

Prostate Diseases and the Metabolic Syndrome

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UNIVERSITY OF GOTHENBURG

Gothenburg in 2015

Cover illustration: Title by Artist name

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ISBN: (Print) 978-91-628-9378-1, (Electronic publication) 978-91-628-9379-8

Printed in Gothenburg, Sweden in 2015

Printer's name

Ineko

To my family

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ABSTRACT

The overall aim of this thesis was to explore the association between the components of the metabolic syndrome (MetS), including vitamin D, and lower urinary tract symptoms (LUTS). The focus was on the relationship between diseases of the prostate, mainly benign prostatic enlargement (BPE) and prostate cancer (PC).

The study cohort consisted of 3,014 Swedish men aged 69-81 enrolled in the Swedish arm of the Osteoporotic Fractures in men (MrOs) study. The study participants were randomly selected using population registries and then contacted and asked to participate in the study. The selected men were asked to fill in questionnaires regarding a variety of items such as daily activities and physical exercise, eating-, drinking- and smoking habits, medication, past and present history of diseases, voiding habits, surgery and current treatment. They underwent investigations regarding bone mineral density, measurements of body composition such as height, weight, body mass index, body fat mass and lean mass and blood samples were obtained for analyses of a variety of variables. In a subgroup of this cohort (1,010 individuals) more

extended analyses were performed. Furthermore, in a small cohort (184 individuals), the prostate gland volumes were measured through transrectal ultrasonography. Lower urinary tract symptoms were measured by the International Prostate Symptom Score and urinary incontinence (UI) was evaluated by a questionnaire. The cohort has been followed for more than 10 years. The MrOs register was coordinated with the Swedish Death Register, the Swedish Cancer Register, and the National Prostate Cancer Register.

This investigation showed that LUTS and UI were neither associated with any major component of the MetS, nor associated with serum levels of vitamin D. However, serum levels of serotonin were negatively associated with LUTS and UI, while fasting glucose and adiponectin were positively associated with LUTS. Benign prostatic enlargement was associated with low levels of vitamin D, serum calcium, sex hormone-binding globulin and high-density lipoprotein cholesterol. Individuals with type 2 Diabetes mellitus (T2DM) had a decreased risk of being diagnosed with incident prostate cancer. Increased levels of vitamin D were associated with increased risk of being diagnosed with PC. Individuals with low serum c-reactive-protein levels and taller individuals had a higher risk of developing PC. Plasma levels of osteocalcin, a protein produced by osteoblasts, were lower in individuals with T2DM, and higher in individuals with incident PC.

The overall conclusions in the present thesis were that vitamin D was negatively associated with BPE and positively associated with PC, however, not associated with LUTS. In addition, the end-point component of MetS, T2DM, was positively associated with BPE but inversely associated with incident PC, and finally, that osteocalcin was positively associated with incident PC.

Keywords: Metabolic syndrome, lower urinary tract symptoms, urinary incontinence, benign prostatic enlargement, prostate cancer, serotonin, vitamin D, osteocalcin

ISBN: (Print) 978-91-628-9378-1, (Electronic publication) 978-91-628-9379-8

SAMMANFATTNING PÅ SVENSKA

BETYDELSEN AV METABOLA FAKTORER FÖR URINVÄGSBESVÄR OCH SJUKDOMAR I PROSTATA

Tidigare studier har visat att det så kallade ”metabola syndromet (MetS)”, med dess hormonella och metabola avvikelser, har samband med prostatasjukdomar. Den betydande geografiska variationen i incidens, de starka tidstrenderna och förändringarna i risk hos folkgrupper som flyttar mellan länder med olika incidens utgör starka belägg för att omgivningsfaktorer och dietära faktorer har betydelse för uppkomst av sjukdomar i prostatan. Epidemiologiska studier har visat att det finns samband mellan vitamin D och prostatasjukdomar. Vitamin D är välkänt för dess roll vid reglering av calcium- och fosfatbalansen i kroppen. Detta vitamin, som ju egentligen inte alls är ett vitamin utan ett hormon, och dess metaboliter, reglerar även tillväxt och differentiering av andra celltyper. Receptorer för vitamin D finns också i prostatakörteln.

Prostatacancer (PC) är den vanligaste cancerformen och den vanligaste orsaken till cancerrelaterad död bland män i Sverige. Orsakerna till prostatacancer är inte helt klarlagda. Hög ålder och ärftlighet är de viktigaste kända riskfaktorerna. Det genetiska underlaget för ärftlig PC är inte heller fullständigt definierat. Till de gener som står i centrum

för intresset hör de som kodar för androgenreceptorn och vitamin D-receptorn.

Syftet med denna avhandling är att belysa sambandet mellan metabola faktorer, däribland Vitamin D och nedre urinvägsbesvär samt sjukdomar i prostatan (godartad prostataförstoring och prostatacancer).

Underlaget för studien är en stor kohort av svenska män, 3 014, samlade från Uppsala, Göteborg och Malmö, MrOs studien. MrOs är en internationell jämförande studie av osteoporos och dess riskfaktorer hos äldre män. I denna studiekohort har bland annat koncentrationerna av vitamin D, kalcium, paratyreoideahormon (PTH), tillväxtfaktorer och könshormoner analyserats. Det identifierades 262 individer med prostatacancer vid starten av studien för drygt 10 år sedan, så kallade ”prevalenta prostatacancerfall”. Under studiens gång fram till december 2013 identifierades ytterligare 252 nya prostatacancerfall, så kallade ”incidenta prostatacancerfall”. Samkörning har gjorts med svenska dödsorsaksregistret, det nationella cancerregistret och det nationella kvalitetsregistret för prostatacancer. Hos 184 individer från Göteborgskohorten mättes volymen av prostatakörteln via transrektal underljudsundersökning (TRUL).

Olika aspekter på relationen mellan vitamin D, BMD, metabolt syndrom och prostatahälsa (godartad förstoring och cancer) har studerats med så kallade ”fall kontroll metodik”.

Resultaten visar att vattenkastningsbesvär hos svenska män inom åldersintervallet 69-81 år är relaterade till prostataförstoring samt

positivt associerad till nivåer av serumglukos och adiponectin men negativt associerat till nivåer av serumserotonin, och att godartad prostataförstoring var relaterad till låga nivåer av: serum vitamin D, SHBG, serum calcium och HDL-kolesterol. Inga samband påvisades mellan LUTS/BPE och övriga komponenter av MetS. Risken att drabbas av PC ökar i takt med stigande nivåer av serumvitamin D upp till 90 nmol/l och individer med diabetes har lägre risk att utveckla PC och har låga nivåer av serumtestosteron och dess metaboliter i serum. Dessutom fann vi att individer med låga serum CRP-nivåer (< 1,58 g/l) och långa individer (över 179 cm) hade högre risk att drabbas av PC. Inga samband påvisades mellan PC och övriga komponenter av MetS. Vidare visades att PC är relaterad till höga nivåer av plasmaosteocalcin (OC), en osteoblastaktivitetsmarkör, vilken kan ha hormonella egenskaper, samt att individer med T2DM har lägre nivåer av OC.

De viktigaste konklusionerna i avhandlingen är att MetS, med diabetes som dess slutstadium, var inte associerad till LUTS, däremot positivt associerad till BPE och negativt associerad till PC, att låga nivåer av vitamin D var associerad till BPE, att risken för PC ökar vid stigande nivåer av serum vitamin D, samt att höga nivåer av osteocalcin var associerad till incident PC och låga nivåer till T2DM.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

- I. M.A. Haghsheno, D. Mellström, R. Peeker, J. Hammarsten, M. Lorentzon, V. Sundh, M. Karlsson, C. Ohlsson and J.E. Damber. *Lower urinary tract symptoms are associated with low levels of serum serotonin, high levels of adiponectin and fasting glucose, and benign prostatic enlargement.*
Scand J Urol, 2014. 25: p. 1-7

- II. M.A. Haghsheno, D. Mellström, C.J. Behre, J.E. Damber, H. Johansson, M. Karlsson, M. Lorentzon, R. Peeker, E. Barrett-Connor, E. Waern, V. Sundh, C. Ohlsson and J. Hammarsten. *Low 25-OH vitamin D is associated with benign prostatic hyperplasia.*
J Urol., 2013. **190**(2): p. 608-14

- III. M.A. Haghsheno, D. Mellström, R. Peeker, J. Hammarsten, M. Lorentzon, Ö. Ljunggren, M. Karlsson, H. Johansson, C. Ohlsson and J.E. Damber. *Is there an Association between Components of the Metabolic Syndrome and incident Prostate Cancer? A prospective cohort study in Sweden.*
Submitted

- IV. M.A. Haghsheno, J-E. Damber, R. Peeker, H. Johansson, M. Karlsson, C. Lewerin, J. Hammarsten, M. Lorentzon, U. Smith U. Lerner, C. Ohlsson and D. Mellström. *High plasma osteocalcin predicts incident prostate cancer.*
Submitted

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ERRATA	FEL! BOKMÄRKET ÄR INTE DEFINIERAT.

ABBREVIATIONS

ALP	Alkaline Phosphatase
BMD	Bone Mineral Density
BMI	Body Mass Index
BOO	Bladder Outlet Obstruction
BPE	Benign Prostatic Enlargement
BPH	Benign Prostatic Hyperplasia
CRP	C-reactive Protein
DHEA	Dehydroepiandrosterone
DHT	Dihydrotestosterone
DRE	Digital Rectal Examination
ED	Erectile Dysfunction
HDL	High Density Lipoprotein
25(OH) D	25-hydroxyvitamin D
HR	Hazard Ratio
IGF-1	Insulin-like Growth Factor 1
IPSS	International Prostate Symptom Score
LDL	Low Density Lipoprotein
LUTS	Lower Urinary Tract Symptoms
MrOs	The Osteoporotic Fractures in Men Study
NOS	Nitric Oxide Synthase
OAB	Overactive Bladder
OAB wet	Overactive Bladder with urge incontinence
OR	Odds Ratio
PC	Prostate Cancer
PSA	Prostate Specific Antigen
PTH	Parathyroid Hormone
RALP	Robotic Assisted Laparoscopic Prostatectomy
RIA	Radioimmunoassay technique
RP	Radical Prostatectomy
SHBG	Sex Hormone Binding Globulin
T2DM	Type 2 Diabetes Mellitus
TRUS	Transrectal Ultrasonography
UI	Urinary Incontinence

Un-OC

UVB

VDR

Undercarboxylated OC

Ultraviolet Beta

Vitamin D Receptor

1 INTRODUCTION

During recent decades, evidence has accumulated suggesting that lower urinary tract symptoms (LUTS) and diseases of the prostate, mainly benign prostatic enlargement (BPE) and prostate cancer (PC) might be associated with the metabolic syndrome (MetS) [1-9]. Individuals with MetS have been shown to have low levels of serum vitamin D [10]. This hormone (vitamin D is in fact a hormone) is an anti-proliferative [11], pro-apoptotic agent [12], with receptors in the prostate [13] as well as elsewhere in the body. Vitamin D has been shown to have anti-inflammatory properties through which it may inhibit benign enlargement of the prostate gland [14]. Low levels of vitamin D in plasma have been shown to be associated with a reduced risk of PC [15]. However, other studies indicate that both low and high levels of vitamin D are related to increased risk of PC [16]. The aim of the present thesis was to elucidate whether there is an association between MetS, with vitamin D at focus, LUTS and diseases in the prostate, mainly BPE and PC.

1.1 Historical Background

Archeological discoveries indicate that our ancestors suffered from the same urological discomforts as those that people deal with on a daily bases in modern urology. A large stone has been found in the urinary bladder of a 5,000- years- old Egyptian mummy, indicating that bladder outlet obstruction (BOO) causing formation of stones over time was a substantial problem even back then. On the Ebers Papyrus, dating from 1550 BC, urinary retention and urinary incontinence (UI) secondary to spinal cord injuries were discussed.

Hippocrates wrote that urinary retention was an incurable disorder and that the prognosis was *pessima* (the worst).

The ancient Greek Herophilus of Chalcedon, who worked in Alexandria in Egypt during the 330 - 260 BC, as a physician and is often called “the father of anatomy,” was the first to name the prostate. The Greek word “*prostate*,” means “protector, guardian or defender.”

Modern urology derives from medieval lithologists, who were healers specialized in the removal of bladder stones. In the *Tabula Anatomica* in 1535, Vesalius described a structure located below the urinary bladder around the urethra, which today is known as the prostate gland. Francisco Diaz, a Spanish surgeon, is considered to be the founder of modern urology. He wrote his thesis on diseases of the bladder, kidneys and urethra in 1588. Niccolo Massa, a Venetian physician in the 16th century, wrote the first authentic paper on the prostate. Jean Riolan suggested in 1649 that the prostate could obstruct the bladder neck, causing dysuria, and Guthrie described the pathology of prostate hyperplasia in 1835. John Hunter wrote about “callosities of the prostate obstructing bladder emptying”, and reported that there must be a relationship between the testicles and prostate gland growth (Weyrauch 1959, Encyclopedia Britannica 2,000). At the same time during this period, different surgical instruments were designed for incision of the bladder neck and prostate gland, catheterization of the urethra and bladder, and open surgery on the prostate. Narcier introduced an “*inciseaur*” for making a transurethral incision of the urethra, and an “*exciseaur*” for excising parts of the prostate gland. In the 18th century, James Miller of Edinburgh (1812 - 1864) wrote in *Principals of Surgery* (1844) and *Practice of Surgery* (1846):

”Hypertrophy of the gland is usually regarded as but one of the many signs of senile degeneracy in the frame. As the eyes grow dim, cartilages ossify and the

arteries change their codes, so the prostate is supposed to grow large and hard..... Treatment is but palliative. We can scarcely hope to retard much less remove the enlargement.” (Encyclopedia Britannica 7th and 8th editions).

Sir Henry Thomson (1820 - 1904), who worked as an urologist in London in the 19th century, wrote in *The Pathology and Treatment of Stricture of the Urethra (1852)*, that habitual catheterization of the bladder was a favorable method of treatment rather than surgery, and Browne wrote in 1893 that “*as long as catheter life was tolerable prostatectomy should not be attempted*”.

The first professor in urology was the French physician, Felix Guyon, and by then, in 1890, urology became a specialty in medicine separated from general surgery. Belfield (1856 - 1929) and MacGill performed enucleation of the adenoma of the prostate. Eugene Fuller (1858 - 1930), professor in venereal and genitourinary diseases in New York, published his famous article titled “Six successful cases of prostatectomy”. Peter Freyer (1851 - 1921) performed prostatectomies, sometimes without gloves when the case was extra- difficult, and he kept the specimens in jars in his office. At the same time, several reports were published on the treatment of an enlarged prostate, from ligating the internal iliac arteries to castration, resulting in the atrophy of the gland. Castration therapy for an enlarged prostate raised the idea of the association between androgenic hormones and growth of the prostate gland.

During the past century, a new phenomenon has occurred. It is now natural for men to live beyond 50 years, and nowadays, in the Scandinavian countries, men live up to 80 years of age on average. With increasing age, comes a rise in the prevalence of a large number of urological diseases such as prostate cancer (PC), benign prostatic enlargement (BPE) causing infravesical obstruction (benign prostatic obstruction, BPO), overactive bladder (OB) and erectile dysfunction (ED). Today, diseases of the lower urinary tract are common

among the elderly population, and treatments of these disorders, from medication to advanced surgery, sometimes assisted by robots, is done routinely by urologists around the world.

1.2 Lower Urinary Tract Symptoms and Prostate Diseases

Symptoms related to the lower urinary tract in men were previously most often termed *prostatism*, mainly owing to the notion that all these problems were caused by age-dependent prostatic enlargement as a result of benign prostatic hyperplasia (BPH). Around twenty years ago, the term *prostatism* was abandoned in favor of the term "*Lower Urinary Tract Symptoms*" (LUTS) [17]. Another modern collective term for these conditions is "*Lower Urinary Tract Dysfunction*" (LUTD), which refers to disorders in the lower urinary tract causing LUTS.

1.2.1 Lower Urinary Tract Symptoms

The term *lower urinary tract symptoms* refers to symptoms such as urgency, increased frequency of micturition, reduced bladder- filling sensation, post micturition dribble, pelvic pain, obstructive symptoms and urinary incontinence (UI). LUTS may in certain cases indicate that BPH is present [18]. The severity of LUTS for each individual is commonly measured by the International Prostate Symptom Score (IPSS). Even if LUTS are often associated with a considerable decrease in quality of life, the existing symptoms are seldom life- threatening. The situation is much more serious if

the bladder does not empty completely. In such cases, the patient may develop bladder stone formation, urinary infection, septicemia, total urinary retention and deteriorated kidney function [19, 20].

International Prostate Symptom Score

The International Prostate Symptom Score (IPSS) is based on the answers to seven questions concerning urinary symptoms and one question concerning quality of life (Table 1). Each question concerning the urinary symptoms allows the patient to choose one of six possible answers indicating increasing severity of the particular symptom. The answers are assigned points from 0 to 5. The total score can therefore range from 0 to 35 (asymptomatic to very symptomatic). The seven questions refer to the following urinary symptoms: (1) incomplete emptying, (2) frequency, (3) intermittency, (4) Urgency, (5) weak stream, (6) straining and (7) urinating at night. The first seven questions of the IPSS are identical to the questions on the American Urological Association Symptom Index, which categorizes symptoms as follows: mild (symptom score less than or equal to 7), moderate (8 - 19) and severe (20 - 35). Question 8 on the IPSS refers to the patient's perceived quality of life. The possible answers to this question range from "delighted" to "terrible" or from 0 to 6. Although this one question may not capture the global impact of LUTS on quality of life, it may serve as a valuable starting point for a doctor-patient conversation.

International Prostate Symptom Score (IPSS)

Name _____ Date _____

Please fill out this short questionnaire to help us find out more about any urinary problems you might have; for questions 1 through 7, circle the number under the column that best describes your situation; for question 6, circle the number in the row which best describes your situation.

	Not at all	Less than 1 time in 5	Less than half the time	About half the time	More than half the time	Almost Always
1. INCOMPLETE EMPTYING: Over the past month, how often have you had a sensation of not emptying your bladder completely after you finished urinating?	0	1	2	3	4	5
2. FREQUENCY: Over the past month, how often have you had to urinate again less than 2 hours after you finished urinating?	0	1	2	3	4	5
2. INTERMITTENCY: Over the past month, how often have you found you stopped and started again several times when you urinated?	0	1	2	3	4	5
3. URGE TO URINATE: Over the past month, how often have you found it difficult to postpone urination?	0	1	2	3	4	5
5. WEAK STREAM: Over the past month, how often have you had a weak urinary stream?	0	1	2	3	4	5
6. STRAINING: Over the past month, how often have you had to push or strain to begin urination?	0	1	2	3	4	5
	None	1 time	2 times	3 times	4 times	5+ times
7. URINATING AT NIGHT: Over the past month, how many times did you most typically get up to urinate from the time you went to bed at night until the time you got up in the morning?	0	1	2	3	4	5

Symptom Score:

1-7 Mild, 8-19 Moderate, 20-35 Severe

Total: _____

BOTHERSOME SCORE DUE TO URINARY SYMPTOMS							
	Delighted	Pleased	Mostly Satisfied	Mixed	Mostly Dissatisfied	Unhappy	Terrible
QUALITY OF LIFE DUE TO URINARY SYMPTOMS: How would you feel if you had to live with your urinary condition the way it is now – no better, no worse – for the rest of your life?	0	1	2	3	4	5	6

Turn over →

Table 1. The IPSS-score as used in its Swedish version in Sweden, for the evaluation of LUTS

The prevalence of LUTS

LUTS are common conditions, but their prevalence varies between studies. The prevalence of LUTS in Dutch men is estimated to be more than 10%, successively increasing from 3% between 45 and 49 years of age, to 24% at

the age of 80 years [18]. In a Swedish population study of more than 2,000 men between the ages of 41 and 80 years, from 10 geographical regions across Sweden, about 30% of the men reported an IPSS of 8 or more, with a considerable percentage of them having an IPSS of 15 or more. There was a strong correlation between a high IPSS and age. One third of the men with LUTS reported a considerable negative influence on their quality of life [21]. In a study from central Sweden, also comprising more than 2,000 men between 40 and 80 years of age, the prevalence of LUTS was 24% [22].

Are all LUTS induced by infravesical obstruction?

It is important to note that LUTS is not synonymous with obstruction or prostatic enlargement. This has led to the term *prostatism* being discarded and the term *LUTS* being used instead. The urinary bladder may primarily be overactive without infravesical obstruction.

In 1989, Tage Hald developed the three-ring diagram to portray the association between BPE, BOO and prostatism / LUTS) (Figure 1). The green area represents where an individual has LUTS and infravesical obstruction due to BPE at the same time. Such a patient might be relieved from symptoms after surgery on the prostate.

Tage Hald's Rings

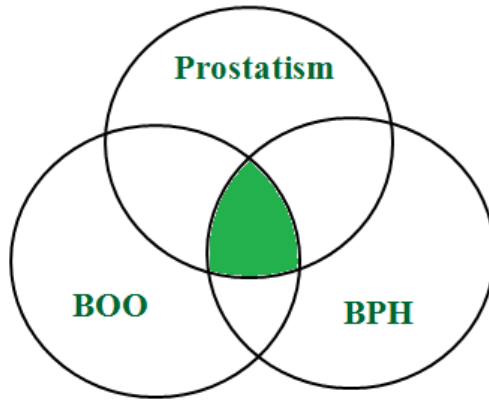


Fig 1. Tage Hald's rings, illustrating the relationship between benign prostatic enlargement (BPH), bladder outlet obstruction (BOO) and urinary tract symptoms (prostatism)

Overactive bladder

Overactive bladder (OAB) is a clinical diagnosis in which the symptoms may vary. The patient may be annoyed by an increased frequency of micturition and severe urgency with a short warning time [23]. As a matter of fact, such urge, or so-called urgency, is a necessary symptom in making the diagnosis of “overactive bladder”.

Many men and women in Sweden suffer from overactive bladder of varying degree, from rather light discomfort to total social disability. Around 35% of the patients suffering from overactive bladder (OAB) are estimated to have so-called “OAB wet”, by which one means that the overactive bladder is associated with urge incontinence.

The causes of overactive bladder can vary from a local change in the bladder due to an infection or inflammatory disease, to neurogenic bladder dysfunction due to stroke, neurological diseases, spinal cord injuries or congenital abnormalities such as myelomeningocele and other types of spinal dysraphism. In some cases of OAB, there is no problem with the bladder itself, the overactivity is instead induced by deficient neurogenic control. The afferent as well as the efferent nerve circuit traffic is under continuous inhibitory control via different types of reflex systems that are highly complex and also incompletely elucidated.

Sometimes it is just a question of increased urinary production without any overactive bladder involvement, even if the patient experiences the same discomfort in increased micturition frequency. By the use of a micturition protocol, it is possible to see the volume of urine the patient eliminates on each micturition occasion.

A recent proposal claims that stress conditions, via increased activity of the sympathetic nervous system and the hypothalamic- pituitary- adrenal axis, are involved in the pathophysiology of LUTS [9, 24, 25]. Bjorntorp presented a number of observations suggesting a link between psychosocial and socioeconomic handicaps via involvement of the central serotonergic system, claiming that the underlying biological mechanisms are anxiety and depression as a basis for stress conditions [26, 27]. A low serum serotonin level has been linked to major depressive disorders among other clinical conditions [28], demonstrating an association between major depression as measured by a depression score and LUTS [29, 30]. Zorn and co-workers also showed a strong association between depression and idiopathic UI [31]. Hence, low levels of serum serotonin might result in excessive stress, causing anxiety, major depressive disorders and LUTS. This hypothesis is in line with recent

reports by McVary and co-workers [24] and Ullrich and co-workers [25]. Using stress tests, both groups found that the activity of the sympathetic nervous system was linked to LUTS.

The diagnosis of neurogenic bladder dysfunction is made by means of cystometry combined with anamnestic data, micturition diaries, the IPSS and other instruments in the hand of a urologist.

Treatment of overactive bladder with per oral drugs

Theoretically, many drugs may lead to resolution of frequency and/or urgency incontinence via receptor interaction at the level of the central nervous system or the peripheral nervous system, but in current clinical practice, muscarinic receptor antagonists (anticholinergics) and a beta-3 agonist are the only compounds available. Anticholinergics exert their function via inhibition of postganglionic parasympathetic cholinergic receptors (muscarinic receptors) on the surface of the smooth muscle cell, while beta-3 agonist functions by selective stimulation of the beta-3 receptors, resulting in relaxation of the detrusor muscle of the bladder. Recent data have demonstrated that the urothelium of the bladder itself expresses muscarinic receptors [32, 33], thus allowing for anticholinergic drugs to interfere, even with the afferent signals from the bladder. Upon treatment with these drugs, the bladder generally reaches a higher volume prior to involuntary contractions. Moreover, the amplitude of the contractions decreases and the functional bladder capacity increases, resulting in relief of some of the symptoms. The beta-3 agonist (Mirabegron), conversely, rather works via facilitation of the adrenergic system that keeps the bladder silent and relaxed in the storage phase [34].

The antidepressant drug duloxetine is used for treatment of urinary stress incontinence in clinical practice [35].

1.2.2 Prostate Diseases

The focus of this thesis was on two conditions in the prostate; benign enlargement of the prostate gland and prostate cancer

Benign prostatic enlargement and benign prostatic hyperplasia

The prostate gland grows during puberty, reaching a volume of 18 - 20 cc, and continues to grow at a different speed for each individual over the years. The size of the prostate gland can be estimated by digital rectal examination or measured by ultrasonography. Benign Prostatic Enlargement (BPE) is a morphological diagnosis, while Benign Prostatic Hyperplasia (BPH) is a histological diagnosis and does not necessarily imply gland enlargement. None of the terms say anything about the degree of the problem they may cause. If the gland is found to be enlarged (probably because of BPH), it does not automatically mean that any problems are perceived or that the enlarged prostate even causes a flow resistance. However, Benign Prostatic Obstruction (BPO) or Bladder Outlet Obstruction (BOO) is a condition where the flow is affected by increased resistance.

Etiology and pathogenesis behind benign prostatic hyperplasia

To date, the etiology for the development of BPH has been unsatisfactorily investigated. However, it is known that eunuchs do not develop prostatic hyperplasia, so to a certain extent a physiological influence of androgens is a prerequisite for age-dependent enlargement of the prostate. A close association between prostate volume and serum levels of glucuronised androgen metabolites has been shown [36]. The so-called benign prostate gland enlargement takes place in the transitional zone (the part of the prostate closest to the urethra). Apart from androgens, heredity plays a role.

Parameters of progression of benign prostatic hyperplasia

Progression of BPH may result in increased infravesical obstruction and onset of symptoms such as impaired urinary flow. Complications of BPH in the final stage, such as urinary retention, urinary tract infection, bladder stone formation and effects on the upper urinary tract, are more important indicators of significant progress. Men with BPE combined with LUTS are at increased risk of experiencing urinary retention [37].

Measurement of the prostate gland volume

The volume of the prostate is measured by means of transrectal ultrasonography (TRUS), and an enlarged gland is considered to have a volume larger than 40 cc.

Treatment of benign prostatic hyperplasia

At the beginning of the 1990s, finasteride (Proscar[®]) was introduced as the first prostate-reducing drug. It is a so-called 5-alpha-reductase inhibitor. The pharmacological mechanism of action is based on the fact that testosterone is metabolized to 5-alpha-dihydrotestosterone. The conversion from testosterone to 5-alpha-dihydrotestosterone is catalyzed by the enzyme 5-alpha-reductase and by inhibiting this enzyme, a significant suppression of 5-alpha-dihydrotestosterone is obtained. There are two types of 5-alpha-reductase, type 1 and type 2, and finasteride inhibits primarily type 2. There is a competitor, dutasteride (Avodart[®]), which inhibits both types. The clinical significance of inhibiting both receptors at the same time is unclear. Both finasteride and dutasteride cause a decrease in the size of the prostate gland, resulting in some regression of symptoms.

The other strategy for pharmacological treatment of BPH is alpha-adrenoreceptor blockade. Here, one takes advantage of the fact that there are alpha-adrenergic receptors at the bladder neck and in the prostate tissue, and stimulation of these receptors may cause contraction of the bladder neck. By blocking these receptors with a competitive alpha-adrenergic inhibitor, the bladder neck relaxes. Worldwide, the most commonly used drug is tamsulosine. In Sweden, alfuzosine (Xatral[®]) is the dominant drug, but the drugs doxazosine (Alfadin-BPH[®]) and terazosin (Hytrinex[®]) are also registered in Sweden. The great advantage of alpha-blockade is that it has a fast onset of action. The effect is often noticeable after only a few weeks. A combination treatment with alpha-adrenoreceptor blockade and a 5-alpha-reductase inhibitor has proved to be more effective than single- drug treatment. The aim of this strategy is to use the alpha-blocker for optimal relief of

symptoms and the 5-alpha-reductase inhibitor for durability and prevention of complications.

Sildosine is the most recently introduced alpha adrenergic inhibitor, with a particularly high affinity for the alpha 1A receptor [38].

Minimally invasive treatment of benign prostatic hyperplasia

Minimally- invasive methods have existed for some 20 years. Some of the methods have been further developed and are now considered good enough to be accepted as standard treatments. Among these, transurethral microwave thermotherapy can be mentioned, a treatment method that requires neither general- nor spinal anesthesia.

Surgical treatment of benign prostatic hyperplasia

The most common surgery for BPH is transurethral resection of the prostate. If the prostate gland is small, a so-called “transurethral incision of the prostate may be done instead. For large prostate glands of more than 100 g, open surgery may be a good alternative. This can be done transvesicallly or transcapsularly, and by this procedure, the growth zones of the prostate are enucleated.

Prostate cancer

Prostate cancer is the most common form of cancer in Swedish men, with around 9000 cases diagnosed annually.

Etiology and pathogenesis behind prostate cancer

The etiology of PC is unknown, but age, hereditary and ethnicity are well known risk factors [39]. The disease is uncommon before the age of 50. For the so-called “hereditary PC (HPC1)”, the gene is known to be localized on chromosome 1 (1q24-25). However, some genetic polymorphisms affect the onset of the disease. Other suggested locations of genes involved in the onset of the disease are 1q42.2-43; 1p36; 5q11.2; 17p11; 19p13; 20q13; and Xq27-28. Men who have several close relatives with PC are recommended to attend to regular checkups from the age of 45. African Americans have a greater incidence of PC, and a more aggressive, and potentially more lethal form of PC than do Caucasian Americans [40].

Diagnosis of prostate cancer

PC is nowadays most commonly diagnosed through biopsy by means of TRUS. It may also be diagnosed clinically or accidentally after surgery for BPE. Usually the patient is referred to a urologist by a general practitioner (GP) or a family doctor, either because the prostate-specific antigen (PSA) level was elevated in a blood sample, or because the GP palpated an unusual mass on rectal digital examination.

Prostate- specific antigen

Prostate- specific antigen (PSA) is a serine protease produced by the luminal cells of the prostatic epithelium and is a member of the kallikreine gene family and the most commonly used tumor marker in oncology. It is secreted into the seminal vesical fluid and mixed with semen during ejaculation. PSA lyses the protein semenogelin, resulting in liquefaction of the ejaculate and improvement in sperm motility. It is measurable in low concentrations (ng / ml) in serum after puberty, since its expression is strongly bound to androgens. PSA in blood circulates in both free form and bound form (bound to proteins such as alfa- 1-anti- chymotrypsin, alfa- 2- macroglobulin and alfa- 1- antitrypsin). Damage to the basal cell layer and the basal membrane of the prostate, caused by, for example, inflammation, trauma or PC allows PSA to leak in to the circulating blood, resulting in an elevation of its concentration. The diagnosis of PC nowadays is mainly driven by elevated levels of serum PSA (PSA > 3.0 ng / ml).

Classification of prostate cancer

The extent of the disease is classified according to the tumor node metastases (TNM) system, where T denotes the tumor size, N the extent of any lymph node involvement and M the presence or absence of distant metastases. Other means of determining the extent of PC are through examination of a specimen after radical prostatectomy by microscopy, or through imaging techniques, the so- called pT- classification (Table 2).

Table 2. TNM classification for prostate cancer

Primary tumor (T)

Clinical

TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
T1	Clinically unapparent tumor, not palpable or visible by imaging
T1a	Tumor incidental histologic finding in ≤5% of tissue resected
T1b	Tumor incidental histologic finding in >5% of tissue resected
T1c	Tumor identified by needle biopsy (because of elevated prostate specific antigen (PSA) level
T2	Tumor confined within prostate; tumors found in one or both lobes by needle biopsy, but not palpable or reliably visible by imaging
T2a	Tumor involves one-half of one lobe or less
T2b	Tumor involves more than one-half of one lobe, but not both lobes
T2c	Tumor involves both lobes
T3	Tumor extends through the prostatic capsule; invasion into the prostatic apex, or the prostatic capsule is classified not as T3 but as T2
T3a	Extracapsular extension (unilateral or bilateral)
T3b	Tumor invading seminal vesicle(s)
T4	Tumor fixed or invades adjacent structures other than seminal vesicles (eg, bladder, levator muscles, and/or pelvic wall)

Pathologic (pT)

pT2	Organ confined
pT2a	Unilateral, involving one-half of one lobe or less
pT2b	Unilateral, involving more than one-half of one lobe, but not both lobes
pT2c	Bilateral disease
pT3	Extraprostatic extension
pT3a	Extraprostatic extension or microscopic invasion of the bladder neck
pT3b	Seminal vesicle invasion
pT4	Invasion of the bladder and rectum

Regional lymph nodes (N)

Clinical

NX	Regional lymph nodes were not assessed
N0	No regional lymph node metastasis
N1	Metastasis in regional lymph node(s)

Pathologic

pNX	Regional nodes not sampled
pN0	No positive regional nodes
pN1	Metastases in regional nodes(s)

Distant metastasis (M)*

M0	No distant metastasis
M1	Distant metastasis
M1a	No regional lymph nodes(s)
M1b	Bone(s)
M1c	Other site(s) with or without bone disease

*If more than one site of metastasis is present, use the most advanced category (pM1c)

There are other methods of determining the aggressiveness and severity of the disease, such as Gleason grading. Gleason grading is a system based on the architectural pattern of the tumor. It consists of the sum (also called score) of two patterns, called grades, ranging from 2 - 10. Each of the grades can vary between 1 (well differentiated) and 5 (poorly differentiated). According to the latest consensus in 2005 reached by the International Society of Urological Pathology, patterns 1 and 2 should rarely, if ever, be used. In prostate biopsies, the Gleason score should be the sum of the most common grades plus the highest (worst) grade, resulting in a Gleason score ranging from 6 to 10. For radical prostatectomy specimens the Gleason score should still be reported as the two most common patterns, but with a comment if small foci of a higher grade are present.

The histopathological differentiating, shown in table 3, is another system summarizing the Gleason grading system in three differentiating grades.

Table 3. Histopathological grade

Histopathological grade (G)

GX	Gleason score cannot be assessed
Gleason ≤6	Well differentiated (slight anaplasia)
Gleason 7	Moderately differentiated (moderate anaplasia)
Gleason 8-10	Poorly differentiated or undifferentiated

A combined method of determining the extent of PC is by dividing the tumor into four risk categories (very low, low, intermediate and high risk). Here one takes into account a combination of several parameters used in the diagnosis of PC. According to a modified version of the National Comprehensive Cancer Network (NCCN) guidelines, these risk categories are defined as low- risk: clinical local stage T1 tumor, PSA < 10 ng/ml and Gleason score ≤ 6; intermediate- risk: ≤ T2 tumor or PSA 10 - 20 ng/ml or Gleason score 7; and high- risk: T3 -T4 tumor or PSA ≥ 20 or Gleason score ≥ 8 or N1 or M1.

Another combined method is division into different anatomical stages as shown in Table 4.

Table 4. Anatomic stage/prognostic groups

Stage	T	N	M	PSA*	Gleason
I	T1a - c	N0	M0	PSA < 10	Gleason ≤6
	T2a	N0	M0	PSA < 10	Gleason ≤6
	T1 - T2a	N0	M0	PSA X	Gleason X
IIA	T1a - c	N0	M0	PSA < 20	Gleason 7
	T1a - c	N0	M0	PSA ≥ 10 but < 20	Gleason ≤6
	T2a	N0	M0	PSA < 20	Gleason ≤7
	T2b	N0	M0	PSA < 20	Gleason ≤7
	T2b	N0	M0	PSA X	Gleason X
IIB	T2c	N0	M0	Any PSA	Any Gleason
	T1 - 2	N0	M0	PSA ≥ 20	Any Gleason
	T1 - 2	N0	M0	Any PSA	Gleason ≥8
III	T3a - b	N0	M0	Any PSA	Any Gleason
IV	T4	N0	M0	Any PSA	Any Gleason
	Any T	N1	M0	Any PSA	Any Gleason
	Any T	Any N	M1	Any PSA	Any Gleason

*If PSA or Gleason is not available, grouping should be determined by T stage and/or either PSA or Gleason, as available.

Progression of prostate cancer

Prostate cancer predominantly metastasizes lymphogenically to lymph nodes in the obturator and pelvic regions and hematogenically to the skeleton. Recent studies suggest that metastatic PC cells are able to mimic osteoblasts, implying that bone markers produced by osteoblasts, such as osteocalcin (OC), may play a part in the progression of PC [41]. In the late stages, metastases of PC may even affect other organs, such as the liver, brain and lungs.

Treatment of prostate cancer

In the treatment of PC, with curative intent, one has to choose between two main possible approaches depending on the degree and severity of the disease on diagnosis, and the patient's age, co-morbidity, preference and wish, together with the doctor's judgment and recommendation.

Curative treatment focuses on treating the disease in order to cure the patient. In this approach one can choose either surgery (radical prostatectomy) or radiation beam therapy (externally, internally or a combination of both). At university hospitals and larger regional hospitals in Sweden, radical prostatectomy is conducted in the majority of the cases by means of robotic-assisted laparoscopic technique.

Whenever cure is not possible, or if the patient's condition does not allow for a more aggressive treatment, one should focus on preventing progression of the disease, increasing the survival time of the patient and relieving him from pain. PC, at least in its early stages, is dependent on androgens for its growth. Manifold substances are available for preventing PC from metastasizing and progression. The basic treatment consists of drugs that either inhibit the action

of androgens on the prostate gland (anti- testosterone) or inhibit the production of androgens (GnRH agonists or antagonists). Treatment with androgen deprivation therapy has side- effects with the symptoms resulting from a lack of blood circulating testosterone. Long- term treatment with such substances results in osteoporosis, with increased risk for osteoporotic fractures. The most important adverse effect of treatment with GnRH-agonists and antagonists is that the treatment may lead to the development of the MetS, as treatment with these drugs has been linked to practically all well-known aspects of the MetS, a constellation of risk factors implicated in the development of T2DM and cardiovascular disease. As time passes, the cancer cells become independent of testosterone. They continue to grow and metastasize predominantly to the skeleton, but also to other regions. This stage of the disease is referred to as the “castration resistant stage”. The focus of the treatment in this stage is to delay the progression of the disease and to prevent further metastases. Bone resorption inhibitors are a group of drugs that are used to prevent osteoporosis and delay the onset of osteoporotic fractures. Chemotherapy and treatment with anti- angiogenic drugs are the drugs of choice at this stage of the disease, where palliation should be the focus.

1.3 Metabolic Syndrome

The metabolic syndrome (MetS) is a disorder of energy utilization and storage, diagnosed by a co-occurrence of three of five of the following medical conditions: abdominal (central) obesity, elevated blood pressure, elevated fasting plasma glucose, high serum triglycerides, and low high-density lipoprotein (HDL) cholesterol levels. The syndrome has been described as one entity characterized by a defect in the insulin-mediated glucose uptake. This

primary metabolic abnormality is mainly localized in the muscle and adipose tissue and the liver, leading to an insulin resistance and a secondary hyperinsulinemia. Thus, MetS increases the risk of developing cardiovascular disease and type 2 diabetes mellitus (T2DM) as the endpoint [42]. The syndrome is prevalent in countries with western lifestyles, and the prevalence increases with age [43]. The principal symptom of MetS is central obesity (also known as visceral, male-pattern or apple-shaped adiposity), overweight with adipose tissue accumulation mainly around the waist and trunk. Over the years MetS in men has been defined in various ways by several health organizations. However, none of these definitions can yet be considered the gold standard, because they all emphasize on different aspects of the syndrome.

Table 5 shows a summarized version of these established organizations definition of MetS applicable on men.

This thesis is based on risk factor analyses linking components of MetS with urological disorders. A surrogate for cardiovascular disease was defined as anamnestic data regarding history of myocardial infarction and stroke, and T2DM was defined through anamnestic data. Other components of MetS have been suggested to be vitamin D deficiency ($< 50 \text{ nmol / l}$), hypogonadism (s-testosterone $< 8 \text{ nM}$), low levels of serum adiponectin and high levels of C-reactive protein (CRP).

	Obesity	TG mmol / L	HDL-C (mmol / L)	BP (mmHg)	FPG (mmol / L)
<i>International Diabetes Federation (IDF)</i>	BMI > 30 kg / m ²	> 1.7 or treatment for HTG	< 1.03 or treatment for HCE	SBP > 130 DBP > 85 or treatment for HT	> 5.6 or diagnosis of T2DM
<i>World Health Organization (WHO)</i>	BMI > 30 kg / m ²	> 1.7 or treatment for HTG	< 0.9 or treatment for HCE	SBP ≥ 140 DBP ≥ 90 or treatment for HT	
<i>The European Group for the Study of Insulin Resistance (EGIR)</i>	Waist circumference ≥ 94 cm	≥ 2.0 or treatment for HTG	< 1.0 or treatment for HCE	SBP ≥ 140 DBP ≥ 90 or treatment for HT	≥ 6.1 or diagnosis of T2DM
<i>US National Cholesterol Education Program (NCEP)</i>	Waist circumference ≥ 102 cm	≥ 1.7 or treatment for HTG	< 40 mg / dL or treatment for HCE	SBP ≥ 130 DBP ≥ 85 or treatment for HT	≥ 6.1 or diagnosis of T2DM

TG: Triglyceride, HDL-C: high density lipoprotein cholesterol, BP: blood pressure, SBP: systolic blood pressure, DBP: diastolic blood pressure, FPG: fasting plasma glucose, BMI: body mass index, HTG: hypertriglyceridemia, HCE: hypercholesterolemia, HT: hypertension

Table 5. Summary of different health organizations definition of MetS for men

1.3.1 Adiponectin

Adiponectin is a protein which is exclusively secreted from adipose tissue (and also from the placenta in pregnancy) [44] into the bloodstream and is very abundant in plasma relative to many other hormones. Adiponectin modulates a number of metabolic processes, including glucose regulation and fatty acid oxidation [45]. It is secreted into the bloodstream where it accounts for approximately 0.01 % of all plasma protein with a concentration of 5-10 $\mu\text{g} / \text{ml}$. Plasma concentrations reveal a sexual difference, with females having higher levels than males. Levels of the hormone are inversely correlated with body fat percentage in adults [46]. Similarly, circulating adiponectin concentrations increase during caloric restriction in animals and humans, such as in patients with anorexia nervosa. This observation is surprising, since adiponectin is produced by adipose tissue. However, a study by Cawthorn and co-workers suggested that adipose tissue within bone marrow, which increases during caloric restriction, contributes to elevated circulating adiponectin [47].

Transgenic mice with increased adiponectin show impaired adipocyte differentiation and increased energy expenditure [48]. Adiponectin plays a role in the suppression of the metabolic derangements that may result in type 2 diabetes [46], obesity, atherosclerosis [45] and non-alcoholic fatty liver disease. Low levels of adiponectin is an independent risk factor for MetS [49]. Adiponectin in combination with leptin has been shown to completely reverse insulin resistance in mice [50]. Levels of adiponectin are reduced in diabetics compared to non-diabetics and weight reduction significantly increases its circulating levels [51].

Adiponectin automatically self-associates into larger structures building low- and high-molecular weight forms. Recent studies showed that the high-

molecular- weight form may be the most biologically active form regarding glucose homeostasis [52]. High-molecular-weight adiponectin was further found to be associated with a lower risk of diabetes with a similar magnitude of association to that for total adiponectin [53]. However, coronary artery disease has been found to be positively associated with high- molecular-weight adiponectin, but not with low- molecular- weight adiponectin [54].

Adiponectin exerts some of its weight reduction effects via the brain. This is similar to the action of leptin [55]. The two hormones perform complementary actions, and can have synergistic effects.

Lewerin and co-workers showed that serum levels of adiponectin, but not leptin, were negatively and independently associated with low levels of hemoglobin, which suggests a possible role of adiponectin in the age-related decline in hemoglobin levels observed in otherwise apparently healthy elderly men [56]. Johansson H and co-workers showed that an increased level of serum adiponectin was a risk factor for fracture. Nevertheless, the predictive value of adiponectin on the risk for fracture, attenuated with time so that its use for the risk assessment in the long term was questionable [57].

Adiponectin binds to a number of receptors. So far, two receptors have been identified with homology to G- protein- coupled receptors, and one receptor similar to the cadherin family [58, 59]. The receptors affect the adenosine- mono- phosphate- kinase, an important cellular metabolic rate control protein. Expression of the receptors, particularly in skeletal muscle and adipose tissue, is correlated with insulin levels [60]. It also results in reduction of the incidence of diabetes in mouse models [61].

Effects

Adiponectin has the following effects:

- Decreased gluconeogenesis
- Increased glucose uptake [45, 55, 62]
- Lipid catabolism [62]
- Beta- oxidation [55]
- Triglyceride clearance [55]
- Protection from endothelial dysfunction (important facet of atherosclerotic formation)
- Insulin sensitivity
- Weight loss
- Low hemoglobin and increased risk for anemia [56]
- Osteoporosis and increased risk for fractures [63]
- Control of energy metabolism [62]
- Impaired adipocyte differentiation [48]
- Reduction of tumor necrosis factor- alpha

A low level of adiponectin is an independent risk factor for developing:

- Metabolic syndrome [49]
- Diabetes mellitus [55, 64-67]
- Obesity
- ADHD in adults [68]

Administration

Extracts of sweet potatoes have been reported to increase levels of adiponectin and thereby improve glycemic control in humans [69]. However, a systematic review concluded that there is insufficient evidence to support the consumption of sweet potatoes to treat T2DM [70]. Since adiponectin is a protein, oral administration is not effective, as enzymes in the digestive system break it down into its constituent amino- acid parts. One way to introduce the hormone into the blood stream is by intravenous administration.

1.3.2 Vitamin D

In 1922, Elmer McCollum tested modified cod liver oil, in which the vitamin A had been destroyed, on dogs sick with osteomalacia (a softening of the bones) and noticed that the modified oil could cure the dogs from the disease, so McCollum concluded that there must be a factor in cod liver oil that cured osteomalacia, and that this compound is distinct from vitamin A. He called it vitamin D because it was the fourth vitamin to be named. Vitamin D refers to a group of fat-soluble secosteroids, i.e. steroids in which one of the bonds in the steroid ring is broken. They originate from cholesterol and are responsible for intestinal absorption of calcium, iron, magnesium, phosphate and zinc. German researcher, Adolf Windaus, who received the Nobel Prize in Chemistry in 1928, first discovered three forms of vitamin D, which he called D_1 , D_2 and D_3 [71]. It was not initially realized that, unlike other vitamins, vitamin D can be synthesized by humans through exposure to ultraviolet light. Later, it was learnt that vitamin D_1 was a mixture of compounds rather than a pure product, so the term D_1 is no longer used. The term "*Vitamin D*" now

refers to several forms. The two most important forms in humans are: ergocalciferol (vitamin D₂) and cholecalciferol (vitamin D₃).

Biosynthesis

In the presence of ultraviolet radiation, many fungi synthesize vitamin D₂ from ergosterol, and many animals synthesize vitamin D₃ from 7-dehydrocholesterol.

The photochemistry of vitamin D biosynthesis in fungi and in animal

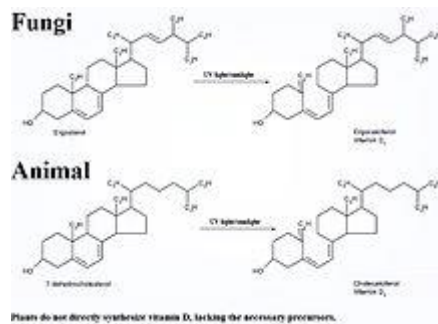


Fig 2. Production of vitamin D₂ and D₃ in fungi and in animals. Wikipedia, the free encyclopedia

The conversion of previtamin D₃ to vitamin D₃ in the skin is about 10 times faster than in an organic solvent [72].

Thermal isomerization of previtamin D₃ to vitamin D₃.

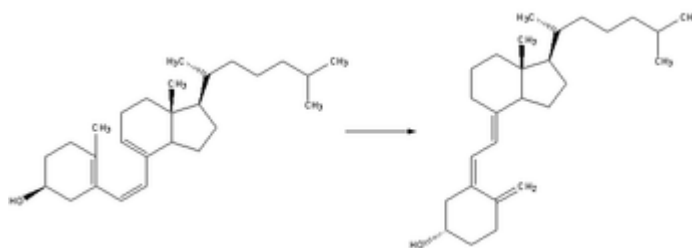


Fig 3. Conversion of previtamin D₃ to vitamin D₃ in the skin. Wikipedia, the free encyclopedia.

Evolution

Photosynthesis of vitamin D in the ocean by phytoplankton (such as *Coccolithophore* and *Emiliana huxleyi*) has existed for more than 500 million years and continues to the present. Although primitive vertebrates in the ocean could absorb calcium from the ocean into their skeletons and eat plankton rich in vitamin D, land animals required another way to satisfy their vitamin D requirement for a calcified skeleton without relying on plants. Land vertebrates have been making their own vitamin D for more than 350 million years. [72].

The skin consists of two primary layers: the inner layer called the dermis, composed largely of connective tissue, and the outer, thinner epidermis. Epidermis on the soles and palms consists of five strata (Fig 4); from outer to inner, they are: the stratum corneum, stratum lucidum, stratum granulosum, stratum spinosum, and stratum basale. Vitamin D is produced in the two innermost strata, the stratum spinosum and the stratum basale.

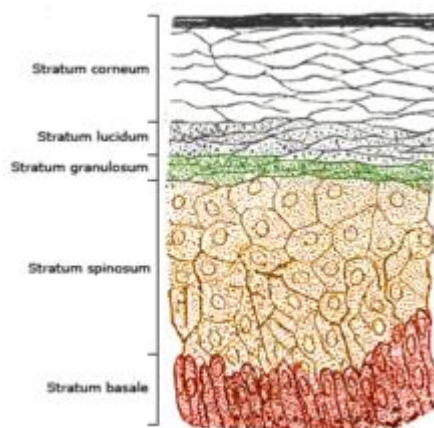


Fig 4. Layers of epidermis in the skin. Wikipedia, the free encyclopedia.

Vitamin D₂ is the synthetic form, while vitamin D₃ is mainly synthesized in the skin from 7-dehydrocholesterol with exposure to sunlight (ultraviolet beta), and also in lower amounts through absorption from the intestine. These vitamins are biologically inactive. Their activation requires enzymatic conversion (hydroxylation) at point 25 in the liver and at point 1 in the kidneys. In the liver ergocalciferol (vitamin D₂) is converted to 25-hydroxyergocalciferol [25(OH) D₂], while cholecalciferol (vitamin D₃) is converted to calcidiol, which is also known as calcifediol [25(OH) D₃]. These two specific vitamin D metabolites are measured in serum to determine a person's vitamin D status [73]. Part of the calcidiol is converted by the kidneys to calcitriol, the biologically active form of vitamin D [74]. Calcitriol circulates as a hormone in the blood, regulating the concentration of calcium and phosphate in the bloodstream and promoting the healthy growth and remodeling of bone. Calcitriol also affects neuromuscular and immune function. These functions of vitamin D are the reasons why vitamin D is nowadays considered to be a hormone rather than a strict vitamin.





Name	Chemical composition	Structure
Vitamin D₁	Molecular compound of ergocalciferol with lumisterol, 1:1	
Vitamin D₂	Ergocalciferol (made from ergosterol)	
Vitamin D₃	Cholecalciferol (made from 7-dehydrocholesterol in the skin).	
Vitamin D₄	22-Dihydroergocalciferol	
Vitamin D₅	Sitocalciferol (made from 7-dehydrositosterol)	

Table 6. Chemical composition and structure of different compounds of vitamin D. Wikipedia, the free encyclopedia.

The hydroxylation of vitamin D in liver is catalyzed by the microsomal enzyme vitamin D 25-hydroxylase [75], which is produced by hepatocytes. Once made, the product (calcidiol) is released into the plasma, where it is bound to α -globulin, vitamin D-binding protein. Calcidiol is then transported to the proximal tubules of the kidneys, where it is hydroxylated at the 1- α position to form calcitriol (1, 25-dihydroxycholecalciferol [1a, 25(OH)₂D₃]). This product is a potent ligand of the vitamin D receptor, which mediates most of the physiological actions of the hormone. The conversion of calcidiol to calcitriol is catalyzed by the enzyme 25-hydroxyvitamin D₃ 1- α -

hydroxylase, the levels of which are increased by the parathyroid hormone (and additionally by low calcium or phosphate).

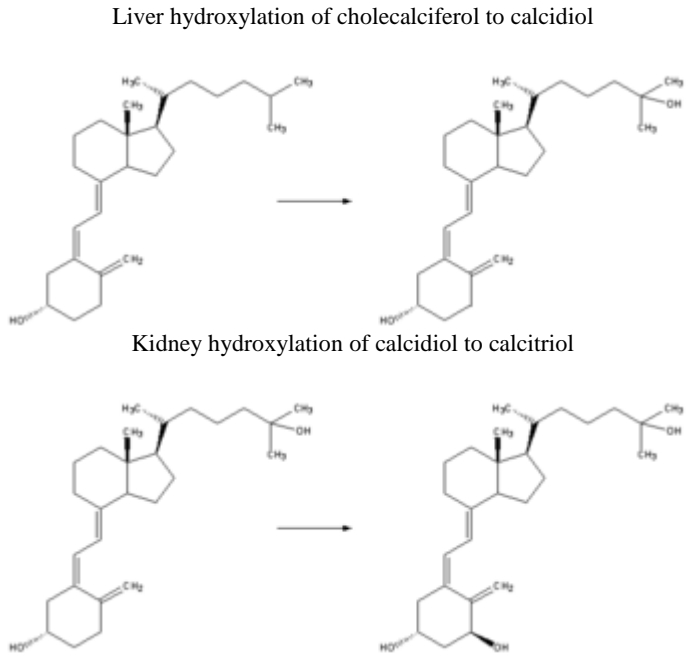


Fig 5. Hydroxylation of vitamin D₃ in liver and in kidney, converting it to active form. Wikipedia, the free encyclopedia.

In addition to the kidneys, calcitriol is synthesized by monocyte / macrophages in the immune system. When synthesized by monocyte / macrophages, calcitriol acts locally as a cytokine, defending the body against microbial invaders by stimulating the innate immune system [76].

Action of vitamin D

The active vitamin D metabolite, calcitriol, mediates its biological effects by binding to the vitamin D receptor (VDR), which is principally located in the nuclei of its target cells. The binding of calcitriol to the VDR allows the VDR to act as a transcription factor that modulates the gene expression of transport proteins (such as TRPV6 and calbindin), which are involved in calcium absorption in the intestine [77]. The vitamin D receptor belongs to the nuclear receptor superfamily of steroid / thyroid hormone receptors, and VDRs are expressed by cells in most organs, including the brain, heart, skin, gonads, prostate, and breast. VDR activation in the intestine, bone, kidney, and parathyroid gland cells leads to the maintenance of calcium and phosphorus levels in the blood (with the assistance of parathyroid hormone and calcitonin) and to the maintenance of bone content [78]. One of the most important roles of vitamin D is to maintain skeletal calcium balance by promoting calcium absorption in the intestines, promoting bone resorption by increasing the number of osteoclasts, maintaining calcium and phosphate levels for bone formation, and allowing proper functioning of the parathyroid hormone to maintain serum calcium levels. The VDRs are known to be involved in cell proliferation and differentiation. Vitamin D also affects the immune system, and VDRs are expressed in several white blood cells, including monocytes and activated T- and B- cells. Vitamin D increases expression of the tyrosine hydroxylase gene in adrenal medullary cells. It is also involved in the biosynthesis of neurotrophic factors, the synthesis of nitric oxide synthase, and an increase in glutathione levels [79].

Sources

Vitamin D is found in only a few dietary sources [80-82]. Other than supplements, exposure to sunlight is the primary source of vitamin D for the majority of people [83]. Vitamin D₂ is mainly found in vegetables like alfalfa, fungi and mushrooms, and vitamin D₃ is found in egg, fish and meat.

Industrial production

Vitamin D₃ (cholecalciferol) is produced industrially by exposing 7-dehydrocholesterol to ultraviolet beta light, followed by purification [84, 85]. The 7-dehydrocholesterol is a natural substance in fish organs, especially the liver [86], and in wool grease (lanolin) from sheep. Vitamin D₂ (ergocalciferol) is produced in a similar way using ergosterol from yeast or mushrooms as a starting material [85].

Vitamin D status levels

According to a combination of recommendations from different organizations on levels of serum vitamin D, the limits are as follows:

- Deficiency: < 25 nmol / l
- Insufficiency: 25 - 50 nmol / l
- Sufficient: 50 - 75 nmol / l
- Optimal: > 75 nmol / l

Deficiency

Vitamin D deficiency may result in decreased bone mineral density and an increased risk of osteoporosis and bone fracture [87]. Thus, although it may initially appear paradoxical, vitamin D is also critical for bone remodeling through its role as a potent stimulator of bone resorption. A diet deficient in vitamin D in conjunction with inadequate sun exposure may cause osteomalacia (or rickets when it occurs in children), which is a softening of the bones. Osteomalacia is usually present when 25-hydroxyvitamin D levels are less than about 10 $\mu\text{g} / \text{l}$ or 10 ng / ml [80]. In the developed world, vitamin D deficiency is a rare disease. However, vitamin D deficiency has become a worldwide issue in the elderly and remains common in children and adults [81, 88]. Vitamin D- fortified milk has helped to eradicate the majority of cases of rickets for children with fat malabsorption conditions.

Health effects of supplementation with vitamin D

The effects of vitamin D supplementation on health are uncertain [89, 90]. Low vitamin D levels may result from diseases rather than cause diseases.

Toxicity

Vitamin D toxicity is rare [81] and is caused by supplementation with high doses rather than by sunlight. The threshold for vitamin D toxicity has not been established; however, the tolerable upper intake level, according to some research, is 4,000 IU / day for ages 9 - 71 [91], whereas another research concluded that in healthy adults, sustained intake of more than 1250 $\mu\text{g} / \text{day}$

(50,000 IU) can produce overt toxicity after several months and can increase serum 25-hydroxyvitamin D levels to 150 ng / ml and greater [81]. Vitamin D overdose causes hypercalcemia. If hypercalcemia is not treated, it results in excess deposits of calcium in soft tissues [81, 92] and organs such as the kidneys, liver, and heart, resulting in pain and organ damage. The main symptoms of hypercalcemia are: anorexia, nausea, vomiting, polyuria, polydipsia, weakness, insomnia, nervousness, pruritus, proteinuria, urinary casts, azotemia, and metastatic calcification (especially in the kidneys). Ultimately renal failure may develop. Other symptoms of vitamin D toxicity include mental retardation in young children, abnormal bone growth and formation, diarrhea, irritability, weight loss, and severe depression [81].

Vitamin D toxicity is treated by discontinuing vitamin D supplementation and restricting calcium intake. Kidney damage may be irreversible. Exposure to sunlight for extended periods does not normally cause vitamin D toxicity [89]. Within about 20 minutes of ultraviolet exposure in light (three to six times longer for pigmented skin), the concentrations of vitamin D precursors produced in the skin reach an equilibrium, and any further vitamin D produced is degraded [93].

Influence of skin pigmentation

It has been shown that dark-skinned people living in temperate climates have lower serum vitamin D levels [94], since melanin in the skin hinders vitamin D synthesis. However, other studies have failed to support this notion [95, 96].

Sunscreen

Sunscreen absorbs or reflects ultraviolet light and prevents much of it from reaching the skin. Sunscreen with a sun protection factor of 8 based on the UVB spectrum has been reported to decrease vitamin D synthetic capacity by 95%, whereas sunscreen with an SPF of 15 can reduce synthetic capacity by 98% [97]. Exposure to light through windows is insufficient because glass almost completely blocks UVB light.

Bone health

In general, evidence cannot support the commonly held belief that vitamin D supplements can help prevent osteoporosis. Their general use for prevention of osteoporosis in individuals without vitamin D deficiency is thus likely not needed [98].

For older people with osteoporosis, taking vitamin D with calcium may help prevent hip fractures, but it also slightly increases the risk of stomach and kidney problems (74). Supplementation with higher doses of vitamin D, in individuals older than 65 years, may decrease fracture risk [99]. This appears to apply more to residents of institutions than to people living independently [100].

Mortality

According to a study by Johansson and co-workers, low serum 25(OH) D is associated with a substantial excess risk of death compared with 25(OH) D

values greater than 50 - 70 nmol / l, but the association attenuates with time [101]. Vitamin D₃ supplementation has been tentatively found to lead to a reduced risk of death in the elderly [102], but the effect has not been certain enough to make taking supplements recommendable. Other forms (vitamin D₂, alfacalcidol, and calcitriol) do not appear to have any beneficial effects with regards to the risk of death [102]. High blood levels appear to be associated with a lower risk of death, but it is unclear if supplementation can result in this benefit [103]. Both an excess and a deficiency in vitamin D, appear to cause abnormal functioning and premature aging [104, 105]. Harm from vitamin D appears to occur at a lower vitamin D level in the Black population than in the White population [91].

Cancer

Vitamin D supplements have been widely marketed on the Internet and elsewhere for their claimed anticancer properties [106], but taking vitamin D supplements has been found not to have significant effect on cancer risk [102]. Some research has suggested that vitamin D₃ decreases the risk of death from cancer, but concerns with the quality of the data were noted [107].

Cardiovascular disease

Taking vitamin D supplements does not meaningfully reduce the risk of stroke, cerebrovascular disease, myocardial infarction, or ischaemic heart disease [108].

Depression

Clinical trials of vitamin D supplementation for depressive symptoms have generally been of low quality and show no overall effect, although subgroup analysis showed that supplementation for participants with clinically significant depressive symptoms or depressive disorder had a moderate effect [89].

Cognition and dementia

A systematic review of clinical studies shows an association between low vitamin D levels, cognitive impairment, and a higher risk of developing Alzheimer's disease [109].

Immune system and infections

In general, vitamin D functions to activate the innate immune system and dampen the adaptive immune system [110]. Deficiency has been linked to increased risk of viral infections, including human immunodeficiency virus (HIV) and influenza [111-113]. Low levels of vitamin D appear to be a risk factor for tuberculosis [114], and historically vitamin D was used as a treatment for it [115].

Autoimmune disease

Although data tentatively link low levels of vitamin D to asthma, evidence to support a beneficial effect from supplementation is inconclusive [116]. Accordingly, supplementation is not currently recommended for treatment or prevention of asthma [117].

Vitamin D deficiency may be a risk factor for multiple sclerosis [118], but no evidence indicates vitamin D has any clinically significant benefit as a treatment [119].

Pregnancy

Low levels of vitamin D in pregnancy are associated with gestational diabetes, pre-eclampsia, and small infants [120]. The benefit of supplements, however, is unclear.

Dietary reference intake recommendations

Different institutions propose different recommendations concerning daily amounts of the vitamin. The commonly recommended daily intake of vitamin D is not sufficient if sunlight exposure is limited.

In the following recommended amounts, the conversion is: $1 \mu\text{g} = 2.5 \text{ nmol} = 40 \text{ IU}$

The recommended daily amount for vitamin D in the European Union is 5 µg. In 2012, the German Society for Nutritional Medicine, a private organization, increased the recommended daily amount to 20 µg.

For postmenopausal women, the European Menopause and Andropause Society recommended 15 µg (600 IU) a day up to 70 and 20 µg (800 IU) for age 71 years and above. This dose should be increased to 4,000 IU/day in some patients with very low vitamin D status or with comorbid conditions [121].

The UK National Health Service recommends that babies and young children aged 6 months to 5 years, pregnant or breastfeeding women, and sun-deprived elderly people should take daily vitamin supplements to ensure sufficient vitamin D intake; the general population receives enough vitamin D from good diets and from sunlight.

The recommended dietary allowances of vitamin D according to the US Institute of Medicine [91] are shown in Table 7.

Age group	RDA (IU / day)
Infants 0 – 11 months	400*
1 – 70 years	600 (15 µg / day)
71+ years	800 (20 µg / day)
Pregnant / Lactating	600 (15 µg / day)

* Indicates adequate intake, as an recommended dietary allowance, has yet to be established for infants

Table 7. The recommended diary allowance of vitamin D according to US Institute of Medicine. Wikipedia, the free encyclopedia.

According to the European Food Safety Authority, the tolerable upper intake levels are:

- 0 -11 months: 25 µg/day (1000 IU)
- 1 - 10 years: 50 µg/day (2000 IU)
- 11 -17 years: 100 µg/day (4000 IU)
- 17+ years: 100 µg/day (4000 IU)
- Pregnant/lactating women: 100 µg/day (4000 IU)

Serum 25-hydroxyvitamin D

US labs generally report 25 hydroxyvitamin D levels as ng / ml. Other countries often use nmol / l.

It has been suggested that serum 25-(OH) D level of 20 ng / ml (50 nmol / l) is desirable for bone and overall health. The dietary reference intakes for vitamin D are chosen with a margin of safety and “overshoot” the targeted serum value to ensure the specified levels of intake achieve the desired serum 25(OH) D levels in almost all persons. No contributions to the serum 25(OH) D levels are assumed from sun exposure, and the recommendations are fully applicable to people with dark skin or negligible exposure to sunlight.

The Institute of Medicine in US concluded that serum 25(OH) D concentrations above 30 ng / ml (75 nmol / l) are "not consistently associated with increased benefit", while serum 25(OH) D levels above 50 ng / ml (125 nmol / l) may be a cause for concern [91]. However, the desired range of serum 25(OH) D is between 20 and 50 ng / ml [91].

Seasonal variation of vitamin D

Vitamin D production varies by season in countries that are far from the equator, because of differences in ultraviolet sun exposure throughout the year. Figure 6 shows this variation in Sweden taken from the MrOs population study cohort. Since these variations in the circulating concentrations of vitamin D exist, a z-score for the population was calculated.

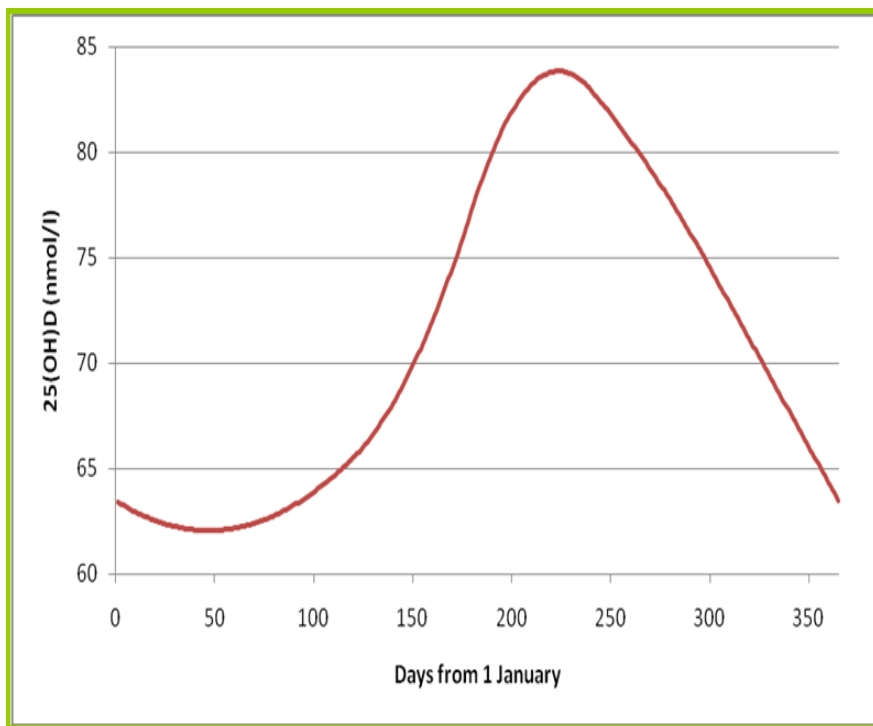


Fig 6. Seasonal variation in levels of serum vitamin D in a Swedish population of elderly men enrolled in MrOs study. Johansson H. *Osteoporos Int.*, 2012 March

Vitamin D and the prostate

It has been shown that prostate epithelial cells can produce the biologically more active 1α -25(OH) D₃ from 25(OH) D₃ [122, 123]. Moreover, it is known that both normal and prostate cancer epithelial cells express vitamin D receptor (VDR) [124, 125]. By binding to the VDR, vitamin D can increase cell differentiation, decrease cell proliferation and increase apoptosis [126-129]. Thus, it is reasonable to postulate that low levels of vitamin D might increase prostate gland volume.

1.3.3 Osteocalcin

Also known as bone gamma-carboxyglutamic acid-containing protein, osteocalcin (OC), is a noncollagenous protein found in bone and dentin. Its synthesis is vitamin K dependent. In humans, OC is encoded by the bone gamma-carboxyglutamic acid protein gene [84, 130]. Its receptor is GPRC6A [131].

Function

Osteocalcin is secreted solely by osteoblasts, is thought to play a role in the body's metabolic regulation, and is pro-osteoblastic, or bone- building, in nature. It is also implicated in bone mineralization and calcium ion homeostasis. OC acts as a hormone in the body, causing beta- cells in the pancreas to release more insulin, and at the same time directing fat cells to release the hormone adiponectin, which increases sensitivity to insulin [132].

Current data suggests a possible role of OC in male fertility [133]. Research from Columbia University Medical Center proposes that OC may enhance the synthesis of testosterone. Although these studies were initially performed by a single laboratory, at least two other groups have independently confirmed the role of OC in insulin secretion [131, 134].

Use as a biochemical marker for bone formation

As osteocalcin is produced by osteoblasts, it is often used as a marker for the bone formation process. It has been observed that higher serum-OC levels are relatively well correlated with increases in bone mineral density (BMD) during treatment with anabolic bone formation drugs for osteoporosis. In many studies, OC is used as a preliminary biomarker for the effectiveness of a given drug on bone formation. For instance, one study that looked at the effectiveness of a glycoprotein called lactoferrin on bone formation used OC as a measure of osteoblast activity [135].

Osteocalcin exists in two forms: γ -carboxylated OC and undercarboxylated OC (un-OC). The γ -carboxylated OC is believed to be involved in bone formation, while the un-OC appears to function as a hormone. Thus, the total OC is believed to be involved in bone turnover and energy metabolism [136-139]. In children and adolescents, levels of OC are high due to high bone metabolism and bone growth, indicating that the osteoblasts are more active than the osteoclasts, resulting in a positive bone formation [140]. Nevertheless, in the elderly, high OC levels seem to be associated with osteoporosis and a higher risk of fractures despite treatment with bisphosphonates, which suggests a high bone turnover in which the osteoclasts are more active, resulting in overall loss of bone mass. [141]. Why and how this change in the

association of OC and bone occurs is still unknown. In addition, altered OC levels can be seen after a meal and after exercise, indicating OC's effects on glucose metabolism [137, 142, 143]. Karsenty and co-workers, as well as other researchers, have found that OC seems to have many physiological functions in mice, where it is suggested to be involved in a pancreas- bone- testis axis, thus, affecting bone formation, glucose metabolism and fertilization [144-147]. In these studies, OC knock-out mice are fat, have glucose intolerance and lower fertility, but higher bone mass. Furthermore, pregnant mice that lack OC have offspring with cognitive dysfunction, suggesting that OC is important in the development of the CNS in the fetus[148].

Our question was whether OC was involved in early diagnosis of PC, and our hypothesis that it might be the case, since even in its early stages, PC might send micrometastases to the skeleton, resulting in a change in bone metabolism and turnover, which in turn would result in increased levels of circulating plasma OC levels. With this assumption, OC could possibly work as an early marker for metastasized PC.

1.4 The Role of MetS in Urology

It has been speculated whether the metabolic syndrome is associated with lower urinary tract symptoms. The complexity of the neural regulation of the lower urinary tract is obvious and some kind of simplification is necessary to be able to describe function disturbances in clinical practice.

1.4.1 Overactive bladder (OAB) syndrome - the role of MetS

In 1988, Bradley proposed a model which divided the voiding cycle in four neurophysiological circuits, one of them being the cerebral voluntary control of micturition, a circuit that is chiefly inhibitory and responsible for the control of the micturition reflex. An injury to this circuit results in an insufficient cortical inhibition and is seen in some common diseases such as brain hemorrhage, cerebral thrombosis, Parkinson's disease, atherosclerosis in the carotid arteries or cerebral small vessel disease. In many cases, the patient may experience bladder overactivity without any overt neurological disease. Using functional magnetic resonance imaging in healthy volunteers, Kuhtz-Buschbeck and associates were able to demonstrate that significant brain activity associated with an increased urge to void was found in the insular cortex, frontal opercula, supplementary motor area, cingulate motor area, right posterior parietal cortex, left prefrontal cortex and cerebellum. They also revealed that suppression of the urge to void significantly activated the left superior frontal lobe [149]. Other authors have contributed to the understanding of cerebral mechanisms and voiding function [150, 151].

Functional magnetic resonance imaging has also been used to determine brain responses to bladder filling in subjects with normal and with poor bladder control (detrusor overactivity). Among those with poor control, cortical responses were exaggerated at larger bladder volumes, except in the orbitofrontal cortex, which remained weakly activated. In one study, the authors concluded that poor bladder control is specifically associated with inadequate activation of orbitofrontal cortex, and that frontal cortical lesions cause bladder control problems [152].

Hence, MetS may cause dysfunction in the endothelium of the vessels in the cerebrovascular system, which in turn may bring about a diminished oxygen tension, with degeneration of neurons with critical inhibitory control functions. Following such degeneration, severe bladder overactivity and frequency including socially incapacitating urinary incontinence, may occur.

1.4.2 Benign prostatic enlargement - the role of MetS

In more recent years, hypotheses have been put forward that growth of the prostate is a partial component of the metabolic syndrome. A connection between the level of insulin as well as estrogen and prostate size has been shown [2-7, 9] . Studies have demonstrated that MetS has similar hormonal divergences. In fact, benign prostatic hyperplasia is related to several conditions that in varying degrees are correlated with MetS: atherosclerosis, hypertension, central obesity, dyslipidemia, hyperuricemia, increased BMI and hyperinsulinemia, which implies that BPH is a part of MetS and thus, belongs to the so-called diseases of affluence. A direct relationship between high fasting insulin levels and BPH has also been noted, indicating that insulin promotes prostate growth [5].

In recent years, a low serum vitamin D level, which also is a component of MetS, has been suggested to be associated with increased gland growth, since treatment with vitamin D-analogues has been shown to prevent prostate cell growth [127, 128, 153].

In short, in recent years, data have shown that the MetS itself and most components of MetS are linked to BPE, however, evidence of causality is lacking as interventional studies have not been performed.

1.4.3 Prostate cancer- the role of MetS

Different theories have been raised through the years regarding the association between MetS and PC. PC is most common among Black Americans, while the disease is unusual in Southeast Asia [154]. This pattern suggests that lifestyle factors and hereditary play a role in the development of PC. Other risk factors for PC that have been proposed are obesity, high intake of animal fat, red meat and dairy products, while a high intake of antioxidants and phytoestrogens such as tomatoes, cabbages, onions, soya, beans and green tea appears to decrease the risk of PC [155-157]. During recent decades, evidence has accumulated suggesting that MetS, which is prevalent in countries with a western lifestyle, might be associated with an increased risk for PC [158-160], while a study by Tande and co-workers and others showed a reduced risk for PC among patients with MetS [161]. As previously described, hypogonadism (serum testosterone < 8 nM) and low levels of serum vitamin D have been suggested to be components of MetS. The protective effect of MetS on the incidence of PC might be mediated through low levels of androgens and low levels of serum vitamin D.

1.4.4 Testosterone deficiency in the aging male- the role of MetS

MetS constitutes an increased risk of developing symptoms of testosterone deficiency in the middle- aged or elderly male. Testosterone levels are inversely correlated to waist circumference [162], and it has been shown that about 50% of obese men with T2DM are hypo- gonadal. Compared with eugonadal men, over an 18 year period, hypogonadal men are at higher risk of dying [163]. Moreover, testosterone deficiency imposes an increased risk of developing T2DM [164], as well as MetS. Substitution therapy with testosterone, on the other hand, has been shown to decrease the levels of pro-inflammatory cytokines and increase the levels of anti-inflammatory cytokines in men with coronary heart disease and diabetes [165].

Thus, several studies have demonstrated that low levels of testosterone are associated with MetS. In the Massachusetts Male Aging Study, it was clearly demonstrated that low levels of testosterone also constituted a risk factor in non-obese men, suggesting that testosterone deficiency in fact predisposes to the pathogenesis of MetS rather than just being a mere consequence of it [166]. This contention is further supported by a recently published paper in which it was shown that men in the lowest tertile of free and bioavailable testosterone had a four- times- higher risk of developing T2DM than men in the highest tertile, adjusted for factors such as obesity, age, race and ethnicity [167].

1.4.5 Erectile dysfunction– the role of MetS

A good erectile function is dependent on a well-functioning interplay between several critical factors. The signaling in the relevant peripheral nerves is of paramount importance, and a disturbance of the afferent signaling is a cardinal feature in T2DM that in turn, can cause the development of erectile dysfunction (ED). MetS is associated with an increased risk of atherosclerosis and T2DM. Furthermore, erectile function is highly dependent on the quality of the vessels in the corpora cavernosa, and a dysfunction in the endothelium of these vessels may cause ED in early stages of ischemic heart disease and atherosclerosis. Today, the literature is generously endowed with reports on the association between risk factors for ED and risk factors for coronary heart disease [168], and this association has also been substantiated by findings that erectile dysfunction in healthy subjects predicts reduced coronary flow velocity reserve [169].

Another mechanism linking MetS with ED is that MetS is characterized by low serum testosterone levels (hypogonadism), which has been shown to be associated with ED [170], possibly via diminished nitric- oxide -synthesis [171].

2 AIMS

The overall aim of this thesis was to elucidate whether components of MetS, with a focus on T2DM (as the endpoint of MetS) and vitamin D, were associated with LUTS, UI, BPE and PC. The specific aim of each paper was as follows:

Paper I

To explore the association between components of MetS (including vitamin D), LUTS and UI

Paper II

To study the association between components of MetS (including vitamin D) and BPE

Paper III

To examine the association between components of MetS (including vitamin D) and PC

Paper IV

To investigate the association between T2DM as the endpoint component of MetS (including vitamin D), OC and incident PC

3 PATIENTS AND METHODS

3.1 Participants

Participants were gathered from the Osteoporotic Fractures in Men (MrOs) study. The MrOs study is a multicenter study of a representative group of elderly men in Sweden (3014), in Hong Kong (2,000) and in the US (6,000). This investigation is an international study of osteoporosis, in men, encompassing clinical, anthropometric, metabolic, endocrine and genetic factors. The study participants were randomly selected using national population registers and they were contacted and asked to participate in the study. To be eligible, the participants had to be able to walk without assistance, provide self-reported data and sign an informed consent. There were no other exclusion criteria. The MrOs Swedish study population consisted of 3014 men, aged 69 - 81 years old, from three centers, Uppsala, Gothenburg and Malmö, each consisting of around 1,000 individuals. Data were gathered between the years 2001 and 2004, and 262 individuals were already diagnosed with PC at the beginning of the study, referred to as “prevalent PC cases”. The follow-up time was more than 10 years, and 252 individuals have developed PC during the study up to now, referred to as “incident PC cases”.

In papers I, II and IV, the study population constituted of the Gothenburg subgroup of the MrOs study. In this cohort, 976 individuals completed the questionnaires for LUTS according to the IPSS (0 - 35), 978 individuals completed questionnaires for UI, and around 950 individuals gave complete blood samples for analysis. Among these 950 individuals, the prostate gland volume was measured in 184 randomly selected subjects stratified by age (70 -

75 years old). After men with a medical history of prostate cancer, prostate surgery or medication for BPE (5-alpha-reductase inhibitors or alpha-blockers) were excluded, 155 men remained for evaluation. In this cohort, 151 individuals were diagnosed with T2DM at the beginning of the study. Excluding those with prevalent PC (91 cases) resulted in 141 individuals remaining. During the study, 97 individuals developed PC.

In paper III, the study population consisted of the total population of the Swedish MrOs study.

3.2 Anthropometric Variables

Height and body weight were measured using standard equipment. Two consecutive measurements of height were performed in the same session, and the average of these measurements was calculated. If there was a discrepancy of ≥ 5 mm between the first two measurements, a third measurement was performed and the average of the two values with the least mutual discrepancy was calculated. Lean body mass, total body fat mass and trunk fat mass were assessed using the DXA Hologic QDR 4500/A-Delphi scanner (Hologic, Bedford, MA, USA).

3.3 Blood Sampling

All tests were done on morning samples after an overnight fast and at least 10 hours of non-smoking. All tests were performed on blood samples that had not been previously thawed. Serum insulin samples were transported directly to

the laboratory. Serum samples for sex hormones were stored at -80 degrees Celsius. Following variables were analyzed.

3.3.1 Gonadal hormones, fasting serum insulin, insulin like growth factor 1, glucose, lipids and adiponectin

A validated gas chromatography mass spectroscopy system was used to analyze testosterone (limit of detection 0.05 ng / ml, intra-assay CV 2.9%, inter-assay CV 3.4%), estradiol (limit of detection 2.00 pg / ml, intra-assay CV 1.5%, inter-assay CV 2.7%), 5 α -androstane-3 α ,17 β -diol-3G (limit of detection 0.50 ng / ml, intra-assay CV 10.3%, inter-assay CV 10.7%), 3 α -androstane, 17 β -diol-17G (limit of detection 0.50 ng / ml, intra-assay CV 4.6%, inter-assay CV 5.3%), dihydrotestosterone and dehydroepiandrosterone (DHEA). Free testosterone and free estradiol were calculated according to the method described by Vermeulen and co-workers [172] and by van den Beld and co-workers [173], taking the concentrations of total testosterone and total estradiol into account and assuming a fixed albumin concentration of 43 g / l. Sex hormone-binding globuline (SHBG) was measured using Immunoradiometric assay (Orion Diagnostics, Espoo, Finland; limit of detection 1.3 nM). All samples were analyzed in duplicate in one laboratory, and the duplicates were averaged for further analyses.

Serum insulin was measured using an immunoelectric method based on chemiluminescence technology on an ADVIA Centaur analyzer (Bayer AB, Gothenburg, Sweden) with a detection limit of 0.1 microU / ml. Fasting plasma glucose was quantified by an enzymatic method using a modular kit

(Roche). Serum levels of insulin-like growth factor (IGF-1) were assayed by a double-antibody IGF-antibody- blocked radioimmunoassay (RIA) using a commercial kit (Mediagnost, Tubingen, Germany) with an intra-assay coefficient of variation of less than 5% and an inter-assay coefficient of variation of less than 8% according to the manufacturer. The median was 161 ng / ml and the 5th and 95th percentiles were 91 and 282 ng / ml, respectively, for men between 60 and 70 years of age, while the median was 98 ng / ml and the 5th and 95th percentiles were 47 and 207 ng / ml respectively for men between 71 and 80 years of age.

Triglyceride levels were determined by fully enzymatic techniques. HDL-cholesterol was determined after precipitation of apolipoprotein (Apo) B-containing lipoproteins with magnesium sulphate and dextran sulphate.

Serum adiponectin was determined using human adiponectin ELISA kit.

3.3.2 Vitamin D, parathyroid hormone, calcium, cystatin C and alkaline phosphatase

Serum 25(OH) D was measured at baseline in men using a competitive RIA-technique (Diasorin, Stillwater, MN, USA) at a single laboratory. Since the serum level of 25(OH) D varied by season, a z-score was constructed. An expected value of 25(OH) D was calculated for each participant according to season (number of days since January 1) [101], using regression with spline functions with knots at 120, 180, 240 and 300 days. Restrictions of equal means at the end and the beginning of the year were applied. This model was calculated for the total Swedish material (n = 2,878). The difference between the observed and the expected 25(OH) D was divided by the standard

deviation around the regression line, which gave a Z-score with a mean of 0 and a standard deviation of 1. For the purposes of presentation, the z-score for 25(OH) D was back-transformed to values in nanomoles per liter standardized to the 25(OH) D values at 150 days (5 months), which gave the mean value of 25(OH) D for the whole Swedish MrOs cohort.

Serum parathyroid hormone (PTH) was analyzed using the Immulite 2,000 Intact PTH assay (Diagnostic Products, Los Angeles, CA, USA; normal 1.1 - 9.9 pmol / L). P-calcium (normal 2.15 - 2.50 mmol / L) and albumin (normal 39 - 45 g / L) were measured by routine techniques. Serum cystatin C was measured by routine techniques in a laboratory in Lund, Sweden, and serum alkaline phosphatase was measured by routine techniques in Gothenburg.

3.3.3 Serotonin

In the Gothenburg cohort of the MrOs study, serum serotonin was measured using an ELISA technique (IBL Immuno Biological Laboratories, Minneapolis, MN, USA).

3.3.4 Osteocalcin

The plasma levels of total osteocalcin (γ -carboxylated + uncarboxylated) were analyzed in the Gothenburg cohort the first time the samples were thawed, using monoclonal antibodies against human osteocalcin, the N-terminal amino acids 1 - 43 and 1 - 49, and detected by electrochemiluminescence (Elecsys N-MID Osteocalcin CalSet; Roche Diagnostics, Indianapolis, IN, USA).

3.4 LUTS

LUTS were measured by the IPSS divided into different categories: no symptoms (IPSS 0), mild symptoms (IPSS 1 - 7), moderate symptoms (IPSS 8 - 19) or severe symptoms (IPSS 20 - 35).

3.5 Urinary Incontinence

UI was measured by the presence of urinary leakage according to two questions regarding the absence or presence of UI (not validated).

3.6 Prostate Gland Volume

The prostate gland was examined using digital rectal examination and ultrasonography equipment (BK Medical 2002 ADI Panther). The gland volume was determined by means of ultrasonography using the ellipsoid method.

3.7 Data Collection regarding PC

Several Swedish registers served as important sources of information for the papers in the present thesis.

The “Swedish population register (*sv. Folkbokföringsregistret*)” is compiled by the Swedish Tax Agency (*sv. “Skatteverket”*), and contains information about Swedish residents, regarding their age, place of birth, residential address, marital status and so on. Whenever a person passes away, the agency receives a death certificate.

The “Swedish Death Register (*sv. Dödsregistret*)” and the “Swedish Cause of Death Register (*sv. Dödsorsaksregistret*)” are two other registers. Following the death of a person, a doctor issues a death certificate, as well as a cause-of-death-certificate. The death certificate is sent to the tax agency, and the cause of death certificate is sent to the “Swedish National Board of Health and Welfare (*sv. Socialstyrelsen*)”. The latter organization publishes statistical summary reports on the causes of death every year.

“The Swedish cancer register (*sv. Cancerregistret*)” was started in 1958. The six regional cancer centers in Sweden are responsible for registering cancer cases in their region and reporting them to the register. The register is generally of high quality, with the overall under-reporting estimated at 3.7% in 1998 [174]. “The Swedish National Prostate Cancer Register (NPCR: *sv. Svenska prostata cancer kvalitetsregistret*)” has all the information regarding the date of the PC diagnosis, the PSA levels at diagnosis, the TNM-classification and the Gleason grading of the tumor.

These registers were coordinated and 252 incident prostate cancer cases were identified up to December 2013, which concluded around 10 years of follow-up. In the Gothenburg subgroup of the MrOs, 91 prevalent and 97 incident prostate cancer cases were identified.

In line with previous studies and according to a modified version of the National Comprehensive Cancer Network guidelines, the cohort with incident

PC was divided into three subgroups depending on the severity of their PC at diagnosis. These risk categories were defined as low- risk: clinical local stage T1 tumor, PSA < 10 ng/ml and Gleason score \leq 6; intermediate- risk: \leq T2 tumor or PSA 10 - 20 ng / ml or Gleason score 7; and high- risk: T3 - T4 tumor or PSA \geq 20 or Gleason score \geq 8 or N1 or M1. The subgroups of PC with local metastases (N1) and with distant metastases (M1) were merged into subgroup 3, the so- called “high-risk” subgroup. “Clinically significant PC” was defined as cancers in the intermediate- risk and high- risk groups.

3.8 Data Collection regarding MetS

A synthesis of different health organizations definitions of MetS worldwide was made. The following criteria were applicable to our data:

Obesity was defined as BMI >30 kg/m².

Instead of HDL-cholesterol, Apo lipoproteins A1 and B were measured. These substances are more specific than HDL-cholesterol for measuring dyslipidemia. Dyslipidemia was defined as: Apo A1 < 1.15 g / l or Apo B \geq 1.2 g / l or Apo B / Apo A1 \geq 0.9. For this study we used the quote Apo B / Apo A 1.

Raised blood pressure was defined as anamnestic data of hypertension.

Hyperglycemia was defined as having fasting blood glucose over 7.0 nmol / l, as in paper II, or anamnestic data of T2DM.

3.9 Data Collection regarding T2DM

Initially, 297 individuals were diagnosed with T2DM when included in the study (anamnesic data) and were either on treatment with anti-diabetic drugs, including insulin, or on a diet. When individuals with prevalent PC (262 individuals) were excluded, the number of participants with T2DM declined to 274 individuals. The diabetic cohort was divided into three subgroups depending on the severity of their disease: mild diabetes (dietary treatment), intermediate diabetes (treatment with per oral anti-diabetic drugs) and severe diabetes (insulin treatment). In the Gothenburg subgroup of the MrOs study, 151 individuals were diagnosed with T2DM initially. After excluding those with prevalent PC, 141 individuals remained.

3.10 Statistical Analysis

The significance level was 0.05. The software used was Stata version 12 and SAS version 9.2 (SAS Institute, Cary, NC, USA) and data analysis programs written at the Department of Public Health and Community Medicine at Mölndals Hospital in Gothenburg, Sweden.

3.10.1 Paper I

Due to the high correlation between HDL-cholesterol and apolipoprotein A1, $r = 0.85$, apolipoproteins were not included in the analysis. Three of the studied variables (insulin, interleukin 6 and CRP) showed a markedly skewed distribution in the total population study and were $\log_{\text{base}10}$ transformed in the

analysis. BPE was defined as a prostate volume larger than 40 ml (median volume). The Pearson correlation coefficient and simple linear regression models measured univariate associations with the IPSS. To find the significant predictors of the IPSS in a multivariable context, a linear regression model was used with backward selection of variables selected from those with the strongest univariate association.

3.10.2 Paper II

Data were analyzed including and excluding subjects with the diagnosis of T2DM (anamnestic data) and those with fasting serum glucose exceeding 7.0 mmol / l, who were considered to have T2DM. By so doing, the influence of serum insulin, which in previous reports had been shown to be an independent risk factor for BPE, was minimized. Thereby, a more independent evaluation of the influence of other risk variables could be done. Three of the studied variables (serum insulin, triglycerides and CRP) were $\log_{\text{base}10}$ transformed in the analysis. Univariate associations between variables and prostate volume were examined using t-tests with the Satterthwaite formula for dichotomized (prostate volume > 40 ml) tests. The independent predictors of large prostate gland volume were tested with binary logistic regression for selection of variables to be entered in the multivariable analysis. Multivariable models were calculated by backwards- stepwise selection of the most important predictors in the univariate analyses. Owing to the high correlation between HDL- cholesterol and Apo A1, $r = 0.85$, Apo A1 was not included in the analysis.

3.10.3 Paper III

Cox proportional hazard regression models were used to test possible predictors of prostate cancer incidence. The variables were divided into quintiles, and comparisons were done separately for the first and fifth quintiles versus quintiles 2 - 4. Further comparisons were made for values below and above the median. A spline Poisson regression model was fitted using knots at the 10th, 50th and 90th percentiles of the z-score of 25(OH) D, to study the association between serum 25(OH) D and PC in more detail. The splines were second-order functions between the breakpoints and linear functions at the tails. A parametric T-test was used to test the association between diabetes and serum testosterone levels. Linear regression was used to test the association between diabetes and vitamin D, adjusted for age and study center.

3.10.4 Paper IV

The Pearson correlation coefficient and simple linear regression models measured univariate associations with PC. Binary logistic regression model, Pearson's correlation, Cochran- Armitage- and chi- square analyses were conducted to assess the association between plasma osteocalcin, BMD, vitamin D levels, levels of other endocrine, metabolic and inflammatory factors, and incident PC in the cohort. A Cox regression model was used to calculate the association between the quintiles of plasma levels of OC and the risk of PC. The distribution of OC was transformed to be as a normal distribution variable using the inverse of the standardized normal distribution function, so comparability could be achieved to other variables described using the hazard ratio / standard deviation (HR / SD). When studying the risk of PC related to plasma OC- levels over time, an interaction term between current

time since baseline and the normalized OC in a Poisson regression model was used. A spline curve using logistic regression with knots fitted at the 10th, 50th and 90th percentiles, was designed to show the association between OC and prevalent T2DM. The splines were second- order functions between the breakpoints and the linear functions at the tails, resulting in a smooth curve. Another figure showed the risk of PC related to OC-levels over time.

4 RESULTS

The results of each paper in this thesis are separately described below.

4.1 Paper I

No significant correlation was found between the major components of MetS such as high blood pressure, serum insulin levels, BMI, levels of serum HDL-cholesterol and LUTS. Vitamin D was not correlated with LUTS. In univariate analysis, serum serotonin levels and serum free testosterone levels showed a negative correlation with LUTS, while serum adiponectin and serum fasting glucose showed a positive correlation with LUTS. Furthermore, the prostate gland volume was significantly correlated with LUTS; the association between the prostate gland volume and the severity of LUTS was linear. Using linear regression with covariates selected as those with univariate correlation ($p < 0.05$), serum serotonin showed a negative correlation with LUTS while serum adiponectin and serum fasting glucose showed a positive correlation with LUTS. Low serum serotonin was an independent risk factor for UI, both in univariate analysis and when controlling for other potential predictors.

4.2 Paper II

The mean prostate volume for the diabetic subgroup was 55 ml (26 individuals) and for the non-diabetic subgroup was 45 ml (127 individuals). The difference between these mean values was significant ($p = 0.036$). Fig 7

shows the relationship between the serum 25(OH) D values (adjusted for season) and the prostate gland volume in a multivariate analysis, adjusting for lean body mass and HDL-cholesterol. In this population of healthy community-dwelling men, only 2 of 155 individuals had serum vitamin D levels lower than 30 nmol / l.

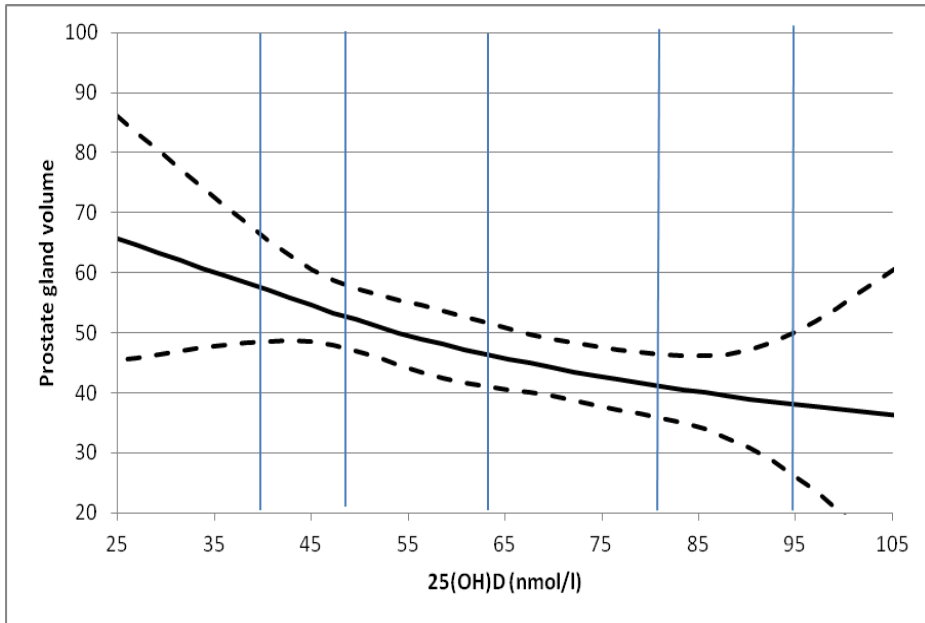


Fig 7. Spline curve describing the correlation between 25-OH-Vitamin D, adjusted for season, and the prostate gland volume

The correlation between the analyzed factors and BPE (prostate gland larger than 40 ml) in two cohorts, i.e. men including individuals with T2DM and men excluding individuals with T2DM, was studied. After excluding subjects with T2DM from the population study, body weight, trunk fat mass, lean body mass and log triglycerides remained positively correlated with BPE, while the positive correlation between BPE and BMI, as well as the positive correlation between BPE and total body fat mass, disappeared. HDL-cholesterol, 25(OH)

D, albumin- corrected serum calcium, adiponectin and serum SHBG remained significantly negatively correlated with BPE, while the negative correlation between total testosterone and BPE disappeared. Furthermore, height showed a positive correlation and log CRP a negative correlation with BPE.

In univariate analyses, subjects with diabetes had an increased risk of having BPE (odds ratio [OR] 3.33). The following factors were positively associated ($p < 0.05$) with BPE, expressed per standard deviation increase: Body weight (OR 1.59), BMI (OR 1.44), trunk fat mass (OR 1.50), total body fat mass (OR 1.40), lean body mass (OR 1.68) and log triglycerides (OR 1.56). The following factors were negatively associated ($p < 0.05$) with BPE, expressed per standard deviation decrease: Seasonally adjusted 25(OH) D (OR 1.87), albumin corrected serum calcium (OR 1.59), HDL-cholesterol (OR 1.88), adiponectin (OR 1.74), total testosterone (OR 1.40) and SHBG (OR 1.81).

After excluding subjects with T2DM, the following factors were positively associated with BPE, expressed per standard deviation increase: Height (OR 1.59), body weight (OR 1.62), trunk fat mass (OR 1.45), lean body mass (OR 1.90) and log triglycerides (OR 1.54). The following factors were negatively associated ($p < 0.05$) with BPE, expressed per standard deviation decrease: Seasonally adjusted 25(OH) D (OR 1.64), albumin- corrected serum calcium (OR 1.87), HDL-cholesterol (OR 1.77), adiponectin (OR 1.51), SHBG (1.82) and log CRP (OR 1.62).

With BPE (prostate volume > 40 ml) as the dependent variable and T2DM, body weight, lean body mass, 25(OH) D, HDL-cholesterol, albumin- corrected s-calcium, adiponectin and SHBG as possible predictors, 25(OH) D, HDL-cholesterol, SHBG and albumin corrected s-calcium came out statistically linked to BPE in a stepwise logistic regression model ($p < 0.05$).

4.3 Paper III

No association was found between major components of MetS (central obesity measured by BMI, dyslipidemia measured by Apo lipoproteins and hypertension) and PC, while individuals with T2DM, as the end point of MetS, showed a tendency to have a lower incidence of PC (HR = 0.61; 95% CI = 0.36 - 1.03; $p = 0.06$).

No linear association between serum levels of vitamin D and incident PC was observed. Individuals with D-vitamin levels below 50 nmol / l (as a component of MetS) had lower risk for incident PC (HR = 0.67; CI = 0.46 - 0.98; $p = 0.04$).

In a spline model (Figure 8), increasing levels of serum vitamin D up to 90 nmol / l were significantly associated with increasing risk of developing PC. The hazard ratio for incident PC per standard deviation increase in serum vitamin D levels was 1.27 (CI:1.05 - 1.53; $p = 0.013$) up to 90 nmol / l, and thereafter no further significant increase or decrease in the risk of developing PC was observed ($p = 0.17$). The positive association between increasing levels of serum vitamin D up to 90 nmol / l and incident PC differed statistically significantly from the neutral association above serum levels of 90 nmol / l ($p = 0.018$), indicating a non- linear association. Individuals with serum vitamin D levels above the median (above 50th percentile = 67 nmol / l) had a higher risk of developing PC than those with serum vitamin D levels below the median (HR = 1.32; CI = 1.02 - 1.70; $p = 0.032$).

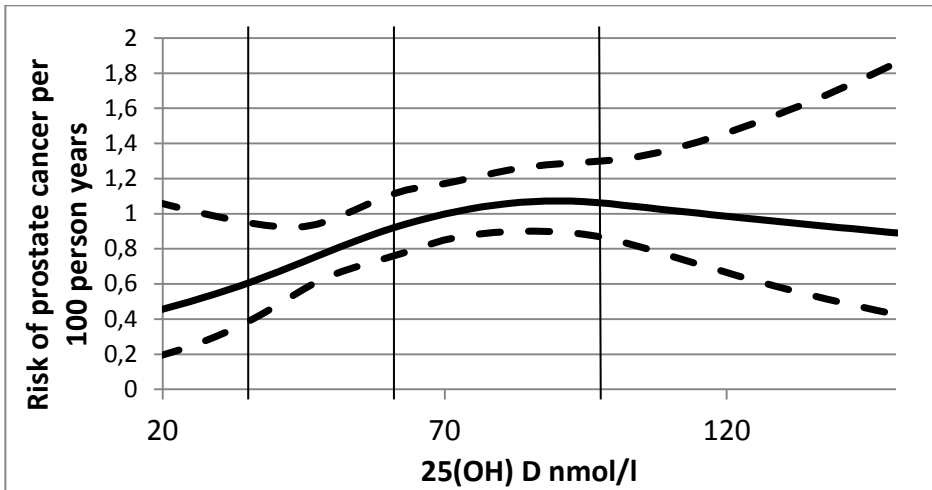


Fig 8. Relationship between seasonally adjusted serum vitamin D levels (z-score) and risk for incident prostate cancer adjusted for age and time. The risk is given as risk of PC per 100 person years for a man aged 80 years after 5 years of follow-up. Knots at the 10th, 50th (median) and 90th percentiles are marked.

Individuals with levels of serum CRP in quintile 1 (below 1.58 g / l) had a significantly higher risk of being diagnosed with PC than those with CRP-levels in quintiles 2 - 4 (HR 0 1.49; $p = 0.008$), and individuals with height above 179 cm (quintile 5) had a significantly higher risk of developing PC (HR = 1.48; $p = 0.010$) than individuals in quintiles 2 - 4.

In an attempt to investigate the combined effect of items included in MetS, a risk score was created by taking the sum of three factors: obesity, dyslipidemia and hypertension. The participants who had all three of the above- mentioned factors were considered to suffer from MetS. A non-significant decreased risk of incident PC in these individuals was found ($p = 0.10$).

Diabetic men who were on drug treatment including insulin tended to have a lower risk of developing PC adjusted for age (HR = 0.581; CI = 0.31 - 1.09; p = 0.09). Men with T2DM seemed to have a lower risk of developing high-risk PC than men without T2DM (RR = 0.596; CI = 0.209 - 1.21; p = 0.10). The diabetic subgroup had significantly lower levels of serum total testosterone, free testosterone, DHEA and serum vitamin D than controls.

4.4 Paper IV

Levels of OC were negatively associated with BMI, and with total fat mass. When adjusted for age and BMI, levels of OC were negatively associated with BMD (at all measured sites) and were positively associated with cystatin C, PTH and alkaline phosphatase (ALP). There was no association between serum levels of OC and androgens (including androgen metabolites), DHEA and SHBG, however a negative association was observed between serum levels of estrone and OC.

Men with T2DM had lower levels of total and free serum testosterone, DHT, SHBG and vitamin D and had increased risk for hypogonadism (s-testosterone below 8.0 nmol / l) (OR = 3.4; 95% CI = 1.91 - 6.10).

Results further showed that levels of OC were significantly higher among those with incident PC than controls, predominantly during the first 5 years of follow-up. When the first year of the study was excluded, individuals who had OC-levels in the 5th quintile had an HR of 2.32 (95% CI = 1.19 - 4.54) for developing PC compared with those in quintiles 1 - 4. After adjusting for background variables, the HR for levels of OC among individuals with incident PC compared with controls, changed to 2.50 (95% CI = 1.30 - 4.81).

The association between OC and PC appeared to decline over time, and after around 5 years of follow-up the association was no longer statistically significant ($p = 0.11$). The HR / SD changed over time, from 1.38 (95% CI = 1.04 - 1.83) at 1 year after baseline, to 1.00 (95% CI = 0.75 - 1.32) at 7 years after baseline (Fig 9).

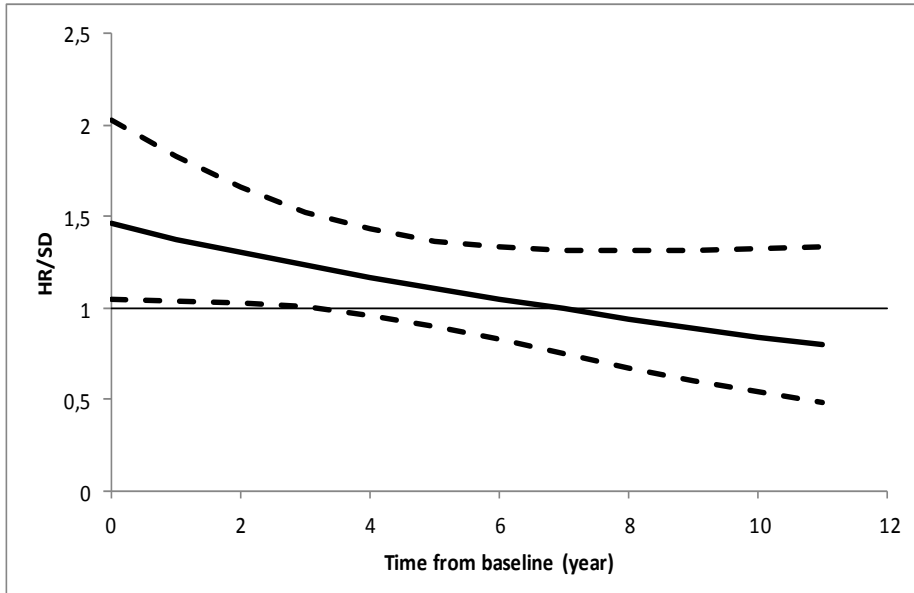


Fig 9. Risk of PC related to levels of plasma Osteocalcin over time adjusted for age

No significant association was observed between serum levels of ALP and PC (HR = 0.92; 95% CI = 0.74 - 1.14). Men with incident PC had mean values of BMD in hip, lumbar spine and total body that were similar to mean values in controls ($p > 0.3$).

In a fully adjusted model, the OR of having T2DM per standard deviation decrease in OC was 2.25 (95% CI = 1.65 - 3.08). Individuals with OC levels in

quintile 1 had higher risk of having T2DM than those in quintiles 2 - 5 (HR = 3.18; 95% CI = 2.03 - 4.96).

A strong relationship between low levels of OC and prevalent T2DM adjusted for age was seen (Fig 8). At the 10th percentile of OC (16 mg / l), the risk of having T2DM was 30% (95% CI = 25 - 35), and at the 90th percentile of OC (38 mg / l), the risk was 8% (95% CI = 5 - 2).

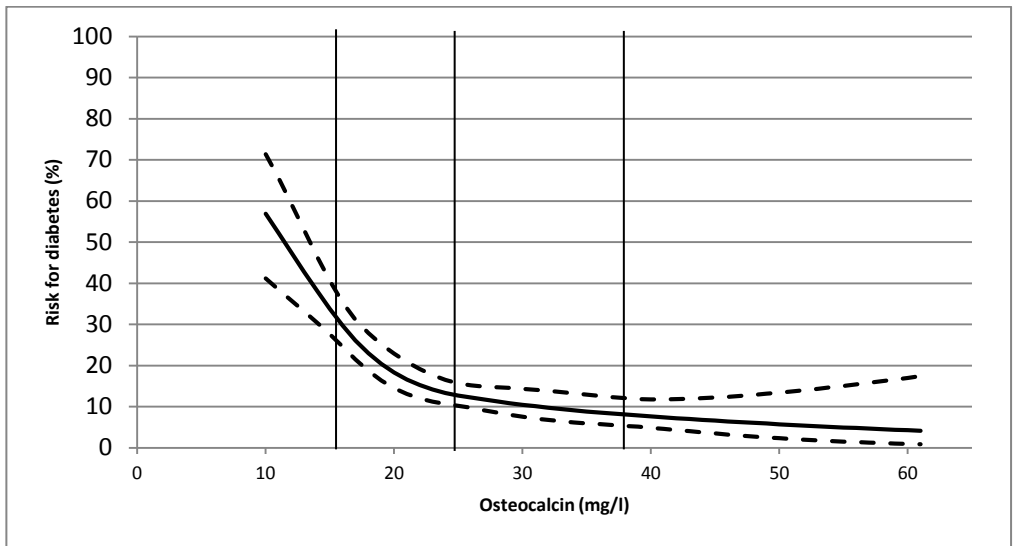


Fig 10. The association between levels of plasma OC and risk of having T2DM adjusted for age. The association is shown for a man aged 75 years. Men with prevalent PC excluded.

5 DISCUSSION

The general idea behind this thesis was to test the hypothesis whether components of MetS were associated with LUTS (including UI) and with prostatic diseases. One component of MetS that was specifically in focus, owing to its anti-inflammatory, anti-proliferative and pro-apoptotic properties, was vitamin D. The fact that this hormone has receptors (VDRs) in the prostate gland further strengthened the idea that it could have its effects locally in the prostate gland, resulting in inhibition of the growth of the gland, and hopefully in inhibition of the onset and progression of PC.

5.1 Paper I

The most important finding was that a low serum serotonin level was an independent risk factor for LUTS and for UI, indicating that a low serotonin level could be part of the pathophysiology of LUTS and UI. The findings are in accordance with two recent reports, demonstrating an association between major depression as measured by a depression score and LUTS [29, 30], and moreover, are in line with another report showing a link between depression and urinary incontinence [31]. Hence, low levels of serum serotonin might result in excessive stress causing anxiety, major depressive disorders and LUTS. Using stress tests, both McVary and co-workers [24] and Ullrich and co-workers [25] found that the activity of the sympathetic nervous system was linked to LUTS.

LUTS was poorly associated with MetS, however, a strong correlation was found between the prostate gland volume and LUTS, which supports the

commonly adopted conception that BPE, presumably via bladder outlet obstruction, might be one of the causative factors for LUTS. However, the association between bladder outlet obstruction and LUTS was not studied. The notion that increased chronic stress is related to LUTS is certainly in line with the clinical findings on the beneficial effect of alpha-receptor blockers in men suffering from LUTS. It is well established that alpha-blockers inhibit the adrenergic activation of alpha-adrenoceptors in the central nervous system as well as in the fibromuscular stroma and capsule of the prostate [175].

Low levels of serum serotonin were associated with UI, which is in line with the use of the antidepressant drug duloxetine in the treatment of urinary stress incontinence in clinical practice [176]. It is well known that stress urinary incontinence is rare in men, with the exception of subjects who have undergone prostatic surgery. Such subjects were excluded from the evaluation and, therefore, it is reasonable to believe that subjects reporting UI were in fact suffering from urgency urinary incontinence.

Increased fasting plasma glucose was associated with LUTS, which is in keeping with the findings of Demir and co-workers [177] and Rohrmann and co-workers. [178]. Animal studies have shown that long-term elevated serum glucose induces a neuronal apoptosis that favors parasympathetic neuron loss over sympathetic neuron loss [179]. Such a loss of parasympathetic neurons might lead to an imbalance and create an oversupply of sympathetic tone over parasympathetic efferent activity, as pointed out by Kupelian and co-workers [180] and Moul and co-workers [181].

Another interesting finding was the inverse relationship between free testosterone and LUTS, suggesting that a low free testosterone level, which is the biologically active serum androgen, might promote pathophysiological mechanisms inducing LUTS. Chang and co-workers also found a statistical

link between a low level of free testosterone and LUTS, in both univariate and multivariate analyses [182]. Testosterone is known to modulate Nitric Oxide Synthase (NOS) activity. Ehren and co-workers have suggested that nitric oxide may influence the dilatation of the bladder neck and urethra during the micturition reflex [183]. Thus, it may be speculated that a low free testosterone level might promote bladder neck constriction and LUTS by reducing NOS activity in the bladder neck and urethra. This hypothesis is certainly in line with the clinical evidence indicating that testosterone treatment [184] and treatment with phosphodiesterase-5-inhibitors such as tadalafil [185] have benefited men suffering from LUTS.

Another finding was the positive correlation between adiponectin and LUTS. This finding is in line with the fact that LUTS as well as levels of serum adiponectin increase with age. High levels of adiponectin are also related to a higher incidence of osteoporotic skeletal fractures, to poorer general health and to death [63, 186]. Hence, high levels of serum adiponectin might be an indicator for early onset of LUTS.

5.2 Paper II

The most important finding is that vitamin D appears to be associated with BPE. A low vitamin D level was associated with BPE and the prostate gland volume decreased with increasing levels of vitamin D, and according to the spline curve, this association was linear. The finding of a link between a low vitamin D level and BPE is interesting, since vitamin D levels could be easily manipulated by oral vitamin D₃ supplementation, artificial UVB radiation and diet. Another epidemiological study examining dietary patterns found a

reduced risk of symptomatic BPE in men using supplemental vitamin D [187]. A low vitamin D level has also been suggested to promote prostate cancer development [188] although epidemiological data are inconsistent with respect to prostate cancer risk [189].

In previous reports, increased serum insulin was an independent risk factor for BPE [2, 190]. However, in this study no association between serum insulin levels and prostate volume >40 ml was observed.

Testosterone is assumed to be an important growth promoting factor for the prostate gland [191]. This assumption is further supported by the clinical efficacy of 5 α -reductase inhibitors in the treatment of BPE [192]. It has been reported that serum levels of specific glucuronidated androgen metabolites predict the prostate gland volume and it has been suggested that glucuronidated androgen metabolites may be potential estimates of intracellular levels of unconjugated steroids and their biological activities in tissues [36]. Parsons and co-workers showed that high levels of serum dihydrotestosterone predicted an increased risk of benign prostatic hyperplasia [193]. Taken together, these data generate the hypothesis that increased androgen activity may be related to the growth of the prostate gland. However, the results did not confirm any relationship between serum levels of total testosterone and BPE, although testosterone was lower in the group with larger prostate volumes, which was balanced by an increased SHBG level. On the other hand, levels of free testosterone, the biologically active androgen, were similar in both groups. SHBG appeared to be an independent reverse risk factor for prostate gland volume, but the rationale for this finding is presently unknown.

It was further shown that the concentration of adiponectin is negatively correlated with BPE. However, adiponectin did not remain as a predictor of

BPE in the multivariate analysis. These findings may suggest that adiponectin is not causally related to BPE.

5.3 Paper III

The most important finding was that established aspects of MetS, such as obesity, hypertension, dyslipidemia and T2DM, did not come out as risk factors for PC. Neither did other components of MetS, such as increased estradiol levels, reduced levels of testosterone [194-196] and low levels of serum vitamin D [197, 198], come out as risk factors for PC. However, men with T2DM showed a tendency to have a lower incidence of PC, which is in line with the results of another report [199]. An altered hormonal milieu in diabetic individuals might be a pathway through which the prostate cells are affected so they less frequently develop into cancer cells. All forms of measured androgens were lower in men with T2DM than in men without T2DM, resulting in low levels of prostate specific antigen (PSA) in serum (since PSA production is under androgen control), which in turn might decrease the likelihood of being diagnosed with PC [200, 201].

The results did not differ after excluding the low-risk subgroup and only taking into account those with clinically significant PC, i.e., the participants with intermediate-risk and high-risk PC.

At variance with the study by Bhindi and coworkers [158], no increased risk of PC in the subgroup of individuals with MetS compared with controls was found. In addition, increased serum vitamin D levels were associated with an increased incidence of PC. These results, however conflicting, are in line with recently published data in the Nordic countries [16, 202] but not in the US arm

of the MrOs study, where no association between vitamin D and PC was found [203].

Using linear regression, no association was shown between CRP and incident PC. However, an increased risk for incident PC was found in individuals with serum CRP levels in the first quintile compared to quintiles 2 - 4. A possible explanation for the association between low levels of CRP and incident PC might be that individuals with PC have higher levels of serum vitamin D and vitamin D is known to have anti-inflammatory effects [204].

Taller individuals (above 179 cm), were shown to have a higher risk of incident PC, which is in line with the studies by MacInnis and English, who reported [205] that height, but not body weight or waist circumference, was positively associated with increased risk of incident PC.

5.4 Paper IV

The most important finding in this study was that levels of plasma OC were significantly higher at baseline among men who developed PC than among controls. After excluding men with prevalent PC (91 individuals), individuals with OC levels in quintile 1 had higher risk of having T2DM than subjects in quintiles 2 - 5 in multivariable analyses adjusted for age, BMI, hip BMD, adiponectin, vitamin D, cystatin C and total testosterone (HR = 3.18; 95% CI = 2.03 - 4.96). It has been shown that individuals with T2DM have low levels of vitamin D and low bone mineral metabolism and turnover [162], indicating low osteoblast activity. This low osteoblast activity could possibly be a mechanistic explanation for why these individuals have low levels of OC. OC was negatively associated with BMI and with total fat mass but not with total

lean mass, indicating that OC is predominantly associated with body fat. Experimental studies have also shown that OC had effects on testis growth and testosterone levels [144]. The association between levels of OC and androgens were studied, but no significant association was found.

In a study by Reichert and co-workers, it was shown that growth of LNCaP cells and PC3 cells on matrices produced by primary human osteoblasts mimics key features of PC metastases to the skeleton, such as induction of genes associated with attachment, migration, increased invasive potential, calcium signaling and osteolysis, resulting in stronger adherence, increased proliferation rate and expression of markers consistence with loss of epithelial phenotype [206]. Nadiminty and co-workers found that PSA produced by metastatic PC cells might participate in bone remodeling through upregulated expression of genes in osteoblasts associated with osteoblast differentiation including osteocalcin genes [207]. Kitagawa and co-workers showed that vascular endothelial growth factor contributes to PC-induced osteoblastic activity [208]. This increased osteoblastic activity might indicate that in its early stages, PC sends micro metastases to the skeleton hematogenically, resulting in higher levels of OC in plasma years before the disease is diagnosed.

5.5 General Discussion

The possibility of a link between MetS and two urological tumors, BPE and prostate cancer has been explored for more than 15 years. Researcher have concluded that a variety of clinical, hemodynamic, anthropometric, metabolic and endocrine aspects of MetS are linked to BPE and prostate cancer, suggesting that these tumors are themselves aspects of the syndrome.

Regarding BPE, the link between MetS and BPE might be considered established knowledge. However, when it comes to prostate cancer the data are conflicting. A more recent notion is that MetS is linked to other important conditions in urology such as male hypogonadism, nephrolithiasis, OAB and ED. However, research into the link between the MetS and these clinical conditions has been going on for a shorter period and evidence is still sparse. Urologists need to be aware of the effect that the MetS has not only on the prostatic diseases, but also on other urological disorders, as well as on overall patient health, and they need to transfer this knowledge to their patients.

Low levels of vitamin D had been suggested to be a component of MetS, which resulted in the hypothesis that low levels of this hormone might result in both benign prostate gland growth and the development and progression of PC. One should keep in mind that studies have been published suggesting that BPE is one of the preliminary stages in the development of PC. During recent years a debate has been under way on whether LUTS is also an aspect of MetS.

In paper I the association between components of MetS and LUTS (including UI) was investigated in general and it was shown that BPE is associated with LUTS, which is in accordance with the current knowledge. BPE may cause BOO, with secondary OAB as a result. However, most components of MetS, including low levels of vitamin D, were not associated with LUTS, which might be due to the mechanism behind the presumed association between MetS and LUTS being somewhat complex with a significant number of confounding factors involved. In summary, the findings did not support the hypothesis that LUTS is an aspect of the MetS. In fact, a high level of adiponectin was associated with LUTS, while a low level of adiponectin is known to be a component of MetS. Instead, the study generated the hypothesis

that a modified form of MetS, characterized by chronic stress and depression via reduced serotonin levels, reduced inhibition of the amygdale nucleus and at the same time increased noradrenergic sympathetic nervous system axis activity and increased bladder neck contraction are parts of the pathophysiology of LUTS.

In paper II the focus was on the association between MetS and BPE, where it was shown that low levels of vitamin D, were indeed associated with BPE. Putting together the results from papers I and II, one could assume that since low levels of vitamin D result in BPE and since BPE is associated with increased LUTS, low levels of vitamin D should have been independently associated with increased LUTS, but this association was not demonstrated, as described above. Individuals with T2DM as the endpoint of MetS had larger prostate glands than nondiabetic controls. Furthermore, the findings confirm previous conclusions by adding four more aspects of the MetS as risk factors for BPE, namely low levels of: vitamin D, calcium, SHBG and HDL-cholesterol). Overall, the present knowledge in this field is consistent with the hypothesis that MetS and its metabolic and endocrine aberrations, promote growth of the prostate gland, suggesting that BPE in itself is an aspect of MetS. On the basis of current knowledge, it is not possible to identify which factors are of most importance for prostate gland growth. It is reasonable to believe that anabolic hormones such as increased fasting plasma insulin, which has been shown to be an independent risk factor for BPE in many reports, and a reduced level of vitamin D may play more significant roles in the promotion of prostate gland growth than other factors.

In paper III the association between components of MetS, with vitamin D at focus, and prostate cancer was studied. Surprisingly it was demonstrate that increased levels of vitamin D up to 90 nmol / l were positively associated with

PC. The mechanism behind these observations is as yet unknown. Individuals with T2DM as the endpoint of MetS had a lower risk of developing PC (presumably partially due to low levels of androgens) and had low levels of serum vitamin D. The results confirm previous reports showing no link or an inverse link between aspects of MetS and incident prostate cancer. Three established aspects of the MetS, namely T2DM, a low vitamin D level and a high CRP level, were inversely related to incident PC.

In paper IV the association between MetS, OC and PC was investigated. T2DM, as the end point of MetS, had previously been shown to be associated with low levels of OC and with a lower incidence of PC. Since PC metastasizes to the skeleton, it was reasonable to assume that there should be an association between OC and PC, and since OC was involved, vitamin D and other skeletally related variables included in MetS should be involved in this complex as well. It was shown that levels of plasma OC were lower among diabetics and that levels of plasma OC were positively associated with incident PC. Speculatively, T2DM, through low levels of OC, prevents the onset and progression of PC. However, no association between levels of serum vitamin D and OC was demonstrated.

5.6 Methodological Considerations and Limitations

The cohort was almost exclusively of Caucasian ethnicity, and, therefore the results may not be valid for other ethnic groups. Data regarding blood samples and some other variables from the entire cohort was lacking, since more detailed measurements were done only on the Gothenburg subpopulation of the MrOs cohort.

5.6.1 Paper I

The cross-sectional study design precludes the ability to make causal inferences from the observations. Urologic dysfunction, as measured by LUTS, could be expected to cause discomfort, with consequential elevations in chronic stress. Another shortcoming is that one might argue that the outcome of the study is due to a mass significance effect, since many parameters were tested in the study. However, the results were in accordance with those of previous studies.

5.6.2 Paper II

The present study has two important shortcomings. First, the study population was, in this context, somewhat small (155 individuals). Second, the age interval of the cohort in this study was quite narrow (70 - 75 years old) owing to the preset inclusion criteria for the MrOs cohort.

5.6.3 Paper III

Since different health organizations have different definitions of MetS, and since data regarding all the major components of MetS was lacking, we made a synthesis of the different health organizations definition of MetS and used surrogates for some items in order to use the criteria that were applicable to our data.

5.6.4 Paper IV

The effect of measurably high levels of OC prior to diagnosis of incident PC, lasted only for 5 years in this study, after which no significant increase was observed in the levels of OC in the subgroup of individuals who further developed PC compared with those without PC. Many biological markers are expected to have the same pattern. Furthermore, this study does not show any causality between OC and PC, but only demonstrates associations.

6 CONCLUSION

The overall conclusion in this thesis is that MetS seems to be associated with BPE, but not with PC and that OC is associated positively with incident PC. The more detailed conclusions in each paper are as follows.

6.1 Paper I

No association among major components of the MetS, LUTS and UI was shown. Furthermore, no association was found among levels of serum vitamin D, LUTS and UI. However, serum serotonin showed an independent negative correlation with LUTS and with UI, while fasting serum glucose and serum adiponectin showed a positive correlation with LUTS. The data on the subgroup of 155 individuals, confirmed the generally held view, that BPE is one of the causative factors for LUTS.

6.2 Paper II

In multivariate analyses, low levels of four components of MetS were independently associated with BPE: 25(OH) D, serum calcium, SHBG and HDL-cholesterol. Individuals with T2DM had increased prostate gland volumes compared with that of nondiabetic controls. The findings support the previously held view that BPE is a component of MetS.

6.3 Paper III

Individuals with T2DM, as the endpoint of MetS, tended to have a lower risk of developing PC and increased levels of serum vitamin D were significantly associated with an increased risk of developing PC. Individuals with low serum CRP levels and taller individuals had a higher risk of being diagnosed with PC over the course of the study.

The findings show that some components of MetS are inversely linked to the risk of being diagnosed with incident PC.

6.4 Paper IV

Levels of OC were higher in individuals with incident PC and lower in individuals with T2DM.

7 FUTURE PERSPECTIVES

Paper I offers a hypothesis on a neurotransmitter mechanism, by which depression and LUTS are linked. The fact that there is a positive association between depression and LUTS is supported by numerous reports. Future studies could focus on a link between serotonin homeostasis and LUTS. It could be speculated that pharmaceutical treatments that increase the serotonin level or lifestyle changes with the same purpose could be of value in the treatment of LUTS.

The results of paper II suggest that irrespective of the exact metabolic mechanism involved in the growth of the prostate gland, measures could be taken to reduce the metabolic perturbations for the purpose of slowing the progression of BPE. Improvement of metabolic perturbations of MetS could be achieved by physical activity and diet, but treatment with pharmaceuticals could also be appropriate. The most convincing evidence regarding the improving effect of diet on metabolic aberration is from studies showing that practically all aspects of MetS could be improved by following a low-carbohydrate, high-fat diet. Certainly, intervention studies in line with these proposals, including supplementation of vitamin D, are viable future perspectives.

Prostate cancer and BPH are clearly two distinct entities, originating from different parts of the prostate gland. Vitamin D seems to be a promising compound, with an established inverse association with BPE. However, its role in the development and progression of PC remains obscure and additional and more reliable studies must be done to elucidate this field.

Osteocalcin appears to be a novel and promising protein in the early diagnosis of PC, plus it seems to react to the phenomenon of micro-metastasaization of PC to the skeleton earlier than ALP. In the future, perhaps this protein could be analyzed more frequently together with PSA and ALP for determining the progression and grade of PC. If this is the case, drugs inhibiting the action of OC might come in handy in the therapy arsenal of PC.

ACKNOWLEDGEMENTS

The work behind this thesis was conducted at the Department of Urology at Sahlgrenska Academy, University of Gothenburg, Sweden.

To Jan-Erik Damber

M.D., professor of urology at Sahlgrenska Academy at the University of Gothenburg, and my main supervisor, whose patience and calmness pushed me through this task, which at times felt impossible to finalize. Thank you for your valuable comments and recommendations in directing this project, letting me grow into it over time.

To Ralph Peeker

M.D., professor of urology at Sahlgrenska Academy at the University of Gothenburg, and my friend, mentor, tutor and co-supervisor. I do not know how my life would have looked without you. You are truly an inspiration to me in every way possible, and I am so happy to have had the privilege of working with you. In my later years, whenever someone asks me about my experiences in life, I can always say, I know Ralph.

To Dan Mellström

M.D., professor of geriatrics at Sahlgrenska Academy at the University of Gothenburg, and my co-supervisor, whom I have known for only a couple of years, yet during this short period, I have come to admire and respect you for

your knowledge in the field, your patience, and your immense support. Thank you for sharing your time and your experience with me.

To Ali Khatami

M.D., PhD, former head of the department of urology at Sahlgrenska University Hospital in Gothenburg, and my friend. Thank you for giving me the opportunity to work at the Department of Urology, and for your support. I admire your determination and persistence at work, knowing that you always desire the best for our patients.

To Jan Hammarsten

M.D., associate professor. Thank you for everything you have done for me throughout this thesis and before it. I am privileged to have known you as my teacher, tutor and co-author. This job would have not been close to being completed without your immense support and engagement.

To Valter Sundh

Our statistician, whose knowledge in statistics has been invaluable for accomplishment of this task. Thank you for your patience, tolerance and for your hard work with the statistics of our studies.

To co-authors professor Claes Ohlsson, professor Ulf Lerner, professor Mattias Lorentzon, Catharina Lewerin (MD, PhD), and Helena Johansson (PhD)

Thank you for sharing your knowledge with me, for your support and for your wise comments. Special thanks to Helena for her statistical work on designing the spline curves in the studies.

To co-authors in MrOs study professors Magnus Carlsson and Östen Ljunggren

Thank you for your engagement and your wise comments

To my Colleagues at Sahlgrenska and other hospitals

Thank you for your support, encouragements and tolerance. I feel privileged to have been working with you all.

To my long departed father

Who was always a source of inspiration to me and a person I always looked up to. I know today he would have been proud of me, and it would have been a great honor for me to celebrate my dissertation with him present. He will always be in my heart.

To my mother,

Who never stops worrying about me and my brother, no matter how old we are. Thank you for always being there for me and for your tolerance, patience and unconditional love.

To my daughter, Matilda

The star of my life. I am so proud to have the privilege of having you as my daughter. I learn so much from you every day, my dear. Thank you for being there.

To my brother

Living far away from me. Although incidents in life have separated us from each other since we were young, I have always felt your presence close to me. You have always been there for me, as my older brother, in times of difficulty and I am grateful for that.

To my relatives and friends

Some of whom have traveled through cities and countries to celebrate my dissertation with me. I thank you all for being there for me. Each one of you has your own special place in my heart.

FUNDING

This thesis was funded by the Swedish Research Council, the Swedish Cancer Society, the ALF / LUA research grant in Gothenburg, the King Gustav V and Queen Victoria's Freemason Foundation and the Anna-Lisa and Bror Björnsson Foundation.

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