk did not differ from risk in the general Dutch population.

An increased prostate cancer risk shortly after a first cancer diagnosis strongly suggests an effect of active screening or incidental detection, resulting from either an increased awareness or anxiety of the patient, or active medical surveillance indicated by the supervising specialist. It has been shown that incidental prostate cancers are frequently detected in cystoprostatectomy specimens[12, 21]. Furthermore, urological patients in particular might request their urologists for PSA testing as a consequence of anxiety or persisting urological complaints. In these situations, the prostate cancer stage distribution would presumably be more favourable in comparison to prostate cancer detected among the general Dutch population. Concurrently, our data showed that unfavourable tumour stages (III and IV) were less common in patients with prostate cancer diagnosed as a second rather than a first primary cancer. The larger percentage of prostate cancers with an unknown tumour stage might indicate that staging is considered less important or is less accurate in patients with prostate cancer diagnosed as a second cancer.

As expected, the risk of prostate cancer was reduced 1-10 years after an invasive bladder cancer diagnosis, probably because the patients who underwent a cystoprostatectomy were no longer at risk of developing prostate cancer. Notably, this finding was not applicable to patients with non-invasive bladder cancer, whose increased prostate cancer risk persisted up to ten years following diagnosis. The more expectant treatment strategy which is often applied to these patients may have resulted in prolonged detection effects.

For patients with a previous diagnosis of melanoma as well as non-melanoma skin cancer, an increased prostate cancer risk was mainly found during later years of follow-up. Contrary to our findings, a previous study with data from one of the regional cancer registries in the Netherlands showed that patients with (non-melanoma) skin cancer had a reduced risk of prostate cancer[22]. It was hypothesized that patients with skin cancer might have relatively high levels of vitamin D as a consequence of sun exposure, which may protect them against the development of prostate cancer[22]. Several other studies, however, did not confirm these findings[23], or showed an increased prostate cancer risk following a skin cancer diagnosis[17, 24]. Focusing on melanoma, data from the Surveillance, Epidemiology, and End Results (SEER) program showed an increased prostate cancer risk up to ten years following melanoma diagnosis[25, 26], which is consistent with our findings. Possible explanations for the increased prostate cancer risk in melanoma patients might refer to shared environmental or genetic aspects. A recent study demonstrated that at least two prostate cancer risk alleles were associated with an increased risk of melanoma in prostate cancer patients (*rs1512268*, odds ratio (OR) 3.9, 95% CI 1.4-10.9 and *rs5759167* OR 2.6, 95% CI 1.2-5.6)[27]. We cannot fully exclude the possibility that increased awareness and screening also contributed to the excess of prostate cancer in melanoma patients, although the lack of an effect during the first year of follow-up argues against this.

We observed a reduced risk of prostate cancer, especially after a follow-up period of more than ten years, among patients who were diagnosed with a head or neck cancer. The possible mechanisms for the reduced prostate cancer risk in these patients are unclear. So far, there is no indication that the main established risk factors for head and neck cancers, such as smoking, alcohol intake, and infections with the human papillomavirus[28] are likely to protect against the development of prostate cancer. Future studies should focus on the possible associations between prostate cancer and different specific tumours in the head and neck region as well as the possible underlying mechanisms.

Table 4.2 Risk of prostate cancer as a second primary cancer according to first cancer site and time since first cancer diagnosis

	Time since first cancer diagnosis (years)											
	0-1				1-10				10+			
First cancer site	**PY at risk**	**Obs.**	**SIR (95% CI)**	**AER**	**PY at risk**	**Obs.**	**SIR (95% CI)**	**AER**	**PY at risk**	**Obs.**	**SIR (95% CI)**	**AER**
All sites[a]	392,642	3388	**2.1 (2.0-2.2)**	45	1200,526	4945	1.0 (0.98-1.0)	0.2	203,319	910	1.1 (0.99-1.1)	2
All sites without bladder cancer[b]	342,395	2003	**1.5 (1.4-1.5)**	19	998,673	3872	1.0 (0.96-1.0)	-0.2	169,079	713	1.1 (0.99-1.1)	3
Invasive bladder	23,166	1090	**9.2 (8.7-9.8)**	419	76,628	361	**0.9 (0.8-0.98)**	-6	12,156	64	0.9 (0.7-1.1)	-6
Non-invasive bladder	27,078	295	**2.4 (2.1-2.6)**	63	125,226	712	**1.2 (1.1-1.2)**	8	22,084	133	1.1 (0.9-1.3)	6
Renal pelvis & ureter	1921	16	**1.8 (1.0-2.9)**	36	4785	28	1.2 (0.8-1.7)	9	727	5	1.3 (0.4-2.9)	14
Other urinary tract	207	8	**7.3 (3.1-14)**	333	536	2	0.7 (0.08-2.5)	-16	106	0	0 (0-5.8)	-60
Penis, testis & other male genital organs	10,872	11	1.2 (0.6-2.1)	1	59,997	51	1.1 (0.8-1.4)	1	16,640	18	1.1 (0.7-1.8)	1
Kidney	11,904	124	**3.0 (2.5-3.5)**	69	41,997	232	**1.4 (1.3-1.6)**	17	7555	39	1.1 (0.8-1.5)	6
Colorectal	66,093	521	**1.7 (1.6-1.8)**	32	221,187	1053	0.9 (0.9-1.01)	-3	33,453	196	1.1 (0.9-1.2)	3
Digestive tract without colorectal	34,328	195	**1.3 (1.1-1.5)**	13	50,522	203	0.9 (0.8-1.05)	-4	6969	40	1.1 (0.8-1.5)	6
Male Breast	1021	11	**2.6 (1.3-4.6)**	66	4113	17	0.9 (0.5-1.5)	-4	606	3	1.0 (0.2-2.8)	-2
Skin (non-melanoma)[c]	33,254	175	0.9 (0.8-1.1)	-3	132,240	792	**1.1 (1.003-1.2)**	4	19,510	126	1.2 (0.97-1.4)	9
Melanoma	18,160	49	1.2 (0.9-1.6)	5	78,968	256	**1.4 (1.3-1.6)**	10	16,455	69	**1.5 (1.2-1.9)**	14
Lung, bronchus & trachea	71,739	458	**1.4 (1.3-1.5)**	18	96,178	390	**0.8 (0.8-0.9)**	-8	11,289	70	1.1 (0.8-1.4)	5
Head or neck	25,069	73	0.9 (0.7-1.1)	-5	93,491	332	0.9 (0.8-1.001)	-4	16,062	53	**0.7 (0.5-0.9)**	-17
Eye & adnexa	1450	8	1.9 (0.8-3.8)	26	6194	19	1.1 (0.6-1.7)	2	1397	5	1.2 (0.4-2.7)	5
Haematolymphopoetic	43,146	229	**1.6 (1.4-1.8)**	20	149,260	399	0.9 (0.8-1.004)	-3	25,296	62	1.0 (0.8-1.3)	-0.3
Bone, joint & soft tissue	6637	18	1.5 (0.9-2.3)	9	25,862	55	1.1 (0.9-1.5)	3	6365	14	1.1 (0.6-1.8)	1
Central nervous system	6772	13	1.2 (0.6-2.1)	3	15,532	10	0.9 (0.4-1.6)	-1	3139	1	0.3 (0.004-1.7)	-7
Endocrine glands	2096	6	1.7 (0.6-3.7)	11	9954	11	0.7 (0.3-1.2)	-5	2611	8	1.6 (0.7-3.2)	12
Primary site unknown	7247	88	**2.8 (2.3-3.5)**	78	6426	17	0.7 (0.4-1.1)	-12	659	3	1.0 (0.2-3.0)	1
Other (e.g. thymus)	472	0	0 (0-2.5)	-31	1396	5	1.3 (0.4-3.1)	9	224	1	1.3 (0.02-7.0)	9

Abbreviations: *AER* absolute excess risk per 10,000 person-years, *CI* confidence intervals, *Obs*. number of observed cases, *PY* person-years, *SIR* standardized incidence ratio.

[a] All invasive primary cancer sites, excluding prostate cancer and including non-invasive bladder cancer. [b] All primary cancer sites, excluding prostate cancer, invasive bladder cancer and non-invasive bladder cancer. [c] Basal cell carcinomas were not included. [d] Restricted to patients with first primary cancers of the: sigmoid colon, rectum, anus and anal canal, penis, testis, other male genital organs, renal pelvis, ureter, and other urinary tract (without bladder cancer).

Table 4.3 Risk of prostate cancer as a second primary cancer according to age at first cancer diagnosis, treatment and time since first cancer diagnosis

	Time since first cancer diagnosis (years)											
	0-1				1-10				10+			
	PY at risk	Obs.	SIR (95% CI)	AER	PY at risk	Obs.	SIR (95% CI)	AER	PY at risk	Obs.	SIR (95% CI)	AER
Age at first cancer diagnosis (years)												
< 50	61,121	27	**12 (8.0-17)**	4	280,993	54	1.2 (0.9-1.6)	0.4	79,658	86	1.2 (0.9-1.5)	2
50-74	241,611	1989	**2.2 (2.1-2.3)**	45	732,833	3584	1.0 (0.998-1.07)	1	115,057	765	1.0 (0.96-1.1)	2
75+	89,910	1372	**1.9 (1.8-2.0)**	73	186,701	1307	**0.9 (0.9-0.98)**	-5	8604	59	1.3 (0.97-1.6)	15
Pelvic radiotherapy for first primary cancer[a]												
Yes	16,294	104	**1.9 (1.6-2.3)**	31	56,558	82	**0.5 (0.4-0.6)**	-17	9381	16	**0.6 (0.3-0.98)**	-11
No	41,460	305	**1.8 (1.6-2.0)**	33	159,662	653	1.0 (0.9-1.1)	0	30,045	138	1.2 (0.97-1.4)	6

Abbreviations*: AER* absolute excess risk per 10,000 person-years, *CI* confidence intervals, *Obs*. number of observed cases, *PY* person-years, *SIR* standardized incidence ratio. [a] Restricted to patients with first primary cancers of the: sigmoid colon, rectum, anus and anal canal, penis, testis, other male genital organs, renal pelvis, ureter, and other urinary tract (without bladder cancer).

Consistent with previous literature[8-11], we showed an overall reduced prostate cancer risk following primary pelvic radiotherapy. Nevertheless, for the first year of follow-up, the subsequent prostate cancer risk was increased among patients who received pelvic radiotherapy. Similar findings were observed for patients who did not receive pelvic radiotherapy, suggesting once more the effects of detection. A possible explanation for the consistently reported reduced prostate cancer risk following pelvic radiotherapy might be that early and indolent prostate tumours are suppressed by the irradiation exposure. Another hypothesis[9, 10] refers to the possibility that radiotherapy initially increases, but in the long term lowers PSA production[29, 30]. In theory, the incidence of screen-detected prostate cancers would then, due to masked PSA levels, be lower as compared to non-irradiated patients. As a consequence, the prostate tumours in irradiated patients are detected later, and hence are more advanced or high-grade as compared to tumours in non-irradiated patients[9]. Others, however, did not find an effect of previous pelvic radiotherapy on prostate cancer stage or grade[10], neither on the decline in PSA levels after irradiation of the non-malignant prostate[31]. We did not compare clinical tumour stages for patients treated with or without pelvic radiotherapy, because we cannot exclude the possibility that period-specific changes in treatment regimens during our extended follow-up period (1989-2008) will account for possible differences between these two groups.

Strengths of our study include the comprehensive approach, which allows a simultaneous evaluation of all specific first cancers sites in relation to subsequent prostate cancer risk during a long follow-up period. Furthermore, we used population-based data of a high-quality cancer registry with a large number of first and second cancer patients. Possible limitations of our study are the relatively small number of patients in some of the subgroup analyses and the limited data on delayed or secondary cancer treatment. As a consequence, patients who were classified as 'not having pelvic radiotherapy' in the subgroup analyses, might still have undergone pelvic radiotherapy as secondary therapy[32]. Although this limitation might have influenced the risk estimates for this subgroup, it is not likely to bias the findings for the subgroup of patients who were classified as 'having pelvic radiotherapy'.

In conclusion, our data showed that cancer survivors have an increased risk of being diagnosed with prostate cancer as a second primary cancer. The effects were mostly restricted to the first year following the first cancer diagnosis, which might implicate an effect of active screening or incidental detection.

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Conflict of interest

The authors declare no conflict of interest.

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Chapter 5a

Body mass index is not a predictor of biochemical recurrence after radical prostatectomy in Dutch men diagnosed with prostate cancer

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Purpose To determine the effect of body mass index (BMI) on clinical and pathological characteristics at time of diagnosis and on risk of biochemical recurrence after radical prostatectomy among Dutch men diagnosed with prostate cancer.

Subjects and methods In total, 1,116 prostate cancer patients with known BMI, diagnosed between 2003 and 2006, were identified from the population-based cancer registry held by the Comprehensive Cancer Centre East, The Netherlands. Of these, 504 patients underwent a radical prostatectomy. Patients were categorized as normal weight (BMI < 25 kg/m^2), overweight (BMI 25–30 kg/m^2), or obese (BMI ≥ 30 kg/m^2). Multivariable proportional hazards regression models, adjusted for age, prediagnostic PSA levels, and pathological characteristics were used to evaluate BMI as a prognostic factor for biochemical recurrence after radical prostatectomy.

Results Overall, clinical and biopsy characteristics did not significantly differ among BMI groups. Pathological characteristics after radical prostatectomy did not significantly differ among BMI groups, except for tumor stage, which was highest in obese patients ($P = 0.017$). For patients treated with radical prostatectomy, 5-year risk (95% confidence intervals) of biochemical recurrence was 30% (23–37%) for normal weight, 32% (25–39%) for overweight, and 25% (9–41%) for obese patients (log rank $P = 0.810$). BMI was not an independent prognostic factor for biochemical recurrence in multivariable proportional hazards regression analyses (hazard ratio 0.99 per kg/m^2, 95% CI: 0.93–1.06).

Conclusion Compared with non-obese men, pathological tumor stage tended to be higher in obese men. Clinical relevance of this finding is unclear, because BMI was not an independent predictor of biochemical recurrence after radical prostatectomy.

Introduction

It has been hypothesized that obesity is a risk factor for the development and progression of prostate cancer (PC), although results are inconsistent. Most studies focusing on body size and PC were conducted in the United States, where a rapidly growing epidemic of obesity is reported with over 66% of adult Americans being overweight or obese[1]. In Europe, incidence of overweight and obesity is also increasing substantially[2]. Whether body size predisposes to adverse PC characteristics or outcome in European men is a matter of debate. Only few European studies examined effects of body mass index (BMI) on adverse pathological findings after biopsy or radical prostatectomy (RP)[3-6]. Gallina and colleagues suggested that high-grade PC at RP might be more prevalent among obese men; however, adding BMI to the multivariable model failed to increase predictive accuracy for high-grade PC[6]. Other studies did not find an association for BMI and tumor grade or stage, extracapsular extension, seminal vesicle invasion, lymph node involvement or positive surgical margins either[3-5]. Results for PC outcome are inconclusive as well. One study from Germany reported BMI as independent predictor of biochemical recurrence (BCR) after RP, although it did not improve predictive accuracy[7], while we and others did not find any effect of BMI on BCR rates after RP[5, 8] or brachytherapy[9].

Since results are conflicting and the epidemic of obesity is growing, additional evidence on the effects of body size on PC risk and prognosis in Europe are needed. Aim of the present study was to determine effects of BMI on clinical and pathological findings at time of diagnosis and on risk of BCR after RP among Dutch men with PC.

Subjects and methods

Patients diagnosed with PC were identified from the population-based cancer registry held by the Comprehensive Cancer Centre East, The Netherlands. From 2003 to 2006, 1,668 patients with PC were identified in this region. Only patients with known BMI data were included in our analyses (*n*=1,116). For all patients, clinical data were collected retrospectively by review of the clinical charts. Part of the patients (*n*=951), who were diagnosed before the age of 76, participated in the POLYGENE project[10] and filled out a postal questionnaires as part of it. Self-reported weight and length were collected either from the POLYGENE questionnaire (*n*=943) or from the clinical charts (*n*=173) and were used to calculate BMI. For 278 patients, BMI was available from the questionnaire as well as from the charts (Spearman r=0.81, P <0.001). For these patients, BMI from the questionnaire was used in the analyses. BMI categories were defined according to the WHO criteria: BMI <25 kg/m^2 (normal weight), BMI 25-30 kg/m^2 (overweight), and BMI ≥30 kg/m^2 (obesity). The institutional review board

approved the study, and all participants of the POLYGENE project provided written informed consent.

Primary treatments were categorized as radical prostatectomy (RP) with or without neoadjuvant androgen-deprivation therapy (ADT), radiotherapy (RT, including external beam radiation and brachytherapy) with or without ADT, active surveillance (AS), androgen-deprivation therapy (ADT), and others (such as cryotherapy and chemotherapy). In total, 517 patients who underwent RP as primary therapy were identified. Patients treated with neoadjuvant ADT (*n*=13) were excluded, leaving 504 patients for analysis. BMI was evaluated as prognostic factor for BCR, which is defined as two consecutive PSA levels ≥0.2 ng/ml. For these analyses, 11 patients were excluded, because data on post-operative PSA levels or BCR status were missing. After RP, patients were generally seen after 6 weeks, 3, 6, 9, and 12 months and then every 6 months, according to the national guidelines for PC follow-up[11]. RP specimens were processed according to protocols from the institutes where patients were submitted to. Gleason grade was presented as the sum of two main Gleason scores. Clinical and pathological stages were classified according to the 2002 TNM classification based on the American Joint Committee on Cancer guidelines (AJCC)[12].

We used Kruskal-Wallis tests to assess the association between BMI categories and continuous clinical and pathological variables, while Chi-square tests were applied to categorical variables. Risk of BCR was calculated with the Kaplan-Meier method, using the log-rank test to compare BMI groups. Univariable and multivariable proportional hazards regression analyses adjusted for age, prediagnostic PSA levels, and pathological variables (Gleason score at RP, pathological stage, surgical margin status, and lymph node status) were performed to evaluate whether BMI is a prognostic factor for BCR after RP. The significance level was set at P <0.05, and all P values were two-tailed. Statistical Package of Social Sciences (SPSS, version 16.0, Chicago, Illinois) was used for all analyses.

Results

Patient characteristics are shown in **Table 5a.1**. Among all PC patients included in the analyses (*n*=1,116), median age at diagnosis was 66.3 (inter-quartile range: 61.2-70.5) years. Median BMI was 25.3 (IQR: 23.9-27.0) kg/m^2, with 47% of this population being overweight and 7% obese. Overall, no statistically significant differences for clinical or pathological findings were observed among the BMI groups. Although not statistically significant, obese patients were somewhat less likely to be referred for RP compared to normal weight and overweight patients (38% versus 46% and 48%, respectively).

Table 5a.1 Demographic, clinical, and pathological characteristics of Dutch patients diagnosed with prostate cancer according to BMI categories

	Total group	BMI <25 kg/m^2	BMI 25-30 kg/m^2	BMI ≥30 kg/m^2	*P* value
Number of patients (%)	1,116 (100%)	510 (46%)	530 (47%)	76 (7%)	–
Age at diagnosis (years)	66.3 (61.2-70.5)	66.1 (61.5-71.0)	66.2 (61.0–70.2)	65.1 (61.1-69.6)	0.753
BMI (kg/m^2)	25.3 (23.9-27.0)	23.7 (22.9-24.4)	26.6 (25.8–27.8)	31.6 (30.7-33.6)	–
BMI at age 18 (kg/m^2)[a]	22.2 (21.0-23.7)	21.5 (20.2-22.6)	23.0 (21.8–24.2)	24.4 (23.1-28.1)	–
Height (cm)	177 (172-182)	178 (173-183)	176 (172–180)	175 (172-179)	–
Weight (kg)	80 (74-85)	75 (70-80)	83 (80–90)	100 (92-104)	–
Smoking (%)					
Never	170 (15%)	86 (17%)	74 (14%)	10 (13%)	0.035
Former	641 (57%)	285 (56%)	321 (61%)	35 (46%)	
Current	136 (12%)	80 (16%)	50 (9%)	6 (8%)	
Family history of prostate cancer (%)					
Yes	228 (20%)	101 (20%)	117 (22%)	10 (13%)	0.285
No	736 (66%)	357 (70%)	336 (63%)	43 (57%)	
Prediagnostic PSA level (ng/ml)[b]	10 (6–20)	9 (6–20)	10 (7–21)	10 (7–27)	0.187
Gleason score biopsy (%)					
<7	689 (62%)	331 (65%)	316 (60%)	42 (55%)	0.128
7	226 (20%)	88 (17%)	120 (23%)	18 (24%)	
>7	120 (11%)	49 (10%)	61 (12%)	10 (13%)	
Clinical stage (cTNM) (%)					
cT1	447 (40%)	200 (39%)	224 (42%)	23 (30%)	0.229
cT2	418 (38%)	196 (38%)	194 (37%)	28 (37%)	
cT3 or cT4	233 (21%)	107 (21%)	104 (20%)	22 (29%)	
Primary treatment (%)					
Active surveillance (AS)	121 (11%)	58 (11%)	57 (11%)	6 (8%)	0.585
RP without ADT	504 (45%)	230 (45%)	245 (46%)	29 (38%)	
RP with ADT	13 (1%)	4 (1%)	9 (2%)	–	
RT without ADT	115 (10%)	60 (12%)	46 (9%)	9 (12%)	
RT with ADT	210 (19%)	94 (19%)	100 (18%)	16 (21%)	
ADT	138 (12%)	58 (11%)	66 (12%)	14 (18%)	
Others	10 (1%)	5 (1%)	4 (1%)	1 (1%)	

Data presented as median (IQR) or number (%). Percentages may not add up to 100% because of missing values. Abbreviations: *ADT* androgen-deprivation therapy, *AS* active surveillance, *BMI* body mass index, *cTNM* clinical tumor-node-metastasis, *PSA* prostate specific antigen, *RP* radical prostatectomy, *RT* radiotherapy. [a]Missing *n*=243; [b]Missing *n*=12.

Characteristics of patients with PC who underwent RP are shown in **Table 5a.2**. Median age and BMI of patients treated with RP were 63.3 (IQR: 58.8-67.1) years and 25.3 (IQR: 23.7-26.9) kg/m^2. Pathological characteristics after RP did not significantly differ between BMI groups, except for tumor (pT) stage which was somewhat higher in obese patients (*P*=0.017).

Table 5a.2 Demographic, clinical, and pathological characteristics of Dutch patients with prostate cancer treated with radical prostatectomy (RP)

	Total group	BMI <25 kg/m^2	BMI 25-30 kg/m^2	BMI ≥30 kg/m^2	*P* value
Number of patients (%)	504 (100%)	230 (46%)	245 (49%)	29 (6%)	–
Age at RP (years)	63.3 (58.8-67.1)	63.4 (58.7-66.7)	63.2 (58.8-67.4)	63.0 (59.3-67.8)	0.961
BMI (kg/m^2)	25.3 (23.7-26.9)	23.7 (22.9-24.4)	26.6 (25.8-27.7)	31.3 (30.5-34.3)	–
Prediagnostic PSA (ng/ml)[a]	8 (6-12)	7 (5-10)	8 (6-13)	8 (5-12)	0.004
Follow-up (months)	40.3 (19.5-53.1)	40.9 (24.7-53.8)	39.4 (17.0-52.4)	40.6 (17.1-57.0)	0.502
Surgery (%)					
Open	284 (56%)	123 (53%)	145 (59%)	16 (55%)	0.251
Laparoscopic	195 (39%)	99 (43%)	88 (36%)	8 (28%)	
Missing	25 (5%)	8 (3%)	12 (5%)	5 (17%)	
PSA nadir < 0.2 ng/ml (%)					
Yes	461 (91%)	213 (93%)	221 (90%)	27 (93%)	0.853
No	36 (7%)	15 (7%)	19 (8%)	2 (7%)	
Missing	7 (1%)	2 (1%)	5 (2%)	0	
Biochemical recurrence (%)					
Yes[b]	142 (28%)	65 (28%)	70 (29%)	7 (24%)	0.874
No	351 (70%)	163 (71%)	167 (68%)	21 (72%)	
Missing	11 (2%)	2 (1%)	8 (3%)	1 (3%)	
Gleason score RP (%)					
<7	348 (69%)	165 (72%)	162 (66%)	21 (72%)	0.148
7	111 (22%)	44 (19%)	60 (24%)	7 (24%)	
>7	30 (6%)	10 (4%)	20 (8%)	0	
Missing	15 (3%)	11 (5%)	3 (1%)	1 (3%)	

To be continued on the next page

Furthermore, obese patients tended to have higher prediagnostic PSA levels compared to overweight and normal weight patients (*P*=0.004). BMI presented as a continuous variable, however, was only weakly correlated with prediagnostic PSA levels (Spearman *r*=0.13, *P*=0.004).

Median follow-up of patients treated with RP was 40.3 (IQR: 19.5-53.1) months. In total, 142 patients developed BCR after RP. The 5-year risk (95% CI) of BCR was 30% (23-37%), 32% (25-39%), and 25% (9-41%) for normal weight, overweight, and obese patients, respectively (log rank *P*=0.810) (**Figure 5a.1**).

As presented in **Table 5a.3**, BMI was not a significant prognostic factor for BCR after RP in univariable (HR 1.02 per kg/m^2, 95% CI: 0.97-1.07) or multivariable (HR 0.99 per kg/m^2, 95% CI: 0.93-1.06) analyses after adjustment for age, prediagnostic PSA, Gleason score at RP, positive surgical margins, positive lymph nodes, and pathological stage. Higher Gleason score, pathological stage, and positive surgical margins were all statistically significant predictors of risk of BCR after RP.

Table 5a.2 Continued

	Total group	BMI <25 kg/m^2	BMI 25-30 kg/m^2	BMI ≥30 kg/m^2	*P* value
Pathological stage (pTNM) (%)					
pT2	349 (69%)	170 (74%)	165 (67%)	14 (48%)	0.017
pT3 or pT4	143 (28%)	54 (23%)	76 (31%)	13 (45%)	
Missing	12 (2%)	6 (3%)	4 (2%)	2 (7%)	
Surgical margins (%)					
Positive	211 (42%)	93 (40%)	102 (42%)	16 (55%)	0.341
Negative	270 (54%)	125 (54%)	133 (54%)	12 (41%)	
Missing	23 (5%)	12 (5%)	10 (4%)	1 (3%)	
Extracapsular extension (%)					
Yes	175 (35%)	72 (31%)	89 (36%)	14 (48%)	0.204
No	221 (44%)	102 (44%)	110 (45%)	9 (31%)	
Missing	108 (21%)	56 (24%)	46 (19%)	6 (21%)	
Invasion seminal vesicles (%)					
Yes	45 (9%)	15 (7%)	25 (10%)	5 (17%)	0.068
No	446 (88%)	211 (92%)	214 (87%)	21 (72%)	
Missing	13 (3%)	4 (2%)	6 (2%)	3 (10%)	
Lymph node dissection (%)	230 (46%)	83 (36%)	131 (53%)	16 (55%)	0.001
Positive lymph nodes (%)	17 (3%)	6 (3%)	9 (4%)	2 (7%)	0.726

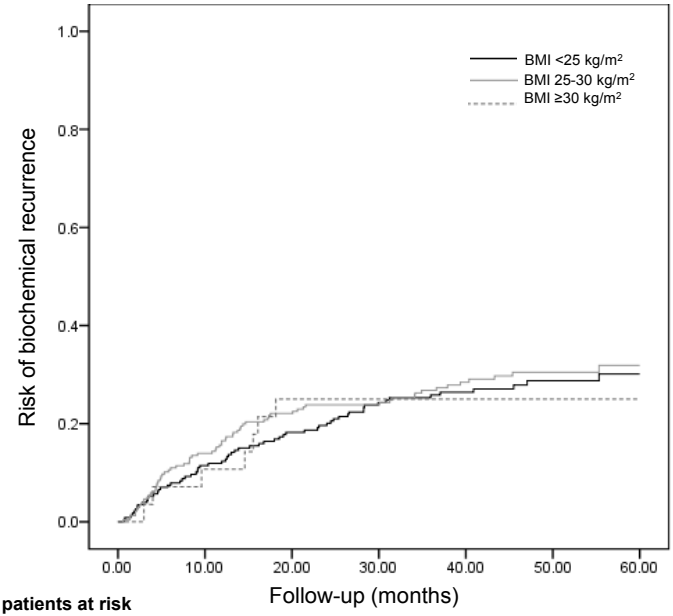
Data presented as median (IQR) or numbers (%). Abbreviations: *BMI* body mass index, *PSA* prostate specific antigen, *pTNM* pathological tumor-node-metastasis staging criteria, *RP* radical prostatectomy.
[a]Missing *n*=5, [b]Including 36 patients who did not reach post-operative PSA levels <0.2 ng/ml.

Discussion

In the present study among Dutch men diagnosed with PC, BMI was weakly associated with higher pathological tumor (pT) stage and higher prediagnostic PSA levels in patients treated with RP. Gleason score, pathological stage, and positive surgical margins were independent predictors of BCR, whereas BMI did not add any prognostic value in multivariable proportional hazards regression analyses. Our findings are consistent with other European studies which did not find a prognostic effect of BMI in patients treated with RP[5, 8]. Only one study reported a trend toward statistical significance for BMI as independent prognostic factor for BCR[7]. Addition of BMI to a multivariable model, however, did not significantly increase predictive accuracy[7]. Whereas most European studies so far were not able to find an association between BMI and any clinical or pathological characteristics, several studies from the United States did report BMI as predictor of BCR and adverse pathological findings after RP[13-15]. These inconsistent results might be explained by the lower rates of obesity and severe obesity in Europe compared to the United States[1, 2].

A remarkable observation in this study is the weak positive association between BMI and prediagnostic PSA levels among RP patients, which was not observed in the overall

study population. Several studies observed an inverse association between BMI and prediagnostic PSA levels[16, 17]. Based on the theory of haemodilution, it has been hypothesized that obese patients have larger plasma or serum volumes, which may lead to lower PSA concentrations[16]. It has also been suggested that lower PSA levels in obese patients might result from decreased androgenic activity[18]. Our results indicated a weak correlation between BMI and PSA levels and were limited to a small subpopulation of patients treated with RP; therefore, we cannot rule out that our result was a chance finding.



No. of patients at risk							
BMI <25 kg/m²	228	199	178	153	118	67	34
BMI 25-30 kg/m²	237	203	179	161	120	69	24
BMI ≥30 kg/m²	28	25	21	19	15	10	3

Figure 5a.1 The 5-year risk of biochemical recurrence in normal weight, overweight, and obese prostate cancer patients treated with radical prostatectomy (*n*=493). Log rank *P*=0.810.

Another finding of our study was the association between BMI and pathological tumor (pT) stage among RP patients, suggesting that advanced-staged tumors were more common among obese patients. As reviewed by others, obesity might indeed play a role in PC aggressiveness, i.e. high stage, high grade, and increased risk of recurrence or mortality[19]. It has been hypothesized that both non-biological and biological mechanisms can be responsible for the association between tumor aggressiveness and body size. Firstly, it might be more difficult to detect (early) PC in obese men, due to lower PSA levels[16, 17] and difficult digital rectal examinations[19]. Secondly, difficulties related to treatment might be responsible for an aggressive type of PC in obese men.

Pathological findings related to technical aspects of surgery like positive surgical margins, however, would be more likely to be affected than tumor grade or stage. Finally, alterations in levels of steroid hormones, adipokines, and inflammatory mediators might also drive PC towards a more aggressive form in obese men[19, 20].

Table 5a.3 Univariable and multivariable proportional hazards regression models predicting biochemical recurrence after radical prostatectomy (RP)

		Univariable			Multivariable (n = 444)[a,b]		
	n	HR	95% CI	*P* value	Adjusted HR	95% CI	*P* value
BMI (kg/m^2)	493	1.02	0.97–1.07	0.525	0.99	0.93–1.06	0.732
BMI	493						
<25 kg/m^2		1.00	–	–			
25–30 kg/m^2		1.08	0.77–1.51	0.658			
≥30 kg/m^2		0.90	0.41–1.96	0.789			
Age at RP (years)	493	1.05	1.02–1.08	0.003	1.02	0.98–1.05	0.396
Surgery	472						
Open		1.00	–	–			
Laparoscopic		1.15	0.81–1.62	0.429			
Year of RP	493	1.07	0.90–1.27	0.441			
Prediagnostic PSA level (ng/mL)	489	1.03	1.01–1.05	0.002	1.00	0.98–1.02	0.866
Prediagnostic PSA level	489						
<4 ng/ml		1.00	–	–			
4–10 ng/ml		3.02	0.95–9.58	0.061			
≥10 ng/ml		4.99	1.57–15.89	0.006			
Gleason score at RP	478						
<7		1.00	–	–	1.00	–	–
7		2.55	1.77–3.68	<0.001	1.71	1.13–2.60	0.012
>7		4.39	2.64–7.31	<0.001	2.55	1.43–4.52	0.001
Pathological stage (pTNM)	483						
pT2		1.00	–	–	1.00	1.00	–
pT3 or pT4		2.50	1.79–3.50	<0.001	1.68	1.13–2.49	0.010
Extracapsular extension	386	2.41	1.62–3.58	<0.001			
Positive surgical margins	470	4.33	2.94–6.38	<0.001	2.85	1.87–4.35	<0.001
Invasion seminal vesicles	480	2.58	1.64–4.04	<0.001			
Positive lymph nodes[c]	489	2.93	1.54–5.58	0.001	1.57	0.80–3.07	0.186

Abbreviations: BMI body mass index, *PSA* prostate specific antigen, *pTNM* pathological tumor-node-metastasis staging criteria, *RP* radical prostatectomy. [a]Variables in the multivariable model are adjusted for each other; [b]Replacing pathological stage by extracapsular extension and seminal vesicles invasion in the multivariable model resulted in adjusted hazards ratios (95% CI) of 1.45 (0.92-2.28, *P*=0.106) for extracapsular extension and 1.06 (0.59-1.91, *P*=0.845) for seminal vesicles invasion, while the adjusted hazards ratios for the remaining variables hardly changed; [c]The reference category is: no lymph node dissection performed or no positive lymph nodes.

Evidence is growing that differentiation between total adiposity and distribution of adipose tissue is relevant in the studies related to obesity and PC. Recent studies suggested that measures of body fat distribution might be better predictors of PC risk

and prognosis when compared to BMI[21, 22]. Fat distribution measurements usually distinct subcutaneous fat from visceral fat depots or simply indicate the location of adipose tissue. Skin fold measurements, waist circumference, and waist-to-hip ratios are frequently used estimates for the amount and location of adipose tissue. Magnetic resonance imaging (MRI) and computed tomography (CT) are considered more reliable methods for assessing subcutaneous and visceral fat content[23]. Von Hafe et al.[22] examined the relation between abdominal visceral fat accumulation, as measured by CT, and PC incidence within a case-control study. They found that visceral fat area and visceral to subcutaneous fat ratio were strongly associated with increased PC risk (crude OR 4.6, 95% CI: 2.6-8.2 and OR 6.0, 95% CI: 2.3-11.0, respectively).

Unfortunately, we did not have data on any measures of fat distribution. Other potential limitations of our study might be its retrospective data collection, self-reported BMI, relatively short follow-up (median 40.3 months), and small number of patients, especially in the obese group. Results therefore need to be interpreted with some caution. We cannot exclude the possibility that the relatively large number of missing values for BMI might have been a source of selection bias, although the observation that patients with missing BMI did not have more advanced tumor characteristics compared to the patients with evaluable BMI in our RP cohort (data not shown) argues against this. The absence of an association between BMI and BCR might also be explained by treatment-related selection. If obese patients tend to have more advanced tumor characteristics at diagnosis, and therefore have other types of treatment (e.g. ADT), while mainly normal weight, low-risk patients will have surgery, a possible association between BMI and BCR could be missed. Our aim was, however, to study the association between BMI and BCR in an average population-based RP cohort. We conclude from our results that in this cohort, BMI does not have any prognostic value for risk of BCR. Whether BMI is associated with risk of recurrence in other treatment groups should be verified in future studies.

In summary, BMI did not affect clinical or pathological characteristics of PC patients at time of diagnosis. Compared with non-obese men, pathological stage tended to be higher in obese men treated with RP. Clinical relevance of these findings with respect to risk of BCR, however, needs to be further elucidated, since BMI itself was not an independent predictor of BCR after RP.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Chapter 5b

Body mass index as a prognostic marker for biochemical recurrence in Dutch men treated with radical prostatectomy

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Purpose To investigate whether body mass index (BMI) is a prognostic factor for biochemical recurrence (BCR) in Dutch men after radical prostatectomy (RP), as although epidemiological studies of obesity in relation to prostate cancer have provided conflicting results, recent studies from the USA suggest that a higher BMI is a risk factor for progression of prostate cancer.

Subjects and methods Of the 1417 patients with prostate cancer who had RP at two University hospitals, 1302 were included in the present study. BMI (kg/m2) classes were defined as normal (<25), overweight (25-30) and obese (≥30). The median follow-up was 59 months and clinical data were obtained retrospectively from charts. BCR was defined as two consecutive prostate-specific antigen (PSA) levels of >0.1 ng/mL.

Results In all, 600 patients were classified as having normal weight (43.9%), 665 as overweight (48.6%) and 103 as obese (7.5%). Overall, 297 patients developed BCR after RP; the 10-year risk (95% confidence interval) of BCR was 31.9% (26.6-37.2%), 30.5% (25.8-35.2%) and 23.9% (14.9-32.9%) for patients in the three categories, respectively (P=0.836). Multivariable proportional hazard regression analyses of BMI and established prognostic factors for BCR did not change these results.

Conclusion BMI appeared to have no prognostic value for BCR in Dutch patients with clinically localized prostate cancer and treated with RP.

Introduction

Prostate cancer and obesity are among the most common health problems currently affecting European men. Obesity is now so common among the world's population that it is beginning to replace undernutrition as the most significant contributor to ill health[1]. Moreover, obesity has enormous public health consequences because it not only increases the risk of several chronic diseases like diabetes, hypertension, coronary heart diseases and certain cancers, but it also imposes a large burden on healthcare use and costs.

The relationship between obesity and prostate cancer is debatable, with some studies indicating that obesity is associated with a decreased risk of prostate cancer[2, 3] and other studies suggesting an increased risk[4, 5]. However, recently two large American multi-institutional studies addressed a significantly higher biochemical recurrence (BCR) rate among obese men treated with radical prostatectomy (RP)[6, 7].

Almost all studies on obesity and the risk of death from prostate cancer or BCR are conducted in the USA; this might be important, because not only is the incidence of obesity much higher in the USA than in Europe, but the mean body mass index (BMI) of obese patients is also higher[8]. In addition, the USA population partly consists of African-Americans, who are more prone to be obese and have higher-grade prostate cancers. Therefore, the USA population has a distinct composition and characteristics compared to the European population. Thus, we analysed men who had RP for clinically localized prostate cancer at two university hospitals (Radboud University Medical Centre, Nijmegen and Erasmus MC, University Medical Center, Rotterdam) in the Netherlands to evaluate the relationship between obesity and risk of BCR.

Subjects and methods

The study population consisted of patients who had RP and for whom the medical records were reviewed retrospectively; 542 patients were treated at the Radboud University Medical Centre between 1992 and 2005, and 875 at the Erasmus University Medical Center between 1988 and 2007. Excluded were patients who had preoperative androgen deprivation or radiotherapy, and those with missing data for height or weight; in all, 49 patients were excluded. For analysis of the risk of BCR, patients with incomplete follow-up data, positive lymph nodes or PSA levels that did not reach a nadir of <0.1 ng/mL were also excluded (n=66). This resulted in a study population of 1302 patients. Preoperative height and weight data were collected retrospectively by reviewing anaesthesia records. The BMI was calculated as usual (kg/m^2), and according to the WHO categories[9], patients were stratified into three groups, i.e. normal weight (<25) overweight (25–30) and obese (≥30).

In general, patients were seen every 3 months during the first year, every 6 months during the second and third year, and yearly thereafter unless there was evidence of cancer recurrence, in which case more frequent follow-up visits were necessary. The serum PSA level was obtained before surgery and at every follow-up visit. BCR was defined as two subsequent PSA levels of >0.10 ng/mL or if a second treatment after RP was needed. The time to BCR was measured from the date of RP until the date of the first PSA level of >0.10 ng/mL.

All RP specimens were fixed overnight, inked, embedded and processed according to well-established protocols[10]. Pathological staging and examination (seminal vesicle invasion, extracapsular extension, margin status and Gleason scores) were done by two specialized genitourinary pathologists (CAHK and GJL). The presence of tumour cells in the inked resection margin was considered a positive surgical margin. All stages were converted to the TNM staging criteria, using the 2002 American Joint Committee on Cancer classification.

Associations between the predefined BMI subgroups and clinical or pathological characteristics were examined using chi-square tests for categorical characteristics and Mann-Whitney U-tests or Kruskal-Wallis tests for continuous characteristics. The risk of BCR was assessed with the Kaplan-Meier method, using the log rank-test to compare subgroups. A Cox proportional hazard model was used for multivariable analyses. Differences were considered to be statistically significant if $P < 0.05$.

Results

Table 5b.1 summarizes the clinical and pathological characteristics of the study population stratified by preoperative BMI groups. The median (range) age of the patients at the time of RP was 63.1 (42.4-75.0) years, and the median BMI was 25.5 kg/m2. In all, 600 patients were classified as having normal weight (43.9%), 665 as overweight (48.8%) and 103 as obese (7.5%). The median follow-up was 59.3 months and the median Gleason score was 6.

Overall, 297 patients developed BCR after RP. The 10-year Kaplan-Meier risk (95% CI) of BRC was 30.5% (27.2-33.8%). Patients in the obese group had slightly lower recurrence rates than those in the normal weight group, but this was not statistically significant. The 10-year risk (95% CI) of BCR was 31.9% (26.6-37.2%), 30.5% (25.8-35.2%) and 23.9% (14.9-32.9%) for patients in the normal, overweight and obese groups, respectively (P=0.836; **Figure 5b.1**).

Table 5b.1 Patients and pathological characteristics

	Normal weight	Overweight	Obese	*P value*
Number of patients (%)	600 (43.9)	665 (48.6)	103 (7.5)	
Age (years)	63.0 (59.0-67.1)	63.3 (58.6-67.1)	62.1 (58.3-65.0)	0.127
BMI (kg/m^2)	23.5 (22.5-24.3)	26.9 (25.8-27.8)	31.4 (30.5-32.3)	
Pre-operative PSA level (ng/mL)	7.1 (4.8-11.1)	7.1 (4.6-11.8)	7.2 (4.6-10.2)	0.815
Follow-up (months)	58.9 (23.7-100.4)	52.9 (18.0-98.2)	54.8 (21.5-102.8)	0.191
Pathological stage				0.402
T2	404 (67.4)	419 (63.3)	68 (66.0)	
T3	167 (27.9)	213 (32.3)	28 (27.2)	
T4	28 (4.7)	30 (4.5)	7 (6.8)	
Pathological Gleason score				0.310
2-6	278 (56.2)	299 (52.7)	39 (47.6)	
7	170 (34.3)	221 (39.0)	37 (45.1)	
8-10	47 (9.5)	47 (8.3)	6 (7.3)	
Positive margins	208 (34.9)	256 (38.7)	42 (40.8)	0.276
Seminal vesicle involvement	67 (11.2)	55 (8.3)	10 (9.8)	0.776
Extracapsular extension	174 (29.5)	217 (33.3)	32 (31.7)	0.347
Lymph node involvement	18 (3.0)	11 (1.7)	2 (2.0)	0.270

Data presented as median (interquartile range) or number (%). Percentages may not add up to 100% because of missing values. Abbreviations: *BMI* body mass index, *PSA* prostate specific antigen.

Using Cox proportional hazards regression models, prognostic factors for the risk of BCR were evaluated by using univariable and multivariable analyses (**Table 5b.2**). Univariable regression analysis showed no significant association between the risk of BCR and obesity in obese (P=0.78) and overweight patients (P=0.67) compared with normal-weight patients. Likewise, in multivariable regression analysis for the risk of BCR, BMI did not appear to have any independent prognostic value.

Discussion

Obesity is a growing problem in Western countries; in Europe, the prevalence of obesity has more than doubled during the last two decades. Obesity accounts for up to 6% of direct health costs and >12% of indirect health costs of shortened lives, reduced productivity, and lowered incomes in Europe[11]. Simultaneously, since the introduction of PSA testing and the growing awareness of prostate cancer in men, the incidence and prevalence of localized prostate cancer has substantially increased and become a major health problem[12].

Several biological mechanisms have been proposed to explain the relationship between adiposity and the risk of prostate cancer. Adipose tissue is an active endocrine and metabolic organ. Beyond alterations in sex steroid hormones (increased serum concentrations of oestradiol and decreased serum concentrations of testosterone) it produces adipokines like leptin, IGF-1, interleukin-6 and vascular

endothelial growth factor. Alterations in sex steroid hormones and adipokines might contribute to the molecular basis of the association between obesity and prostate cancer. However, the exact role of obesity as related to the development of prostate cancer is not yet clear, although recent evidence suggests a particular role for obesity in prostate cancer progression. Mechanisms and the effect of obesity on prostate cancer have been reviewed in more detail elsewhere[13, 14].

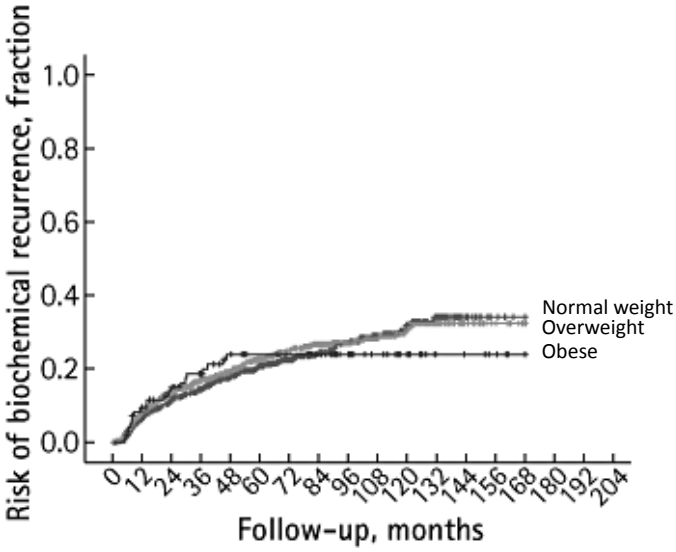

Figure 5b.1 Risk of biochemical recurrence following radical prostatectomy represented by Kaplan-Meier curves for normal weight, overweight and obese patients (P=0.836).

Two large European historical cohort studies reported a positive relationship between BMI and risk of prostate cancer[4, 5], while others found a protective influence of obesity on the development of prostate cancer[3, 15]. Most studies examined incident prostate cancer cases regardless of stage or grade. However, prostate cancer has a highly variable natural history, which can range from fast disease progression in months to a more indolent tumour in which survival can be measured in decades.

Although the relationship between obesity and prostate cancer risk has been indistinct, recently more consistent results were published on the positive association between obesity and prostate cancer mortality[16, 17]. Recent reports, all from the USA, showed poorer cancer control after RP, with a significantly greater propensity for higher grade disease, positive margins, and nodal and seminal involvement than in non-obese patients; the results of these studies are summarized in **Table 5b.3**[6, 7, 18-20].

Table 5b.2 Univariable and multivariable Cox proportional hazard analyses

	Hazard ratio (95% CI)	
	Univariable	**Multivariable**
Age (continuous)	1.03 (1.01–1.05)	
Preoperative PSA (continuous)	1.05 (1.04–1.07)	
Gleason score		
≤6	1	1
7	3.11 (2.28–4.25)	2.33 (1.68–3.22)
≥8	7.72 (5.24–11.38)	3.90 (2.54–5.98)
Pathological stage		
T2	1	1
T3 (vs T2)	3.44 (2.69–4.40)	1.21 (0.87–1.70)
T4 (vs T2)	5.49 (3.75–8.02)	2.03 (1.23–3.36)
Positive surgical margins, yes (vs no)	3.95 (3.12–5.00)	
Seminal vesicle invasion, yes (vs no)	5.46 (4.15–7.19)	
Extracapsular extension, yes (vs no)	3.50 (2.77–4.42)	
BMI, kg/m^2 (categorical)		
25	1	1
25–30	1.05 (0.83–1.34)	0.98 (0.74–1.29)
≥30	0.94 (0.59–1.48)	0.72 (0.40–1.30)
BMI (continuous)	1.00 (0.97–1.04)	
Year of surgery (continuous)	0.94 (0.91–0.97)	

Abbreviations: *BMI* body mass index, *CI* confidence interval, *PSA* prostate specific antigen.

This suggests that obesity has a more prominent role in aggressiveness and progression rather than in the development of prostate cancer. These five studies from the USA reported a higher risk of BCR in obese than in non-obese patients within the first 5 years. Combining these studies, the risk of BCR within 5 years was 23.1% for the non-obese and 31.8% for the obese patients.

The present study could not confirm this relationship, as the 5-year risk of BCR was 23.9% for obese patients vs 20.7% in the normal-weight patients. There are several explanations for this difference. First, all five previous studies cited were in the USA; by contrast with these studies, in which 20-25% of men were obese, the present study had only 7% of obese patients in the study population. This is not surprising, when assessing the National Health and Nutrition Examination Survey; in 2004 the prevalence of obesity among American men was ≈32%[21]. According to the Netherlands Health Interview Survey the prevalence of obesity in Dutch men was only 9%[22]. The present study population had not only fewer obese patients, but more importantly, the obese patients in the study weighed less than those in the American studies listed in **Table 5b.3**. The higher degree and frequency of obesity might translate into 'fatter' fat cells, which might produce a greater quantity of adipokines[23]. This in their turn might result in a higher risk of BCR.

Table 5b.3 A comparison of American studies reporting a relationship between BCR and obesity

Reference	6	7	18	19	20
Number of patients	3162	1106	2131	5631[a]	526
Mean follow-up (months)	31	46	23	54	54
Mean BMI (kg/m^2)	NA	27.5	27	NA	27.8
African American (%)	21.4	25.9	24.9	7.0	13.7
Obese (%)	19.0	22.4	22.0	33.3	24.9
Positive margins (%)					
normal	29.3[b]	28.5	NA	11.1	15.0
obese	35.0	36.3	NA	18.0	18.6
Positive nodes (%)					
normal	3.0[b]	1.8	NA	2.3	3.1
obese	3.8	0.4	NA	2.5	2.3
Positive seminal vesicles (%)					
normal	8.0[b]	8.8	NA	2.8	7.8
obese	10.0	8.5	NA	4.0	13.8
Gleason score ≥8 (%)					
normal	44.3[b c]	3.9	5.8	5.3	20.3
obese	52.4[c]	8.1	5.1	6.8	19.2
BCR at 5 years (%)					
non-obese	32.5	32.0	22.0	10.0	18.3
obese	37.5	40.0	30.0	22.0	28.5
Cox proportional hazard ratio (95% CI) for BMI					
≥30 vs <30 kg/m^2	1.20 (1.02-1.42)[d]	-	1.31 (1.00-1.71)[d]	-	-
P value	0.028	-	0.046	-	-
at diagnosis (continuous)		1.03 (1.01-1.06)[d]	1.20 (1.02-1.41)[e]	-	1.07 (1.02-1.13)[e]
P value		0.014	0.028	-	0.001
30–35 vs <25 kg/m^2		1.19 (0.86-1.64)[d]	-	-	-
P value		0.296	-	-	-
>35 vs <25 kg/m^2		1.99 (1.21-3.27)[d]	1.69 (1.01-2.84)[e]	-	-
P value		0.006	0.047	-	-
≥30 vs <25 kg/m^2		-	-	2.04 (1.61-2.58)[d]	2.35 (1.43-3.86)[df]
		-	-	1.91 (1.51-2.44)[e]	
P value		-	-	<0.001	0.001

Abbreviations: *BCR* biochemical recurrence, *BMI* body mass index, *CI* confidence interval, *NA* not available.
[a] Patients in the normal weight and overweight cohorts were matched 1:1 to the cohort of obese patients on the basis of propensity scores; [b] Normal and overweight groups are combined; [c] Pathological Gleason score ≥7; [d] univariable; [e] multivariable; [f] at age 40 years.

Second, by contrast with the Dutch population, the population of the USA includes African-American men. As an ethnic group, African-American men, who are also more obese, have a significantly higher incidence of prostate cancer and of mortality rates than white men[24]. For example, in the study of Amling *et al.*[6] black race and BMI were

associated with higher BCR in the univariable analysis, but in multivariable analysis only black race remained significant. It is tempting to speculate that increased rates in African-American men might in part explain the differences in BCR after RP, especially because African-Americans are on average more obese and are more prone to have aggressive tumours.

In the present study, obese men more often had (although not significantly) positive margins (40.8%) and T4 tumours (6.8%) than had men with a normal weight (34.9% and 4.7%, respectively). Nevertheless, the risk of BCR was not higher in the obese men. This might be related to the rather few obese patients in the present study. Interestingly, if there was BCR, the mean time to develop BCR was much shorter in the obese men, at 19.9 months, than in normal weight men, at 37.7 months.

The limitations of the present study are, first, the use of BCR as a surrogate of cancer-specific survival. This is important because it was reported previously that BCR can occur late in the postoperative course, and that the presence of BCR is not always a good predictor of prostate cancer-specific death[25]. A 10-year cohort study by Siddiqui *et al*.[26] reported that despite worse pathological features at the time of RP in obese patients, the long-term cancer-specific survival remained the same regardless of BMI. Second, additional quantitative measures of obesity, such as waist-to-hip ratio (calculated as the ratio of waist circumference, at the level midway between the lower rib margin and iliac crest, over the hip circumference at the maximum circumference over the buttocks) and waist circumference were not available. In clinical practice and epidemiological studies, body fat is most commonly estimated using BMI. Abdominal fatness, which is more metabolic active, is best measured by the waist-to-hip ratio or waist circumference. This is particularly true in patients aged >75 years[27]; in the present study most patients were much younger. Third, the height and weight data used were recorded from the anaesthesia records, as reported by the patients at the time of surgery, and introduced the possibility of a bias.

In conclusion, obese patients undergoing RP in two Dutch academic hospitals had no worse pathological characteristics, and had no significantly greater risk of developing BCR.

Conflict of interest

None declared.

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Chapter 6

Selenium affects expression of genes implicated in inflammation and epithelial-to-mesenchymal transition in the prostate

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Purpose Selenium has been considered a chemopreventive agent for prostate cancer. The exact mechanisms of chemoprevention by selenium, however, are not fully understood. We conducted a randomized, placebo-controlled intervention trial to examine the effects of a short-term intervention with selenium on gene expression profiles in non-malignant prostate tissue.

Subjects and methods Twenty-three men scheduled for a prostate needle biopsy were randomly assigned to take 300 µg selenized yeast per day (n=12) or a placebo (non-selenized yeast, n=11) during a median intervention period of 35 (interquartile range: 31-35) days. Prostate biopsy specimens of 15 participants, collected from the transition zone before and after the intervention period, were available for whole-genome microarray expression analyses (n=8 selenium, n=7 placebo).

Results Pathway analyses revealed that the intervention with selenium resulted in a down-regulated expression of genes involved in signalling pathways related to inflammation. Furthermore, expression changes were observed for genes involved in cellular growth, proliferation and development. More specifically, expression of several epithelial markers was up-regulated, whereas expression of mesenchymal markers was down-regulated after the intervention with selenium. The latter finding implies a possible effect of selenium on the epithelial-to-mesenchymal transition.

Conclusion Our data showed that selenium intake induced an anti-inflammatory gene expression profile and affected expression of genes implicated in epithelial-to-mesenchymal transition in non-malignant prostate tissue.

Introduction

Prostate cancer is the most common cancer and an important cause of death among men in Western countries[1, 2]. Effective prevention strategies may be considered the optimal approach to reduce the incidence and mortality of prostate cancer. Selenium has been previously suggested as a likely chemopreventive agent[3-5], although more recent observational and intervention studies did not consistently report a protective effect of selenium for prostate cancer[6-8]. The Nutritional Prevention of Cancer (NPC) trial showed that a daily intake of 200 µg selenium (as selenized yeast) reduced the incidence of prostate cancer by 52% as compared to the placebo group[4]. Contrary, results of the Selenium and Vitamin E Cancer Prevention (SELECT) trial, which aimed to determine whether selenium, vitamin E or a combination of both prevents the development of prostate cancer among the general population, demonstrated that selenium (200 µg per day) did not decrease the incidence of prostate cancer as compared to the placebo group[7, 9]. The provided form of selenium (*L*-selenomethionine) and the relatively high serum selenium levels (~135 µg/L) at baseline might explain the null-findings of the SELECT trial[7, 10, 11].

In parallel with the inconsistency in observational studies and chemoprevention trials, the molecular mechanisms by which selenium might possibly lower prostate cancer risk have not been elucidated and warrant further research. It is known that supplementation with *L*-selenomethionine[12] or selenized yeast[13, 14] results in increased levels of selenium in the prostate. Furthermore, it has been shown that supplementation with 200 µg *L*-selenomethionine per day induces changes in the gene expression profile in prostate tissue[15]. Whether these changes in gene expression underlie a possible chemopreventive effect is not clear, because *L*-selenomethionine itself did not show preventive effects for prostate cancer in the SELECT trial[7]. Other forms of selenium, such as selenized yeast, have been proven to be effective in intervention trials[3, 4], however, detailed information on the molecular effects in prostate tissue is lacking. The aim of the current study was therefore to obtain more insight into the molecular effects of selenium in the prostate by examining gene expression profiles in non-malignant prostate tissue before and after a short-term intervention with selenized yeast in a randomized, double-blind and placebo-controlled intervention trial.

Subjects and methods

Subjects

We recruited participants from the departments of Urology and Radiation Oncology of the Radboud University Medical Centre, an academic tertiary referral centre in the

Netherlands. Men scheduled for diagnostic prostate biopsies, and subsequent treatment with radical prostatectomy (RP) or radiotherapy (RT) for prostate cancer were invited for this study. Also, men scheduled for re-biopsies because of high-grade prostatic intraepithelial neoplasia (HGPIN) or suspicion of malignancies in previous biopsies were eligible for inclusion.

Exclusion criteria were current use of dietary supplements providing more than the recommended daily allowance of 55 µg selenium per day[16], any malignancy in the preceding five years (except for non-melanoma skin cancer), current hepatic or renal disease or inflammatory bowel disease, and neo-adjuvant therapies for prostate cancer. From June 2007 through October 2010, 281 men were assessed for their eligibility to participate in this study. Of these, 23 men were enrolled (**Figure 6.1**).

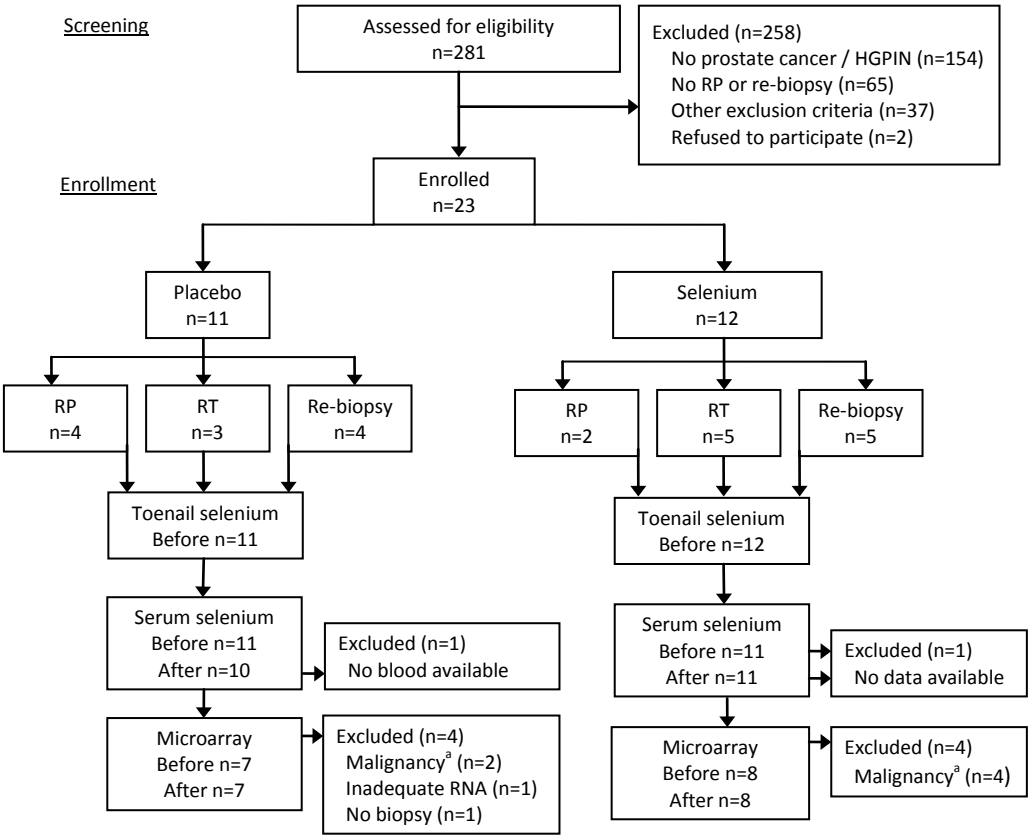

Figure 6.1 Study flow chart

Abbreviations: *HGPIN* high-grade prostatic intraepithelial neoplasia, *RP* radical prostatectomy, *RT* radiotherapy [a] Malignancy in the study biopsy.

Design of the study

Before start of the intervention, prostate tissue, blood samples and toenail clippings were collected and weight and height were measured. Body mass index (BMI) was calculated as weight (kg) divided by squared height (m^2). Furthermore, participants filled out a baseline questionnaire on sociodemographic characteristics, medical history, medication use and dietary supplements.

During the intervention period with an intended duration of five weeks, participants were asked to take daily supplements with selenium or placebo. At the end of the intervention period, prostate tissue and blood samples were collected for 22 participants. Sample collection for one participant failed because of logistic reasons. All participants returned remaining blisters and their completed research diaries, in which details concerning the use of study supplements were registered. The institutional review board approved this study and all participants provided written informed consent. This trial has been registered at clinicaltrials.gov with identifier NCT00446901.

Intervention

Participants were randomly assigned using a permuted-block design (blocks of four participants) to take 300 µg selenized yeast per day (SelenoPrecise®, Pharma Nord, Vejle, Denmark) or a placebo (non-selenized yeast tablet, Pharma Nord, Vejle, Denmark). These tablets were previously used in the PRECISE Trial pilot studies[17, 18]. Compliance was assessed by checking research diaries, counting returned tablets and measuring serum selenium levels before and after intervention. The intervention period ranged from 9 days to 6 weeks, depending on the time between enrollment and final treatment or re-biopsy.

Blood sampling

Blood samples were collected before and after the intervention period. All samples were processed within two hours after withdrawal and analysed directly or stored for further analyses. For selenium analyses, blood was collected into 10-ml serum tubes (Becton Dickinson B.V., Breda, the Netherlands). Serum was collected after centrifugation and stored at -20°C until analyses.

Selenium analyses

Serum selenium levels were measured using an atomic absorption spectrometer (model 4100ZL, PerkinElmer, Groningen, the Netherlands) coupled with a graphite furnace and using Zeeman background correction[19]. The detection limit for the method was 0.10 µmol/L. For each analytical run, a series of standards (CertiPur® AAS standards, no. 1197960100, Merck Chemicals, Darmstadt, Germany) and a control

(Pathonorm-High^{TM}, SERO AS, Billingstad, Norway) were included. All samples were analysed in triplicate. Mean serum selenium levels were reported in µmol/L. During our study, serum selenium analyses in the study centre were discontinued and outsourced to an external laboratory (Algemeen Medisch Laboratorium, Antwerpen, Belgium). As a result, a few samples (n=3) were analysed using a different method, i.e. inductively coupled plasma mass spectrometry (ICP-MS). One participant for whom both serum samples (before and after the intervention) were analysed using ICP-MS was included in the analyses. Another participant, for whom only the sample collected after intervention was analysed using ICP-MS, was excluded.

Baseline toenail selenium levels were assessed using Instrumental Neutron Activation Analyses (INAA)[20] at the Reactor Institute of Delft University, Delft, the Netherlands. Briefly, the specimens were irradiated for 17 seconds in a thermal flux (3.2E+12 $cm^{-2}s^{-1}$). After a decay of 3 seconds, γ-radiation of Se-77 was measured for 17 seconds. This measurement is repeated 6 times (cyclic NAA) with 3 seconds between each cycle. Levels of toenail selenium were reported in mg/kg. A certified bovine liver standard (Standard Reference Material 1577b, National Institute of Standards and Technology, Gaithersburg, USA) was analysed together with the toenail samples. For this standard, a mean selenium concentration (standard uncertainty) of 0.71 (0.04) mg/kg was observed against a mean certified value (standard deviation) of 0.73 (0.06) mg/kg.

Collection of prostate tissue

Prostate tissue was collected using an 18-gauge biopsy needle (Bard Biopsy Systems, Tempe, USA) during regular prostate needle biopsy series guided by transrectal ultrasound (TRUS) or during RP. A biopsy was taken from the superior, ventral region of the left lobe of the prostate, which is specified as the transition zone. The biopsy was embedded in Optimal Cutting Temperature (O.C.T.) Compound (Sakura Finetek Europe B.V., Zoeterwoude, the Netherlands) and frozen in liquid nitrogen. All samples were stored at -80°C.

Histology

The biopsies embedded in O.C.T. Compound were sectioned at -20°C. Representative sections of 5 µm were used for histological examinations; at least two sections were used for a Haematoxilin-Eosin (HE) staining, while three sections were stored at -80°C for additional immunohistochemical research. The remainder of the biopsy was sectioned at 20 µm. These sections were stored in a 12-ml polystyrene tube (Greiner Bio-One, Alphen aan den Rijn, the Netherlands) and used for RNA extraction. All HE-stained slides were reviewed for malignancy, HGPIN and inflammation by an independent and experienced uropathologist (CHK).

RNA extraction

Total RNA was extracted from the sectioned prostate biopsies using TRIzol Reagent according to the manufacturer's instructions (Invitrogen, Breda, the Netherlands). Isolated RNA was purified using RNeasy Micro columns (Qiagen, Venlo, the Netherlands). RNA integrity (RNA 6000 Nanochips for the Agilent 2100 Bioanalyzer, Agilent Technologies) and total RNA yield (Nanodrop ND 1000, Nanodrop products, Wilmington, USA) were assessed for all samples. The mean RNA integrity (RIN) score was 8.2 (standard deviation: 0.66). Prostate biopsies with inadequate RNA yield (<20 ng/µl, n=1) or histological evidence of adenocarcinoma (n=6) were excluded, leaving biopsies of 15 participants available for microarray analyses (n=8 selenium, n=7 placebo).

Microarray analyses

A total of 30 RNA samples were processed for microarray analyses. Briefly, 100 ng of total RNA per sample was labeled using an Ambion WT Expression kit (Austin, TX, USA) and hybridized to Affymetrix GeneChip Human Gene 1.0 ST Arrays. Probe sets were redefined according to Dai et al.[21] using the remapped chip description files (CDF) version 13.0.1 based on the Entrez Gene database. The signal intensities were expressed as Robust Multichip Average (RMA) expression values[22, 23]. Genes with RMA expression values >20 in at least 4 arrays were considered as expressed in prostate tissue and were selected for further analyses. Ratios of the log(base2) transformed intensity signals were used to compare the individual microarray data before and after the intervention. Changes in gene expression within the intervention groups were considered statistically significant if the p-value derived from a two-tailed, paired *t* test with Bayesian correction (Limma) was below 0.05[24]. Differentially changed genes between the intervention groups were identified using a one-way ANOVA with Bayesian correction (Limma p-value <0.05). Regulated pathways were identified through the use of IPA version 9.0 (Ingenuity® Systems, www.ingenuity.com). Canonical pathways with a p-value <0.01 in the Fisher's exact test were considered as significant to the data. Gitools software was used to construct correlation matrices showing the Pearson correlation coefficients for the individual signal-log-ratios of selected genes within each of the intervention groups[25].

Statistical analyses

Since levels of selenium after intervention, duration of intervention and a number of other participants' characteristics were not normally distributed, data were summarized as median and interquartile ranges (IQR) or numbers and percentages. Baseline serum and toenail selenium levels were compared for the selenium group and the placebo group using the Mann-Whitney U test. Serum selenium levels after

intervention were compared to baseline values using the Wilcoxon Signed Rank test. All statistical tests were two-sided and p-values below 0.05 were considered as statistically significant. Statistical Package for Social Sciences (SPSS version 19, Chicago, Illinois) was used for all analyses unless otherwise stated.

Results

In total, 23 participants were enrolled in this study; 12 participants were randomized to the selenium group and 11 participants to the placebo group **(Figure 6.1)**. Baseline characteristics of the participants are presented in **Table 6.1.** The median duration of the intervention period was 35 days (IQR: 31-35) and ranged from 9-42 days. Median compliance, as assessed by checking pill count and diaries, was 100% (range 94-100%).

Table 6.1 Characteristics of the participants at baseline

	All	Placebo	Selenium
Sociodemographic			
Number of participants	23	11	12
Age at start intervention (years)	67.5 (65.0-72.3)	69.5 (63.0-72.6)	67.1 (65.2-71.2)
Body mass index (kg/m^2)	26.2 (24.2-28.1)	26.2 (24.7-28.5)	26.4 (23.8-28.0)
Smoking			
Current	4 (17%)	1 (9%)	3 (25%)
Former	13 (57%)	6 (55%)	7 (67%)
Never	6 (26%)	4 (36%)	2 (8%)
Use of dietary supplements (current)	7 (30%)	4 (36%)	3 (25%)
Clinical			
Prediagnostic PSA levels (ng/mL)	8.0 (4.5-10.3)	7.7 (3.8-11.0)	9.4 (6.0-10.2)
Diagnosis			
No evidence of malignancy	1 (4%)	-	1 (8%)
HGPIN	5 (22%)	4 (36%)	1 (8%)
Prostate cancer	17 (74%)	7 (64%)	10 (83%)
Gleason score at biopsy[a]			
<7	10 (44%)	3 (27%)	7 (58%)
7	3 (13%)	2 (18%)	1 (8%)
>7	4 (17%)	2 (18%)	2 (17%)
Type of treatment / clinical follow-up			
Re-biopsy	9 (39%)	4 (36%)	5 (42%)
Radical prostatectomy	6 (26%)	4 (36%)	2 (17%)
Radiotherapy	8 (35%)	3 (27%)	5 (42%)
Intervention			
Duration of intervention period (days)	35 (31-35)	35 (34-35)	33 (28-35)
Time between collection of prostate tissue (days)	64 (35-98)	65 (36-98)	64 (33-96)

Data presented as median (interquartile range) or numbers (%) Abbreviations: *HGPIN* high-grade prostatic intraepithelial neoplasia, *PSA* prostate specific antigen. [a] Only for patients with prostate cancer.

Table 6.2 Median selenium levels in toenail and serum at baseline and after intervention

		Placebo (n=11)	Selenium (n=12)
Toenail selenium (mg/kg)	Baseline	0.45 (0.37-0.50)	0.43 (0.37-0.48)
Serum selenium levels (µmol/L)	Baseline[a]	1.06 (0.92-1.18)	1.00 (0.92-1.08)
	After intervention[a,b]	1.11 (0.95-1.25)	2.36 (1.74-2.98)[c]

Data presented as median (interquartile range). [a] One participant from the selenium group was excluded because serum levels at baseline and after intervention were measured using two different analytical methods, [b] Ten participants in the placebo group, because sample collection after the intervention period failed for one participant, [c] Statistically significant if compared to baseline levels (p=0.004, Wilcoxon Signed Rank test).

Levels of selenium

Baseline levels of serum (p=0.562) or toenail (p=0.449) selenium did not differ between the two intervention groups **(Table 6.2)**. As compared to baseline values, the levels of serum selenium after intervention were increased in the selenium group (median increase 1.44 µmol/L, IQR: 0.66-1.92, p=0.004), but not in the placebo group (median increase 0.02 µmol/L, IQR: -0.04-0.18, p=0.314).

Microarray analyses

Good quality RNA from prostate biopsies without evidence of malignancy was available before and after the intervention period for 15 participants, resulting in a total of 30 microarrays used in this study **(Figure 6.1)**. After RMA normalization and filtering, 18,398 genes were considered expressed and were included in further analyses **(Figure 6.2)**. Comparisons between the two intervention groups, based on a one-way ANOVA, showed that 2740 genes were differentially changed between the selenium and placebo group (ANOVA Limma p-value <0.05). Subsequent within-group comparisons of individual gene expression profiles before and after intervention revealed that of these genes, expression of 910 genes (522 down-regulated and 388 up-regulated) changed in the selenium group (**Supplementary table 6.A**). In the placebo group expression changes were observed for 1368 genes, of which 660 were down-regulated and 708 up-regulated (**Supplementary table 6.B**).

Pathway analyses

In order to elucidate the roles of the differentially expressed genes, pathway analyses were conducted. As shown in **Figure 6.2**, expression changes in the selenium and placebo group were observed for genes involved in 49 and 77 pathways, respectively (Fisher's exact p-value <0.01). The top-10 of the up- and down-regulated pathways is presented in **Figure 6.3**.

The intervention with selenium resulted in a down-regulated expression of genes involved in signaling pathways related to inflammation, cellular immune response and cellular growth, proliferation and development, while a number of metabolic pathways related to amino acid metabolism or carbohydrate metabolism were up-regulated. In the placebo group, opposite effects were observed; expression of genes involved in pathways related to inflammation was up-regulated, while metabolic pathways were down-regulated.

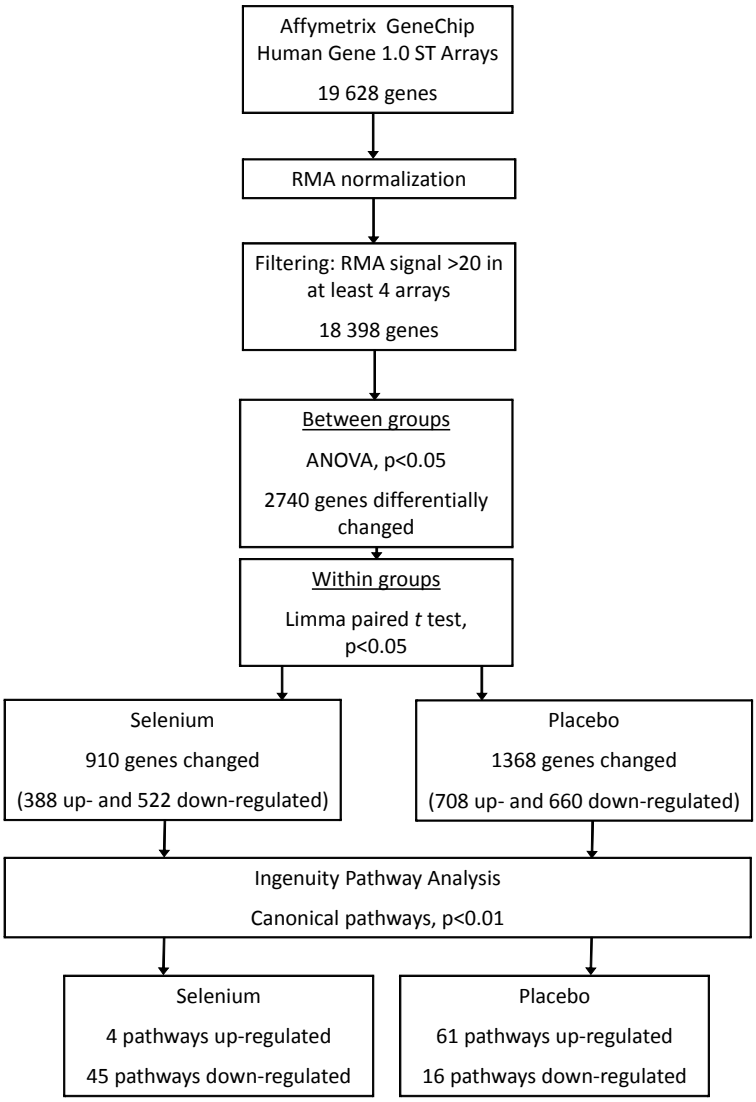

Figure 6.2 Flow chart of gene selection in the microarray analyses

Abbreviations: *ANOVA* analysis of variance, *RMA* robust multichip average. Up- or down-regulated pathways were defined as canonical pathways with a p-value <0.01 in the Fisher's exact test.

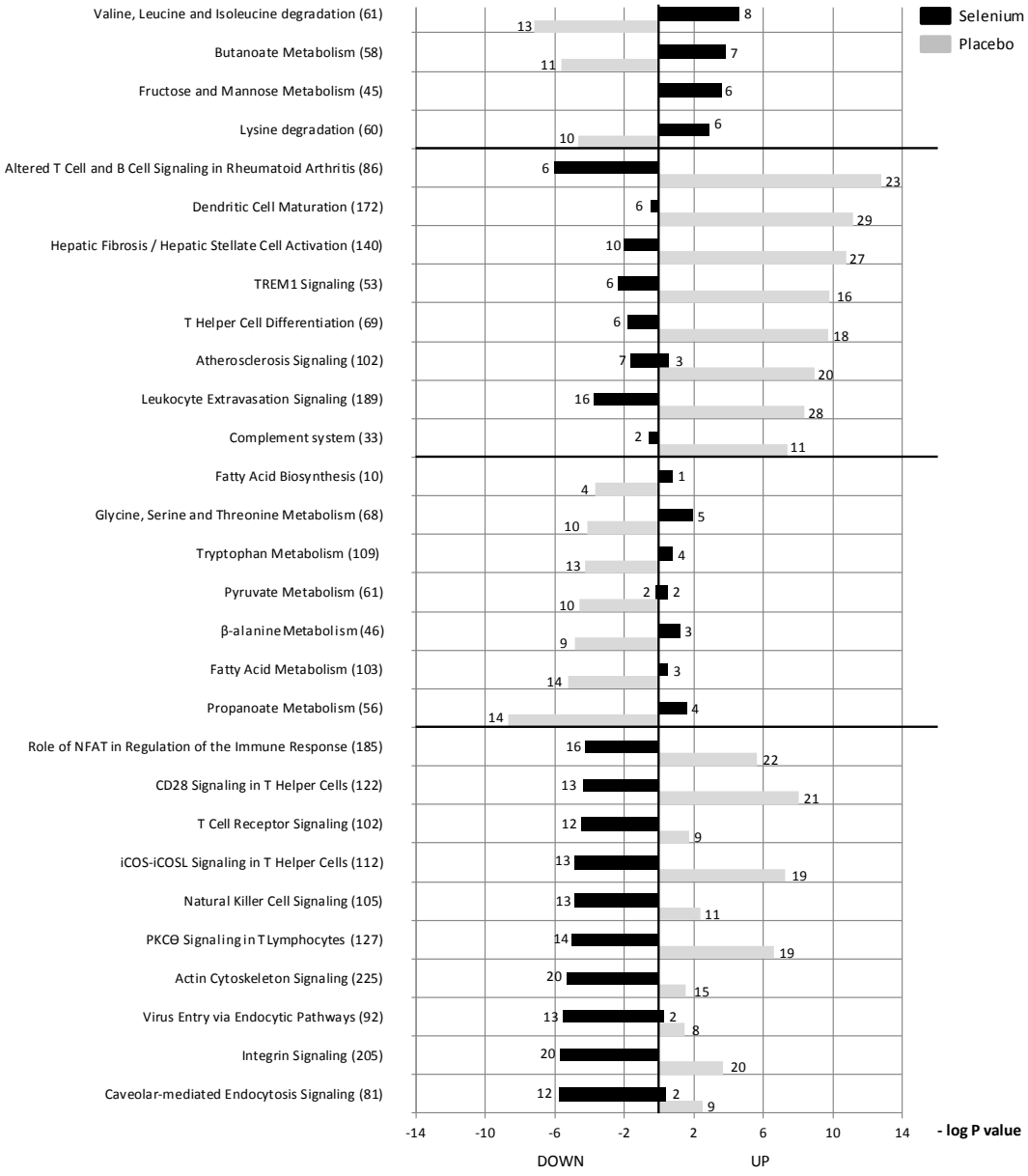

Figure 6.3 The top-10 pathways, identified by Ingenuity Pathway Analysis, which are most significantly up- or down-regulated by the intervention with selenium or placebo. The numbers behind the pathways indicate the number of genes that belong to that pathway and the numbers behind the bars represent the number of differentially expressed genes within that pathway (these genes had a p-value <0.05 in the within group and between group comparisons). Pathways with a p-value >0.01 in the Fisher's exact test were not listed in this figure.

One of the major pathways down-regulated by selenium was the integrin signalling. Further focus on integrin-related genes in the dataset revealed expression changes in other cell adhesion molecules, such as cadherins, selectins and members of the immunoglobulin superfamily as well. An overview of these cell adhesion molecules and their corresponding expression changes is visualized in **Table 6.3**. Specific cell adhesion molecules are suggested to be involved in the epithelial-to-mesenchymal transition (EMT), a process which is characterized by the transition of an epithelial phenotype towards a mesenchymal phenotype and is implicated in cancer progression. The intervention with selenium resulted in an up-regulation of E-cadherin expression and a down-regulation of N-cadherin (not statistically significant) and OB-cadherin expression. In line with these findings, we also found a down-regulated expression of other mesenchymal markers, such as vimentin and fibronectin, and an up-regulation of other epithelial markers (syndecan-1) after the intervention with selenium. An overview of the expression changes of several genes implicated in EMT is presented in **Table 6.4**.

In order to explore the consistency of our findings, we correlated the expression changes (signal-log-ratios) of selected cell adhesion molecules and EMT-related genes amongst each other. The corresponding Pearson correlation matrix (**Figure 6.4**) showed that expression changes in epithelial markers were inversely correlated to the expression changes in mesenchymal markers and the majority of the cell adhesion molecules in the selenium group.

Discussion

Our study demonstrated that a short-term intervention with selenium induced an anti-inflammatory gene expression profile and affected expression of genes implicated in EMT in non-malignant prostate tissue.

Inflammation

Several pathways associated with cellular immune response signalling were down-regulated by selenium, while these and other inflammatory pathways were up-regulated in the placebo group. Possible explanations for the inflammatory gene expression profile observed in the placebo group are a progressive inflammatory or tumour environment in the prostate, or a persisting effect of the first series of prostate biopsies. For example, increased expression of *BCL2* or *PTGS2 (*which encodes cyclooxygenase 2) is often associated with chronic inflammation in prostate tissue[28, 29]. In our study, both *BCL2* and *PTGS2* are strongly up-regulated in the placebo group, while there is a non-significant down-regulation of the expression of these genes in the selenium group, suggesting that in the latter group the inflammatory process is attenuated.

Table 6.3 Effects of selenium or placebo on gene expression of selected cell adhesion molecules

				Selenium			Placebo			Between groups
Cell adhesion molecules	Member	Entrez ID	Description / Location/ Synonym[1]	Individual SLR's	mean SLR	p-value	Individual SLR's	mean SLR	p-value	ANOVA p-value[2]
Integrins	ITGA1 (CD49a)	3672	Integrin α1 subunit		-0.51	0.008		0.009	0.96	0.05
	ITGA3 (CD49c)	3675	Integrin α3 subunit		-0.03	0.78		0.27	0.03	0.07
	ITGA4 (CD49d)	3676	Integrin α4 subunit		-0.32	0.05		0.45	0.01	0.003
	ITGA5 (CD49e)	3678	Integrin α5 subunit		-0.27	0.09		0.35	0.04	0.01
	ITGA6 (CD49f)	3655	Integrin α6 subunit		0.22	0.03		-0.02	0.87	0.10
	ITGA7	3679	Integrin α7 subunit		-0.57	0.03		0.14	0.58	0.05
	ITGA8	8516	Integrin α8 subunit		-1.00	0.007		0.5	0.17	0.005
	ITGA9	3680	Integrin α9 subunit		-0.43	0.02		0.23	0.23	0.02
	ITGAL (CD11a)	3683	Integrin α subunit		-0.37	0.01		0.31	0.04	0.003
	ITGAM (CD11b)	3684	Integrin α subunit		-0.18	0.05		0.4	<0.001	<0.001
	ITGAX (CD11c)	3687	Integrin α subunit		-0.14	0.39		0.58	0.003	0.006
	ITGB1 (CD29)	3688	Integrin β1 subunit		-0.20	0.01		0.16	0.05	0.004
	ITGB2 (CD18)	3689	Integrin β2 subunit		-0.10	0.40		0.65	<0.001	<0.001
	ITGB3 (CD61)	3690	Integrin β3 subunit		-0.47	0.01		0.3	0.08	0.003
	ITGB4 (CD104)	3691	Integrin β4 subunit		0.09	0.45		0.4	0.003	0.07
	ITGB6	3694	Integrin β6 subunit		-0.18	0.30		0.7	<0.001	0.003
	ITGB7	3695	Integrin β7 subunit		-0.19	0.03		0.28	0.006	0.001
Selectins	Selectin L (CD62L)	6402	Leukocytes		-0.49	0.02		0.42	0.06	0.005
	Selectin P (CD62P)	6403	Platelets and endothelial cells		-0.32	0.06		-0.05	0.74	0.27
	Selectin E (CD62E)	6401	Endothelial cells		-0.55	0.03		0.18	0.47	0.04
Cadherins	Cadherin E (CDH1)	999	Epithelial		0.36	0.02		-0.17	0.26	0.02
	Cadherin N (CDH2)	1000	Neuronal		-0.80	0.08		0.97	0.05	0.01
	Cadherin P (CDH3)	1001	Placental		0.24	0.08		0.30	0.04	0.73
	Cadherin 7 (CDH7)	1005			0.19	0.24		0.37	0.04	0.43
	Cadherin OB (CDH11)	1009	Osteoblast		-0.47	0.01		0.36	0.07	0.004
	Cadherin H (CDH13)	1012	Heart		-0.30	0.18		0.62	0.02	0.01
	Cadherin LI (CDH17)	1015	Liver/Intestine		0.29	0.30		-0.63	0.04	0.03
Immunoglobulin superfamily	PECAM1 (CD31)	5175	Platelet endothelial cell adhesion molecule		-0.18	0.27		0.5	0.01	0.01
	MCAM (CD146)	4162	Melanoma cell adhesion molecule		-0.43	0.03		0.23	0.26	0.02
	NCAM2 (CD56)	4685	Neural cell adhesion molecule		0.16	0.37		-0.54	0.01	0.01
	ICAM1(CD54)	3383	Intercellular cell adhesion molecule		-0.09	0.49		0.47	0.003	0.009
	EPCAM (CD326)	4072	Epithelial cell adhesion molecule		0.48	0.04		-0.29	0.22	0.03

Individual signal-log-ratios (SLRs) for each participant in the selenium group (n=8) or the placebo group (n=7) are presented in heatmaps. Down-regulation or up-regulation of gene expression is presented on a colour-scale ranging from green (down-regulated, SLR ≤ -0.5) to red (up-regulated, SLR ≥ 0.5). Abbreviations: *ANOVA* analysis of variance, *SLR* signal-log-ratio. [1]Based on NCBI Entrez Gene database (http://www.ncbi.nlm.nih.gov/gene) and references 26 and 27. [2]Comparisons between the selenium and placebo group are based on a one-way ANOVA with Bayesian correction (Limma).

Table 6.4 Effects of selenium or placebo on gene expression of selected genes involved in epithelial-to-mesenchymal transition

				Selenium			Placebo			Between groups
			Entrez ID	Individual SLR's	mean SLR	p-value	Individual SLR's	mean SLR	p-value	ANOVA p-value[1]
Epithelial markers	Cadherin E	CDH1	999		0.36	0.02		-0.17	0.26	0.02
	Syndecan-1	SDC1	6382		0.21	0.05		0.36	0.002	0.28
	Desmoplakin	DSP	1832		0.31	0.05		-0.12	0.47	0.06
Mesenchymal markers	Cadherin N	CDH2	1000		-0.8	0.08		0.97	0.05	0.01
	Cadherin OB	CDH11	1009		-0.47	0.01		0.36	0.07	0.004
	Vimentin	VIM	7431		-0.15	0.04		0.25	0.002	<0.001
	Fibronectin	FN1	2335		-0.2	0.33		0.58	0.01	0.02
	Alpha-actin-2	ACTA2	59		-0.24	0.009		0.07	0.42	0.02
Inducers of EMT - growth factors	Transforming growth factor	TGFβ1	7040		-0.25	0.04		0.32	0.01	0.002
		TGFβ2	7042		-0.37	0.05		-0.10	0.61	0.30
	Hepatocyte growth factor	HGF	3082		-0.53	0.006		0.39	0.04	0.002
	Fibroblast growth factor	FGF2	2247		-0.27	0.02		0.21	0.08	0.006
		FGF7	2252		-0.32	0.04		0.11	0.49	0.06
		FGF9	2254		-0.47	0.04		0.17	0.45	0.05
		FGF11	2256		0.12	0.03		0.12	0.04	0.94
Inducers of EMT - transcription factors	Snail homolog 1 (Drosophila)	SNAIL	6615		0.06	0.45		0.02	0.78	0.76
	Snail homolog 2 (Drosophila)	SLUG	6591		0.14	0.43		-0.17	0.36	0.24
	Twist homolog 1 (Drosophila)	TWIST	7291		-0.007	0.94		0.18	0.10	0.21
	Zinc finger E-box binding homeobox 1	ZEB1	6935		-0.30	0.10		0.10	0.60	0.13
	Zinc finger E-box binding homeobox 2	ZEB2	9839		-0.19	0.18		0.17	0.25	0.08
Inducers of EMT - other	Type 1 collagen	COL1A1	1277		-0.04	0.84		0.52	0.01	0.04
		COL1A2	1278		0.16	0.28		0.34	0.04	0.03
Pathways involved in EMT	Ras	MRAS	22808		-0.32	0.03		0.13	0.36	0.03
		RRAS	6237		-0.18	0.04		0.03	0.74	0.10
	Nuclear factor kappaB	NFκB2	4791		-0.08	0.39		0.26	0.02	0.02
		RELB	5971		-0.07	0.31		0.28	<0.001	0.002
		REL	5966		-0.11	0.23		0.19	0.07	0.04
	Integrin-linked kinase	ILK	3611		-0.25	0.03		0.05	0.65	0.07
	PIK3-AKT	PIK3CA	5290		-0.2	0.007		0.02	0.76	0.03
		PIK3CG	5294		-0.29	0.02		0.28	0.03	0.003
		AKT3	10000		-0.19	0.006		-0.04	0.61	0.10

Individual signal-log-ratios (SLRs) for each participant in the selenium group (n=8) or the placebo group (n=7) are presented in heatmaps. Down-regulation or up-regulation of gene expression is presented on a colour-scale ranging from green (down-regulated, SLR ≤ -0.5) to red (up-regulated, SLR ≥ 0.5). Abbreviations: *ANOVA* analysis of variance, *EMT* epithelial-to-mesenchymal transition, *SLR* signal-log-ratio. [1]Comparisons between the selenium and placebo group are based on a one-way ANOVA with Bayesian correction (Limma).

A wide range of previous studies described possible anti-inflammatory properties of selenium[30, 31]. A recent study showed that oral administration of selenium reduced prostatic inflammatory cell infiltration and interstitial fibrosis in a rat model for chronic bacterial prostatitis[32]. The exact mechanisms by which selenium might prevent against inflammation remain poorly understood. Traditionally, it has been suggested that antioxidant capacity of the selenoproteins, which attenuate oxidative stress and the formation of reactive oxygen species (ROS), play a pivotal role in the regulation of the anti-inflammatory effects[33, 34]. The nuclear factor kappaB (*NFκB*) pathway might play a central role in the effects of selenium and the selenoproteins on the immune system[35]. Selenium has been shown to inhibit activation of *NFκB* in various animal and human cell types,[36-39] among which prostate cancer cells[40]. In our study, expression of *NFκB* or any of its major down-stream targets was not changed after the intervention with selenium. Expression of a number of *NFκB*-related genes (e.g. *NFκB2, RELB, PTGS2),* however, was increased in the placebo group. This finding suggests that during the intervention period certain stimuli, such as a progressive inflammatory or tumour environment or persisting effects of the prostate biopsies, activated the *NFκB* pathway. It seems that selenium was able to effectively prevent activation of this pathway. Previous studies demonstrated that the selenium-dependent glutathione peroxidases were responsible for blocking *NFκB* activation through inhibition of IκBα phosphorylation and degradation[41]. In our study, however, expression of the selenium-dependent glutathione peroxidases was down-regulated (*GPX3, GPX7*) or unchanged after the intervention with selenium, although we cannot rule out the possibility that activity of these selenoproteins was increased. Others showed that *NFκB* activation can also be regulated by other selenoproteins, such as the thioredoxin reductases[42]. In our study, expression of the mitochondrial thioredoxin reductase 2 (*TXNRD2*) was significantly up-regulated after the intervention with selenium, while down-regulated in the placebo group.

Epithelial-to-mesenchymal transition

Besides the suggested induction of an anti-inflammatory gene expression profile, selenium also affected expression of genes involved in EMT. EMT is characterized by the transition of an epithelial phenotype towards a mesenchymal phenotype[43]. During EMT, the unique characteristics of epithelial cells are replaced by mesenchymal properties such as non-polarity and ability to migrate[43]. Some important molecular events that come along with the transition are loss of epithelial E-cadherin expression[44] and aberrant expression of vimentin, fibronectin and mesenchymal N-cadherin[43]. It is beyond the scope of this paper to describe the complete EMT process, however, excellent reviews have been published previously[43, 45, 46]. EMT plays an important role during embryogenesis and tissue repair or inflammation, however, it is

also considered as a critical process in cancer progression since the loss of cellular adhesion, the reorganization of the cytoskeleton and increased motility might result in tumour cell invasion and metastasis[47].

Several findings from our study suggest that selenium is able to prevent, inhibit or reverse the transition of the epithelial to the mesenchymal phenotype. First, expression of numerous well-established epithelial cell markers[45] was increased (E-cadherin, syndecan-1, desmoplakin), while expression of mesenchymal cell markers (N-cadherin, vimentin, OB-cadherin, α-actin2) was decreased after the intervention with selenium. Second, expression of inducers of EMT (transforming growth factor-β, hepatocyte growth factor, fibroblast growth factor, type 1 collagen) was down-regulated among the participants supplemented with selenium. Third, the canonical pathway related to actin cytoskeleton signalling, which is closely related to EMT[46], was strongly down-regulated by selenium. Our microarray data did not provide compelling evidence that expression of transcriptional regulators of E-cadherin, such as *SNAI1*, *SNAI2*, *ZEB1*, *ZEB2*, and *TWIST1*[46, 48], was changed after the intervention with selenium, although this finding does not rule out a regulatory role of these factors.

To the best of our knowledge, the role of selenium in the regulation of EMT has not been described in detail previously. One *in-vitro* study examined the effects of selenium (as sodium selenite) on features of EMT in human hepatoblastoma (C3a) cells treated with TGFβ1. The authors did not find changes in expression of vimentin or E-cadherin, although expression of type 1 collagen (*COL1A1*) was reduced in response to sodium selenite[49]. Others showed that sodium selenite inhibits the expression of *TGFβ1* in LPS-stimulated prostate cancer (PC3) cells[50]. Tsavachidou and colleagues examined gene expression profiles in distinct anatomical zones and cell-types of the prostate after a 3-6 week intervention with *L*-selenomethionine[15]. Results of their study indicated that genes related to the androgen receptor, tumour protein 53 (*p53*) and *NFκB* signalling pathways were differentially expressed between the patients supplemented with a daily dose of 200 μg *L*-selenomethionine or a placebo[15]. The authors did not explicitly report gene expression changes that could be linked to EMT. Prostate tissue, however, was only collected at a single time point, that is after the intervention period of 3-6 weeks[15]. In our study, we collected prostate tissue before and after the intervention period, which allowed us to compare changes in gene expression within individuals over time. This aspect can be considered as a major strength of our study, because variation due to inter-individual differences does not hinder the interpretation of our microarray data. Another strength of our study includes the ability to perform histological and gene expression analyses from one single prostate biopsy.

Potential limitations of our study are the limited number of participants and the collection of prostate tissue from the transition zone. We aimed to obtain non-malignant tissue in order to study preventive effects of selenium and to avoid possible aberrant effects from tumour cells. The transition zone was chosen, because only 20% of the prostatic adenocarcinomas arise from this zone, in comparison to 68% from the peripheral zone[51]. Furthermore, the transition zone is easily accessible for tissue collection during TRUS-guided biopsies as well as surgical biopsies during RP. The transition zone and the peripheral zones have been shown to differ in gene expression profiles[52, 53]. Therefore, the observed effects of selenium in the transition zone may differ from the effects in the peripheral zone, which is considered the primary zone of interest for chemoprevention.

Our finding that selenium is able to regulate expression of genes implicated in EMT might as such yield important therapeutic implications for prostate cancer, but also other EMT-related conditions such as organ fibrosis. However, it should be considered that EMT is involved in the progression of cancer, but is also initiated as a required response during tissue injury and inflammation[45]. Therefore, the relevance of our findings with respect to prostate cancer and other pathologies needs to be established. Furthermore, the underlying mechanisms need to be clarified and our data have to be validated. The latter also highlight the need for future studies focusing on the functional effects of selenium related to inflammation (e.g. inflammatory cell infiltration) and EMT (e.g. cellular migration or invasion).

In conclusion, our data suggest that selenium induces an anti-inflammatory gene expression profile and might be able to interfere in the process of EMT in non-malignant prostate tissue.

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Conflict of interest
The authors declare no conflicts of interest.

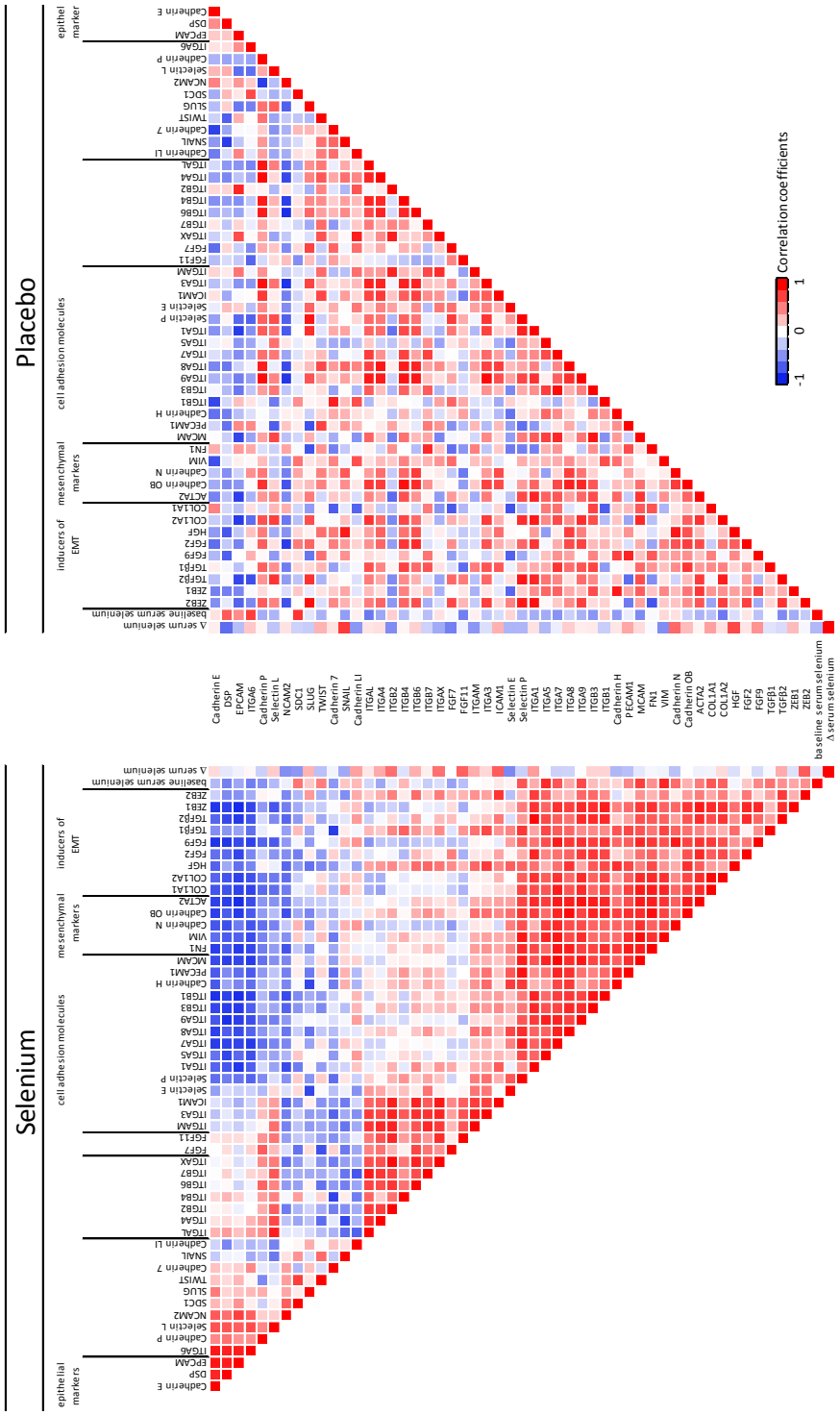

Figure 6.4 Correlation matrices of expression changes for selected genes involved in epithelial-to-mesenchymal transition and cell adhesion. Calculation of the Pearson correlation coefficient is based on the individual signal-log-ratios per intervention group. Abbreviations: *EMT* epithelial-to-mesenchymal transition.

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Supplementary Table 6.a

Entrez ID	Gene symbol	Description	Fold change	p-value
4057	LTF	lactotransferrin	-3.2	0.019
11075	STMN2	stathmin-like 2	-2.2	0.033
8549	LGR5	leucine-rich repeat-containing G protein-coupled receptor 5	-2.2	0.017
8516	ITGA8	integrin, alpha 8	-2.0	0.007
84189	SLITRK6	SLIT and NTRK-like family, member 6	-1.9	0.002
389658	FAM150A	family with sequence similarity 150, member A	-1.9	0.026
3624	INHBA	inhibin, beta A	-1.8	0.023
81610	FAM83D	family with sequence similarity 83, member D	-1.8	0.012
387758	FIBIN	fin bud initiation factor homolog (zebrafish)	-1.7	0.041
10371	SEMA3A	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, 3A	-1.7	0.012
343450	KCNT2	potassium channel, subfamily T, member 2	-1.7	0.006
50507	NOX4	NADPH oxidase 4	-1.6	0.019
9358	ITGBL1	integrin, beta-like 1 (with EGF-like repeat domains)	-1.6	0.034
3598	IL13RA2	interleukin 13 receptor, alpha 2	-1.6	0.017
5139	PDE3A	phosphodiesterase 3A, cGMP-inhibited	-1.6	0.021
157869	C8orf84	chromosome 8 open reading frame 84	-1.6	0.019
57575	PCDH10	protocadherin 10	-1.6	0.009
26002	MOXD1	monooxygenase, DBH-like 1	-1.6	0.013
2487	FRZB	frizzled-related protein	-1.6	0.010
4915	NTRK2	neurotrophic tyrosine kinase, receptor, type 2	-1.6	0.021
10129	FRY	furry homolog (Drosophila)	-1.6	0.029
27295	PDLIM3	PDZ and LIM domain 3	-1.6	0.009
10631	POSTN	periostin, osteoblast specific factor	-1.6	0.050
9369	NRXN3	neurexin 3	-1.6	0.026
27129	HSPB7	heat shock 27kDa protein family, member 7 (cardiovascular)	-1.6	0.008
7111	TMOD1	tropomodulin 1	-1.5	0.008
953	ENTPD1	ectonucleoside triphosphate diphosphohydrolase 1	-1.5	0.001
91624	NEXN	nexilin (F actin binding protein)	-1.5	0.003
2823	GPM6A	glycoprotein M6A	-1.5	0.044
8842	PROM1	prominin 1	-1.5	0.027
5648	MASP1	mannan-binding lectin serine peptidase 1 (C4/C2 act. comp. of Ra-reactive factor)	-1.5	0.003
2669	GEM	GTP binding protein overexpressed in skeletal muscle	-1.5	0.005
9729	KIAA0408	KIAA0408	-1.5	0.008
11259	FILIP1L	filamin A interacting protein 1-like	-1.5	0.007
254228	FAM26E	family with sequence similarity 26, member E	-1.5	0.003
23213	SULF1	sulfatase 1	-1.5	0.015
1490	CTGF	connective tissue growth factor	-1.5	0.016
5350	PLN	phospholamban	-1.5	0.003
79750	ZNF385D	zinc finger protein 385D	-1.5	0.025
57188	ADAMTSL3	ADAMTS-like 3	-1.5	0.046
7881	KCNAB1	potassium voltage-gated channel, shaker-related subfamily, beta member 1	-1.5	0.000
6401	SELE	selectin E	-1.5	0.026
2273	FHL1	four and a half LIM domains 1	-1.5	0.014
9595	CYTIP	cytohesin 1 interacting protein	-1.5	0.015
58189	WFDC1	WAP four-disulfide core domain 1	-1.5	0.003
161436	EML5	echinoderm microtubule associated protein like 5	1.5	0.042
8349	HIST2H2BE	histone cluster 2, H2be	1.5	0.027
57512	GPR158	G protein-coupled receptor 158	1.5	0.037
440905	LOC440905	hypothetical LOC440905	1.5	0.006
3817	KLK2	kallikrein-related peptidase 2	1.5	0.043
4824	NKX3-1	NK3 homeobox 1	1.5	0.016
51109	RDH11	retinol dehydrogenase 11 (all-trans/9-cis/11-cis)	1.5	0.035
84084	RAB6C	RAB6C, member RAS oncogene family	1.5	0.021
54566	EPB41L4B	erythrocyte membrane protein band 4.1 like 4B	1.5	0.002
131034	CPNE4	copine IV	1.5	0.031
285175	UNC80	unc-80 homolog (C. elegans)	1.5	0.008
55503	TRPV6	transient receptor potential cation channel, subfamily V, member 6	1.5	0.014
57475	PLEKHH1	pleckstrin homology domain containing, family H (with MyTH4 domain) member 1	1.5	0.006
54898	ELOVL2	elongation of very long chain fatty acids (FEN1/Elo2, SUR4/Elo3, yeast)-like 2	1.5	0.028
2786	GNG4	guanine nucleotide binding protein (G protein), gamma 4	1.5	0.020
51280	GOLM1	golgi membrane protein 1	1.5	0.014
440689	HIST2H2BF	histone cluster 2, H2bf	1.5	0.001
6695	SPOCK1	sparc/osteonectin, cwcv and kazal-like domains proteoglycan (testican) 1	1.5	0.005
11013	TMSB15A	thymosin beta 15a	1.5	0.010

4986	OPRK1	opioid receptor, kappa 1	1.6	0.035
6296	ACSM3	acyl-CoA synthetase medium-chain family member 3	1.6	0.033
1803	DPP4	dipeptidyl-peptidase 4	1.6	0.013
23657	SLC7A11	solute carrier family 7, (cationic amino acid transporter, y+ system) member 11	1.6	0.004
85414	SLC45A3	solute carrier family 45, member 3	1.6	0.019
6319	SCD	stearoyl-CoA desaturase (delta-9-desaturase)	1.6	0.022
3081	HGD	homogentisate 1,2-dioxygenase	1.6	0.041
2537	IFI6	interferon, alpha-inducible protein 6	1.6	0.011
57126	CD177	CD177 molecule	1.6	0.023
148823	C1orf150	chromosome 1 open reading frame 150	1.6	0.018
80157	CWH43	cell wall biogenesis 43 C-terminal homolog (S. cerevisiae)	1.6	0.030
6019	RLN2	relaxin 2	1.7	0.040
957	ENTPD5	ectonucleoside triphosphate diphosphohydrolase 5	1.7	0.004
79054	TRPM8	transient receptor potential cation channel, subfamily M, member 8	1.8	0.022
10417	SPON2	spondin 2, extracellular matrix protein	1.8	0.012
11012	KLK11	kallikrein-related peptidase 11	1.8	0.007
283651	C15orf21	Dresden prostate cancer 2	1.8	0.023
2346	FOLH1	folate hydrolase (prostate-specific membrane antigen) 1	1.8	0.027
84419	C15orf48	chromosome 15 open reading frame 48	2.0	0.012
3158	HMGCS2	3-hydroxy-3-methylglutaryl-CoA synthase 2 (mitochondrial)	2.0	0.017

Selection (fold change ≤-1.5 or ≥1.5) of genes which are up-regulated or down-regulated in the selenium group. The reported mean fold change and p-value represent expression changes within the selenium group and were calculated using the two-tailed, paired Limma t test (p-value <0.05). Furthermore, all genes were differentially changed between the selenium and placebo group (one-way ANOVA Limma p-value <0.05).

Supplementary Table 6.b

Entrez ID	Gene symbol	Description	Fold change	p-value
6476	SI	sucrase-isomaltase (alpha-glucosidase)	-2.9	0.000
23671	TMEFF2	transmembrane protein with EGF-like and two follistatin-like domains 2	-2.6	0.004
6013	RLN1	relaxin 1	-2.6	0.044
542767	PCOTH	prostate collagen triple helix	-2.4	0.001
119694	OR51F2	olfactory receptor, family 51, subfamily F, member 2	-2.3	0.004
440905	LOC440905	hypothetical LOC440905	-2.2	0.000
7033	TFF3	trefoil factor 3 (intestinal)	-2.2	0.003
4224	MEP1A	meprin A, alpha (PABA peptide hydrolase)	-2.1	0.014
1807	DPYS	dihydropyrimidinase	-2.0	0.000
341883	LRRC9	leucine rich repeat containing 9	-2.0	0.003
56667	MUC13	mucin 13, cell surface associated	-2.0	0.050
6019	RLN2	relaxin 2	-1.9	0.016
54474	KRT20	keratin 20	-1.9	0.033
54860	MS4A12	membrane-spanning 4-domains, subfamily A, member 12	-1.8	0.031
50940	PDE11A	phosphodiesterase 11A	-1.8	0.003
2044	EPHA5	EPH receptor A5	-1.8	0.001
23310	NCAPD3	non-SMC condensin II complex, subunit D3	-1.8	0.032
338596	ST8SIA6	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 6	-1.8	0.009
729171	ANKRD20B	ankyrin repeat domain 20B	-1.8	0.001
157310	PEBP4	phosphatidylethanolamine-binding protein 4	-1.8	0.019
54898	ELOVL2	elongation of very long chain fatty acids (FEN1/Elo2, SUR4/Elo3, yeast)-like 2	-1.8	0.008
6581	SLC22A3	solute carrier family 22 (extraneuronal monoamine transporter), member 3	-1.7	0.007
33	ACADL	acyl-CoA dehydrogenase, long chain	-1.7	0.003
1135	CHRNA2	cholinergic receptor, nicotinic, alpha 2 (neuronal)	-1.7	0.032
3899	AFF3	AF4/FMR2 family, member 3	-1.7	0.006
55504	TNFRSF19	tumor necrosis factor receptor superfamily, member 19	-1.7	0.002
4583	MUC2	mucin 2, oligomeric mucus/gel-forming	-1.7	0.041
493913	PAPPAS	PAPPA antisense RNA (non-protein coding)	-1.7	0.005
4045	LSAMP	limbic system-associated membrane protein	-1.7	0.009
11148	HHLA2	HERV-H LTR-associating 2	-1.7	0.013
885	CCK	cholecystokinin	-1.7	0.004
2980	GUCA2A	guanylate cyclase activator 2A (guanylin)	-1.7	0.005
2168	FABP1	fatty acid binding protein 1, liver	-1.6	0.004
10223	GPA33	glycoprotein A33 (transmembrane)	-1.6	0.016
9576	SPAG6	sperm associated antigen 6	-1.6	0.005
57554	LRRC7	leucine rich repeat containing 7	-1.6	0.028
154091	SLC2A12	solute carrier family 2 (facilitated glucose transporter), member 12	-1.6	0.001
1138	CHRNA5	cholinergic receptor, nicotinic, alpha 5	-1.6	0.012

353322	ANKRD37	ankyrin repeat domain 37	-1.6	0.001
5558	PRIM2	primase, DNA, polypeptide 2 (58kDa)	-1.6	0.018
4285	MIPEP	mitochondrial intermediate peptidase	-1.6	0.002
29091	STXBP6	syntaxin binding protein 6 (amisyn)	-1.6	0.005
6695	SPOCK1	sparc/osteonectin, cwcv and kazal-like domains proteoglycan (testican) 1	-1.6	0.006
81796	SLCO5A1	solute carrier organic anion transporter family, member 5A1	-1.6	0.010
9687	GREB1	growth regulation by estrogen in breast cancer 1	-1.6	0.006
90288	C3orf25	chromosome 3 open reading frame 25	-1.6	0.008
10739	RFPL2	ret finger protein-like 2	-1.6	0.029
22843	PPM1E	protein phosphatase, Mg2+/Mn2+ dependent, 1E	-1.6	0.011
1573	CYP2J2	cytochrome P450, family 2, subfamily J, polypeptide 2	-1.6	0.003
375611	SLC26A5	solute carrier family 26, member 5 (prestin)	-1.6	0.000
220965	FAM13C	family with sequence similarity 13, member C	-1.6	0.012
1015	CDH17	cadherin 17, LI cadherin (liver-intestine)	-1.6	0.039
1767	DNAH5	dynein, axonemal, heavy chain 5	-1.6	0.014
57185	NIPAL3	NIPA-like domain containing 3	-1.5	0.049
1047	CLGN	calmegin	-1.5	0.038
4133	MAP2	microtubule-associated protein 2	-1.5	0.027
84125	LRRIQ1	leucine-rich repeats and IQ motif containing 1	-1.5	0.002
2651	GCNT2	glucosaminyl (N-acetyl) transferase 2, I-branching enzyme (I blood group)	-1.5	0.028
125981	ACER1	alkaline ceramidase 1	-1.5	0.013
1491	CTH	cystathionase (cystathionine gamma-lyase)	-1.5	0.000
10846	PDE10A	phosphodiesterase 10A	-1.5	0.020
29968	PSAT1	phosphoserine aminotransferase 1	-1.5	0.007
57715	SEMA4G	sema domain, (Ig), transmembrane domain (TM) and short cytoplasmic domain, 4G	-1.5	0.011
39	ACAT2	acetyl-CoA acetyltransferase 2	-1.5	0.015
79846	C7orf63	chromosome 7 open reading frame 63	-1.5	0.013
81033	KCNH6	potassium voltage-gated channel, subfamily H (eag-related), member 6	-1.5	0.013
11123	RCAN3	RCAN family member 3	-1.5	0.016
11001	SLC27A2	solute carrier family 27 (fatty acid transporter), member 2	-1.5	0.044
64757	MOSC1	MOCO sulphurase C-terminal domain containing 1	-1.5	0.005
2786	GNG4	guanine nucleotide binding protein (G protein), gamma 4	-1.5	0.037
23566	LPAR3	lysophosphatidic acid receptor 3	-1.5	0.040
84084	RAB6C	RAB6C, member RAS oncogene family	-1.5	0.028
79748	LMAN1L	lectin, mannose-binding, 1 like	-1.5	0.017
27284	SULT1B1	sulfotransferase family, cytosolic, 1B, member 1	-1.5	0.028
79986	ZNF702P	zinc finger protein 702, pseudogene	-1.5	0.021
2104	ESRRG	estrogen-related receptor gamma	-1.5	0.006
23245	ASTN2	astrotactin 2	-1.5	0.002
89944	GLB1L2	galactosidase, beta 1-like 2	-1.5	0.003
54733	SLC35F2	solute carrier family 35, member F2	-1.5	0.022
116328	C8orf34	chromosome 8 open reading frame 34	-1.5	0.000
3977	LIFR	leukemia inhibitory factor receptor alpha	-1.5	0.006
55964	SEPT3	septin 3	-1.5	0.033
3638	INSIG1	insulin induced gene 1	-1.5	0.010
10020	GNE	glucosamine (UDP-N-acetyl)-2-epimerase/N-acetylmannosamine kinase	-1.5	0.003
57181	SLC39A10	solute carrier family 39 (zinc transporter), member 10	-1.5	0.011
56171	DNAH7	dynein, axonemal, heavy chain 7	-1.5	0.000
202151	RANBP3L	RAN binding protein 3-like	-1.5	0.033
4685	NCAM2	neural cell adhesion molecule 2	-1.5	0.010
3781	KCNN2	potassium intermediate/small conductance calcium-activated channel, subfam N,2	-1.5	0.009
220416	LRRC63	leucine rich repeat containing 63	-1.5	0.002
728606	LOC728606	hypothetical LOC728606	-1.5	0.006
4983	OPHN1	oligophrenin 1	-1.5	0.000
128344	C1orf88	chromosome 1 open reading frame 88	-1.5	0.022
4929	NR4A2	nuclear receptor subfamily 4, group A, member 2	1.5	0.007
5920	RARRES3	retinoic acid receptor responder (tazarotene induced) 3	1.5	0.006
714	C1QC	complement component 1, q subcomponent, C chain	1.5	0.000
3880	KRT19	keratin 19	1.5	0.006
10261	IGSF6	immunoglobulin superfamily, member 6	1.5	0.003
5328	PLAU	plasminogen activator, urokinase	1.5	0.001
4233	MET	met proto-oncogene (hepatocyte growth factor receptor)	1.5	0.031
25903	OLFML2B	olfactomedin-like 2B	1.5	0.001
3399	ID3	inhibitor of DNA binding 3, dominant negative helix-loop-helix protein	1.5	0.003
4064	CD180	CD180 molecule	1.5	0.006
8875	VNN2	vanin 2	1.5	0.026

27286	SRPX2	sushi-repeat-containing protein, X-linked 2	1.5	0.018
5396	PRRX1	paired related homeobox 1	1.5	0.009
3119	HLA-DQB1	major histocompatibility complex, class II, DQ beta 1	1.5	0.003
79750	ZNF385D	zinc finger protein 385D	1.5	0.035
2122	MECOM	MDS1 and EVI1 complex locus	1.5	0.000
1075	CTSC	cathepsin C	1.5	0.000
963	CD53	CD53 molecule	1.5	0.003
56938	ARNTL2	aryl hydrocarbon receptor nuclear translocator-like 2	1.5	0.006
7474	WNT5A	wingless-type MMTV integration site family, member 5A	1.5	0.003
58475	MS4A7	membrane-spanning 4-domains, subfamily A, member 7	1.5	0.001
633	BGN	biglycan	1.5	0.006
8870	IER3	immediate early response 3	1.5	0.000
3772	KCNJ15	potassium inwardly-rectifying channel, subfamily J, member 15	1.5	0.004
2335	FN1	fibronectin 1	1.5	0.015
5270	SERPINE2	serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), 2	1.5	0.007
3687	ITGAX	integrin, alpha X (complement component 3 receptor 4 subunit)	1.5	0.003
54209	TREM2	triggering receptor expressed on myeloid cells 2	1.5	0.003
27242	TNFRSF21	tumor necrosis factor receptor superfamily, member 21	1.5	0.001
9332	CD163	CD163 molecule	1.5	0.011
64231	MS4A6A	membrane-spanning 4-domains, subfamily A, member 6A	1.5	0.000
1043	CD52	CD52 molecule	1.5	0.003
56925	LXN	latexin	1.5	0.002
196	AHR	aryl hydrocarbon receptor	1.5	0.001
80896	NPL	N-acetylneuraminate pyruvate lyase (dihydrodipicolinate synthase)	1.5	0.001
1012	CDH13	cadherin 13, H-cadherin (heart)	1.5	0.016
1999	ELF3	E74-like factor 3 (ets domain transcription factor, epithelial-specific)	1.5	0.008
2524	FUT2	fucosyltransferase 2 (secretor status included)	1.5	0.022
3394	IRF8	interferon regulatory factor 8	1.6	0.001
3397	ID1	inhibitor of DNA binding 1, dominant negative helix-loop-helix protein	1.6	0.001
7434	VIPR2	vasoactive intestinal peptide receptor 2	1.6	0.011
7482	WNT2B	wingless-type MMTV integration site family, member 2B	1.6	0.026
1536	CYBB	cytochrome b-245, beta polypeptide	1.6	0.002
55107	ANO1	anoctamin 1, calcium activated chloride channel	1.6	0.004
6337	SCNN1A	sodium channel, nonvoltage-gated 1 alpha	1.6	0.002
3689	ITGB2	integrin, beta 2 (complement component 3 receptor 3 and 4 subunit)	1.6	0.000
1959	EGR2	early growth response 2	1.6	0.000
3726	JUNB	jun B proto-oncogene	1.6	0.000
10154	PLXNC1	plexin C1	1.6	0.000
3934	LCN2	lipocalin 2	1.6	0.019
9052	GPRC5A	G protein-coupled receptor, family C, group 5, member A	1.6	0.009
11006	LILRB4	leukocyte immunoglobulin-like receptor, subfamily B (with TM / ITIM domains), 4	1.6	0.001
2207	FCER1G	Fc fragment of IgE, high affinity I, receptor for /// gamma polypeptide	1.6	0.003
9120	SLC16A6	solute carrier family 16, member 6 (monocarboxylic acid transporter 7)	1.6	0.011
388325	C17orf87	chromosome 17 open reading frame 87	1.6	0.001
11010	GLIPR1	GLI pathogenesis-related 1	1.6	0.001
3694	ITGB6	integrin, beta 6	1.6	0.001
1462	VCAN	versican	1.6	0.027
6366	CCL21	chemokine (C-C motif) ligand 21	1.6	0.023
51338	MS4A4A	membrane-spanning 4-domains, subfamily A, member 4	1.6	0.001
6423	SFRP2	secreted frizzled-related protein 2	1.6	0.001
1846	DUSP4	dual specificity phosphatase 4	1.6	0.004
7127	TNFAIP2	tumor necrosis factor, alpha-induced protein 2	1.6	0.005
4688	NCF2	neutrophil cytosolic factor 2	1.7	0.002
8832	CD84	CD84 molecule	1.7	0.000
3075	CFH	complement factor H	1.7	0.002
11326	VSIG4	V-set and immunoglobulin domain containing 4	1.7	0.006
241	ALOX5AP	arachidonate 5-lipoxygenase-activating protein	1.7	0.004
121506	ERP27	endoplasmic reticulum protein 27	1.7	0.007
1520	CTSS	cathepsin S	1.7	0.000
7805	LAPTM5	lysosomal protein transmembrane 5	1.7	0.000
4481	MSR1	macrophage scavenger receptor 1	1.7	0.006
445	ASS1	argininosuccinate synthase 1	1.7	0.000
9547	CXCL14	chemokine (C-X-C motif) ligand 14	1.7	0.004
1958	EGR1	early growth response 1	1.7	0.001
6347	CCL2	chemokine (C-C motif) ligand 2	1.8	0.041
4582	MUC1	mucin 1, cell surface associated	1.8	0.004

7052	TGM2	transglutaminase 2 (C polypeptide, protein-glutamine-gamma-glutamyltransferase)	1.8	0.000
7058	THBS2	thrombospondin 2	1.8	0.039
9076	CLDN1	claudin 1	1.8	0.001
1118	CHIT1	chitinase 1 (chitotriosidase)	1.8	0.005
5918	RARRES1	retinoic acid receptor responder (tazarotene induced) 1	1.8	0.014
6036	RNASE2	ribonuclease, RNase A family, 2 (liver, eosinophil-derived neurotoxin)	1.8	0.008
2191	FAP	fibroblast activation protein, alpha	1.8	0.020
2359	FPR3	formyl peptide receptor 3	1.8	0.001
51442	VGLL1	vestigial like 1 (Drosophila)	1.8	0.002
2212	FCGR2A	Fc fragment of IgG, low affinity IIa, receptor (CD32)	1.8	0.000
968	CD68	CD68 molecule	1.8	0.000
4023	LPL	lipoprotein lipase	1.8	0.025
5265	SERPINA1	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1	1.8	0.007
7057	THBS1	thrombospondin 1	1.9	0.000
284340	CXCL17	chemokine (C-X-C motif) ligand 17	1.9	0.003
1490	CTGF	connective tissue growth factor	1.9	0.001
6363	CCL19	chemokine (C-C motif) ligand 19	1.9	0.001
10457	GPNMB	glycoprotein (transmembrane) nmb	1.9	0.000
346389	MACC1	metastasis associated in colon cancer 1	1.9	0.002
1382	CRABP2	cellular retinoic acid binding protein 2	1.9	0.000
3491	CYR61	cysteine-rich, angiogenic inducer, 61	2.0	0.001
3855	KRT7	keratin 7	2.0	0.026
1311	COMP	cartilage oligomeric matrix protein	2.1	0.003
22943	DKK1	dickkopf homolog 1 (Xenopus laevis)	2.1	0.000
6523	SLC5A1	solute carrier family 5 (sodium/glucose cotransporter), member 1	2.1	0.002
4318	MMP9	matrix metallopeptidase 9 (92kDa gelatinase, 92kDa type IV collagenase)	2.2	0.007
5743	PTGS2	prostaglandin-endoperoxide synthase 2 (prostaglandin synthase/ cyclooxygenase)	2.2	0.003
2568	GABRP	gamma-aminobutyric acid (GABA) A receptor, pi	2.2	0.000
5054	SERPINE1	serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), 1	2.3	0.000
4069	LYZ	lysozyme	2.3	0.002
266977	GPR110	G protein-coupled receptor 110	2.4	0.001
1356	CP	ceruloplasmin (ferroxidase)	2.5	0.024
4316	MMP7	matrix metallopeptidase 7 (matrilysin, uterine)	2.5	0.039
6590	SLPI	secretory leukocyte peptidase inhibitor	3.0	0.000
1116	CHI3L1	chitinase 3-like 1 (cartilage glycoprotein-39)	3.1	0.003

Selection (fold change ≤-1.5 or ≥1.5) of genes which are up-regulated or down-regulated in the placebo group. The reported mean fold change and p-value represent expression changes within the placebo group and were calculated using the two-tailed, paired Limma t test (p-value <0.05). Furthermore, all genes were differentially changed between the selenium and placebo group (one-way ANOVA Limma p-value <0.05).

7 Discussion

The overall aim of this thesis was to obtain insight into nutritional and clinical aspects relevant to different stages of prostate cancer. In the following paragraphs, the main findings presented in this thesis will be summarized. Next, implications and future perspectives, which might be of relevance to the researcher, (future) patient or urologist will be discussed.

Table 7.1 Overview of the studies presented in this thesis

Chapter	Design of the study	Population	Exposure	Outcome	Main findings
2	Descriptive study (retrospective)	Patients diagnosed with PCA (n=956)	-	Perceived causes of their PCA	Established causes of PCA were not commonly perceived, not even among patients with these risk factors
3	Population-based cohort study	General male population (n=2,118)	Serum levels of triglycerides and total, HDL and LDL cholesterol	PCA incidence (43 cases)	Higher LDL and HDL cholesterol increased the risk of aggressive PCA and non-aggressive PCA, respectively
4	Population-based cohort study	Patients with cancer identified through the population-based cancer registry (n=551,553)	Type of cancer (versus the general population)	PCA incidence (9,243 cases)	Cancer survivors had an increased PCA risk in the first year following their first cancer diagnosis
5a	Population-based cohort study	Patients with PCA and treated with RP identified through the population-based cancer registry (n=493)	BMI	Risk of biochemical recurrence after RP (142 cases)	BMI was not a predictor of biochemical recurrence after RP
5b	Hospital-based cohort study	Patients with PCA and treated with RP identified from two academic hospitals (n=1,302)	BMI	Risk of biochemical recurrence after RP (297 cases)	BMI was not a predictor of biochemical recurrence after RP
6	Randomized, placebo-controlled intervention trial	Patients with a suspicion or diagnosis of PCA and scheduled for prostate biopsies (n=23)	300 ug selenized yeast or placebo during a 5-week intervention period	Changes in gene expression profiles in non-malignant prostate tissue (n=15)	Selenium affected expression of genes implicated in inflammation and EMT in the prostate

Abbreviations: *BMI* body mass index, *EMT* epithelial-to-mesenchymal transition, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *PCA* prostate cancer, *RP* radical prostatectomy.

7.1 Outline

The aim of this thesis was to evaluate nutritional, lifestyle and clinical factors involved in different stages of prostate cancer. Reflecting on this main aim, I focused on a number of specific research objectives, which were also described in **Chapter 1**;

1. Identification of leads for research on potential risk factors
2. Evaluation of risk factors for incident prostate cancer
3. Evaluation of factors that might influence risk of recurrent disease
4. Studying the molecular mechanisms of chemoprevention

In order to address these research objectives, this thesis described a relatively broad spectrum of studies, ranging from a descriptive, qualitative study to a randomized clinical trial with molecular endpoints. An overview of the design of these studies is presented in **Table 7.1**.

In the following paragraphs, the main findings will be briefly summarized and I will reflect on the scientific implications and future perspectives of each of the research objectives. Relevance of the findings from the point of view of the (future) patient and urologist will be discussed in **paragraph 7.3** and **7.4**.

7.2 Implications for researchers

Methods for evaluation of scientific quality are widely described[1, 2] and frequently applied in systematic literature reviews or meta-analyses. Therefore, these issues will not be discussed in detail in this chapter. Instead, I will focus on the questions: "*How can we develop studies that provide useful answers to our research questions? What have we learned from our studies and how can we implement this knowledge or experience to improve future studies?*"

7.2.1 Identification of leads for research on potential risk factors

The identification of new risk factors for prostate cancer is important in order to understand the aetiology, to identify high-risk groups for early detection and to develop effective prevention strategies. **Chapter 2** described the results from an explorative study in which patients with prostate cancer were asked about perceived causes of their disease. Results of this study showed that the majority of the patients

was not able to mention any cause that might have contributed to the development of their prostate cancer. Patients who did report perceived causes often referred to heredity, specific environmental factors, nutrition or physical activity, and stress. Although we were not able to identify outstanding leads for research on new risk factors, this study was valuable from the perspective that possible gaps were identified in the perception and knowledge of both patients and scientists.

The odd one out

At the time of writing this discussion, the paper described in **Chapter 2** was submitted to eight different peer-reviewed journals in the field of oncology or urology. Only one journal has sent this paper for peer-review, all others directly indicated that the paper was outside the scope of the journal. There might be a few possible underlying reasons for the repeated rejections. First, the topic of the paper was indeed outside the scope of all the approached journals or other papers had priority for these journals. Second, the quality of the paper was believed to be poor. Third, the objective or design of the study was perceived as inadequate. Or fourth, a qualitative paper with mainly quotes rather than numbers or graphs is rather exceptional in this field. As a number of independent editors explicitly stated that the paper was clear and well-written, I tend to assume that quality was not the main reason, although we are of course willing to accept other opinions. Also, the topic of the paper seems to be adequate as prostate cancer is a rather common topic in the fields of oncology and urology. Therefore, it was likely the approach, the design and the nature of our paper that were critically perceived. Since I still think that the results of our study are interesting in a way that they reflect the reasoning and perceptions of the patients, we critically reconsidered its limitations and looked for improvements. A few methodological aspects and implications will be discussed below.

How to find a needle in a haystack?

In this study, over 65 unique, possible causes of prostate cancer were identified. All of these causes were reported by experts in the field; namely the patients who more or less know what happened during their lives, who experienced all important events by themselves and who might be aware of the different aspects that preceded their prostate cancer development. However, out of all these answers, how should we exactly find the interesting and promising leads for future research? Is the use of felt-tip pens a possible cause of prostate cancer, or is the Chernobyl disaster a more likely candidate?

Ideally, we would like to observe a ‘pattern’ in the answers of the patients. If several patients would have mentioned the use of felt-tip pens as a possible cause of their

prostate cancer, this would have been an interesting lead, apart from a low prior in the sense of biological plausibility. Nevertheless, the fact that only one patient mentioned felt-tip pens does not necessarily mean that this is not a possible risk factor for prostate cancer. In our study, we found some general causes or broad categories, such as stress and heredity, which were reported by several patients. Majority of the causes, however, referred to highly-specific factors reported by individual patients. Therefore, the most efficient and adequate approach to analyse these data seemed to be a combination of a systematic strategy and common sense.

First, we intended to group the reported causes in categories. Based on the available literature, we then aimed to judge whether these categories are likely risk factors for prostate cancer or not. Finally, we tried to provide insight into the reasoning and perceptions of the patients by citing and emphasizing various specific causes within each category. Although we realize that this approach may not be the most conventional strategy to find new and outstanding risk factors, it helped us to reveal unprompted beliefs of cancer causation, to identify common misperceptions, and to recognize gaps in the available literature.

The question has partly determined the answer

Various studies have already evaluated perceptions about causes of prostate cancer[3-5] and also other forms of cancer[6-8]. Most of these studies asked their participants or patients the question: *"Do you know what causes cancer?"* In our study, we used a similar question, however, we just added a personal touch by asking: *"Do you known what may have been the cause of your cancer?"* There is a substantial difference in the interpretation of both questions. The first question determines knowledge, while the second question refers to a personal situation. If we asked our patients: *"Do you know what causes prostate cancer?"* we assume that various patients would have reported age, heredity and black race, because these are established risk factors for prostate cancer[3]. In our study, however, we did not ask the patients about their knowledge, but about their personal situation. This might explain why relatively few patients reported causes related to established risk factors for prostate cancer; our patients were merely white men who possibly did not perceive themselves as old or did not have a positive family history. However, we cannot rule out the possibility that lack of knowledge also contributed to the relatively low response rate.

A few other studies from the United States and Australia examined perceived causes of prostate cancer among patients recently diagnosed with this disease[4, 5, 9]. In these studies it was demonstrated that patients with prostate cancer were least likely (42%) to report perceived causes, as compared to other cancer patients (48-75%), which was

explained by the authors by the lack of scientific evidence available for factors involved in prostate carcinogenesis[5]. Although we were not directly able to provide useful data on the knowledge of our patients, we clearly demonstrated that the vast majority of the patients was not able to identify any cause of their disease and that some overestimations and misconceptions persist about factors that are unlikely to influence prostate cancer risk.

Furthermore, the established risk factors for prostate cancer were not commonly perceived, not even among patients with these risk factors, which suggests that there might be a key role for effective cancer education and prevention programmes. In order to provide detailed insight into the reasoning of the patients, we suggest combining data on knowledge and perception of cancer causes. A suggestion for future studies is therefore to include the following questions: *"Do you know what causes prostate cancer?", "Can you report which of these causes might have contributed to the development of your prostate cancer?",* and *"Are there any other possible factors that might have contributed to the development of your prostate cancer?"* By using this approach it will be possible to determine either the knowledge about general risk factors, as well as the perception of individual risk factors.

In conclusion

Cancer is a multifactorial disease with a complex aetiology. The identification and confirmation of new risk factors for cancer is complicated by the lack of a standardized approach, the limitations and challenges of most study designs, and the possible interactions between risk factors and biological factors. Asking patients about perceived causes of their cancer might help to identify leads for aetiological research. Combining information on patients' perceptions and knowledge will improve insight into reasoning of the patients and at the same time indicate whether effective education and prevention programs are required.

7.2.2 Evaluation of risk factors for incident prostate cancer

Two potential risk factors for incident prostate cancer were evaluated in this thesis. In **Chapter 3**, the association between blood lipid levels and prostate cancer incidence was studied. Results showed that higher serum levels of total cholesterol and LDL cholesterol were associated with an increased risk of prostate cancer. Furthermore, higher levels of LDL cholesterol were also associated with an increased risk of aggressive prostate cancer, while higher levels of HDL cholesterol were associated with an increased risk of non-aggressive disease. Based on these findings, we confirm that blood lipid levels might be considered as a possible risk factor for prostate cancer, although the potential underlying mechanisms should be examined in more detail in future studies.

The risk of prostate cancer as a second primary cancer was examined in **Chapter 4**. We showed that cancer survivors had an increased risk of being diagnosed with prostate cancer as compared to the general Dutch population. For most of the specific first cancer sites, the increased prostate cancer risk was limited to the first year of follow-up, which implies a main effect of active screening or incidental detection.

The incidence of prostate cancer depends on screening

Incident prostate cancer, registered by the Dutch population-based cancer registry, is used as an endpoint in both studies described in **Chapter 3 and 4.** Incident prostate cancer is defined as a registered diagnosis of prostate cancer after start of a study or exposure measurement. A possible limitation of incident prostate cancer as an endpoint is that active screening influences the incidence rates, especially in studies with disease-related exposures. PSA tests are widely used to detect prostate cancer. Although the specificity of this method for prostate cancer is debatable[10, 11], active searching and screening for prostate cancer will lead to more detected cases of prostate cancer; i.e. higher incidence rates. A substantial number of the detected prostate cancers might lack clinical relevance, because most insignificant tumours will never cause symptoms or increase morbidity, but may lead to overtreatment and related complications[12, 13]. This observation also explains why PSA testing is not implemented as a population-wide screening method for prostate cancer and why the intensive screening policies for the general population in the USA have been recently tempered[14, 15].

Nevertheless, men who were previously diagnosed with any disease or experienced health problems might, because of anxiety or medical indications, tend to apply for

PSA testing or other screening or diagnostic methods relatively frequently. As a consequence, the prostate cancer incidence might be higher in these men as compared to the general, healthy population. With respect to scientific studies, this does not necessarily affect study outcomes as long as the likelihood of detecting prostate cancer is the same for all study participants. However, if only specific subgroups are far more likely to undergo screening, which in theory could be the patients with an unfavourable blood lipid profile, or previously diagnosed urological cancers in our studies, this might influence the risk estimates for prostate cancer.

Besides, the diagnosis of mainly non-aggressive, or insignificant prostate tumours detected by screening might hinder the generalizability and clinical relevance of the study findings. An example of this issue is also provided by the Reduction by Dutasteride of Prostate Cancer Events (REDUCE) trial (see also **Chapter 1**). The extensive REDUCE trial aimed to evaluate the effects of dutasteride on prostate cancer incidence in high-risk men[16, 17]. As expected due to the high-risk profile, but also due to the protocol-directed biopsies for all participants at the end of the study, the incidence of prostate cancer was extremely high (25%)[17], even compared to men with a comparable prostate cancer risk (8-10%)[18, 19]. Because of the intervention with dutasteride, the prostate cancer incidence decreased from 25% to 20%[17]. Given the much lower expected incidence rates for the general population, the relevance of this risk reduction remains debatable[19]. In parallel, caution should be paid to the possible effects of a detection bias in our studies. Although our patients were not intensively screened for prostate cancer, there might be a tendency to relatively frequent PSA testing among some specific subgroups. Ideally, future studies should take into account the frequency and reasons for PSA testing, for instance by linking incidence data to electronic patient files with laboratory applications and results.

It is time to shift the focus from association to causation

Our studies provided useful insight into the associations between exposures and incidence of prostate cancer. The outcomes of the studies can be interpreted in terms of chance or risk: *"does the studied exposure influence the risk of getting prostate cancer?"* However, information about risk usually does not explain the underlying mechanisms. Therefore, the next step is to gain insight into the causality. It is important to determine whether there is a biological, clinical or technical explanation for the suggested association. The risk of getting prostate cancer after a previous cancer diagnosis can, for instance, be higher because both cancers share specific aetiological factors such as a common genetic background. Another option is that treatment for the first cancer results in an increased or decreased risk of prostate cancer. Third, patients with a previous cancer might opt for PSA screening more often

as compared to a general population. Also reverse causality should be considered in our studies. Reverse causality refers to the phenomenon that the outcome influenced the exposure. Our study suggests that higher levels of serum cholesterol increased the risk of getting prostate cancer. However, we cannot exclude the possibility that the opposite happened; i.e. that the preclinical prostate tumour affected blood lipid levels. This issue of reverse causality might be partly solved by excluding the incident prostate cancer cases diagnosed during the first years of follow-up. However, because prostate cancer is often a slow-growing and indolent cancer, reverse causality cannot be fully excluded.

Adding another level of evidence might provide more insight into the issues described above. An option is to continue with mechanistic-oriented research. Experiments with cell lines or animal models could for example elucidate whether high levels of cholesterol will increase the synthesis of androgens from cholesterol, whereas clinical studies are needed to confirm the suggested association between availability of cholesterol and androgen synthesis in malignant prostate tissue[20]. Another level of evidence might be provided by the randomized trials. This applies in particular to the studies with high-risk patients or modifiable exposures, such as the blood lipid levels which can be altered by diet and lifestyle changes or statin use. Nevertheless, the advantages of these approaches in contrast to the observational studies should not be overestimated, because the limited generalizability of most mechanistic studies and the ethical considerations of the randomized trials should be kept in mind.

In conclusion

Within the scope of the previously described research aim, we evaluated two possible risk factors for incident prostate cancer. As in other epidemiological studies, we cannot provide much information about the causality of the suggested association, which highlights the relevance of future studies. These studies should focus in particular on confirmation and validation of the findings and examination of aetiological mechanisms.

7.2.3 Evaluation of factors that might influence risk of recurrent disease

For this research objective, we focussed on the evaluation of body mass index (BMI). In **Chapter 5a** and **Chapter 5b**, BMI was examined as a risk factor for biochemical recurrence following radical prostatectomy among patients with prostate cancer identified from the population-based cancer registry and two academic referral hospitals, respectively. BMI was not an independent predictor of biochemical recurrence in these two studies.

Differentiation can make the difference

BMI is often used to classify overweight (BMI 25.0-30.0 kg/m^2) and obesity (BMI ≥30 kg/m^2)[21]. However, it has been extensively reviewed by others that the use of these categories has some limitations, because BMI in itself does not reflect body composition and does not differentiate between visceral and subcutaneous fat[22-24]. Other measures of overweight and body fat distribution might therefore provide additional information. There are some indications that waist circumference or the amount of visceral or periprostatic fat might predict prostate cancer risk[25, 26] or aggressiveness[27]. Evidence for an effect on biochemical recurrence following radical prostatectomy or brachytherapy is scarce[28]. Therefore, it is suggested to include measures of fat distribution in future studies focussing on overweight and the risk of prostate cancer recurrence. As imaging techniques, such as magnetic resonance imaging (MRI), are nowadays often used for diagnosis and staging purposes[29], it might be worthwhile to implement a standard protocol for the assessment of the amount of different types of fat on these images in clinical practice.

Body size early in life warrants special attention

Another relevant issue regarding the exposure in our studies is the timing of exposure measurement. Weight and height were collected retrospectively from questionnaires which were distributed among the prostate cancer patients, or from the clinical charts. In the questionnaire, patients reported average weight during adult life. In most cases, clinical data from the charts were recorded during an anaesthetic intake for the radical prostatectomy, which indicates that the patients were already diagnosed with prostate cancer at time of exposure measurement. From a clinical perspective, insight into the association between BMI at time of diagnosis and risk of biochemical recurrence can be useful for treatment-related considerations. From a biological perspective, however, rather than focussing on BMI at diagnosis, it might be interesting to evaluate whether overweight or obesity earlier in life predisposes towards more aggressive

prostate cancer or an increased risk of recurrence. Some cohort studies showed that a higher BMI at young age (18-29 years) was associated with either a decreased[30, 31] or an increased[32] risk of developing prostate cancer later in life[33]. Possible explanations for these findings might refer to hormonal conditions[31], the ability to control homeostasis due to prolonged exposure, or specific lifestyle habits which explain some residual confounding. Although published results are conflicting and the exact mechanisms are not elucidated, these findings at least suggest that body size early in life might influence prostate carcinogenesis. To what extent this finding can be extrapolated to the risk of prostate cancer recurrence is not yet clear. For our study described in **chapter 5a**, we had self-reported data on weight at age 18. However, we did not include this variable in our univariable or multivariable proportional hazards regression models, as these data were only available for part of the patients who completed the questionnaires (n=399/504) and data on height at age 18 was not available for any of the patients. It might be interesting to include measures on weight and height during childhood, adolescence and adult life in future studies. Ideally, future comprehensive, prospective cohort studies should start at birth or even during pregnancy in order to follow body size at regular time points during life. Already a few of these extensive (sub)cohorts starting at relatively young age were initiated in the Netherlands (e.g. the PRIDE study[34]). Another option is to look into the possibility to link the patient data to registries of the Well Baby Clinics (0-4 years) and the Community Health Services (GGD, 4-19 years), who record weight and height during childhood and adolescence. As most of these registries use digital files since 2009, this approach is probably not effective until the current generation reaches the age of fifty and becomes at risk for prostate cancer.

Biochemical recurrence as endpoint; does it really matter?

As described above, we were interested in the effects of BMI on prostate cancer recurrence following radical prostatectomy. As radical prostatectomy is considered as a radical and curative treatment during which the entire prostate is removed, PSA levels should ideally become undetectable within six weeks following surgery[35]. Rising PSA levels, after an undetectable PSA, are often indicated as biochemical recurrence. In clinical practice, the regular follow-up of PSA levels and determination of biochemical recurrence can be used to consider options for secondary therapies. The use of biochemical recurrence as an endpoint in scientific studies, however, is characterized by some limitations. First, the cut-off levels to define rising PSA levels are not uniformly implemented. Over fifty different criteria were described to define biochemical recurrence following radical prostatectomy[35]. Also in our studies, two different approaches were used, which is due to the differences in registry strategies by the population-based cancer registry and the academic centres. In **chapter 5a**, we

defined biochemical recurrence as two consecutive PSA levels ≥0.2 ng/mL, while in **chapter 5b** we have chosen for two consecutive PSA levels ≥0.10 ng/mL. Although such subtle differences in definitions for biochemical recurrence does not necessarily influence the results of a study[36], it hinders comparisons between studies. Therefore, I suggest that, if biochemical recurrence is used as an endpoint, a consensus about a uniform definition should be accepted. As suggested by others, a cut-off point between ≥0.2 ng/mL and ≥0.4 ng/mL seems to be most relevant with respect to clinical outcome[35, 37].

Second, although biochemical recurrence might reflect disease recurrence, it has been argued that it does not necessarily predict prognosis or prostate cancer-specific mortality[38, 39]. Alternative approaches would be to include 'more clinically relevant' endpoints such as prostate-cancer specific mortality. Potential disadvantages of this approach are the requirement of a relatively long follow-up, and the possible interference of secondary therapies, which can make it rather complicated to deduce the direct association between exposure and outcome. Already a few prospective studies showed suggestive associations for BMI and prostate-cancer specific mortality following various types of treatment[40]. Another option is to verify disease recurrence using additional methods. According to the guidelines of the European Association of Urology it is recommended to follow patients for at least ten years after treatment[41]. In case of rising PSA levels (≥20 ng/mL) or complaints of bone pain, prostate biopsies and MRI/CT or bone scans are indicated to verify disease recurrence and presence of metastases. Combining the information regarding rising PSA levels (biochemical recurrence), a palpable nodule, and presence of metastases detected by biopsy or imaging techniques (clinical recurrence) might improve the clinical relevance of future studies.

Mechanistic research is needed to understand the role of adiposity in prostate carcinogenesis

In the previous paragraphs, we already discussed topics related to the exposure and endpoint of our current studies. Optimization and extension of the observational studies will hopefully contribute to new insights and compelling evidence. However, it does not add an extra level of evidence. It is now time to move forward and to have a look from a different perspective. One approach is to focus on the tissues of interest itself: adipose tissue and prostate tissue. Especially for patients treated with radical prostatectomy, it is relatively easy to collect small samples of subcutaneous fat and pre-peritoneal fat or periprostatic fat[42]. Also small prostate needle biopsies can be easily collected during surgery. By collecting these tissues from all patients who provide written informed consent, a biobank of surgical samples can be initiated.

Combined with routinely collected information about weight, height (anaesthetic records) and body fat distribution (from a staging MRI), this biobank can be a starting point for mechanistic research on the association between body size, metabolic health and prostate carcinogenesis.

As adipose tissue behaves like a metabolically-active organ[43], it might be interesting to study the secretory profile of the adipose tissue surrounding the prostate. Circulating levels of several adipokines have already been associated with the development and progression of prostate cancer[44, 45]. For colorectal cancer, a recent study showed that the expression of inflammatory adipokines in the visceral adipose tissue was higher in patients as compared to healthy controls[46]. These findings carefully suggest that the inflammatory adipokines might facilitate the development of cancer, possibly by maintaining a microenvironment which is favourable to the tumour cells. In parallel, adipose tissue surrounding the prostate might influence prostate carcinogenesis. Van Roermund and colleagues showed that the periprostatic fat content as measured by CT did correlate with prostate cancer aggressiveness in patients treated with radiotherapy or brachytherapy[27]. Furthermore, levels of the proinflammatory adipokine interleukin 6 (IL-6) secreted by the periprostatic adipose tissue were correlated with increasing tumour grade in the prostate[47]. From these studies, however, it cannot be concluded whether the characteristics of the adipose tissue influenced prostate cancer aggressiveness or vice versa. Therefore, it would be interesting to study the role of the adipokines in the development and progression of prostate cancer in more detail. A possible starting point can be to isolate and culture periprostatic adipose tissue from a wide range of subjects. Exposure of prostate (cancer) cells to the conditioned culture medium of the periprostatic adipose tissue might explain how the secreted adipokines modulates proliferation of the prostate cancer cells.

Mechanistic studies with prostate tissue, rather than adipose tissue, offer the opportunity to link body size to molecular signatures in the prostate. A recent study by Sharad and colleagues described the gene expression profiles in tumour (relative to normal) cells of patients with a high (mean ± SD; 27.6±1.67 kg/m^2) or normal BMI (mean ± SD; 21.9±2.20 kg/m^2)[48]. Although the authors did not extensively describe comparisons of high versus normal BMI, they showed that processes related to lipid metabolism and cholesterol homeostasis were altered in tumour cells of patients with a high BMI[48]. A next step would be to compare the prostatic gene expression profiles for patients with normal weight, overweight and obesity, or even correlate the gene expression profiles to a metabolic signature including BMI, fat distribution, the levels of circulating adipokines and characteristics of adipose tissue surrounding the prostate.

In conclusion

In the previous paragraphs, some methodological aspects of our studies were discussed and at the same time suggestions or improvements for future research were provided. Ideally, future studies focussing on the effects of overweight and obesity on the risk of disease recurrence or progression should innovate by extending and collecting a broad spectrum of data. By using the current methodologies in clinical practice in an efficient way, these 'new' types of studies will not necessarily lead to complicated logistics or financial limitations. Diagnostic MRI or CT images can be used for measurements of fat distribution or periprostatic fat content, samples from adipose tissue and prostate tissue can be collected during radical prostatectomy and routinely collected data on disease recurrence or progression can be used to define clinically relevant endpoints. Hopefully, such new approaches will not only clarify whether or not, but also how and why, the presence of (abundant) adipose tissue is associated with prostate cancer development, recurrence and progression.

7.2.4 Studying the molecular mechanisms of chemoprevention

In order to study the molecular mechanism underlying the possible chemopreventive effect of selenium, we examined changes in gene expression profiles in non-malignant prostate tissue in a randomized, placebo-controlled trial with selenized yeast. Results of this study showed that selenium induced an anti-inflammatory gene expression profile in the prostate. Furthermore, our data showed that supplementation with selenized yeast affected expression of genes implicated in the process of epithelial-to-mesenchymal transition (EMT).

The use of transcriptomics is a powerful tool to identify potential mechanisms

As described above, the aim of our study presented in **Chapter 6** was to examine the molecular mechanism underlying a possible chemopreventive effect of selenium. By using whole-genome microarrays, we were able to examine the expression of over 19,000 annotated genes in one prostate needle biopsy simultaneously. The use of this, so called, 'transcriptomics' approach provided complete and comprehensive insights into the overall effects of selenium on gene expression profiles in the prostate. The main advantage of this approach is that we were not limited to a group of *a priori* selected genes which might be involved in cancer prevention according to previous literature or common hypotheses. Although these experiments are sometimes called 'fishing expeditions', the whole-genome microarrays as such provide good opportunities for the development of new, pioneering ideas about molecular mechanisms. There are also a few methodological limitations considering the use of whole-genome microarrays. Meaningful genes might be missed due to the enormous amount of data, while biologically irrelevant processes could be overestimated as a consequence of prior knowledge or overrepresentation in the available literature.

Since statistical testing for the identification of differentially expressed genes is performed for more than 19,000 genes simultaneously, there is a plausible chance of finding false-positives[49]. The use of q values, which is based on false discovery rates (FDR)[50] and takes into account multiple-testing, is therefore often indicated in microarray analyses. However, the use of q values is complicated in human dietary intervention studies with relatively few participants and small effects as extensively described by others in our group[51]. We did not select genes based on q values in our studies for the reasons described earlier[51]. Instead, rather than highlighting expression changes of individual genes, we focussed on the identification of biological pathways that were differentially regulated within and between the experimental groups. Furthermore, the expression changes were highly consistent among the patients in the

two experimental groups, implicating that the observed effects were robust and truly reflect the effects of the intervention.

Another possible difficulty related to the microarray experiments is the biological interpretation. As an example, the effects of selenium on the inflammatory processes will be discussed. Based on the observation that pathways involved in inflammation were down-regulated after the intervention with selenium, it was hypothesized that selenium induces an anti-inflammatory effect. From a biological point of view, one could argue that a down-regulation of inflammatory pathways is beneficial (less inflammation) or harmful (inadequate response to inflammation). Since it is well-studied that a down-regulation of inflammatory pathways is associated with less inflammation in many conditions and tissues (e.g. references[52, 53]), we have no reason to assume that the anti-inflammatory effect of selenium is harmful. However, this example illustrates the difficulties related to the 'two-way reasoning'. A possibility to improve the biological interpretability of the microarray data is again to add multiple levels of evidence. The microarray data we have used only reflect gene expression. Ideally, adding data related to, for instance, histology, protein expression, microRNA expression, and DNA methylation might result in a better understanding of the exact mechanisms and thereby improve clinical relevance.

Studying differences in gene expression changes gets the most out of your microarray experiment

Our intervention study is relatively unique in a way that we have collected prostate tissue at two time points, that is before and after the intervention period, and that we have included a placebo group. There have been a few studies which examined the effects of a dietary or lifestyle intervention on whole-genome gene expression profiles in the prostate[54-58]. Some have used a 'preoperative' model which means that after the intervention, prostate tissue was collected during surgery[57]. By using the preoperative model, it is only possible to compare experimental groups after intervention. Our 'two-biopsy' model, however, also allowed comparisons within a person and thereby reflects changes in gene expression over time. Other studies, which did also use a 'two-biopsy' model, did not include a placebo or control group[54, 56]. The use of a placebo or control group is crucial for the identification of effects that are independent of the intervention. By including a placebo group, we were able to identify the effects of the repeated biopsy, although we cannot fully exclude the possibility that the composition of the placebo pills or a progressive inflammatory or tumour environment contributed to the gene expression changes in the placebo group as well. There is one limitation of the 'two-biopsy' model in comparison to the 'preoperative' model. Since the biopsies were routinely collected during ultrasound-guided prostate needle biopsies, there was

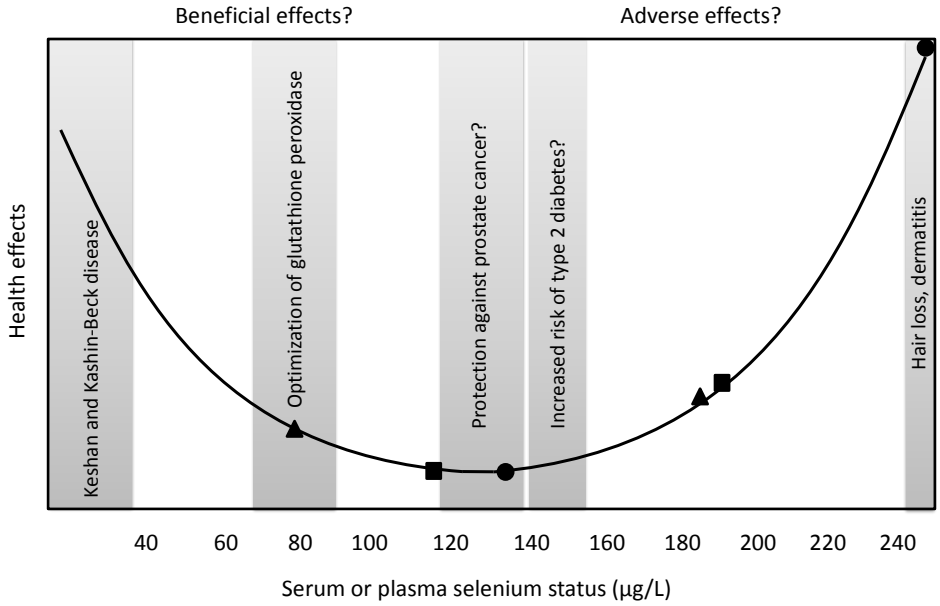
limited amount of tissue available. In theory, this problem can be solved by extending the number of collected biopsies and thereby increasing the amount of tissue. The burden of these additional biopsies should be balanced against the well-being of the patients, because most patients already provide eight to ten prostate biopsies for diagnostic purposes. Future studies should therefore carefully consider whether the 'two-biopsy' model, the 'preoperative' model or a combination of both will be most accurate to answer their research questions.

Deficiency might explain efficiency

The inconsistent findings from previous intervention studies focussing on the effects of selenium on prostate cancer incidence may be, amongst other reasons, explained by the baseline selenium status of the participants. In the Nutritional Prevention of Cancer (NPC) trial, mainly the participants with relatively low selenium status at baseline (plasma selenium <123.2 µg/L) seem to benefit from the daily intervention with 200 µg selenized yeast[59]. The median baseline serum selenium levels of the participants of the Selenium and Vitamin E Cancer Prevention (SELECT) Trial, which did not confirm a protective effect of selenium on prostate cancer risk, were relatively high at 135 µg/L[60]. After the daily intervention with 200 µg *L*-selenomethionine, serum selenium levels of the SELECT participants even increased to 252 µg/L[60], which might exceed an optimal selenium status. Based on experimental studies in dogs, and a number of observational studies in humans, a U-shaped dose response curve for selenium status and several health outcomes was suggested[61-63]. This U-shaped dose response curve indicates that men with low selenium status might benefit from selenium supplementation, while men with a higher status might experience adverse effects of an additional selenium intake.

What are the implications of this U-shaped dose response curve for the findings of our study? Since the selenium intake in most European countries is relatively low as compared to the United States[62], it was expected that the selenium status of our patients was low in comparison to participants of the NPC trial and the SELECT trial. As shown in **Figure 7.1**, median serum levels of selenium of our patients were 78 µg/L and increased to 185 µg/L after a five week intervention with 300 µg selenized yeast per day. While overlaying the U-shaped dose response curve with these data, it indeed seemed that our patients started at a 'low status' and ended with an 'optimal to high selenium status'. Based on these data and the results of our study, we hypothesize that our patients may benefit from a prolonged intervention with selenium. However, we do not have any data on prostate cancer risk and other health outcomes or occurrence of long-term adverse effects. Some[60, 64-66], but not all[67] previous studies suggested that serum selenium levels above 120-150 µg/L may already be associated

with adverse effects such as an increased risk of type 2 diabetes. It seems that the optimal range of selenium intake and status is narrow and strongly depend on various factors such as baseline status, genotype[68-70], and metabolic capacity[62]. At this moment, the exact role of all these factors is unclear and there is insufficient evidence to justify recommendations for supplemental selenium intake.



▲ SePros study (Kok et al., submitted)
● SELECT trial (Lippman et al., JAMA 2009; 301(1): 39-51)
■ NPC trial (Clark et al., JAMA 1996; 276(24): 1957-63)

Figure 7.1 The U-shaped dose response curve for selenium status and health outcomes illustrated by the risk of prostate cancer. Based on figures presented by Chiang et al.[61] and Fairweather-Tait et al.[62]. To convert µg/L to µmol/L multiply by 0.0127.

The beauty of the yeast

The different forms of selenium used in the NPC trial and the SELECT trial might also explain the conflicting findings of these studies. The NPC trial has used selenized yeast. Selenized yeast is produced by growing yeast in a selenium-enriched medium, which result in the binding of selenium compounds to yeast components, such as cell wall proteins[71, 72]. Although the exact composition might vary by batch and manufacturer, selenized yeast normally contains a mixture of selenium compounds, with the organic selenomethionine being the most abundant form (~70%)[72, 73]. Based on the advice of a panel of selenium experts, there is consciously chosen for the use of *L*-selenomethionine within in the SELECT trial[74]. The preference of *L*-selenomethionine

over selenized yeast was based on the suggested batch-to-batch variation in selenized yeast, and the ability to link possible health effects to one well-defined selenium compound[60, 74]. Because we aimed to explore the mechanisms underlying the chemopreventive effect as observed in the NPC trial, we have chosen for the use of selenized yeast in our study. Our selenized yeast tablets (SelenoPrecise, Pharma Nord, Vejle, Denmark) were also used in other intervention trials[75] and were characterized by their stable batch-to-batch quality profile. As long as the exact selenium compound which might be responsible for potential chemopreventive properties has not been identified, the use of selenized yeast, because of its variety in active selenium compounds, seems to provide best opportunities to study the chemopreventive effects of selenium.

The design of the SELECT trial was too efficient

As described previously, the transition to a mesenchymal phenotype is considered as a critical process in cancer progression, because the loss of cellular adhesion, the reorganization of the cytoskeleton and increased motility might result in invasion and metastasis. Our study suggested that selenium affects expression of genes implicated in the process of epithelial-to-mesenchymal transition (EMT). Although this hypothesis needs to be confirmed in future studies, this finding might implicate that selenium prevents the progression rather than the development of prostate cancer. This suggestion might also explain the contradicting findings of the NPC trial and the SELECT trial. The recruitment and the blinded intervention of the NPC trial took place between 1983 and 1996[76]. At that time, the use of PSA testing was just introduced. Since prostate cancer was a secondary endpoint in the NPC trial, there were no standardized prostate cancer detection methods, such as PSA tests or digital rectal examinations (DRE), implemented in the study protocol[76, 77]. Plasma PSA levels were available for 75% of the male participants, however, these analyses were performed retrospectively from samples stored for research purposes. Therefore, the diagnosis of prostate cancer during the study was mainly based on pathology reports that were available for patients who visited their urologist on their own initiative[77]. In conclusion, men participating in the NPC trial were representative for a general, non-screened population and the detection of prostate cancer was part of normal clinical practice. In contrast, the SELECT trial has, in parallel with the current trends in the USA, more intensively screened its participants for the diagnosis of prostate cancer. Participants visited their study centre every 6 months and although PSA tests and DREs were not mandatory except for the baseline measures[60], the majority of the participants underwent one or more PSA tests (~85%) or DREs (~72%) during the first years of the study[60]. The biopsy rates of both trials also illustrate the differences in the probability to detect prostate cancer (NPC trial 3-7%[59] and SELECT trial 12%[60]). In theory, prostate

cancers might be diagnosed not at all or at a later stage in the NPC trial as compared to the SELECT trial. This hypothesis is supported by the observation that 24% of the prostate cancers were advanced (stage T3-T4) in the NPC trial[59], whereas in the SELECT trial almost exclusively (99.4%) localized (stage T1-T2) tumours were found[60]. If selenium indeed influences the progression of prostate cancer, there was no opportunity to express its effects in the SELECT trial where all cancers were already detected and strictly controlled or treated in an early stage. Primary analyses within the NPC trial showed that the preventive effects of selenium were most pronounced for advanced prostate cancer (relative risk compared to placebo; RR 0.27, p=0.03) compared to localized cancer (relative risk compared to placebo; RR 0.42, p=0.02)[77]. This finding, however, was not described in subsequent analyses after additional follow-up[59]. Previous observational studies already suggested that a high selenium status was associated with a decreased risk of advanced, but not localized prostate cancer[78, 79]. After years of ambiguity, the results of our study might provide a possible explanation for the specific effects of selenium on prostate cancer progression rather than development.

Taking together all the evidence described above, combined with the findings from our intervention study described in **Chapter 6**, it might be worthwhile to shift the focus from prostate cancer as one general disease to the recognition of more specific defined subclasses of clinically relevant prostate cancer. Not only selenium, but also a variety of other factors such as BMI[80, 81], statins[82], and blood lipid levels[83, 84], seem to have an effect on advanced, high-grade or aggressive forms of prostate cancer in particular. Apparently, prostate cancer is a disease with two, or maybe more, faces with respect to aetiology, treatment, and prognosis and it should be considered as such in future studies.

In conclusion

In theory, adequate chemopreventive strategies might help to lower the incidence and mortality of prostate cancer. Extensive research on biological mechanisms and the long-term effects on cancer biology and other health outcomes, however, is required before any chemopreventive strategy can be implemented. Selenium has been considered a chemopreventive candidate for prostate cancer. Although the exact biological mechanisms remain to be elucidated, previous studies suggest that the effects of selenium depend on baseline selenium status, provided form of selenium and the stage of the disease. So far, there is insufficient evidence to justify recommendations for supplemental selenium intake. Future studies should specifically focus on the (preventive) role of selenium in prostate cancer progression in high-risk populations, rather than in the general population.

7.3 Implications for (future) patients

Prostate cancer is a very common disease among elderly men. Although prostate cancer is relatively well-studied, various aspects of this disease remain poorly understood. The aim of the research described in this thesis was to obtain insight into factors that might be involved in the development and recurrence of prostate cancer. Furthermore, factors that might play a role in the prevention of prostate cancer were studied. Insight into these factors is important for researchers and health professionals in order to optimize diagnostic methods and to develop new and effective treatment or prevention strategies. For men, patients, their family and friends, knowledge on these factors also has important implications and might help to answer questions such as: "W*hat can I do to lower my prostate cancer risk and how can I prevent the progression or recurrence of the disease?*"

What can I do to lower my prostate cancer risk?

In theory, lowering prostate cancer risk can be achieved by avoidance of factors (risk factors) that increase the risk of getting cancer or exposure to factors (preventive factors) that protect against the disease. Only a few risk factors for prostate cancer have been clearly identified; age, a positive family history, black race and some genetic variants. All of these risk factors are non-modifiable, i.e. that they cannot be avoided. So far, there is no strong and consistent evidence that any modifiable factors, apart from a suggestive role for blood lipid levels, influence the risk of prostate cancer. The study described in **Chapter 3** confirmed that high levels of cholesterol might increase the risk of prostate cancer. As levels of cholesterol can be altered by adapting a healthy lifestyle or by the use of cholesterol-lowering drugs; this factor can be considered as a modifiable risk factor. Although larger studies presented similar findings[84-87], it should be realized that relatively few patients were included in our study. At this moment, it is not clear whether actively lowering cholesterol levels by adapting a healthy lifestyle will indeed result in a lower prostate cancer risk. Nevertheless, adapting a healthy lifestyle is recommended since a balanced diet, adequate levels of physical activity and maintenance of a healthy weight may not only lower prostate cancer risk, but also reduce the burden of many other diseases.

There is no consensus about the effectiveness and safety of active prevention strategies for prostate cancer. Many pharmacological, dietary and lifestyle interventions have been investigated during the past years, however, at this moment there is insufficient evidence to recommend any of these strategies. More and more people are using dietary supplements on their own initiative. Data from a national

survey in the Netherlands indicated that in the period 2007-2010 up to 36% of all men aged 51-69 years took dietary supplements[88]. The widespread use of dietary supplements might be facilitated by the fact that they are easily available in supermarkets, drugstores and internet shops, and that some of them have been associated with beneficial health effects. There might be indeed a few promising candidates for prostate cancer prevention. However, although dietary supplements seem to be relatively innocent, recent studies suggested that a prolonged intake of some specific supplements might result in serious adverse effects and even an increased risk of getting prostate cancer[89]. Therefore, the use of dietary supplements is not recommended for the prevention of prostate cancer until the safety, long-term effects and biological mechanisms are carefully evaluated.

Unfortunately, it seems that there is not much that can be done to lower the risk of getting prostate cancer at the moment. Although the beneficial effects with respect to prostate cancer are not proven, the general recommendations provided by the World Cancer Research Fund[90] might be a good starting point to adapt a healthy lifestyle; maintain a healthy body weight and be physically active, consume mainly plant-based foods and limit the consumption of energy-dense foods, red meat, processed meat, salt and alcoholic drinks. Furthermore, it might be worthwhile to recognize possible symptoms related to prostatic disorders in an early stage, and to be aware of the non-modifiable risk factors for prostate cancer such as a positive family history. It should be noted that active screening with PSA tests without having symptoms or medical indications is not indicated, because the burden of the diagnostic tests might not balance the benefits of finding tumours in the general population[14, 15].

How can I prevent the progression or recurrence of the disease?

For patients who are already diagnosed with prostate cancer, the question how to lower risk of progression or recurrence might be extremely important. Compared to incident (newly diagnosed) prostate cancer, there are relatively few studies which addressed modifiable factors that might influence the course of the disease in prostate cancer patients. Overweight is the main modifiable risk factor studied in relation to risk of prostate cancer recurrence. In our studies described in **Chapter 5**, we did not find any evidence that overweight influences the course of the disease after surgery for prostate cancer. However, we did follow the patients only for a period of five years and the study population was limited to patients treated with surgery for prostate cancer. The findings might therefore not apply to other (overweight) patients with prostate cancer. Overweight is associated with complications during surgery, increased risk of recurrence and more aggressive prostate cancers in other studies[40, 91, 92]. Therefore, there are some indications that maintaining or adapting a healthy weight

after the diagnosis is beneficial and might possibly help to prevent adverse side-effects, and improve prostate cancer outcome, in addition to other possible beneficial effects.

A few dietary and lifestyle interventions are evaluated in patients diagnosed with prostate cancer. For some of these strategies, it is suggested that they have beneficial effects because they influence levels of PSA[93], or disease progression[94]. However, the findings of these studies need to be confirmed and validated and the long-term effects on prostate cancer outcome, safety, quality of life and feasibility need to be examined. At this moment, there is insufficient evidence to recommend any of the specific dietary and lifestyle programs, and therefore patients mainly adhere to the advices of their physicians, or follow their own intuition. The World Cancer Research Fund recommends also patients already diagnosed with cancer to follow the general guidelines as described above. Of special importance are the recommendations regarding avoidance of self-prescribed supplement use, because of the unexplored effects of these supplements on cancer growth, and the possibility that specific dietary supplements might interfere with some cancer therapies[95-97].

In conclusion

There is an unfortunate lack of information about modifiable risk factors for prostate cancer. This implies that there is hardly anything what patients can do to lower their risk of getting prostate cancer or to prevent progression or recurrence of this disease. In line with this, there is insufficient evidence to implement recommendations for the prevention of prostate cancer development, progression or recurrence. The recommendations for the general population as well as cancer patients refer to adapting a lifestyle with a balanced diet, adequate levels of physical activity and a healthy weight. The use of self-prescribed dietary supplements for the prevention of prostate cancer is explicitly discouraged.

7.4 Implications for the urologist

Above anyone else, the urologist recognizes the increasing incidence, and thereby the clinical and social burden, of prostate cancer. Although a major progress has been made in the past decades, there is a strong need for better understanding of prostate cancer aetiology and for the identification of targets for effective prevention or treatment strategies. The aim of this thesis was to evaluate potential risk factors for incident and recurrent prostate cancer and to study the molecular mechanisms possibly involved in the aetiology of prostate cancer. From this perspective, a few implications, which might be of relevance in clinical practice, will be discussed below.

Incident prostate cancer

There is a growing body of evidence that high levels of serum cholesterol are associated with an increased risk of aggressive prostate cancer[84-87, 98]. In theory, the identification of patients with an unfavourable blood lipid profile at high-risk of developing aggressive prostate cancer might provide implications for early detection and targeted prevention or treatment strategies. One of the suggested prevention strategies in these high-risk patients is the use of statins[83, 85, 99]. However, before such clinical prevention strategies can be implemented, the long-term effects on cancer biology and other health outcomes should be carefully determined. Furthermore, there is not yet an indication to screen men for unfavourable blood lipid profiles, as the benefits of early prostate cancer detection in these men are not established.

In the first year after their diagnosis, patients diagnosed with a previous cancer (and previous urological cancers in particular) seem to have an increased risk of prostate cancer. Nevertheless, this finding does not necessarily indicate that these patients are more likely to develop prostate cancer; it might also mean that prostate cancers are detected more frequently, possibly as a consequence of active screening or early detection. As there is no data on long-term effects and cancer-specific mortality, it is not possible to advice or dissuade active screening for prostate cancer in cancer survivors. In order to elucidate the role of screening and detection in cancer patients, however, it might be extremely useful to register the reasons for PSA testing (e.g. anxiety, complaints, regular checks), whether the diagnosis of prostate cancer was incidental, and the method used for the prostate cancer diagnosis (e.g. imaging techniques, biopsy, surgical specimens) for research purposes in the medical files.

Recurrent prostate cancer

Overweight and obesity are the main modifiable risk factors studied in relation to risk of prostate cancer recurrence. Contrary to most studies performed in the United

States, our studies did not provide evidence that overweight or obesity influence the risk of biochemical recurrence following radical prostatectomy. Based on our findings, there is no strong indication, apart from technical issues or other co-morbidities, to refuse overweight and obese patients for radical prostatectomy. Overweight, however, is associated with complications during and after surgery, increased risk of recurrence and more aggressive prostate cancers in other studies[40, 91, 92]. Therefore, patients may be advised to maintain or adapt a healthy weight, which might help to prevent side-effects, and improve prostate cancer outcome, in addition to other possible beneficial effects.

Chemoprevention

There has been considerable interest in the role of nutrition and lifestyle factors in prostate cancer prevention. Men at risk for prostate cancer might find information on suggested candidates for prostate cancer prevention as a consequence of the easy access to health information on the internet[100]. The 'innocent character' of dietary supplements might persuade these men start taking specific dietary supplements focussing on prostate health. So far, there is no consensus about the effectiveness and safety of dietary supplements for prostate cancer prevention in the general population. In contrast, it is suggested that a prolonged intake of some dietary supplements (e.g. a high dose of vitamin E as α-tocopherol) might result in an increased prostate cancer risk[89]. The use of dietary supplements for the prevention of prostate cancer should be discouraged until the safety, long-term effects and biological mechanisms are carefully evaluated. Also patients already diagnosed with prostate cancer should be advised to avoid the use of self-prescribed dietary supplements. Although some dietary and lifestyle interventions are suggested as appropriate candidates for the prevention of prostate cancer progression, the unexplored effects on cancer biology and the possible interference with cancer therapies[95-97] should be carefully determined.

In conclusion

Patients suspected for or diagnosed with prostate cancer may be advised to adapt a lifestyle with a balanced diet, adequate levels of physical activity and maintenance of a healthy weight. The use of self-prescribed dietary supplements is explicitly discouraged.

7.5 Future research agenda

The results of this thesis show that specific nutritional and clinical factors might influence risk of prostate cancer or have an effect on gene expression in the prostate. A future challenge is the confirmation and 'translation' of these findings into the development and implementation of effective treatment or prevention strategies for prostate cancer.

Suggested research agenda

- The current knowledge and perception of risk factors among cancer patients and the general population should be taken into account while developing effective cancer education and awareness programs.
- It should be determined whether men with unfavourable blood lipid profiles might benefit from screening and early detection of prostate cancer.
- The effects of lowering (either by lifestyle or pharmaceutical interventions) circulating levels of cholesterol in high-risk men need to be established with respect to prostate cancer risk, cancer biology and other long-term health outcomes.
- The mechanisms underlying the suggested effects of high levels of circulating cholesterol on (aggressive) prostate cancer risk warrant clarification.
- Detailed insight into prognosis and cancer characteristics of screen-detected prostate cancers among cancer survivors is warranted.
- Given the increasing number of patients with overweight and obesity, the effect of body mass index (BMI) on risk of biochemical recurrence needs to be monitored over time. Weight and BMI earlier in life should be taken into account whenever possible.
- It might be interesting to explore the potential interaction between gene expression profiles in the prostate and the 'metabolic signature' of the patient (including body mass index, fat distribution, levels of circulating adipokines and characteristics of the adipose tissue surrounding the prostate).
- The hypothesis that selenium might prevent against the development of advanced prostate cancer needs to be tested.
- Potential effects of selenium on functional processes (e.g. cellular invasion and migration) related to the epithelial-to-mesenchymal transition need to be determined.

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Samenvatting

(Summary in Dutch)

Prostaatkanker is de meest voorkomende kanker bij mannen in Nederland. Er is weinig bekend over de oorzaken van prostaatkanker. Tot op heden zijn slechts een paar risicofactoren voor prostaatkanker vastgesteld, namelijk hogere leeftijd, zwart ras en bepaalde genetische factoren. Helaas geldt voor al deze risicofactoren dat ze niet te beïnvloeden zijn. Hierdoor zijn er weinig tot geen opties voor preventie (ofwel het voorkómen) van prostaatkanker. Voor de ontwikkeling van effectieve preventie-programma's, maar ook voor het opsporen van mannen met een hoog risico en het optimaliseren van behandelmethoden, is het van groot belang dat er meer inzicht wordt verkregen in risicofactoren voor prostaatkanker in verschillende stadia van de ziekte.

Het doel van dit proefschrift is het bestuderen van factoren die een rol kunnen spelen bij het ontstaan of de terugkeer van prostaatkanker. Dit proefschrift richt zich hiervoor in het bijzonder op factoren die te maken hebben met voeding, leefstijl en bepaalde medische condities.

Allereerst is een inventarisatie gemaakt van factoren die interessant kunnen zijn om te bestuderen in relatie tot prostaatkanker (**hoofdstuk 2**). Hiervoor hebben we 956 mannen met prostaatkanker met behulp van een vragenlijst gevraagd om mogelijke oorzaken voor hun ziekte te noemen. Uit dit onderzoek bleek dat de grote meerderheid (85%) van deze patiënten niet in staat was om een mogelijke oorzaak te noemen. De patiënten die wel een verklaring hadden, noemden zeer diverse oorzaken variërend van fietsen tot het gebruik van viltstiften en van sterilisatie tot de Tsjernobyl-ramp. Over het algemeen wijten relatief veel patiënten hun prostaatkanker aan factoren gerelateerd aan erfelijkheid, specifieke omgevingsfactoren, voeding of leefstijl en stress. Opvallend is dat er vaak oorzaken (zoals alcohol-inname) werden genoemd die zeer waarschijnlijk geen rol spelen bij het ontstaan van prostaatkanker. De eerder genoemde 'bewezen' risicofactoren voor prostaatkanker werden daarentegen niet regelmatig genoemd, zelfs niet door patiënten die tot de risicogroepen behoren. Concluderend kan dus gesteld worden dat veel patiënten zich niet bewust zijn van mogelijke oorzaken van hun prostaatkanker. In hoeverre dit te wijten is aan een gebrek aan kennis, of doordat er simpelweg weinig eenduidige risicofactoren voor prostaatkanker zijn, is niet bekend. Wel is duidelijk dat er verkeerde opvattingen bestaan over risicofactoren voor prostaatkanker en dat 'bewezen' risicofactoren niet goed herkend worden.

In navolging van eerdere onderzoeken zijn de volgende twee hoofdstukken gericht op specifieke factoren die mogelijk een rol kunnen spelen bij het ontstaan van prostaatkanker. In de studie beschreven in **hoofdstuk 3** is bestudeerd of cholesterol-

concentraties in het bloed verband houden met het risico om prostaatkanker te ontwikkelen. Voor dit onderzoek zijn de gegevens onderzocht van 2.118 mannen die allen afkomstig zijn uit de regio Nijmegen en deelnemen aan de Nijmegen Biomedische Studie. Bij aanvang van de studie is bloed afgenomen en zijn de cholesterolconcentraties gemeten. Vervolgens zijn deze mannen gedurende een langere periode (gemiddeld ruim 6,5 jaar) gevolgd. Na afloop van deze periode bleek dat 43 mannen prostaatkanker hadden gekregen. De resultaten van dit onderzoek laten zien dat mannen met een hoge cholesterolconcentratie in het bloed een hoger risico hebben om prostaatkanker te ontwikkelen. Dit verhoogde risico geldt voornamelijk voor de agressieve vormen van prostaatkanker.

Door middel van een onderzoek met een vergelijkbare opzet is vervolgens het risico op prostaatkanker bepaald voor mannen die al eerder een andere soort kanker hebben gehad (**hoofdstuk 4**). Aanleiding voor dit onderzoek is de hypothese dat het vóórkomen van prostaatkanker mogelijk geassocieerd is met andere kankersoorten door gemeenschappelijke risicofactoren, door blootstelling aan eerdere kankerbehandelingen, ofwel doordat er intensiever gezocht wordt naar andere tumoren als iemand al kanker heeft. Voor dit onderzoek heeft de Nederlandse Kankerregistratie de gegevens beschikbaar gesteld van 551.553 mannen die in de periode 1989-2008 voor de eerste keer met kanker zijn gediagnosticeerd. Bij 9.243 van deze mannen is vervolgens prostaatkanker gevonden. Het bleek dat met name in het eerste jaar na een eerdere kankerdiagnose het risico op prostaatkanker verhoogd was. Waarschijnlijk is dit te wijten aan een toegenomen alertheid bij patiënt of arts en intensieve medische controles, waardoor veel prostaattumoren opgespoord werden. Op de langere termijn was er voor de meeste kankerpatiënten echter geen verhoogd risico op prostaatkanker. Alleen voor mannen die eerder huidkanker hadden, lijkt er na tien jaar nog steeds een iets verhoogd risico op prostaatkanker te zijn, terwijl mannen met een kanker aan hoofd of hals dan juist een verlaagd risico op prostaatkanker hadden. Tot slot is met dit onderzoek ook aangetoond dat prostaatkanker iets minder vaak voorkomt bij mannen die eerder een bestraling in het bekkengebied hebben ondergaan.

Overgewicht wordt ook regelmatig in verband gebracht met prostaatkanker. Omdat overgewicht in eerdere studies voornamelijk geassocieerd is met prostaatkanker in een laat stadium of terugkeer van de ziekte na een behandeling, zijn de **hoofdstukken 5a** en **5b** specifiek gericht op de vraag of het hebben van overgewicht een risicofactor is voor de terugkeer van de tumor na een operatie voor prostaatkanker. Hiervoor zijn de gegevens bekeken van patiënten met prostaatkanker die een operatie hebben ondergaan waarbij de prostaat in zijn geheel is verwijderd. Na de operatie is er bij deze

patiënten, volgens de standaard richtlijnen in de betreffende ziekenhuizen, regelmatig bloed afgenomen. In het bloed is de concentratie van het prostaat-specifiek antigeen (PSA) bepaald; dit is een eiwit dat in hoge concentraties kán duiden op de aanwezigheid van prostaatkanker. In het geval van stijgende PSA concentraties na de prostaatoperatie wordt er gesproken van terugkeer van de ziekte, ook wel recidief genoemd. Wij hebben niet kunnen aantonen dat het hebben van overgewicht de kans op een dergelijk recidief beïnvloedt.

Hoofdstuk 6 van dit proefschrift is gewijd aan de mogelijkheden voor preventie, ofwel het voorkómen van prostaatkanker. Selenium is een voedingsstof die in het verleden regelmatig in verband is gebracht met de preventie van prostaatkanker. Er is echter maar weinig bekend over de biologische mechanismen en de daadwerkelijke effecten van selenium in de prostaat. Dit onderzoek is uitgevoerd om te bestuderen welk effect selenium heeft op de activiteit van de genen, ofwel de genexpressie, in de prostaat. Wij hebben hiervoor aan 23 mannen gevraagd om gedurende een periode van vijf weken dagelijks een voedingssupplement met 300 µg selenium of een placebo in te nemen. Bij aanvang en na afloop van de studie is bij deze mannen een klein stukje prostaatweefsel (prostaatbiopt) afgenomen. Na controle van dit weefsel bleken de biopten van 15 deelnemers geschikt om te analyseren op een zogenoemde microarray. Met behulp van een dergelijke microarray waren we in staat om de expressie van meer dan 19.000 genen in de prostaat tegelijkertijd te meten. De resultaten van deze metingen suggereren dat genen die betrokken zijn bij ontstekingsreacties (inflammatie) verminderd tot expressie komen na inname van selenium. Dit zou kunnen duiden op een mogelijk ontstekingsremmend effect van selenium. Ook voor genen die betrokken kunnen zijn bij de ontwikkeling van agressieve vormen van prostaatkanker waren veranderingen in genexpressie waarneembaar na inname van selenium. In hoeverre deze bevindingen relevant zijn met betrekking tot de preventie van prostaatkanker, en welke biologische mechanismen er verantwoordelijk zijn voor deze effecten zal bestudeerd worden in vervolgonderzoeken.

Tot slot zijn de belangrijkste bevindingen van dit proefschrift kort samengevat in **hoofdstuk 7**. In dit hoofdstuk zijn ook verschillende aspecten genoemd die belangrijk zijn voor de interpretatie van de resultaten én zijn speerpunten voor toekomstig onderzoek beschreven.

Concluderend kan gesteld worden dat zowel bij de patiënt als de onderzoekers, vrij weinig bekend is over factoren die betrokken zijn bij de ontwikkeling van prostaatkanker in verschillende stadia van de ziekte. Op basis van de resultaten van dit proefschrift wordt bevestigd dat een hoge cholesterolconcentratie waarschijnlijk een risicofactor is voor (agressieve) prostaatkanker. Op de vraag óf en waarom het hebben van een eerdere kanker het daaropvolgende risico op prostaatkanker beïnvloedt, is nog geen eenduidig antwoord te geven, al lijkt het erop dat de meeste kankerpatiënten zich op lange termijn geen extra zorgen hoeven te maken. Op basis van onze resultaten is er geen overtuigend bewijs om aan te nemen dat overgewicht het risico op de terugkeer van prostaatkanker na een prostaatoperatie beïnvloedt. Echter, gezien het sterk stijgende aantal patiënten met overgewicht is het wellicht interessant om deze vraagstelling ook vanuit een meer mechanistisch oogpunt te bestuderen. Wat betreft de preventie van prostaatkanker kunnen we concluderen dat selenium een aantal duidelijke effecten heeft op de genexpressie in de prostaat. In hoeverre deze veranderingen in genexpressie daadwerkelijk kunnen bijdragen aan de preventie van prostaatkanker, in het bijzonder bij mannen met een verhoogd risico op prostaatkanker, moet blijken uit toekomstig onderzoek.

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Dieuwertje.

About the author

Curriculum Vitae

Dieuwertje Kok was born on the 17th of April 1983 in Oss, the Netherlands. In 2001, she finished her secondary school at the St. Ludgercollege (Athenaeum) in Doetinchem. In September of that year, she entered the program of Biology and Medical Laboratory Research at the Saxion University of Applied Sciences in Enschede. She graduated with honours with specializations in Clinical Chemistry as well as Medical Microbiology in 2004. In the same year, Dieuwertje started an MSc program in Medical Biology at the University of Groningen, specializing in the field of Neuroendocrinology. In 2006, she graduated with honours and started her PhD project which was funded by the World Cancer Research Fund. The aim of this project was to identify factors which are related to prostate cancer risk and recurrence. During her time as a PhD fellow at the division of Human Nutrition of Wageningen University, she joined the educational program of the Graduate School VLAG. In 2008, Dieuwertje received an exchange grant from the Nutrigenomics Organisation and visited the laboratory of the Human Nutrition Research Centre of Newcastle University (UK) for six weeks. During this visit, she optimized assays to examine gene-specific hypermethylation in human prostate tissue. In 2011, Dieuwertje was involved in the development of the research program 'Nutrition and Cancer' within the collaborative network of Wageningen University and Hospital Gelderse Vallei. Currently, she is a postdoctoral fellow at Wageningen University within the program 'Cancer and Nutrition' of the Alpe d'HuZes Foundation.

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Book contributions

DEG Kok, LA Afman, P van 't Veer. Handboek Prostaataandoeningen. Chapter 12, Voeding, levensstijl en chemopreventie (Nutrition, Lifestyle and Chemoprevention), page 201-215. ISBN 9058981371 / 9789058981370

Overview of completed training activities

Discipline-specific activities		
Courses		
Masterclass 'Nutrigenomics'	Graduate School VLAG, Wageningen (NL)	2007
Masterclass 'Diet and Cancer'	Graduate School VLAG, Wageningen (NL)	2007
Course 'Nutritional & Lifestyle Epidemiology'	Graduate School VLAG, Wageningen (NL)	2009
Course 'Advanced Microarray Data Analysis'	Nutrigenomics Organisation NuGO, Maastricht (NL)	2009
Course 'Advanced visualization, integration and biological interpretation of ~omics data'	Graduate School VLAG, Wageningen (NL)	2011
Conferences and meetings		
Conference of the Netherlands Epidemiology Society (WEON)	Maastricht (NL) Groningen (NL)	2007 2008
Conference of the Nutrigenomics Consortium ('NuGO week')	NuGO, Oslo (Norway) NuGO, Wageningen (NL)	2007 2011
NWO Nutrition meeting	NWO, Deurne (NL)	2007 2011
National conference on Nutrition	Hospital Gelderse Vallei / WUR, Ede (NL)	2008
3rd ECNIS (Environmental Cancer Risk, Nutrition and Individual Susceptibility) Annual Meeting	ECNIS / NuGO, Barcelona (Spain)	2008
Wageningen Nutritional Sciences Forum	Wageningen University, Arnhem (NL)	2009
Symposium 'Epigenetics and Disease'	Nijmegen Centre for Molecular Life Sciences, Nijmegen (NL)	2009
Symposium 'From molecule to population'	Nijmegen Centre for Evidence Based Practice, Nijmegen (NL)	2011

General activities		
PhD Introduction Week	Graduate School VLAG, Bilthoven (NL)	2007
NuGO Introduction Course	NuGO, Wageningen (NL)	2007
PhD competence assessment	Wageningen Graduate Schools, Wageningen (NL)	2007
Talent day 'Write it right' and 'Grant application'	NWO, Utrecht (NL)	2007
Course 'Tutoren Training'	Educational Staff Development, Wageningen University, Wageningen (NL)	2007
Course 'Academic Writing'	Radboud University Nijmegen, Nijmegen (NL)	2009
Course 'Techniques for Writing and Presenting a Scientific Paper'	Wageningen Graduate Schools, Wageningen (NL)	2010
Career assessment	Wageningen Graduate Schools, Wageningen (NL)	2011
Masterclass 'Hands on grants'	Dutch Cancer Society (KWF), Amsterdam (NL)	2011
Course 'Good Clinical practice'	Hospital Gelderse Vallei, Ede (NL)	2011
Masterclass 'Multilevel analyses'	Graduate School VLAG, Wageningen (NL)	2011
Masterclass 'Analysis in R'	Graduate School VLAG, Wageningen (NL)	2012
Optional activities		
Preparation research proposals	Wageningen University, Wageningen (NL)	2006
Literature and Discussion groups 'Journal Club', ' Oldsmobiles', 'NMG group', 'Methodology Meetings' and 'Rothman Lunches'	Wageningen University, Wageningen (NL)	2006-2010
Participating PhD Study Tour to USA	Wageningen University	2007
A 6-week visit to the Human Nutrition Research Centre, Newcastle University, supported by a NuGO exchange grant	Newcastle University, Newcastle (UK)	2008

Colophon

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