Defining the role of lipids and metabolic factors as risk factors for prostate cancer incidence and aggressiveness

Adel Tahseen Aref

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Abstract

Prostate Cancer (PCa) is the second most common cancer and among the top causes of cancer-related mortality among men. In recent years there has been a growing debate about the effect of metabolic factors (diabetes, hypertension, and hyperlipidaemia) and obesity on prostate cancer risk and aggressiveness.

This thesis aims to uncover the role of metabolic factors, sex hormones, and obesity as risk factors for prostate cancer incidence and aggressiveness, and how the metabolic factors and sex hormones are important confounders in prostate cancer risk assessment and screening models. It also aims to review how assessing different aspects of obesity including peri-prostatic fat influences prostate cancer incidence and aggressiveness. The thesis consists of five chapters:

- **Chapter 1** is a literature review and it includes;
    - **Section 1** an introduction to prostate cancer risk factors.
    - **Section 2** a summary of prostate cancer as a metabolic disease.
    - **Section 3** a summary of the debate around prostate cancer screening.
    - **Section 4** a summary of the debate around the role of sex hormones in prostate cancer pathogenesis.
    - **Section 5** (published review) a review on the role of periprostatic versus subcutaneous fat and its association with prostate cancer risk.
- **Chapter 2** (published research): describes the inverse relationship between obesity and PSA levels and the underlying mechanisms
- **Chapter 3** (research submitted for publication) describes how obesity attenuates prostate cancer screening and leads to an underestimation of prostate cancer risk.

- **Chapter 4** (research prepared for publication) describes how metabolic factors may attenuate prostate cancer screening efficacy.
- **Chapter 5** (research submitted for publication) describes the association between sex hormones and prostate cancer characteristics at the time of diagnosis.

The research work done through this project and as part of this thesis, have shown that:

- Obesity leads to lower PSA levels through two main mechanisms, first; the change in the sex hormone levels among men with obesity (mainly the increase in the estradiol-to-testosterone ratio), second; the increase in the plasma volume among men with obesity.
- The lowers levels of PSA among men with obesity (especially moderate and severe obesity) could lead to underestimation of prostate cancer risk and potentially delay prostate cancer diagnosis.
- Metabolically healthy men (those without diabetes, hypertension or obesity) appear to benefit from prostate cancer screening in terms of reducing their risk of prostate cancer-specific mortality, in comparison to those who have one or more of these conditions.
- Sex hormones (mainly the higher estradiol-to-testosterone ratio) are associated with a higher Gleason score at the time of diagnosis.

The results of this project give opportunities to introduce and identify new risk reduction modalities and interventions, as well as identify factors that may attenuate the efficacy of prostate cancer screening. The effect of these factors needs to be confirmed in randomised controlled trials. Longer term, applying the results of this

project in clinical practice may refine the implementation and/or interpretation prostate cancer screening, and improve the available risk reduction interventions.

Dedication

To my Mother; Ms Aida Saad

To my Sister; Dr. Amira Aref

To my Wife; Dr. Reham Fayed

To my Sons, Abdulrahman & Omar

To the loving memory of my Father; Mr Tahseen Aref
(1944 - 2008)

You are the reason of my life, of everything I have done or will do in the future

Thank you all for being in my life

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Quraan 7:43

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2. Nassar, ZD*, **Aref, AT***, Miladinovic, D, Mah, CY, Raj, GV, Hoy, AJ & Butler, LM 2018, 'Peri-Prostatic Adipose Tissue: The metabolic microenvironment of prostate cancer', *BJU International*, Feb 20. * Co-Authors

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1. **Aref A.** Obesity and PSA, South Australian Health and Medical Research Institute (SAHMRI), Research show case, Adelaide, SA, 2016
2. **Aref A.** Obesity and prostate cancer screening, Freemasons Foundation Centre for Men's Health Research Seminar, the University of Adelaide, Adelaide, SA, April 2017
3. **Aref, A.,** Vincent, A. D., O'Callaghan, M., Martin, S., Sutherland, P., Hoy, A., Lisa M. Butler & Wittert, G. The inverse relationship between prostate-specific antigen (PSA) and obesity, Australian and New Zealand Urogenital and Prostate (ANZUP), Melbourne, Australia, July 2017
4. **Aref A.** Obesity and PSA (Podium)- 3 min Thesis competition, The University of Adelaide, Adelaide, SA, July 2017
5. **Aref A.** Prostate cancer and obesity (podium) Cancer in South Australia Translational Research Meeting at SAHMRI, September 2017
6. **Aref A**. Obesity reduces serum PSA and leads to underestimation of prostate cancer risk assessment, Asia Pacific Prostate Cancer Conference, Brisbane, August 2018
7. **Aref** A. Session Chair, Cancer in South Australia Translational Research Meeting at SAHMRI, September 2018
8. **Aref** A, Poster session Judge, Florey Undergraduates Research Conference, October 2018
9. **Aref, A.,** Vincent, A. D., O'Callaghan, M., Martin, S., Sutherland, P., Hoy, A., Lisa M. Butler & Wittert, G. Obesity attenuates PSA based prostate cancer risk assessment, Cancer Metabolism Meeting, Sydney, May 2019

10. **Aref, A.,** Vincent, A. D., O'Callaghan, M., Martin, S., Sutherland, P., Hoy, A., Lisa M. Butler & Wittert, G. Abstract presentation - Having one or more of the metabolic syndrome factors attenuates the efficacy of prostate cancer screening, Poster Session - Cancer Metabolism Meeting, Sydney, May 2019

Abbreviations

5AR	5-alpha reductase
5ARIs	5-alpha reductase inhibitors
ADT	Androgen deprivation therapy
Akt-1	Alpha serine/threonine-protein kinase
AR	Androgen receptor
BMI	Body mass index
BPH	Benign prostatic hyperplasia
CI	Confidence interval
CRPC	Castration Resistant Prostate Cancer
DHT	Dihydrotestosterone
DM	Diabetes
DPB	Diastolic blood pressure
E/T	Estradiol to testosterone ratio
E1	Estrone
E2	Estradiol
EMLT	Estimated Mean Lead Time
EHPCCG	Endogenous Hormones and Prostate Cancer Collaborative Group
ER	Estrogen receptor
ERSPC	European Randomised Study of Screening for Prostate Cancer
FAMAS	Florey Adelaide Male Ageing Study
FASN	Fatty acid synthase
FPG	Fasting plasma glucose
GS	Gleason score
HDL	High-density lipoprotein cholesterol
HTN	Hypertension
IDF	International Diabetes Federation
M	Metastasis status
MIALES	The Men Androgen Inflammation Lifestyle Environment and Stress
MLT	Mean Lead Time
MS	Metabolic syndrome
N	Lymph node status
NCEP-ATPIII	Adult Treatment Panel III from the National Cholesterol Education Program
NICE	The National Institute for Health and Care Excellence
NWAHS	North West Adelaide Health Study
PCa	Prostate Cancer
PCPT	Prostate Cancer Prevention Trial
PCSM	Prostate cancer specific mortality
PI3K	Phosphatidylinositol 3–kinase
PLCO	Prostate, Lung, Colon, Ovarian-screening trial
PSA	Prostate Specific Antigen
ROC	Receiver Operating Characteristics
SBP	Systolic blood pressure
SCD1	Stearoyl CoA desaturase-1
SEER	The Surveillance, Epidemiology, and End Results
SHBG	Sex hormone binding globulin

SREBP	Sterol regulatory element-binding proteins
T	Testosterone
T stage	Tumour stage
TG	Triglycerides
USPSTF	United States Preventive Services Task Force
WC	Waist circumference
WHO	World Health Organization
WHR	Waist hip ratio

Chapter 1. Introduction and literature review

Introduction

Prostate Cancer (PCa) is the second most common cancer, and one of the top causes of cancer-related mortality, among Western men (Bashir 2015; Bray et al. 2018). Because of worldwide population growth and aging, there are likely to be 1.7 million new prostate cancer cases and an estimated 500,000 prostate cancer-related deaths by 2030 (Ferlay et al. 2015). The burden of prostate cancer in Australia is approximately equal to that of breast cancer among females, with an estimated 19,500 new cases to be diagnosed in 2019, representing 25% of all new cancer cases among men, and 3300 prostate cancer-specific deaths, representing 12% of all men's cancer related deaths (Cancer Australia 2019).

In recent years there has been a growing debate about the association between metabolic factors (Karzai, Madan & Dahut 2016) including obesity (Allott, Masko & Freedland 2013) and prostate cancer. While laboratory-based research and mice models have provided evidence for a role of lipid synthesis and obesity in prostate cancer aggressiveness (*Appendix 1: prostate cancer staging and definition of high risk and aggressive prostate cancer*) (Allott, Masko & Freedland 2013; Butler, Centenera & Swinnen 2016; Chen et al. 2018; Kobayashi et al. 2008), epidemiological studies have not to date been conclusive regarding the effect of metabolic factors (including diabetes, hypertension and hyperlipidaemia) and obesity on prostate cancer incidence and aggressiveness. Addressing this gap in knowledge will provide opportunities to introduce and identify novel risk reduction modalities and interventions to decrease the burden of prostate cancer.

This thesis aims to uncover (i) the role of metabolic factors, sex hormones and obesity as risk factors for prostate cancer incidence and aggressiveness; (ii) the confounding effect of metabolic factors and sex hormones on prostate cancer risk

assessment models and prostate cancer screening models; and (iii) to review how the assessment of obesity including peri-prostatic fat measurement influences prostate cancer incidence and aggressiveness. **Chapter 1** is a literature review, and it includes; **section 1**, an introduction to prostate cancer risk factors and what the underlying mechanisms that may be contributing to increased prostate cancer incidence and aggressiveness. **Section 2** examines prostate cancer as a metabolic disease and how metabolic factors and their association with sex hormones affect prostate cancer incidence and aggressiveness. **Section 3** covers the debate regarding prostate cancer screening and the possible factors attenuating the efficacy of screening to reduce prostate cancer-specific mortality. **Section 4** is a summary of the debate around the role of sex hormones in prostate cancer pathogenesis. **Section 5** (published review article) reviews the role of periprostatic fat and its potential association with prostate cancer risk. **Chapter 2** (published research) describes how obesity affects PSA levels and examines the underlying mechanisms; **chapter 3** (research submitted for publication) describes how obesity attenuates prostate cancer screening and prostate cancer risk assessment models and leads to an underestimation of prostate cancer risk; **chapter 4** (research prepared for publication) describes how metabolic factors may attenuate prostate cancer screening efficacy; and finally **chapter 5** (research submitted for publication) is about examining the association between sex hormones and the pathological features of prostate cancer at time of diagnosis.

1. Prostate cancer risk factors

The established risk factors for prostate cancer include age, ethnicity, and a family history of prostate cancer. Other risk factors, such as western lifestyle and obesity, are still under debate. The following section is a description of how some of these established risk factors are possibly mediated by underlying metabolic and hormonal pathways.

1.1 Age and prostate cancer

The cumulative risk of all types of cancer increases with age (mainly up to the age of 70 years) (White et al. 2014). Regarding prostate cancer, age is the most established risk factor. The peak rate of a prostate cancer diagnosis is in the age group 65 to 69 (Cancer Australia 2018). The probability of a prostate cancer diagnosis is 2.3% in men aged 50 to 59, 6.3% in men aged 60 to 69, and 11% in men aged 70 or more (Siegel, RL, Miller & Jemal 2015). Different mechanisms may explain why prostate cancer risk increases with age. Although debatable, the decline in testosterone levels with age was suggested to be associated with an increase in the risk of prostate cancer incidence and aggressiveness (Michaud, Billups & Partin 2015). Older men are more often obese (have more fat mass) and more likely to be diabetic, hypertensive, and have altered lipid profile. These conditions are related to reductions in testosterone levels and relative increases in the conversion of testosterone to estradiol. These alterations in metabolic profile may be related to the observed increased prostate cancer incidence in older men (Karzai, Madan & Dahut 2016). Alternatively, an increase in somatic mutations in the mitochondrial DNA, a process that is believed to increase oxidative stress in the cells and lead to further damage to the nuclear DNA is another suggested mechanism for increasing the risk of prostate

cancer with age (Khandrika et al. 2009). The fact that prostate cancer risk increases with age may reflect complex crosstalk between metabolic factors, obesity and the sex hormonal milieu that may promote prostate cell proliferation and malignant transformation (Khandrika et al. 2009), although this does not rule out common risk factors or causality between prostate cancer, aging, and an alteration in the hormonal and metabolic profile.

1.2 Racial variation in risk of prostate cancer incidence and aggressiveness

There is an ethnic difference in regards to both the risk of prostate cancer incidence and risk of having aggressive prostate cancer (Kumar et al. 2018). The racial difference in prostate cancer may be in part due to the access to health care systems (Dess et al. 2019; Moses et al. 2017), the application of prostate-specific antigen (PSA) based screening (Ito et al. 2019), as well as the maturity of data registries; African and developing countries, have less mature and underdeveloped cancer registries meaning that the precise magnitude of prostate cancer problem is not well represented (Rebbeck et al. 2013).

African Americans have more than double the risk of developing prostate cancer in comparison to other races (Siegel, R et al. 2014). They are also diagnosed at a younger age with more aggressive tumour characteristics and more advanced stages (He, T & Mullins 2017). Prostate cancer-related mortality is also the highest among those of African descent (Age-standardized prostate cancer mortality rate in; South Africa 26.8/100K; Middle Africa 22.7/100K; Western Africa 18.7/100K) (Bray et al. 2018; Ferlay et al. 2015). African Americans also have a higher risk of disease recurrence (recurring of prostate cancer after first-line treatment) (Latini et al. 2006). Socio-economic factors may explain part of this racial difference. African-Americans

were found to have a lower socio-economic level (Latini et al. 2006), less frequent follow-up while on watchful waiting (Shavers et al. 2004), and less general education than non-Hispanic White (Albano et al. 2007). Furthermore, African American race is an independent risk factor for prostate cancer incidence after adjusting for age, PSA level, digital rectal examination (DRE), year of biopsy and the total number of core biopsies taken (Yanke et al. 2006). They also have higher rates of prostatic intraepithelial neoplasia in the prostatic biopsies (Tewari et al. 2005), findings that are unlikely to be explained by access to medical services. Alternatively, there are biological factors that may explain this racial difference. In comparison to white Caucasian men, African Americans have higher expressions of the androgen receptor (AR) (Nwaneri, McBeth & Hinds 2016), higher frequencies of the CYP3A4 allele (a gene belonging to the cytochrome p450 family involved in testosterone metabolism and prostate cancer aggressiveness) (Bhardwaj et al. 2017), and higher expression of epidermal growth factor receptor (EGFR), which is involved in androgen-independent prostate cancer growth (Kumar et al. 2018). Another biological mechanism that may explain racial differences in prostate cancer risk is the differences in estradiol (E2) and estrogen receptor beta (ERß) activity. African American men have higher levels of E2 and increased activity of ERß in comparison to white Caucasian men, both of which are associated with increased prostate cancer risk (Abd Elmageed et al. 2013).

On the other side of the spectrum the incidence of prostate cancer appears to be lower among Asians (Age-standardized incidence rate in; South-Central Asia 5.0/100K; South-Eastern Asia 12.7/100K; Eastern-Asia 13.9/100K and Western-Asia 26.9/100K) (Bray et al. 2018). Genetic and socio-economic factors, as well as the availability of prostate cancer screening, are some of the suggested reasons for this racial discrepancy (Kimura & Egawa 2018). However, during the last decade, there

has been an increase in prostate cancer incidence among Asians possibly due to the adoption of prostate cancer screening strategies in addition to a change in lifestyle (Ito et al. 2019). The effect of lifestyle factors on prostate cancer risk among Asians will be discussed in more details in the next section.

1.3 Effect of lifestyle on prostate cancer worldwide variation

There is a wide variation in prostate cancer incidence worldwide. Many factors influence this variation, including the existence of reliable registry data (Figure 1), prostate cancer screening programs, lifestyle factors as well as genetic factors (as mentioned previously).

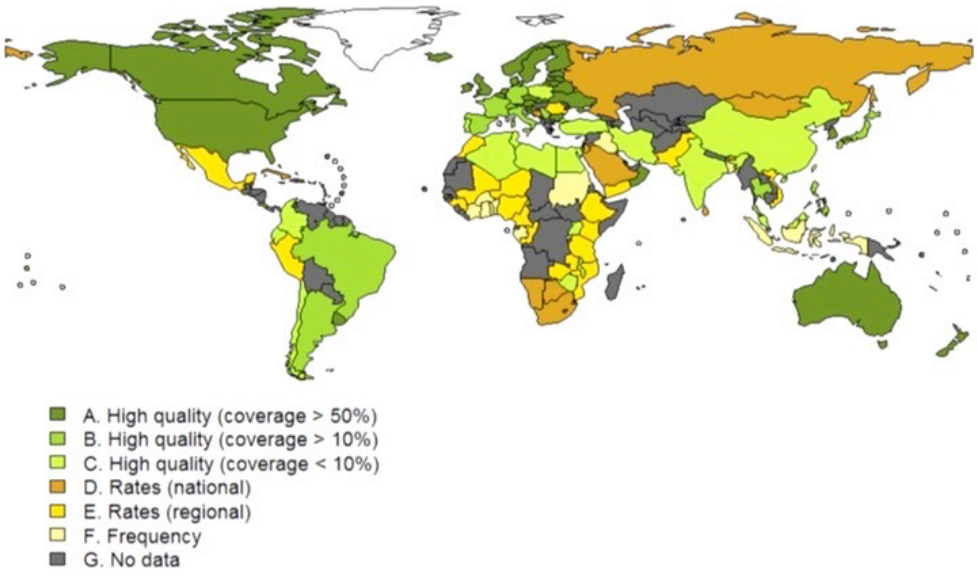

Figure 1: Quality of Cancer incidence data registry worldwide
The figure is used with permission from Cancer Incidence and mortality worldwide, GLOBOCAN 2012 (Ferlay et al. 2015)

As per GLOBOCAN 2018 cancer incidence (Figure 2), the highest incidence of prostate cancer is in Australia/New Zealand (Age-standardized rate 86.4/100K), Northern Europe (Age-standardized rate 85.7/100K), Western Europe (Age-standardized rate 75.5/100K) and Northern America (Age-standardized rate 73.7/100K), while the lowest incidence is among Asian populations (South-Eastern Asia (Age-standardized rate 12.7/100K) and South Central Asia (Age-standardized rate 5.0/100K)) (Bray et al. 2018).

The influence of western lifestyle and its association with metabolic disorders and obesity was suggested to affect prostate cancer incidence. This was observed among Asian immigrants to western countries as well as among Asian countries that adopted a western lifestyle diet (higher intake of saturated fat, red meat, and dairy products). The rate of prostate cancer among Asian immigrants to New South Wales, Australia was higher than that in their countries of origin (1.7/100K versus 16.3/100K for Chinese versus Chinese immigrants; 7.6/100K versus 30.0/100K for Hong Kong; 6.9/100K versus 28.3/100K for Indian; 11.0/100K versus 33.3/100K for Singapore), with a similar trend in change in rates of colorectal cancer, which support the hypothesis of western lifestyle and diet-related effect (Grulich, McCredie & Coates 1995). A similar trend of increasing prostate cancer incidence overtime was observed among Asian immigrants to the United States of America (Gomez et al. 2013). The incidence of prostate cancer among Asian Americans is almost equal to that among non-Asian Americans, after adjusting for access to health care systems (Raymundo et al. 2011). Part of this increase in prostate cancer incidence among Asian immigrants may be attributed to prostate cancer screening programs. However, over the past years, there has been an observed increase in the incidence of prostate cancer in Asian countries, even among those countries that did not widely adopt national prostate

cancer screening programs (Kitagawa & Namiki 2015; Namiki et al. 2010; Park et al. 2006). This has been accompanied with an increase in the prevalence of obesity in Asian countries (Prevalence of obesity increased in; West Pacific from 0.8 in 1980 to 4.9% in 2015; South-Eastern Asia from 1.7% in 1980 to 6.8% in 2015) (Chooi, Ding & Magkos 2019), which may reflect the effect of the adoption of western lifestyle accompanied by increasing rates of obesity.

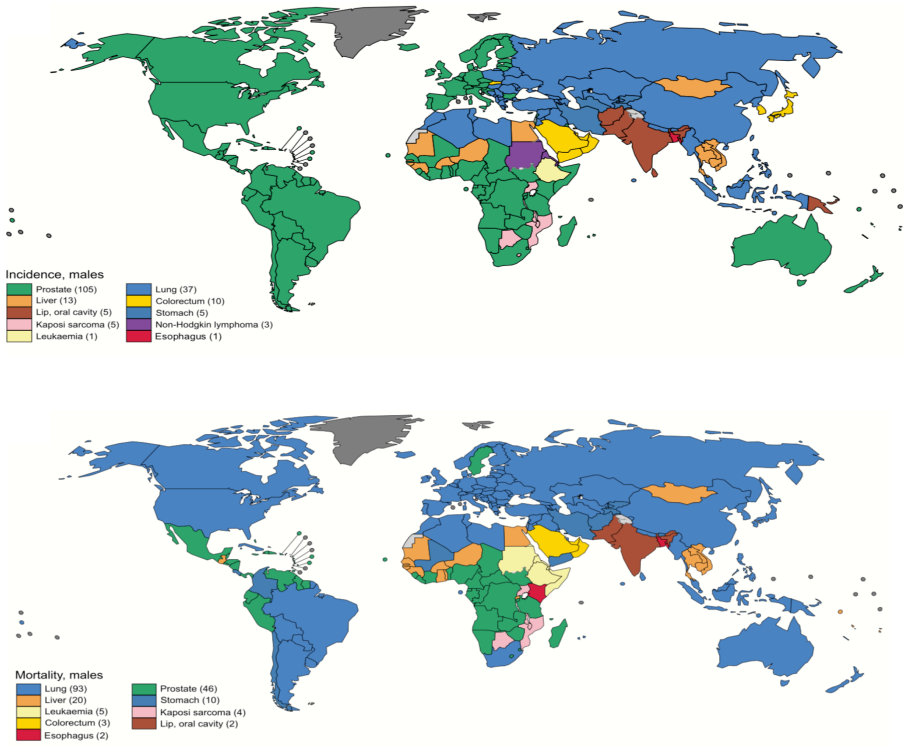

Figure 2: Prostate Cancer global (A) incidence and (B) mortality
The figure is used with permission from Cancer Incidence and mortality worldwide, GLOBOCAN 2018 (Bray et al. 2018)

1.4 Other risk factors

In addition to the previously mentioned risk factors, there are other risk factors that may contribute to increasing risk of prostate cancer. Some of these factors have a well-established evidence base e.g. family history and inherited germline mutations (BRCA1/2 and Lynch syndrome) (Na et al. 2017), while others are still under investigation, including diet, smoking, infections and sexual factors (Patel & Klein 2009).

Summary

Prostate cancer is a major health problem among Western countries, including Australia, with the expectation of an increase in its burden as population age. There is some evidence to suggest that the metabolic and hormonal factors may mediate, at least partially, the established prostate cancer risk factors such as age and race. By understanding how the underlying mechanisms affect prostate cancer pathogenesis, we may be able to identify and improve the risk reduction modalities, and design interventions to reduce the burden of this disease.

2. Prostate cancer as a metabolic disease

2.1 Introduction to metabolic syndrome and obesity

Metabolic syndrome is a cluster of metabolic conditions that include central obesity, insulin resistance, hypertension, and dyslipidaemia. Together metabolic syndrome represents a risk factor for the development of numerous metabolic and non-metabolic conditions, including type 2 diabetes mellitus, cardiovascular disease, and numerous cancers (O'Neill & O'Driscoll 2015).

During the last 20 years, there have been competing definitions of metabolic syndrome (Blanc-Lapierre et al. 2015) (summarized in table 1). One main difference between these definitions is whether obesity should be included to define metabolic syndrome.

Table 1: Definitions of metabolic syndrome

	Criteria for defining metabolic syndrome among men
WHO 1999	Type 2 diabetes (FPG >150 mg/dl)
	Plus any two of the following:
	• Hypertension (SBP≥130 mmHg or DBP ≥85 mmHg)
	• Dyslipidaemia (Low HDL <40 ml/dl and high TG >150mg/dl)
	• Obesity (BMI>30 or WHR >0.9)
NCEP-ATPIII 2005	***At least three of the following:***
	• Type 2 diabetes (FPG >150 mg/dl)
	• Hypertension (SBP≥130 mmHg or DBP ≥85 mmHg)
	• Dyslipidaemia (Low HDL <40 ml/dl and high TG >150mg/dl)
	• Obesity (WC>102 cm or BMI>30 or WHR >0.5)
IDF 2006	Obesity (WC >94 cm or BMI >30)
	Plus any two of the following:
	• Type 2 diabetes (FPG >150 mg/dl)
	• Hypertension (SBP≥130 mmHg or DBP ≥85 mmHg)
	• Dyslipidaemia (Low HDL <40 ml/dl and high TG >150mg/dl)

IDF: International Diabetes Federation; WHO: World Health Organization, WHR: Waist hip ratio; NCEP-ATPIII: Adult Treatment Panel III from the National Cholesterol Education Program; TG: Triglycerides; WC: Waist circumference; BMI: Body mass index; HDL: High-density lipoprotein cholesterol; SBP: Systolic blood pressure; DPB: Diastolic blood pressure; FPG: Fasting plasma glucose

There has been a parallel increase in the global incidence of prostate cancer and metabolic syndrome during the past two decades. The global incidence of prostate cancer has increased by 3.7 fold in the period from 1990 to 2015 (Pishgar et al. 2018). This change is primarily due to population growth, the population aging, and the increase in the incidence rates. There was a total of 40% global increase in prostate cancer incidence in the period from 2006 until 2016, of which 7% was due to the increase in the incidence rate (Fitzmaurice, Christina et al. 2018). However, this contribution differs according to the countries' sociodemographic index. Among the middle sociodemographic index countries, there is a 74% increase in the incidence of prostate cancer, of which 34% was due to the increase in incidence rate (Fitzmaurice, Christina et al. 2018).

On the other hand, the global prevalence of obesity has doubled in the period between 1980 and 2015, and the global prevalence of type II diabetes is expected to increase from 8.8% in 2015 to 10.8% by 2040. This translates to a 25% current global prevalence of metabolic syndrome among adults (Saklayen 2018). Over the past 25 years, there has been a global increase in obesity-related deaths and disabilities, with cardiovascular, diabetes, chronic kidney diseases, and cancers being the most common obesity-related comorbidity and cause of mortality (Afshin et al. 2017). Obesity is associated with an increased risk of a number of cancers (including esophagus, colon and rectum, liver, gallbladder and biliary tract, pancreas, breast, uterus, ovary, kidney, thyroid and leukemia), poorer survival, worse prognosis, poorer treatment tolerance and treatment outcomes (Parekh, Chandran & Bandera 2012). There are biological factors that relate obesity to carcinogenesis and cancer progression. Obesity is associated with chronic inflammation, lower levels of adipokines (adiponectin), insulin resistance (which includes hyperinsulinaemia and

hyperglycaemia), a change in the gut microflora, and immune system impairment (Font-Burgada, Sun & Karin 2016). The parallel trends of increasing incidence and prevalence of prostate cancer and metabolic syndrome may also suggest common risk factors or causality between these two conditions (Blanc-Lapierre et al. 2015).

2.2 Prostate cancer as a metabolic disease

Prostate cancer can be considered a metabolic disease due to two aspects of dependency. The first aspect relates to prostate cancer cell metabolism and its dependence on lipid synthesis (section 2.2.1), while the second relates to the effect of metabolic factors on prostate cancer diagnosis, aggressiveness and progression, and how these characteristics relate to changes in sex hormones (sections 2.2.2 and 2.2.3).

2.2.1 Prostate cancer and lipid synthesis

Prostate cancer cells do not use aerobic glycolysis (Warburg effect) as a source of energy; instead, they depend on *de novo* lipogenesis (Deep & Schlaepfer 2016; Wu, X et al. 2014). The role of lipids in prostate cancer includes, amongst others, energy production through fatty acid beta-oxidation and cell membrane formation. The synthesis of unsaturated lipids plays a crucial role in both cell survival and signaling (Butler, Centenera & Swinnen 2016). Lipogenesis in prostate cancer cells was found to be dependent on both increasing *de novo* fatty acid synthesis and the use of extracellular fatty acid availability (Zadra, Giorgia & Loda 2019).

Increased fatty acid synthesis and overexpression of the related enzymes is associated with prostate cancer incidence and progression. Stearoyl CoA desaturase-1 (SCD1) (a rate-limiting step enzyme in the formation of the mono-unsaturated fatty acids, a critical component of the membrane phospholipids) is overexpressed in prostate cancer cells, and its action is essential for the proliferation of prostate cancer

cells but not for normal prostate cells (Fritz et al. 2010). Fatty acid synthase (FASN) is a key enzyme in fatty acid synthesis from acetyl CoA. FASN is involved in the initiation of prostate cancer and the progression to the castrate-resistant stage (CRPC; progression after starting hormonal treatment) (Rossi et al. 2003). A recent study showed that a novel FASN inhibitor (IPI-9119) could antagonise castrate-resistant prostate cancer cell growth and enhance the efficacy of second-line hormonal treatment (Enzalutamide) (Zadra, Giorgia et al. 2019).

There is complex cross-talk between lipid synthesis and androgen signalling pathways in prostate cancer cells. The phosphatidylinositol 3–kinase (PI3K) pathway has an essential role in prostate cancer, being involved in regulatory pathways inside the cells, including metabolism, survival, and proliferation (Chalhoub & Baker 2009). FASN expression is associated with the activity of Akt in prostate cancer tissue (Akt is a serine/threonine protein kinase that is activated through the PI3K pathway) (Yang et al. 2002). Inhibition of FASN leads to a decrease in Akt expression, which subsequently leads to the down regulation of Akt (reviewed in (Zadra, G., Photopoulos & Loda 2013)). Inhibition of androgen receptors leads to a decrease in expression and activity of FASN through the inhibition of SREBP-1 activity(Swinnen et al. 1997; Swinnen et al. 2000). SREBPs are transcription factors that control lipid synthesis by controlling the expression of several essential enzymes that are required for fatty acid and cholesterol synthesis (Eberle et al. 2004). The SREBP family has 3 isoforms (SREBP-1a, SREBP-1c, SREBP-2). SREBP-1 is stimulated by androgens in prostate cancer cells, while simultaneously AR expression is regulated by SREBP-1 (Butler, Centenera & Swinnen 2016; Huang et al. 2012). At the cellular level, it, therefore, appears that prostate cancer cells depend on the bi-directional cross-talk

between the lipid/fatty acid synthesis and the androgen pathways for proliferation and progression.

2.2.2 Obesity and prostate cancer

Obesity, or excess body fat, is estimated to be the primary cause of cancer in about 13% to 20% of all obesity-related cancers (including breast in postmenopausal women, ovary, endometrium, colon, esophagus, gallbladder, pancreas, kidney and prostate) (Byers & Sedjo 2015). The different pathways through which excess body fat leads to an alteration in cell proliferation and carcinogenesis include, among others, chronic inflammation, an alteration in the sex hormonal milieu, and an increase in serum insulin and insulin-like growth factor 1 (IGF-1) (Byers & Sedjo 2015).

The association between obesity and prostate cancer is complex (Figure 2). This is primarily due to further interacting factors that are unique to prostate cancer: **first**, prostate cancer is a highly prevalent cancer especially among elderly, an age group at which there is a high prevalence of obesity and metabolic related conditions (metabolic syndrome). Men with obesity and metabolic complications have poorer overall survival and thus may die from other causes before they are diagnosed with prostate cancer, i.e. a competing risk effect (Grossmann, M. & Wittert 2012). **Second**, prostate cancer is an androgen-dependent tumour. Obesity is usually accompanied by lower serum testosterone levels (Gautier et al. 2013). The lower testosterone levels may be associated with lower prostate cancer incidence due to lower intra-prostatic testosterone and DHT (Thompson, IM, Jr. et al. 2013), albeit, it was suggested that lower testosterone levels might be associated with aggressive prostate cancer (Thompson, IM, Jr. et al. 2013). (This will be discussed in more

details in **section 4** below and **chapter 5**.) Lower serum testosterone results in a reduction in PSA levels that may lead to missed diagnoses and thus a decrease in cumulative age-specific incidence, but at the same time, may lead to a more advanced prostate cancer stage due to the delay in diagnosis (Chow et al. 2018). Besides, obesity is associated with alteration in other biological pathways that can lead to aggressive prostate cancer. This includes the deregulation of the insulin and insulin-like growth factor pathways; the cross-talk between insulin pathway and sex hormones; and the paracrine effect of peri-tumour adipose tissue that facilitates tumour vascularisation, growth and cell migration through chemokine secretion (Bandini, Gandaglia & Briganti 2017).

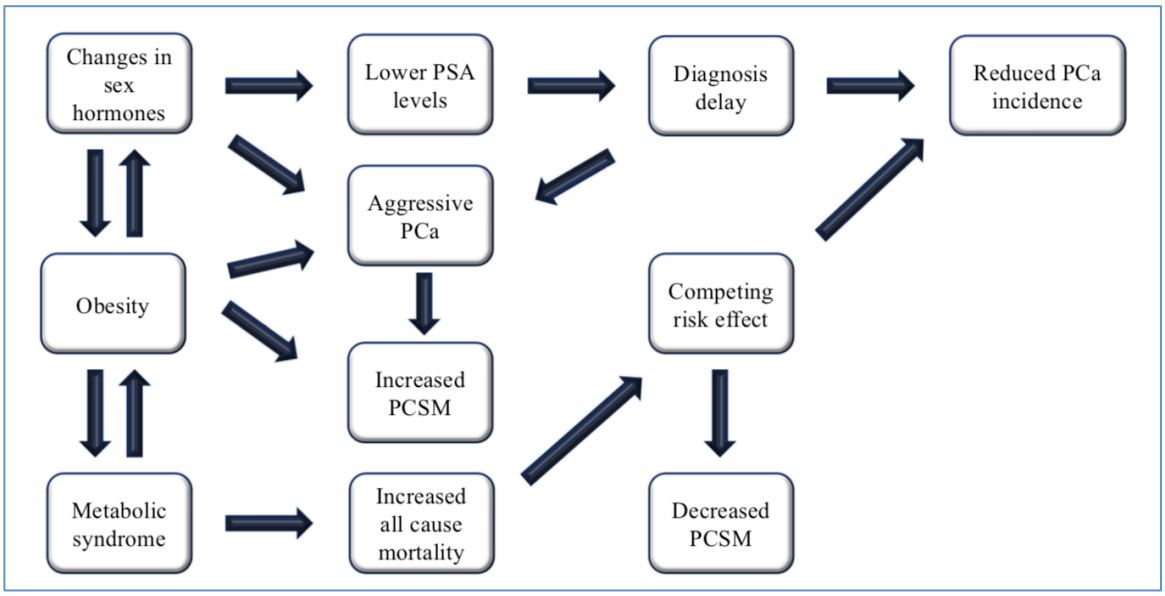

Figure 3: Causal diagram relating obesity and metabolic syndrome with prostate cancer development, aggressiveness, and mortality

PCa: Prostate cancer; PCSM: prostate cancer specific mortality; Sex hormones include estradiol, testosterone and sex hormone binding globulin; Aggressive PCa: Advanced stage and/or high Gleason score

2.2.2.1 Central obesity and prostate cancer

A large number of population-based and nested-case control studies have explored the associations between obesity and the risk of prostate cancer incidence and aggressiveness. This has led to ten published meta-analyses relating obesity to prostate cancer incidence, the incidence of localised, and incidence of aggressive prostate cancers (Table 2).

These meta-analyses found that the association between obesity and risk of prostate cancer (total) incidence is either weak (Bergstrom et al. 2001; MacInnis & English 2006; Renehan et al. 2008) or undetectable (Choi et al. 2018; Hu, M. B. et al. 2014). There was a weak inverse association between obesity and risk of localised or non-aggressive prostate cancer (Discacciati, Orsini & Wolk 2012; Fang et al. 2018; Xie et al. 2017). Of note, there was heterogeneity among the studies within each meta-analysis. The association between obesity and aggressive prostate cancer was more consistent and robust, with a positive association between obesity and aggressive prostate cancer (Table 2).

2.1.1.1.1 The associations between obesity and prostate cancer – differences between studies

The direction of the association between obesity and total prostate cancer incidence is unclear. Obesity may be associated with a delay in prostate cancer diagnosis due to lower PSA levels in men with higher BMI (Chow et al. 2018). This would lead to a reduced age-specific prostate cancer incidence, and reduced localised prostate cancer incidence. There is some evidence to support this hypothesis; first, the inverse association between obesity and prostate cancer incidence seems to be affected by the presence of more intense PSA screening practice (Allott & Hursting

2015). By the early 2000s, the annual prevalence of PSA testing among men aged over 50 was 57% in the USA, versus 6% in the UK and 11% in Australia (Baade, Youlden & Krnjacki 2009). Studies that are conducted in the United States, (where PSA screening is widespread, and thus prostate cancer diagnosis is driven by PSA results), did not detect an association between obesity and prostate cancer incidence, while the studies conducted in either Europe or Australia, (where there are lower rates of PSA screening), showed a positive association between prostate cancer incidence and obesity (Renehan et al. 2008). The effect of obesity on PSA and how this may affect PSA based screening models is presented in **section 3**. Second, the positive association between obesity and high risk or aggressive prostate cancer may dilute the association between obesity and total prostate cancer incidence if there is a true negative association with localised/early prostate cancer. This was apparent in meta-analyses that differentiate between the incidence of localised and advanced (aggressive) prostate cancer (Fang et al. 2018; Xie et al. 2017) (Table 2).

The time of obesity assessment may also lead to different conclusions in terms of the association between obesity and prostate cancer risk or mortality. In the meta-analyses by Zhong *et al.* and Cao *et al*., pre-diagnosis BMI was associated with an increased risk of prostate cancer specific mortality, but not at diagnosis or post-diagnosis BMI (Cao, Y & Ma 2011; Zhong et al. 2016). This may indicate a cumulative effect of obesity on prostate cancer aggressiveness. The overall change in BMI and the duration of being obese may lead to metabolic and hormonal changes that change prostate cell biology and hence increase prostate cancer cell proliferation and progression. However, some of the studies included in the Cao *et al.* and Zhong *et al.* meta-analyses are cohort studies, and thus it is unclear if there is a treatment effect on BMIs assessed post cancer diagnosis.

Finally, differences in how aggressive prostate cancer is defined (whether Gleason score alone is used or a combination of the stage, Gleason score, and PSA) also affects the conclusions of analysis, as will be shown in **Chapter 5**.

Table 2: Associations between prostate cancer and obesity - summary of meta-analyses

Author name & year	Predictor of interest	Number of studies	Outcome(s)	Number of cases	RR [95%CI]	Comments
(Bergstrom et al. 2001)	BMI	6	Prostate cancer incidence	4592	1.01 [1.00- 1.02]	2 trials contribute 80% of the number of patients One trial was using the BMI at the age of 21
(MacInnis & English 2006)	BMI	56	Prostate cancer incidence	68753	1.05 [1.01- 1.08] For incidence	
			Localised prostate cancer		0.96 [0.89- 1.03] For localised disease	
			Advanced prostate cancer		1.12 [1.01 – 1.23] For advanced disease	
(Renehan et al. 2008)	BMI	27	Prostate cancer incidence	70421	1.03 [1.00- 1.07]	14.4 years median follow up years
		12			1.00[0·96–1·03]	North American studies
		10			1.04[1·01–1·07]	European and Australian studies
		5			1·15 [0·95–1·39]	Asia–Pacific studies
(Cao, Y & Ma 2011)	BMI	6	Prostate cancer specific mortality	6817	1.15[1.06–1.25]	Case control studies
		6		932	1.20 [0.99–1.46]	Post diagnosis BMI assessment
(Discacciati, Orsini & Wolk 2012)	BMI	12	Localised prostate cancer	19130	0.94[0.91–0.97] For localised disease	Dose-response meta-analysis
		13	Advanced prostate cancer	7067	1.09 [1.02–1.16] For advanced disease	

Author name & year	Predictor of interest	Number of studies	Outcome(s)	Number of cases	RR [95%CI]	Comments
(Hu, M. B. et al. 2014)	BMI	11	Prostate cancer incidence	29464	1.15 [0.98–1.34] For prostate cancer incidence	
			Advanced prostate cancer		1.37 [1.19–1.57] For advanced disease	
(Zhong et al. 2016)	BMI	38	Prostate cancer specific mortality	2738000	1.15 [1.07–1.23] For Pre-diagnosis BMI	Pre-diagnosis or post-diagnosis BMI showed no effect on all-cause mortality in prostate cancer patients.
					1.10 [0.99–1.22] For post-diagnosis BMI	
(Xie et al. 2017)	BMI	17 Localised prostate cancer	Localised prostate cancer	51307	0.96 [0.92 – 1.00]	A dose response meta-analysis Sensitivity analysis indicated that results for non-aggressive PCa are not robust and steady
		21 Aggressive prostate cancer	Aggressive prostate cancer		1.07 [1.03 – 1.12]	
(Choi et al. 2018)	BMI	27	Prostate cancer incidence	70391	1.02 [1.00 - 1.05]	Re-analysis of dose-response meta-analysis by adding big data or missed individual studies
(Fang et al. 2018)	BMI	43	Prostate cancer incidence	144687	1.02 [1.00 – 1.04]	
		15	Incidence of localised prostate cancer	29493	0.97 [0.95 – 0.99]	
		16	Incidence of advanced prostate cancer	8410	1.06[1.00 – 1.12]	

2.2.2.2 Visceral obesity and prostate cancer

The role of visceral obesity has emerged as a confounder that may be affecting the association between obesity and prostate cancer. There are two main types of fat in the human body. White adipose tissue (the white fat) that is mainly located in subcutaneous and visceral fat sites, and is associated with obesity and metabolic comorbidities; and brown adipose tissue (the brown fat) that is responsible for thermogenesis and energy dissipation, and is negatively associated with obesity and the related metabolic complications (Gonzalez et al. 2017). Excess visceral fat is associated with cardiovascular complications and the development of diabetes irrespective of the subcutaneous fat deposition; however, subcutaneous fat is usually used to assess obesity (Ding et al. 2017; Hiuge-Shimizu et al. 2012). This raises the question, is excess peri-prostatic fat (an example of visceral fat) causally associated with prostate cancer incidence and aggressiveness? **Section 5** reviews the role of peri-prostatic fat in prostate cancer from a preclinical and clinical perspective.

2.2.3 Metabolic syndrome and prostate cancer

There is a debate regarding healthy obesity (obesity that is not complicated with other metabolic conditions) versus non-healthy obesity (obesity that is complicated with other metabolic conditions) (Jung, Lee & Song 2017). Using metabolic syndrome instead of obesity as the variable of interest has been used in several studies to elicit whether having one or more of metabolic comorbidities increase the risk of prostate cancer incidence or aggressiveness. Several meta-analyses have tried to uncover these associations (Table 3). In summary, the association between metabolic syndrome and risk of prostate cancer incidence appears to be very weak, however the positive association between metabolic syndrome and

aggressive (high risk) prostate cancer appears to be more consistent, albeit, still debatable due to the heterogeneity of the studies, the way metabolic syndrome was defined and individual effect of each of the metabolic syndrome factors on prostate cancer (will be discussed in details in the subsequent section).

2.2.3.1 Reasons for inconsistency among studies

There are different reasons for this inconsistency; some may be related to study designs, while others are related to the effect of each component of metabolic syndrome on prostate cancer risk.

2.2.3.1.1 Heterogeneity of the studies

There was moderate to high heterogeneity between the studies included in these meta-analyses (based on the difference in studies' design, risk of bias and the clinical characteristics) (I^2= 60% to 78% (Esposito et al. 2013), I^2= 74% to 85% (Gacci et al. 2017)).

2.2.3.1.2 Definition of metabolic syndrome

The factors used to define metabolic syndrome may have an impact on the conclusions. The IDF definition of metabolic syndrome included obesity as an essential component in the definition, while the WHO, and the NCEP ATP III definitions do not include obesity as an essential component to define metabolic syndrome (Table 1). In the Gacci *et al*. meta-analysis there was a slightly detectable association between metabolic syndrome and prostate cancer incidence (RR= 1.17, 95%CI = [1.0- 1.36]), but when restricted to studies using only the NCEP ATP III definition, no association was detected (OR= 1.09, 95%CI = [0.93, 1.27]) (Gacci et al. 2017). However, the definition of metabolic syndrome did not have an impact on the

association between metabolic syndrome and prostate cancer in the meta-analysis by Esposito *et al*. (Esposito et al. 2013).

2.2.3.1.3 Epidemiological and demographical factors

Epidemiological, social, and factors related to prostate cancer screening may have also influenced the results of these meta-analyses. In the meta-analysis by Esposito *et al*., there was an association between metabolic syndrome and prostate cancer incidence among the studies conducted in Europe, but not amongst studies conducted in Asia or the United States (Esposito et al. 2013). This may be due to epidemiologically related confounders, including differences in metabolic syndrome prevalence between Europe, Asia, and the United States, differences in prostate cancer screening and detection rates, and differences in population ethnic composition.

2.2.3.1.4 Medications

One crucial factor often not assessed is the medication used for the treatment of diabetes, hyperlipidaemia, and hypertension and whether those conditions were controlled by the medication or not. There is growing evidence of risk reduction and an improvement in prostate cancer outcomes among men using metformin (the first-line treatment for diabetes) (He, K et al. 2019; Pircher et al. 2018; Saini & Yang 2018) and statins (medications used in the treatment of hyperlipidaemia) (Meng et al. 2016; Papadopoulos et al. 2011; Raval et al. 2016; Zhang, Y & Zang 2013). The usage of antihypertensive medication mainly the angiotensin converting enzyme inhibitors were also suggested to reduce the risk of prostate cancer. However, the results of the studies are still controversial (Azoulay et al. 2012; Cao, L et al. 2018;

Mc Menamin et al. 2012; Rotshild et al. 2019). Not accounting for medications may, therefore, confound study results.

2.2.3.1.5 Effect of metabolic syndrome components on prostate cancer

The distinct effect of the individual metabolic syndrome components on prostate cancer incidence and aggressiveness is another possible reason for the differences between study findings. As mentioned above, obesity may be associated with detection bias and thus have both an inverse association with prostate cancer incidence, and a positive association with prostate cancer aggressiveness.

The association between hyperlipidaemia and prostate cancer appears at most very weak. In two meta-analyses, the associations between prostate cancer incidence and each of serum triglycerides (RR =0.95, 95%CI = [0.97, 1.04]) (Ma, HQ et al. 2016) and serum cholesterol (RR =1.05, 95%CI = [0.97, 1.14]) (YuPeng et al. 2015) were not detected, however this may be confounded by the usage of lipid-lowering agents. The presence of diabetes is inversely associated with prostate cancer incidence, as shown in several meta-analyses (Bansal et al. 2013; Jian Gang et al. 2015; Zhang, F et al. 2012). Many confounders may influence this finding, first, the competing risk effect, in that diabetic men have worse overall survival (Lee, Giovannucci & Jeon 2016) and thus may die before being diagnosed with prostate cancer (Grossmann & Wittert 2012). Second, the duration of diabetes may influence the risk of prostate cancer. Prolonged diabetes is associated with a state of hypoinsulinaemia, and consequently lower levels of leptin, higher levels of insulin growth factor (IGF) binding protein and thus lower levels of circulating IGF-I (a growth regulator and prostate carcinogenic promoting factor) (Kasper & Giovannucci 2006). In a study on 51,529 men (4511 with prostate cancer), the risk of prostate cancer incidence was lower in those who have diabetes for a longer duration (HR=

0.75, 95%CI= [0.61, 0.93]) (Kasper, Liu & Giovannucci 2009). Third, the effect of medication, especially metformin, is suggested to be associated with decreased prostate cancer incidence (Deng et al. 2015; Wang et al. 2016). Finally, diabetes is also associated with lower PSA levels, which may lead to diagnosis bias and lower in prostate cancer detection rate among men with diabetes (Dankner et al. 2016; Sarma et al. 2015).

Acting in the opposite direction, the presence of hypertension was associated with an increased risk of prostate cancer incidence in three meta-analyses ((Gacci et al. 2017) RR= 1.10, 95%CI = [1.01, 1.19], (Esposito et al. 2013) RR= 1.15, 95%CI = [1.01, 1.30] and (Liang et al. 2016) RR= 1.08, 95%CI = [1.02, 1.15]). One of the suggested mechanisms is the activity of the sympathatic nervous system that has been linked to androgen-dependent prostate cancer cell growth (De Nunzio et al. 2012).

Thus the overall effect of the association between metabolic syndrome and prostate cancer incidence will be an amalgam of the opposing effects of diabetes and obesity from one side and hypertension from the other side. Added to this, the influence of missing information regarding the duration of metabolic syndrome components, the medical control of the metabolic syndrome components and the biological effect of the medications together may confound the association between metabolic syndrome and prostate cancer incidence.

2.2.3.1.6 Effect of metabolic factors on sex hormones

Another confounder for metabolic syndrome and prostate cancer studies is serum sex hormone status. Prostate cancer is an androgen-dependent tumor, and the sex hormone milieu and the balance between serum estradiol and serum testosterone play a role both in the detection of prostate cancer (through effects on serum PSA

levels) and on prostate cancer biology and progression (presented in detail in **section 4**).

Sex hormones are affected by metabolic syndrome, mainly obesity, and to a lesser extent by the presence of diabetes and hyperlipideamia. Obesity causes an increase in the aromatase dependent conversion of testosterone to estradiol (in the adipose tissue), thus increasing serum estradiol and leading to a negative feedback effect on the hypothalamic-pituitary hormonal axis, and thereby a decrease in serum testosterone and an increase in the serum estradiol to testosterone ratio (Gautier et al. 2013). Besides, with obesity, there are higher levels of leptin, which in large concentrations lead to a decrease in the responsiveness of the testicular Leydig cells to gonadotropin hormone stimulation (Saboor Aftab, Kumar & Barber 2013). Weight loss is associated with an increase in serum testosterone in men and the restoration of a balanced sex hormone milieu (Corona et al. 2013; Escobar-Morreale et al. 2017). On the other hand, lower levels of testosterone lead to a decrease in total lean body mass and an increase in the total fat mass (Kelly, DM & Jones 2015). Testosterone therapy is associated with a decrease in the fat mass, primarily subcutaneous fat (Corona et al. 2016); however, there is still a debate regarding the effect of restoring testosterone levels on visceral fat (Grossmann, Mathis et al. 2015).

Reduced levels of testosterone is a risk factor for metabolic syndrome development (Li, C et al. 2010), which has been observed among prostate cancer patients after initiation of androgen deprivation therapy (Bosco et al. 2015). Interestingly lower testosterone levels appear to be a risk factor for diabetes independent of obesity, suggesting a direct effect on glucose metabolism and insulin resistance (Selvin et al. 2007). However, this association may still be mediated, at least partially, through the effect of testosterone on body fat composition (Gates et al.

2013; Grossmann, Mathis et al. 2015). Reduced testosterone levels were also associated with an increased risk of hypertension, and elevated levels of serum cholesterol and triglycerides (Cheung et al. 2015). This complicated relationship between sex hormones, obesity, and metabolic syndrome makes identifying their associations with prostate cancer challenging. In the next sections, the associations between sex hormones and risk of prostate cancer incidence and aggressiveness will be discussed in more detail.

Table 3: Meta-analyses of the association between metabolic syndrome and prostate cancer

Author name	Predictor of interest	Number of studies	Outcome	RR [95% CI]	Comments
(Esposito et al. 2013)	Metabolic syndrome ≥3 versus <3 components	14 (total studies)	Prostate cancer incidence	1.12 [0.93- 1.60]	- 27 years follow up - Some studies use metabolic syndrome ≥ 3 versus 0 components - Effect of obesity defined using waist circumference was significant in all trials - Dyslipidaemia is not associated with risk of prostate cancer
		8 European studies		1.20 [1.02- 1.66]	
		4 US studies		1.03 [Not reported]	P=0.39
		2 Asian studies		0.99 [Not reported]	P=0.93
(Xiang et al. 2013)	Metabolic syndrome (Different definitions were used)	9 Prostate cancer incidence	Prostate cancer incidence	0.96 [0.85-1.09]	- Different definitions of MS were used. - Included only longitudinal cohorts in the meta-analysis - Follow up time range from 2 to 30 years
		7 High Gleason score		1.36 [0.90-2.06]	
		4 Advanced stage	Advanced prostate cancer	1.37 [1.12 - 1.68]	

Study	Variable of interest	Number of studies	Outcome	OR [95% CI]	Comments
(Gacci et al. 2017)	Metabolic syndrome (different definitions)	18 studies for PCa incidence	Prostate cancer incidence	1.17 [1.0- 1.36]	- In sub-analysiss using only NCEP ATP III definition of metabolic syndrome, no association was detected between metabolic syndrome and prostate cancer incidence. (OR= 1.09, 95%CI = [0.93, 1.27])
		6 studies for aggressive PCa	Aggressive prostate cancer	1.77 [1.34 – 2.34]	

Summary

Prostate cancer is a metabolic disease with cell proliferation and progression being highly dependent on lipid and fatty acid synthesis, an androgen-dependent process. Metabolic syndrome components (including obesity, diabetes, hypertension, and hyperlipidaemia) and obesity per se have been associated with prostate cancer aggressiveness. However, the association with prostate cancer incidence remains unclear, albeit modest. The effect of metabolic syndrome components on developing comorbidities and all-cause mortality may attenuate the influence of metabolic syndrome on prostate cancer incidence through a competing risk effect. Also, obesity and diabetes are associated with lower PSA levels and thus, a possible detection bias, which adds to the complexity of the association with prostate cancer incidence. The effect of sex hormones on metabolic syndrome and vice versa and the effect of medication and the control of metabolic syndrome components may be influencing the development and aggressiveness of prostate cancer (at least partially).

3. The effect of metabolic factors on prostate cancer screening models

3.1 Prostate specific antigen (PSA) and prostate cancer screening

Prostate specific antigen (PSA) is a protease that is produced by the secretory epithelial cells of the prostate gland. Any condition that leads to a disruption of the normal prostate epithelial architecture will increase the diffusion of PSA into the prostatic tissue and from there into circulation (Ahn & Ku 2006; Gray et al. 2004). PSA is organ-specific, but it is not disease-specific; as such, levels in the blood can be elevated due to other pathological conditions of the prostate, such as benign hyperplasia or prostatitis. Measurement of PSA in the blood was approved in the United States of America in 1986 to monitor prostate cancer and in the 1990s for prostate cancer screening (Heidegger et al. 2015).

In contrast to case-finding (which is selecting sub-group of population with some high risk features for further screening tests), prostate cancer screening (the term used through this thesis) is a population wide testing of asymptomatic men aiming for early diagnosis of prostate cancer (i.e. at earlier stages) (Ranson et al. 2018). Hypothetically, screening should lead to more effective treatment outcomes and a reduction in mortality. Initially, PSA screening led to a 21% reduction in the incidence of advanced stages and metastatic prostate cancer in the United States of America (Etzioni et al. 2008) and a 6% reduction in prostate cancer-specific mortality by 1997 (Barnholtz-Sloan et al. 2003), however this was offset by a 29% increase in the rate of detection of low grade and very early prostate cancer cases (over-diagnosis) (Cooperberg et al. 2004; Etzioni et al. 2002). The screening-related over-diagnosis is comparable to the prevalence rate of incidental prostate cancer during regular autopsy (36%) for men unknown to have had prostate cancer (in the pre-PSA screening era) (Etzioni et al. 2002). This has raised the question as to whether the

detection of very early prostate cancer is of any clinical value. However, there is a debate around the methods of calculating the rate of over-diagnosis, how to define over-diagnosis and the population used to estimate the effect of screening on advancing time to prostate cancer diagnosis (Draisma et al. 2009). There are different definitions of over-diagnosis with no current consensus on what is the best definition. Over-diagnosis can be defined as the number of men diagnosed with prostate cancer due to PSA testing who otherwise would not have been clinically diagnosed during their lifetime (Etzioni et al. 2002). It can also be defined as the number of men in whom diagnosis through PSA screening would not lead to extension of their lifespan (Etzioni et al. 2002; McGregor et al. 1998). An alternatively-used definition is detecting clinically insignificant tumours (smaller than 0.2 cm^3 or with Gleason score less than 7) (Draisma et al. 2009). Depending on how over-diagnosis is defined, the impact of screening on rates of over-diagnosis will potentially differ.

Longer follow-up showed that the effect of screening on reducing prostate cancer specific mortality is at best modest (IRR = 0.79, 95%CI [0.69, 0.91]) (Ilic et al. 2018). The trade-off between the higher rates of over-diagnosis and modest reductions in prostate cancer-specific mortality is the main issue underpinning the debate regarding the value of prostate cancer screening.

3.2 Prostate cancer screening studies

During the past 20 years, five large randomised clinical trials have attempted to examine the effectiveness of prostate cancer screening in reducing prostate cancer-specific mortality. However, there is variation between the results of these studies.

The Quebec Prospective Randomised Controlled Trial (The Canadian Trial) was a two-arm randomised controlled clinical trial in which 46,486 men from the

electoral roll of the Quebec City area, Canada were randomised (2:1) in the period between 1988 and 1999 to either be invited to screening or not to be invited to screening (usual care). The study aimed to explore the effect of prostate cancer screening on prostate cancer-specific mortality. In this study, a PSA level of 3ng/ml was used as an indication for transrectal ultrasonography (TRUS). A biopsy was indicated based on PSA and the TRUS findings. Only 23.6% of the men invited to screening underwent the initial PSA testing and DRE, while 7.3% of men in the control arm had PSA screening test during the study after randomisation (contamination rate). After 11 years of follow-up, there was a 64% reduction in prostate cancer-specific mortality in the screening arm in comparison to the control arm (RR=0.36, 95%CI =[0.19, 0.65]) (Labrie et al. 2004).

The European Randomised Study of Screening for Prostate Cancer (ERSPC) is a European multicentre randomised two-arm screening trial that explored the effect of prostate cancer screening in 162,387 participants on reducing mortality (Schroder et al. 2009). Men were recruited during the period between 1991 and 2003 (however, some centres started recruiting later (Netherlands, Finland, Italy, Spain, and Switzerland) or stopped recruitment earlier (Netherlands, Finland, Italy, and Spain)). The screening interval was designed to be every 4 years, with a PSA level of ≥3.0 ng/ml providing the indication for biopsy. The overall compliance with the study protocol was 85.6%, and on average men were screened 2.3 times during the study duration. The rate of contamination in the control arm (having a PSA test) was estimated to be in the range of 23 to 40%. After 13 years of follow-up, the ERSPC reported that screening increased the diagnosis of prostate cancer by 57% (RR=1.57, 95%CI=[1.51,1.62]) and decreased risk of prostate cancer-specific mortality by 21% (RR=0.79, 95%CI = [0.69, 0.91]). However, all-cause mortality was not affected by

screening (RR=1.00, 95%CI = [0.98, 1.02]) (Schroder et al. 2014). The 16 year follow-up mortality report was recently published and it showed a similar reduction in the prostate cancer specific mortality with screening (RR=0.80, 95%CI=[0.72, 0.89]) with an increase in the absolute difference in prostate cancer specific mortality from 0.14% at 13 years to 0.18% at 16 years (Hugosson et al. 2019). An important finding in this study is that the absolute risk reduction was increasing in magnitude over time, with one death avoided per 570 screened at 16 years of follow-up versus 781 screened at 13 years of follow-up, versus 979 screened at 11 years and 1410 at 9 years, suggesting a cumulative benefit of screening over time.

The Prostate, Lung, Colorectal, and Ovarian (PLCO) cancer screening trial is a multi-center randomised (1:1) controlled two-arm trial across the United States of America, which enrolled 76,685 men between 1993 and 2001. Men enrolled in the screening arm were offered annual PSA tests over six years, and annual digital rectal examinations for four years (Andriole, Gerald L et al. 2009). A PSA level of 4ng/ml was considered a positive result, however, there was no protocol for further investigation after a positive test result. The decision for any subsequent investigation was left to the patient and his health care provider. After 13 years of follow-up, the cumulative incidence of prostate cancer was 12% higher in the screening arm (RR=1.12, 95%CI=[1.07, 1.17]), while there was no effect on prostate cancer-specific mortality (RR=1.10, 95% CI= [0.87, 1.36]), a marginal reduction in all-cause mortality was observed in the screening arm (RR=0.96, 95% CI= [0.93, 1.00]) (Andriole, G. L. et al. 2012). A report after 15-years of follow-up showed no effect of screening on prostate cancer-specific mortality (RR=1.04, 95%CI= [0.87, 1.24]) (Pinsky, Paul F et al. 2016). The average compliance rate for PSA screening was 84.1% in the screening arm. However, it was noted that 46% of men in the control

arm had had at least one PSA test in the year preceding randomisation (contamination rate), and a total of 86% of the control arm had at least one PSA test during the study (post-randomisation) and around 50% having had annual screening. After 17 years of follow-up there was still no detectable reduction in prostate cancer-specific mortality with screening (RR=0.93, 95%CI=[0.81, 1.08]), however, there were a reduction in high Gleason score (≥8) tumours at diagnosis (RR=0.89, 95%CI=[0.88, 0.90],]) (Pinsky, P. F. et al. 2018).

The CAP Randomised Clinical Trial is a primary care-based cluster-randomised trial that assessed the effect of low-intensity PSA-based screening (single – one time PSA test) on prostate cancer-specific mortality (Martin et al. 2018). 415,357 participants from the United Kingdom were included in the study. A PSA level of 3ng/ml or higher was considered positive, and further biopsy was offered. The compliance with the study protocol was 40%, with a cumulative contamination rate of 10 to 15 % over ten years. After a median of 10 years follow-up there was no detectable effect on prostate cancer-specific mortality (RR= 0.96, 95%CI= [0.85, 1.08]), although there was a 19% increase in prostate cancer diagnosis in the screening arm (RR=1.19, 95%CI=[1.14, 1.25]), and prostate cancer tumours detected in the screening arm were less likely to be high grade (OR=0.68, 95%CI=[0.64, 0.73]) or of advanced stage (OR=0.68, 95%CI=[0.62, 0.75]).

The ***Swedish Prostate Cancer Randomised Screening Study*** (Lundgren et al. 2018) randomly selected 2400 men from a population of 27,464 men to the screening arm using a PSA test, DRE, and TRUS. In this study, compliance with screening was 74%. PSA level of 10ng/ml, notably higher than the other studies above, was used as an indication for biopsy. After 20 years of follow-up, there was no effect of a single screening intervention on prostate cancer-specific survival (RR=0.97, 95%CI=[0.71,

1.23]). However, there was an increase in prostate cancer diagnosis with screening (RR=1.12, 95%CI=[1.08, 1.38]). In this study, there was an overall survival benefit with screening (RR=0.92, 95%CI=[0.86, 0.98]).

There is disagreement between the results of the screening trials, with the Canadian and the ERSPC studies showing an effect of screening on reducing prostate cancer-specific mortality, while the other studies failed to show such an effect. This may be due to differences in study design including different PSA threshold levels used to trigger further investigations (the PLCO used PSA level of 4 ng/ml, the Swedish study used PSA level of 10 ng/ml, while the other studies used PSA level of 3 ng/ml); PSA screening intensity (the CAP study and the Swedish study were using low-intensity screening (single PSA test), while the Canadian, the ERSPC and the PLCO were using more intense screening schedules); the "post PSA test" diagnosis protocol (the PLCO study has no pre-specified post-diagnosis protocol); and differences between studies in terms of compliance (the adherence to study protocol; the Canadian and the CAP studies had low compliance, while the PLCO, the ERSPC, and the Swedish studies have higher compliance rates (70% to 85%)), or screening contamination in the control arms (contamination rates of 7.3%, 20-40% and >85% in the Canadian, ERSPC and the PLCO studies respectively).

Other factors may have contributed to the observed different results between studies. The positive result of the ERSPC study was suggested to be the result of a more effective treatment for prostate cancer cases diagnosed in the screening arm. In the ERSPC study, men who were diagnosed with prostate cancer in the screening arm were more likely to have radical prostatectomy as the primary treatment in comparison to the control arm (40.3% versus 30.3% for screening versus control respectively). However, this difference in treatment selection in the ERSPC study was

believed to have minimal if any effect on prostate cancer-specific mortality (Carlsson et al. 2019; Wolters et al. 2010). Another difference between studies is in mean lead times (MLT). The MLT reflects the time by which prostate cancer diagnosis was accelerated as a result of screening relative to the estimated time to diagnosis if not screened (Draisma et al. 2009). Using an estimated MLT (EMLT) (which reflects the intensity of screening and diagnosis, with higher values indicating higher attendance for screening, more frequent screening and less conservative criteria for biopsy), the EMLT for the PLCO control arm was estimated to be only 25% less than in the screening arm (EMLT = 4.0 versus 3.1 for screening versus control respectively). Besides, the EMLT of the PLCO control arm was more than twice that of the ERSPC control arm (EMLT =4.0 versus 1.6 for screening versus control, respectively) (Tsodikov et al. 2017). These longer EMLTs in the PLCO control arm are results of more frequent PSA tests, more frequent biopsies (in the control arm of the PLCO versus ERSPC), less conservative criteria for biopsy and lower risk of prostate cancer death (in the PLCO versus ERSPC). After adjusting for the difference in the EMLT (intensity of screening and diagnosis), there was 27 to 32% reduction in the expected risk of prostate cancer death in the PLCO study compared to 25 % to 31% reduction in the expected risk of prostate cancer death in the ERSPC study. A pooled analysis of the PLCO and ERSPC studies found that screening is effective in reducing prostate cancer-specific mortality with an average of 16% (95%CI [4 %, 27%]) reduction in risk (after accounting for the different baseline risk of prostate cancer specific mortality in the PLCO setting relative to the ERSPC setting) (Tsodikov et al. 2017).

The 16% reduction in prostate cancer death shown in the Tsodikov *et al*. pooled analysis is overlapping with the point estimate of the ERSPC study (RR= 0.80) and the lower confidence interval of the PLCO study (CI= [0.87, 1.36]), the CAP study

(CI= [0.85, 1.08]), and the close to lower confidence interval of the Swedish study (CI=[0.71, 1.23]). This may suggest that prostate cancer screening may be actually reducing prostate cancer specific mortality and that the problem is mainly due to the difference in the studies' design, duration of follow up, the intensity of screening and contamination rate of the control arm, which leads to variation in the upper limit of the confidence interval.

3.2.1 Systematic reviews and meta-analyses

Two systematic reviews were published in 2018 to address the effect of prostate cancer screening on prostate cancer-specific mortality.

Ilic *et al.* performed a systematic review and meta-analysis, including the five previously mentioned studies (Ilic et al. 2018). In this meta-analysis, screening had no effect on all-cause mortality (incidence rate ratio (IRR)=0.99, 95%CI=[0.98, 1.01], I^2=0%, moderate risk of bias), and no effect on prostate cancer-specific mortality (IRR=0.96, 95%CI=[0.85, 1.08], I^2=58%, serious risk of bias, inadequate concealment of allocation during randomisation resulting in potential for selection bias; inadequate or lack of blinding of participants and personnel, resulting in potential for performance bias; and some contamination) and inconsistency (ERSPC trial shows significant reduction while all other trials show no significant difference). There was a marginal decrease in the incidence of stage III/IV prostate cancer (IRR=0.87, 95%CI=[0.72, 0.99], I^2=87%, serious risk of bias, and inconsistency). One of the limitations of this meta-analysis is the low grade of evidence of its findings based on the heterogeneity in the studies methodology, as all the studies included were assessed as being potentially susceptible to performance bias (except the ERSPC study) and this has lowered the confidence of their effect size estimates (Ilic et al. 2018). This

meta-analysis also assessed the risk of complications from screening based on the PLCO and CAP studies, and it has shown that screening is associated with an increase in the risk of complications due to biopsy including sepsis (one man for every 1000 men screened), and increase risk of complication due to treatment including urinary incontinence (three men for every 1000 men screened), and erectile dysfunction (25 men for every 1000 men screened).

The United States Preventive Services Task Force (USPSTF) published another systematic review and meta-analysis of 36 studies (including randomized control studies, cohort studies and external validations of pre-biopsy risk calculators to identify aggressive prostate cancer) (Fenton et al. 2018). This systematic review concluded that PSA screening reduces prostate cancer-specific mortality but at the expense of increased false-positive results, complications, and over-diagnosis. Based on this systematic review, the USPSTF changed its recommendation from grade D (against prostate cancer screening) to grade C (advocating for an individualised approach to screening) (Grossman et al. 2018).

In summary, randomised clinical studies have shown that prostate cancer screening has led to an increase in prostate cancer diagnosis, mainly of low stage and low-grade tumours (over-diagnosis), but a reduction in the diagnosis of the advanced stage – high-grade tumours. There was no effect of prostate cancer screening on all-cause mortality, while the effect on prostate cancer specific mortality was modest. There are many differences between the studies in terms of study design, compliance, the intensity of screening, and contamination in control arms, thus pooled analyses that include these studies should be interpreted with caution.

3.2.2 The potential value of screening

As discussed above, screening has led to an increase in the detection of early prostate cancer. However, the long-term benefit of early detection of prostate cancer and the long-term effects on mortality remains unresolved. One difficulty is the indolent course of prostate cancer, which requires screening studies to have a long follow up time to detect changes in prostate cancer-specific mortality. In a Swedish population-based cohort study with 21 years of follow-up and 223 early-stage prostate cancer cases diagnosed during this period, prostate cancer-specific mortality was more than doubled in the period after 15 years of follow-up in comparison to the first 15 years post-diagnosis (mortality rate for first 15 years 15/1000 person-years, 95%CI = [10, 21]; and 44/1000 person-years, 95%CI = [22,88], beyond 15 years of diagnosis) (Johansson et al. 2004). This suggests that detecting an effect on prostate cancer mortality requires very long follow up time to have a sufficient number of events. Another difficulty is that prostate cancer is a disease of older men. Older men are more likely to be offered non-curative treatment when they have localised disease due to frailty, having multiple chronic comorbidities, and reduced life expectancy. Data from the population-based registry, USA (SEER) showed that older men (more than 67 years old) who were diagnosed with localised prostate cancer and offered non-curative treatment options had higher rates of prostate cancer-specific mortality (HR=3.34, 95%CI=[1.97, 5.67]; in a Fine and Gray competing risk regression model adjusted for age at diagnosis, year of diagnosis and PSA level) when compared to men who received curative treatment (Aizer et al. 2014). Thus the under-treatment of older men may increase the risk of mortality from prostate cancer and thus mask any reduction in the prostate cancer-specific mortality due to screening.

Early detection of prostate cancer has another advantage from an economic point of view. Detecting patients in low or intermediate risk stages leads to a reduction in the cumulative cost of prostate cancer treatment of more than 25% (treatment costs include primary treatment and subsequent disease and treatment complication costs) (Gordon et al. 2018).

In summary, prostate cancer screening can be of benefit in terms of detecting prostate cancer in early stages, which may have survival benefits over longer follow up periods in addition to the economic advantages of detecting prostate cancer in early stages.

3.3 Other factors that may attenuate prostate cancer screening

In addition to the previously mentioned factors that are related to screening trial design, other factors may attenuate the efficacy of PSA based prostate cancer screening models. A delay in prostate cancer diagnosis due to PSA based detection bias (for example lower PSA levels as a result of using 5ARIs medications) has been suggested to increase the risk of aggressive prostate cancer and prostate cancer-related mortality (Sarkar et al. 2019). In such a situation, factors that affect circulating PSA levels may subsequently affect the efficacy of current prostate cancer-screening models. Studies have shown that obesity leads to lower PSA levels in the blood (this will be discussed in the next section and **chapter 3**). Thus it is possible that neglecting the effect of obesity may be altering the effectiveness of prostate cancer screening. Other factors that may affect prostate cancer screening are metabolic comorbidities (including diabetes, hypertension, and hyperlipidaemia), which may mask the effect of prostate cancer screening through the competing risk effect (will be discussed in section 3.3.2 and **chapter 4**). Thus identifying factors that may be

altering or masking prostate cancer screening effectiveness and subsequently selecting sub-groups of men who will benefit most from prostate cancer screening is essential to improve the efficacy and design of screening protocols.

3.3.1 Obesity and PSA

The effect of obesity on PSA levels has been widely studied in the past two decades, but several areas of debate remain. Current debates are concerned with (i) the underlying mechanism(s) by which obesity affects PSA levels, (ii) whether this effect is seen only in prostate cancer-free men or also in men with prostate cancer, and (iii) whether or not obesity attenuates the sensitivity of the PSA test to detect prostate cancer.

3.3.1.1 The association between obesity and PSA and underlying mechanisms

Several studies have explored the effect of obesity on PSA levels (Tables 4A and 4B). The majority of studies (13 studies out of 19 in prostate cancer-free men) concluded a negative association between PSA and BMI. Tables 4A and 4B summarise the studies in cancer-free and men with prostate cancer respectively that have explored the relationship between PSA and BMI and presents their explanations for the underlying mechanism through which BMI may affect PSA.

Most studies examined the haemodilution effect (the increase in plasma volume in obese men) as the underlying cause for lower PSA with obesity. However, an increase in BMI leads not only to an increase in plasma volume, but also a decrease in the serum testosterone levels (Woodard et al. 2012). The haemodilution effect and the reduction in serum testosterone may explain the change in PSA with

BMI. In **chapter 2**, these mechanisms will be compared using data from the MAILES study.

3.3.1.2 Prostate cancer diagnosis and the association between BMI and PSA

The inverse association between BMI and PSA levels may be affected by the presence of sub-clinical or clinical prostate cancer. As mentioned previously, obesity is associated with lower PSA levels, which may mask the diagnosis of early (small size) prostate cancer. However, because of the delay in prostate cancer diagnosis, it is expected to have larger tumour volume over time, and thus higher PSA levels, which may mask the effect of obesity on PSA levels among men with advanced prostate cancer.

In a study of 15,827 men who were referred for PSA testing in Sweden between 2010 and 2013 (Bonn et al. 2016), the inverse association between PSA and BMI was only observed in men who were not diagnosed with prostate cancer during the subsequent five years of follow-up. In another retrospective study of 14,293 prostate cancer patients who underwent radical prostatectomy at the Mayo Clinic during the period 1987 to 2007 (Mitchell et al. 2011), there was no detectable association between preoperative PSA and BMI at time of surgery (ß coefficient = 0.003, p= 0.72), nor was there a detectable interaction (p= 0.98) between PSA and BMI for the prediction of tumour volume using PSA levels. On the other hand, a retrospective study using two prostate cancer cohorts (the SEARCH and the DUKE cohorts) including 3390 prostate cancer patients (Freedland et al. 2008) showed that higher BMI at time of diagnosis was associated with lower PSA levels. A combined retrospective analysis of three different prostate cancer cohorts (the SEARCH, the DUKE and the Johns Hopkins cohorts) including data of 13654 prostate cancer

patients found that PSA at time of surgery was negatively associated with increasing BMI (Banez et al. 2007).

3.3.1.3 The clinical significance of the effect of BMI on PSA

The clinical importance of a negative association between BMI and PSA is whether it affects the accuracy of PSA to detect prostate cancer. In a study of 3471 Asian men who were eligible for prostate biopsy because of high PSA levels, the PSA accuracy in predicting prostate cancer (using receiver operating characteristics (ROC)-derived area under the curve (AUC)) was 57% (95%CI =[54% to 61%]) in normal-weight men (BMI 23 to 25 kg/m2), 61% in overweight (95%CI = [58% to 65%]) and 54% (95%CI =[38% to 71%]) in obese men (Oh et al. 2013). However, this difference was not significant (p-value = 0.11 and 0.75 for the difference between normal weight and each of overweight and obese respectively). In a prospective cohort of 917 white European men, the accuracy of PSA as a predictor of prostate cancer did not differ between BMI groups (AUC 0.56 and 0.60 for normal weight and obese respectively) (Banez et al. 2014). An age-adjusted BMI-PSA model designed by Harrison *et al.* did not increase the accuracy to predict prostate cancer in comparison to the NICE guidelines PSA value threshold (sensitivity 0.80 and 0.79 for NICE guidelines thresholds and age-PSA-BMI adjusted model respectively) (Harrison et al. 2016).

There are several limitations in the studies that attempted to adjust the PSA levels according to BMI groups; this includes retrospective study designs, the lack of information on ethnicities, history of other factors that may affect PSA levels or prostate cancer risk which includes diabetes, medications like statins, metformin, and

5 alpha-reductase. Of course, the alternative hypothesis is that BMI adjusted PSA models do not improve the predictability of the PSA test.

Recently Chow *et al.* built a model that predicts the expected level of the tumour PSA secretion based on tumour stage, grade, and tumour volume (Chow et al. 2018). In this model, there was a significant difference between the expected PSA level and the measured PSA level (delta PSA) in men with severe obesity (2.5ng/ml versus 8ng/ml for delta PSA in normal and very obese men respectively, $p<0.01$). Using mediation analysis, BMI was the significant predictor of the difference between the measured and the expected PSA levels ($b= 0.25$, SD = 0.08, $p= 0.002$).

Chow *et al*. analysis suggest that obesity may lead to an underestimation of prostate cancer risk and possibly a delay in prostate cancer diagnosis (detection bias). PSA based detection delay (associated with the use of 5ARIs) was suggested to increase the risk of prostate cancer-related mortality (HR=1.39, 95%CI= [1.27, 1.52]) (Sarkar et al. 2019). There is a possibility that higher BMI may lead to underestimation of prostate cancer risk and thus a delay in diagnosis which may explain partially the positive association between obesity and advanced-stage prostate cancer (Fang et al. 2018; Xie et al. 2017) and prostate cancer related mortality (Cao, Y & Ma 2011; Zhong et al. 2016). However, despite this possibility, there are still no recommendations to consider BMI factors when interpreting the PSA results in clinical practice.

Table 4A: Studies exploring the effect of BMI on PSA in prostate cancer-free men

Studies including prostate cancer-free men								
Author and year	**Population (N)**	**Ethnicity background**	**Range/ Median Age**	**Mean BMI kg/m^2 or % obese**	**Mean PSA (ng/ml)**	**Effect of obesity on PSA**	**Explored mechanism**	**Comments**
(Gray et al. 2004)	1405	Mixed	40 - 69	Not mentioned	Not mentioned	Total PSA was 18% lower in men with BMI >34	Not done	
(Baillargeon et al. 2005)	2779	Mixed	20 - ≥70	34% obese	1.32	PSA decreases linearly with an increase in BMI groups	Not done	
(Barqawi et al. 2005)	4458	Mixed	60 years	19% obese	1.1	Men with BMI≥30 have lower PSA levels across all age groups	Not done	National screening cohort – No data about future PCa diagnosis
(Ochiai, Fritsche & Babaian 2005)	653	Mixed	62	27.2	5.5	No correlation detected between PSA and BMI The result of the multivariable regression model was not statistically significant, although the association was in the negative direction	Not done	Suggest effect of obesity on PSA to be through the effect of body size (BMI) on prostate volume.
(Teas et al. 2005)	77	African American	52.5	28.5 36.3% obese	1.1	No statistically significant interaction detected between PSA and BMI	The suggested effect is through the lower levels of E1 in men with high BMI on PSA levels	Participants were attending PCa screening clinic and have PSA level below the threshold, with no history of PCa – No data on future diagnosis
(Ahn & Ku 2006)	2032	Mixed	36	23.5	0.85	A statistically significant negative association between PSA and BMI	Not done	
(Fowke et al. 2006)	299	White Caucasian and African American	40 - 79	28.2 32% obese	0.73	PSA decreased with increasing BMI, with a statistically significant trend for men with age less than 60 years	Not done	
(Hutterer et al. 2007)	616	White French Canadian	58	26.2 13.3% obese	1.8	No statically significant association between PSA and BMI	Not done	The study cohort is men who underwent prostate cancer screening. No data about screening result

Author and year	Population (N)	Ethnicity background	Range/ Median Age	Mean BMI Kg/m^2 or % of obese	Mean PSA (ng/ml)	Effect of obesity on PSA	Explored mechanism	Comments
(Kim, YJ et al. 2007)	8640	Korean	52.8	25.5 41.1% obese	1.1	A statistically significant negative association between PSA and BMI mainly in age groups less than 60 years old	Not done	Older age group had a small sample size for those in obese groups.
(Chia et al. 2009)	2714	Mixed Asian	59	24.1 36.3 % obese	1.5	A statistically significant negative association between PSA and BMI mainly in the elderly age group	Not done	- No data about the future diagnosis of PCa - Asian BMI was used for categorization
(Grubb et al. 2009)	28,380	Mixed	62.3	27.6 23.5% obese	1.5	A statistically significant negative association between PSA and BMI	The negative association explained by the haemodilution effect	Data from the PLCO study. Included men who are PCa free during the first 6 years of screening.
(Ohwaki et al. 2010)	19,367		50	23.7 3% obese	0.7	Higher BMI was weakly correlated with lower PSA	PSA concentration increase with increasing haematocrit suggesting haemodilution effect with obesity	
(Lopez Fontana et al. 2011)	413	South American	59	28.8	1.4	BMI was negatively correlated with PSA	Haemodilution and serum testosterone effect were explored. Results suggest haemodilution effect based on similar testosterone concentration in all BMI groups.	
(Li, F et al. 2012)	1444	Chinese	40 – 65	17.7 % obese (BMI>27.5)	0.80	A statistically significant negative association between PSA and BMI	Haemodilution effect based on consistent PSA mass with BMI	PSA mass was calculated by two methods using haematocrit and BMI

Author and year	Population (N)	Ethnicity background	Range/ Median Age	Mean BMI Kg/m^2 or % of obese	Mean PSA (ng/ml)	Effect of obesity on PSA	Explored mechanism	Comments
(Kim, JH et al. 2014)	907	Korean	66	24.3 41% obese (≥BMI 25)	5.95	No correlation between PSA and BMI	Suggested haemodilution effect after adjusting for prostate volume	Negative PCa biopsy but serum PSA ≥3ng/ml
(Klaassen et al. 2016)	8122	Mixed	62	21.4% obese	5.6	A statistically significant negative association between PSA and BMI when adjusting for testosterone and DHT.	The effect of testosterone and DHT can explain 19% only of the change in PSA with BMI	Data from the REDUCE study
(Bonn et al. 2016)	15827	White	65	13.5% obese	4.3	A statistically significant negative association between PSA and BMI in men who did not develop PCa	Not done	735 men diagnosed with PCa during follow up

PCa: Prostate Cancer; BMI: Body Mass Index; PSA: Prostate Specific Antigen, DHT: Dihydrotestosterone

Table 4B: Studies exploring the effect of BMI on PSA in prostate cancer cases

Studies including prostate cancer cases								
Author and year	**Population (N)**	**Ethnicity background**	**Range/ Median Age**	**Mean BMI Kg/m^2 or % of obese**	**Mean PSA (ng/ml)**	**Effect of obesity on PSA**	**Explored mechanism**	**Comments**
(Banez et al. 2007)	13,654	Mixed						- This is a retrospective analysis from 3 different data-sets - Plasma volume was positively associated with increasing BMI - The plasma volume effect (PSA mass) was not evident in the John Hopkins cohort
	SEARCH (1373)		61.1	30% obese	6.9	PSA significantly decrease with increasing BMI.	The suggested effect is due to an increase in the Plasma volume in obese men (haemodilution)	
	Duke Prostate Centre (1974)		62.5	28% obese	6.2			
	Johns Hopkins (10,287)		57.8	16% obese	5.9			
(Freedland et al. 2008)	3390	Mixed	61.5	28.2	8.9	A statistically significant negative association between PSA and BMI	Not done	
(Mitchell et al. 2011)	14293	Mixed	62	27.1 26% obese	9.6	No statistically significant association between PSA and BMI at time of surgery	Not done	No interaction detected between PSA and BMI for the predictability of tumour volume

PCa: Prostate Cancer; BMI: Body Mass Index; PSA: Prostate Specific Antigen, DHT: Dihydrotestosterone

3.3.2 Metabolic factors and the competing-risk effect

Prostate cancer is primarily a disease of the elderly, an age group at higher risk of metabolic and cardiovascular-related comorbidities. The increase in the prevalence of comorbidities may represent another challenge for prostate cancer screening studies, as men may be dying from other causes before dying from prostate cancer, resulting in a competing risk effect (Matthes et al. 2018). This raises the question, who will best benefit from prostate cancer screening?

An analysis using the ten-year follow-up data from the PLCO study has shown an interaction between comorbidities and the effect of screening to reduce prostate cancer-specific mortality (p=0.006 for the interaction). Thus screening of men with minimal comorbidities reduced the risk of prostate cancer-specific mortality (HR=0.56, 95%CI=[0.33, 0.95]), in comparison men with at least one significant comorbidity did not benefit from prostate cancer screening (HR=1.43, 95%CI=[0.96, 2.1]) (Crawford et al. 2011). In addition to the competing risk effect, men with significant comorbidities are more often offered non-curative treatment modalities (Berglund et al. 2011; Marr et al. 2006), and the presence of comorbidities may lead to worse treatment outcomes and early progression (Alibhai et al. 2005). Interestingly in the 13-year follow up report of the PLCO study, no interaction was detected between screening and the presence of comorbidities (Andriole, G. L. et al. 2012). In the 13-year follow up report the authors used either "having no comorbidities" versus "having one or more comorbidities" (based on a modified Charlson score), which was different from that used by Crawford *et al*. The authors in the 13-year report have questioned the method used by Crawford *et al.,* and concluded that the interaction between screening and comorbidities sensitive to the definition of comorbidities (i.e.,

the conditions included). However, even when they used the same definition of Crawford *et al.* and combining the relative risk rate, no interaction was observed.

In light of these conflicting results of the 10 and 13 year reports, we will re-examine the interaction between metabolic syndrome components and screening on the PLCO data (using the recently reported 17 year follow-up data) and explore the effect of this interaction on the effectiveness of prostate cancer screening on prostate cancer-specific mortality **(chapter 4)**.

It is worth mentioning that a prevalent limitation of all of these analyses is that most of the studies that explored the competing risk effects on prostate cancer mortality are using historical data, and do not consider the improvement in medical management of cardiovascular diseases. Thus, those findings may not be directly transferrable to contemporary recent prostate cancer cohorts.

Summary

The benefits of prostate cancer screening are still an area of debate. Prostate cancer screening reduces the risk of advanced prostate cancer diagnosis and may affect reducing prostate cancer specific mortality. The effect on reducing prostate cancer specific mortality seems to require a longer duration of follow-up (around 20 years of follow up) to be observed. On the other hand, prostate cancer screening leads to increased over-diagnosis of prostate cancer (mainly early stage and localised) and increase the risk of biopsy related complications.

Prostate cancer screening studies have reported divergent outcomes in regards to the clinical benefit of screening. One reason for this disagreement may be due to the difference in the study designs, rate of contamination in the control arm and rates of compliance with the studies' protocols. Besides, other factors may affect the screening models. Serum PSA decreases with obesity, with some studies suggesting that this may lead to an underestimation of prostate cancer risk and a delay in prostate cancer diagnosis. Also, the presence of comorbidities may attenuate the efficacy of prostate cancer screening due to the competing risk effect. Unfortunately, these factors have not been considered nor explored in most of the prostate cancer screening studies and may explain the disagreement between different studies assessing prostate cancer screening.

4. Prostate cancer and the sex hormone milieu

4.1 The prostate gland and sex hormones

The prostate gland is an androgen-dependent organ. The availability of testosterone (T) is controlled by sex hormone binding globulin (SHBG), and the conversion of testosterone to dihydrotestosterone (DHT) through the 5-alpha-reductase (5AR) enzyme inside the prostate gland. At the time of puberty, the surge in androgen hormone levels leads to an increase in prostate gland volume through binding to the androgen receptor (AR) (Michaud, Billups & Partin 2015). Testicular Leydig cells are the primary source of testosterone production in men (90%), with the remaining 10% being produced by the adrenal glands. Once androgens bind to cytoplasmic AR, the hormone-receptor-complex shuttles into the cell nucleus and stimulate expression of numerous androgen-regulated genes that are responsible for prostate cell growth and survival (Tan et al. 2015; Tindall & Rittmaster 2008).

In addition to androgens, estrogens play a significant role in prostate gland growth, acting mainly through estrogen receptors (ER) α and ß (Usoro et al. 2015). There are three forms of estrogen in males: estrone (E1), estradiol (E2) and estriol (E3). Estradiol is the most potent estrogen in men and is mainly formed via the peripheral aromatisation of testosterone (80%), while the remainder (20%) is formed by Leydig cells (Vermeulen et al. 2002).

The balance between estradiol and testosterone levels plays an important role in prostate gland development and pathogenesis. An increase in estradiol-to-testosterone ratio (E/T) during intrauterine life is responsible for squamous metaplasia within developing prostate epithelium (Zondek et al. 1986). Increased E/T is one of the underlying mechanisms for BPH pathology observed in elderly and African-American men (Prins et al. 2007).

4.2 Prostate cancer and serum sex steroids

The sex hormone milieu may be an important link between the risk of prostate cancer and metabolic factors. In section 2.2.3.1.5, the effect of metabolic factors on sex hormones was summarized. In the current section, evidence linking sex hormones and prostate cancer risk will be presented.

4.2.1 Serum testosterone and the risk of prostate cancer

Since 1941 when Huggins and Hodges showed that prostate epithelial cell growth is androgen-dependent (Huggins, Scott & Hodges 1941), androgen deprivation has become the standard of care in treating men with advanced prostate cancer (Sartor & de Bono 2018). Since the Huggins and Hodges finding, it has been suggested that higher serum testosterone may be associated with an increased risk of developing prostate cancer (Rhoden & Morgentaler 2004), and thus speculated that testosterone replacement therapy for men with prostate cancer and those on androgen deprivation therapy (ADT) could increase the risk of prostate cancer recurrence (Yassin et al. 2019). Testosterone is converted inside the prostate to DHT through the action of the 5-alpha reductase (5AR) enzyme. The levels of intraprostatic DHT are associated with BPH, and thus the decrease in the levels of DHT through the usage of 5AR enzyme inhibitors (5ARIs) has been the treatment of choice for BPH (Parsons et al. 2012; Rittmaster 2008; Roehrborn et al. 2002; Wurzel et al. 2007). This section will summarise available data regarding the association between testosterone and risk of prostate cancer.

4.2.1.1 Endogenous testosterone and risk of prostate cancer incidence and aggressiveness

In the Endogenous Hormones and Prostate Cancer Collaborative Group (EHPCCG) individual-level meta-analysis that included data from 18 prospective studies (matched case-control studies and randomised trials with 3886 prostate cancer cases and 6438 controls), no association was detected between levels of serum total testosterone and the risk of prostate cancer incidence (testosterone RR=1.1, 95%CI=[0.96, 1.3]) nor with risk of high grade prostate cancer (testosterone RR= 0.98, 95%CI =[0.76, 1.25]). Also, no association was detected between serum levels of free testosterone (testosterone that is unbound to either SHBG or albumin in the blood, which may represent the physiologically active form of testosterone) and either risk of prostate cancer incidence or high-grade prostate cancer. There was also no evidence of a dose-response relationship, nor of heterogeneity between the studies (Roddam et al. 2008). A meta-analysis in 2016 that included an additional four studies also did not detect an association between serum total testosterone and risk of prostate cancer incidence (RR=0.99, 95%CI=[0.96, 1.02]), with no evidence of heterogeneity (I^2 = 0%) (Boyle et al. 2016). A more recent meta-analysis including 20 prospective studies (with 6933 prostate cancer cases and 12,088 controls in total) showed that, compared to men in the 2nd to 10th percentiles, men in the lowest 10th percentile for free testosterone have less risk of developing prostate cancer (OR=0.77, 95%CI=[0.69, 0.86]), and less risk of low grade prostate cancer (OR=0.76, 95%CI = [0.67, 0.88]), but no detectable association with high grade prostate cancer (OR=1.56, 95%CI=[0.95, 2.57]) (Watts et al. 2018). Similar to the EHPCCG meta-analysis, no association was detected between the risk of prostate cancer and total testosterone (OR=1.00, 95%CI=[0.90, 1.11]).

4.2.1.2 The effect of low testosterone and testosterone replacement therapy on the risk of prostate cancer

The use of 5ARIs has been associated with a decreased risk of prostate cancer incidence through the reduction of intra-prostatic testosterone and DHT levels. The Prostate Cancer Prevention Trial (PCPT) reported a 24% reduction (95%CI = [18.6%, 30.6%]) (Thompson, IM et al. 2003) and the REDUCE study showed a 23% reduction (95%CI = [9.9%, 35.3%]) (Andriole, G. L. et al. 2010) in risk of prostate cancer incidence with usage of 5ARIs. These results have been confirmed in two meta-analyses (RR=0.66, 95%CI=[0.52, 0.85]) (Monga et al. 2013) and (RR=0.74, 95%CI = [0.55, 1.00]) (Wilt et al. 2008).

In contrast to associations with reduced prostate cancer incidence with 5ARIs, several studies reported an increased risk of high-grade (Gleason score ≥7) prostate cancer among men using 5ARIs. This was shown in the PCPT study (RR=1.62, 95%CI=[1.37, 1.93]) (Thompson, IM et al. 2003), in the long term follow-up of the PCPT study (RR=1.17, 95%CI=[1.00, 1.37]) (Thompson, IM, Jr. et al. 2013), a French trial (increase risk of Gleason ≥8, OR=1.21, 95%CI=[1.00, 3.21]) (Scailteux et al. 2018) and in a recently published population-based cohort study in which men using 5ARIs were more likely to have Gleason score ≥8 (25.2% versus 17% for none 5ARIs users, p<0.001) (Sarkar et al. 2019). However, this has not been replicated among other studies (REDUCE study: RR=1.53, 95%CI=[0.86, 2.73] (Andriole, G. L. et al. 2010) and CombAT study: RR=0.59, 95%CI= [0.26, 1.31] (Roehrborn et al. 2011) or in pooled analysis of the two studies for risk of Gleason score ≥8 (RR=0.99, 95%CI=0.39, 2.53]) (Monga et al. 2013)). It is worth mentioning that the studies that did not detect associations between 5ARIs and high Gleason score tumours have very

wide confidence intervals, mainly in the positive direction. This could suggest a small positive association. Detection bias has been suggested for the increased risk of high-grade tumours in a low androgen environment with lower PSA levels due to 5ARIs delaying prostate cancer diagnosis (Sarkar et al. 2019), however it is also possible that a low androgen hormone milieu may promote aggressive prostate cancer due to differential growth responses or development of high grade tumours *de novo* (Watts et al. 2018).

Interestingly, men with clinical hypogonadism and unstable serum total testosterone over prolonged time duration are at higher risk of developing prostate cancer, in comparison to those with stable serum testosterone (Xu et al. 2018; Zhang, X et al. 2019). Besides, testosterone replacement therapy has not been found to increase the risk of prostate cancer. Meta-analyses of studies on testosterone therapy among men with hypogonadism did not show an increase in the risk of prostate cancer (**Cui *et al***: five randomized controlled studies for short term testosterone therapy: OR=0.74, 95%CI=[0.25, 2.19], I^2 = 0%, three randomized controlled studies for long term therapy: OR=0.99, 95%CI=[0.24, 4.02], I^2 = 0% (Cui et al. 2014); **Boyle *et al***: 11 placebo-controlled studies: OR=0.84, 95%CI=[0.31, 2.25], I^2 = 0% (Boyle et al. 2016); **Elliott *et al***: 13 randomised controlled trials: OR=0.97, 95%CI=[0.43, 2.23], I^2 = 0% (Elliott et al. 2017)). One limitation of those meta-analyses is the low number of events (prostate cancer cases). However, the same conclusion was reached in larger case-control cohorts. In a nested case-control study using the national prostate cancer register of Sweden 38,570 prostate cancer cases were matched to 192,838 prostate cancer-free men; no association was observed between testosterone replacement therapy and risk of prostate cancer development (OR=1.03, 95%CI=[0.90, 1.17]). Interestingly men who were on testosterone replacement therapy have a lower risk of

aggressive (≥T3, Gleason score ≥8, positive lymph node or positive metastasis) prostate cancer (OR=0.50, 95%CI =[0.37, 0.67]) (Loeb et al. 2017). Another study of 174,593 men, of which 58,617 received testosterone replacement therapy due of low serum testosterone, showed that testosterone therapy was not associated with increased risk of prostate cancer incidence (HR=0.90, 95%CI=[0.81, 1.01]) nor with risk of aggressive prostate cancer (HR=0.89, 95%CI=[0.70, 1.13]) (Walsh et al. 2018). In a more recent study of 12,779 men diagnosed with hypogonadism, of which 215 men developed prostate cancer during a median follow-up of 3.7 years, there was no association between testosterone replacement therapy and risk of prostate cancer incidence (HR=0.97, 95%CI=[0.71, 1.32]) (Santella et al. 2019). A recent meta-analysis on testosterone therapy in high-risk prostate cancer patients on androgen deprivation therapy showed that testosterone therapy did not increase the rate of progression (biochemical recurrence) in this group of patients (rate of biochemical recurrence = 0%, 95%CI=[0.00%, 0.05%]) (Teeling et al. 2018).

4.2.1.3 Summary of the association between testosterone and prostate cancer risk

In summary, although prostate cancer is an androgen-dependent tumour, population-based and case-cohort studies have failed to show an association between serum testosterone and risk of prostate cancer incidence. There is some evidence that lower intra-prostatic testosterone levels may be associated with a lower risk of prostate cancer incidence (detection bias cannot yet be ruled out) and at the same time with a higher risk of developing aggressive prostate cancer. To date, there is no evidence that testosterone replacement therapy increases the risk of prostate cancer development. It appears that the stability of serum testosterone over a prolonged duration may be more important than a single time point assessment.

Several factors should be considered when interpreting the results of the studies about the association between serum testosterone and risk of prostate cancer. These factors include the variation in the time between assessment of serum testosterone level and time of prostate cancer diagnosis; the use of different assay methods (radioimmunoassay, chemiluminescence, fluoroimmunoassay) to assess serum testosterone (Roddam et al. 2008), ignoring hormone diurnal variation and long term variation over time and ignoring the presence of comorbidities and chronic illness. Also, not all the studies have examined the difference between both total and free testosterone and the risk of prostate cancer incidence or aggressiveness, nor considered the effect of other hormones on testosterone (mainly SHBG and estradiol), which may influence the associations with prostate cancer. Other factors that should be considered when interpreting the results of those studies are: the variation between serum androgen levels and the intra-prostatic androgen levels, which have been shown to have a low correlation (Cook et al. 2017), and variation in prostate tissue sensitivity to the changes in serum testosterone, which is known as the "Saturation Model". This model suggests that prostate tissue is very sensitive to low testosterone concentrations, but once the testosterone level exceeds a saturation point (usually around 8nmol/l), there are no more androgen-mediated changes in the prostate as androgen-androgen receptor binding has reached a maximum saturation (Khera et al. 2014). Thus it may be intraprostatic androgen levels that are associated with risk of prostate cancer rather than the circulating androgen levels, but this hypothesis requires further confirmation.

The association between serum testosterone and risk of aggressive prostate cancer may be non-linear. A number of studies have shown that high-grade prostate cancer is associated with both the highest and the lowest testosterone percentiles even

after adjusting for other sex hormones (estradiol and SHBG) (Capogrosso et al. 2017; Izumi et al. 2017; Salonia et al. 2012). Thus a U-shaped association may relate serum testosterone to the risk of high-grade prostate cancer.

4.2.2 Estrogens and prostate cancer risk

Estrogen receptors (ER) have an important role in prostate cancer initiation and progression. ERα promotes cancer cell proliferation as well as being involved in the initiation of hyperplasia, inflammation, dysplasia, and squamous metaplasia of prostate cells (Nicholson & Ricke 2011). ERα is expressed in aggressive tumour epithelial cells (high Gleason score) and is involved in the initiation of carcinogenesis of prostate cells (Ricke et al. 2008). ERβ regulates epithelial growth of the prostate and is thought to be protective against neoplastic transformation (Weihua et al. 2001). However, it has been reported as overexpressed in bone and lymph node metastases (Lai et al. 2004). The decrease in levels of ERβ during prostate cancer progression is associated with a decrease in the levels of E-cadherin and thus associated with increases in the metastasis potentiality (Nelson et al. 2014). ERβ has five isoforms, each having a different role in prostate cancer. ERβ2 was found to be the dominant isoform for prostate cancer, followed by ERβ1 and ERβ5 (Leung et al. 2010). The ERβ2 and ERβ5 are associated with a shorter time to metastasis development while ERβ1 has a role in the inhibition of proliferation, decreasing hyperplasia and acting as tumour suppressor gene, its expression decreases with prostate cancer progression (Dey et al. 2012). However, the role of ERß in prostate cancer should be considered with caution primarily because of a lack of standardised guidelines for assessing the specificity and functionality of the ERß antibody (Andersson et al. 2017).

Although estrogen has been previously used as a treatment for prostate cancer (acting via negative feedback action on the hypothalamus-pituitary hormonal axis and thus leading to lower serum testosterone levels), it may also be involved in the pathogenesis of prostate cancer. One of the mechanisms of prostate progression to advanced stages is through androgen receptor mutation (Centenera et al. 2018). Preclinical studies have shown that estradiol can activate both wild-type androgen receptor and mutated androgen receptor (T877A) in a prostate cancer cell line (LNCaP) (Susa et al. 2015). Furthermore, stromal-derived prostate cancer cells can synthesise estradiol from testosterone and secrete cytokines under the regulation of estradiol (Machioka et al. 2015). Animal studies have shown that there is a requirement for both testosterone and estrogen in prostate cancer initiation and progression (long term treatment of mice with estradiol and testosterone leads to 100% rate of prostatic adenocarcinoma) (Bosland, Ford & Horton 1995; Ozten et al. 2010). It has also been suggested that higher estradiol levels can partially explain the observed racial differences in the risk of prostate cancer incidence and aggressiveness in Africans and African-Americans (Abd Elmageed et al. 2013).

4.2.2.1 Studies exploring the association between serum estrogens and risk of prostate cancer incidence and aggressiveness

Several clinical and epidemiological studies have explored the association between serum estrogens, mainly estradiol, and risk of prostate cancer incidence and aggressiveness. In the Endogenous Hormones and Prostate Cancer Collaborative Group (EHPCCG) meta-analysis there was no detected association between serum estradiol and risk of prostate cancer (RR=0.93, 95%CI=[0.77, 1.11]) nor risk of high-grade prostate cancer (RR=0.85, 95%CI =[0.63, 1.15]) (Roddam et al. 2008). Since

this meta-analysis, further studies have explored the relationship between serum estradiol and the risk of developing prostate cancer. In a prostate cancer cohort study on 539 prostate cancer patients who were diagnosed between 2001 and 2005 at Dana-Farber Cancer Institute, no association between highest estradiol quartile and Gleason score was observed (OR=1.01, 95%CI=[0.63, 1.63]) (Sher et al. 2009). Similarly another case-cohort study (within the Osteoporotic Fractures in Men cohort study of community-dwelling men) that included 1652 controls and 275 prostate cancer cases with 5 years follow-up showed no association between serum estradiol and risk of prostate cancer incidence (HR=0.95, 95%CI=[0.67, 1.34]) (Daniels et al. 2010). In a nested case-control study using data from the PCPT trial including 1798 cases and 1798 controls, no association was detected between highest estradiol quartile and the risk of prostate cancer incidence (OR=1.23, 95%CI=[0.95,1.56]) or high-grade prostate cancer (OR=1.11, 95%CI=[0.71, 1.73]) among men not using 5ARIs (the control group) (Yao et al. 2011). Another nested-case control study on a sub-cohort from the PLCO study (with 195 controls and 195 advanced-stage prostate cancer cases) showed no association between highest estradiol quartile and aggressive prostate cancer (OR=0.80, 95%CI=[0.43, 1.48]) (Black et al. 2014).

In summary, cohort and case-cohort studies have failed to detect an association between serum estradiol and risk of prostate cancer development or aggressiveness. Given the associations observed in lab studies, it is surprising that so little has been observed at the population level. If true associations do exist, then there may be some other possible reasons for their lack of detection. One reason may be the unknown status of estrogen receptor activity, which has a dual function on prostate cancer pathogenesis (as mentioned above). Besides, higher levels of estradiol may have a negative feedback effect on the hypothalamus-pituitary hormonal axis, which

may lead to lower levels of serum testosterone and affect the association between estradiol and prostate cancer risk. Other factors are similar to those mentioned above for testosterone, including the method of analysis used, the presence of other factors that may affect estradiol levels (medications, chronic illness) as well as the variation in the time between estradiol assessment and diagnosis of prostate cancer. Of course, there may be no true association between serum estradiol and risk of prostate cancer; however, this can only be concluded after addressing all the limitations in the available study designs.

4.2.3 E/T ratio and prostate cancer risk

The physiological functions of estradiol and testosterone generally act in opposite directions with regards to prostate function. Consequently, assessing the balance between these two sex hormones may provide a better picture of the effects of sex hormones on different physiological and pathological conditions than considering each hormone separately (van Koeverden et al. 2019).

Few studies have explored the association between estradiol-to-testosterone ratio) with prostate cancer incidence and aggressiveness (Table 5 and Figure 4). One study showed an inverse association between E/T and prostate cancer incidence (OR=0.45, 95%CI=[0.26, 0.81]) (Tsai et al. 2006). There was a positive association between E/T and aggressive prostate cancer in one study (OR=3.02, 95%CI= [1.29, 7.04]) (Platz et al. 2005), a negative association between E/T and aggressive prostate cancer in another study (OR=0.27, 95%CI=[0.12, 0.59]) (Black et al. 2014), and a non-linear association with aggressive prostate cancer in third study (significant association with 2^{nd} and 3^{rd} E/T quartiles, but not with the 4^{th} quartile, p=0.38 for

trend) (Schenk et al. 2016). Other studies were not able to detect any association with prostate cancer incidence or aggressiveness (Table 5 and Figure 4).

Certain factors may be confounding the results of these studies. One of those factors is that the evaluation of E/T was done at a single time point, which may not represent the lifetime change of the E/T. The only study that showed a negative association between E/T and prostate cancer incidence assessed the E/T at middle age (median 34 years old) with an average of 34 years follow-up (Tsai et al. 2006). In regards to the association between E/T and prostate cancer aggressiveness, there is heterogeneity in how aggressiveness was defined. Some studies used only Gleason score to define aggressive prostate cancer (Daniels et al. 2010; Schenk et al. 2016; Sher et al. 2009), while other studies used a combination of Gleason score and T stage (Black et al. 2014; Severi et al. 2006; Tsai et al. 2006). Combining T stage with Gleason score may not be optimal as the effect on T stage may be influenced by factors that delay diagnosis (for example lower PSA levels due to lower testosterone, obesity, and/or use of 5ARIs), while the effect on Gleason score may not be influenced by these factors or possibly affected in the opposite direction. Finally, one more factor that may influence the association of prostate cancer aggressiveness with sex hormones is that in most studies, there was no discrimination between Gleason scores 3+4 and 4+3 for defining prostate cancer aggressiveness.

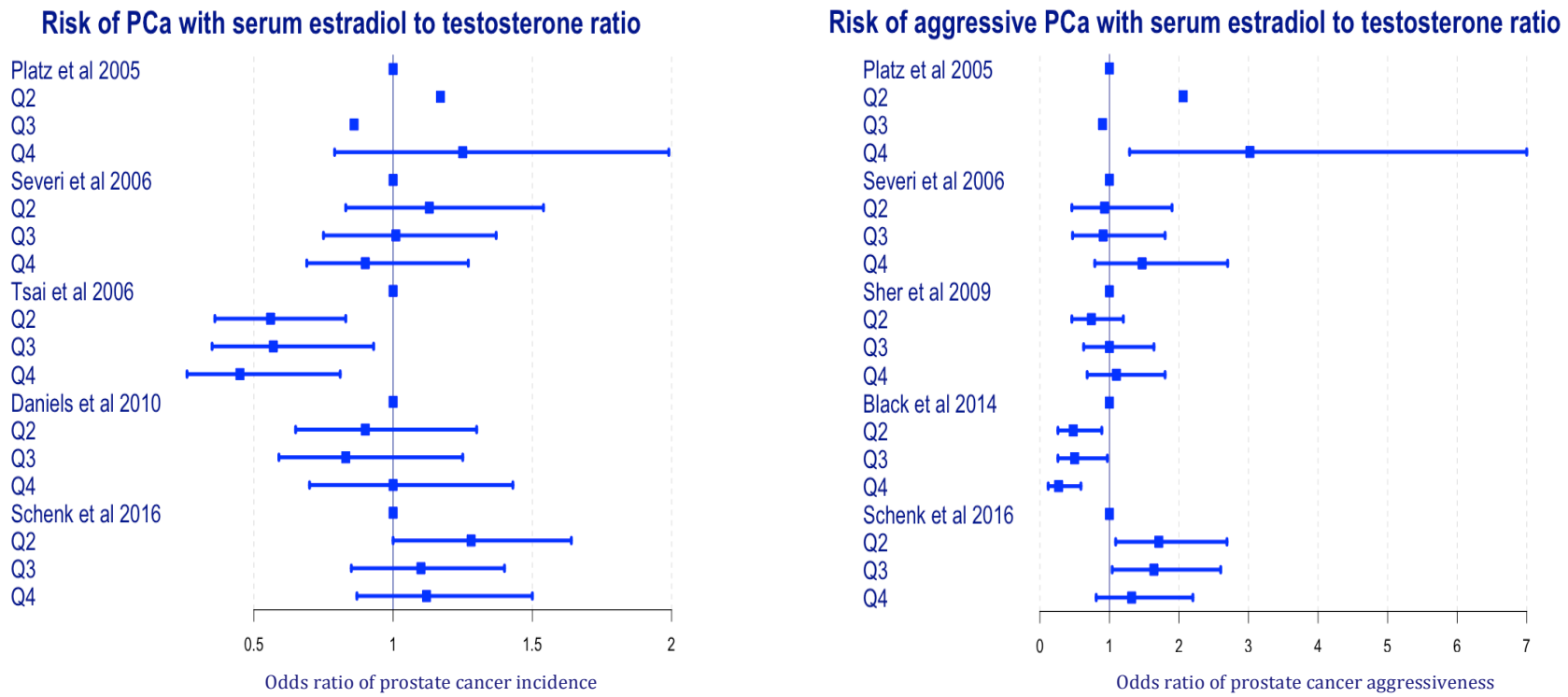

Figure 4: The association between estradiol to testosterone ratio and risk of prostate cancer incidence and aggressiveness.

The frequencies of estradiol to testosterone values divide into four equal groups.

Table 5: Studies exploring the association between estradiol-testosterone ratio and the risk of prostate cancer and prostate cancer aggressiveness

Author	PCa cases	Controls	Outcome	Assessment E/T ratio	Covariate adjustment	Race	Aggressive PCa (%)	Aggressive definition	Associations with E/T Reference = 1st Q	Comments
(Platz et al. 2005)	460	460	PCa incidence	At baseline	Age, time of day, year of blood draw, PSA, SHBG	Mixed	32%	GS≥7	**Incidence** 2nd Q: OR= 1.17 [NR, NR] 3rd Q: OR = 0.86 [NR, NR] 4th Q: OR = 1.25 [0.79, 1.99]	- Nested case-control in a follow-up study. - Used Conditional logistic model.
			Aggressive PCa						**Aggressiveness** 2nd Q: OR= 2.06 [NR, NR] 3rd Q: OR = 0.90 [NR, NR] 4th Q: OR = 3.02 [1.29, 7.04]	
(Severi et al. 2006)	524	1859	PCa incidence	At baseline	Age, country of birth (Adjusting for BMI, smoking and education did not change the results)	White	17%	T3 or T4, N +ve or M +ve or GS>7	**Incidence** 2nd Q: HR = 1.13, [0.83, 1.54] 3rd Q: HR= 1.01, [0.75, 1.37] 4th Q: HR= 0.90, [0.69, 1.27]	- Cox model. - Australian study. - Did not adjust for SHBG. - Follow up cohort study- nested case-cohort.
			Aggressive PCa						**Aggressiveness** 2nd Q: HR= 0.93, [0.46, 1.9] 3rd Q: HR= 0.91, [0.47, 1.8] 4th Q: HR= 1.47, [0.79, 2.7]	
(Tsai et al. 2006)	325	650	PCa incidence	Middle age	Age, and T for E, SHBG and TE and E for T and for E and T with SHBG	Mixed	29%	T3-4 & /or M +ve & /or poorly differentiated or undifferentiated grade	2nd Q: OR = 0.56, [0.36, 0.83] 3rd Q: OR = 0.57, [0.35, 0.93] 4th Q: OR = 0.45, [0.26, 0.81]	- Conditional logistic regression. - NB: ET ratio decrease risk of Pca. - Adjusted for T in E/T model. - Matched – case – control.

Author	PCa cases	Controls	Outcome	Assessment E/T ratio	Covariate adjustment	Race	Aggressive PCa (%)	Aggressive definition	Associations with E/T Reference = 1st Q	Comments
(Sher et al. 2009)	539	0	Aggressive PCa	At diagnosis	Age, SHBG, BMI, and PSA	Mixed	47 %	GS≥7	2nd Q: OR = 0.74, [0.46, 1.2] 3rd Q: OR = 1.0, [0.63, 1.64] 4th Q: OR = 1.10, [0.68, 1.8]	Cases only
(Daniels et al. 2010)	275	1652	PCa incidence	At baseline	Age, race, study site, BMI, person-time	Mixed	50%	GS≥7	2nd Q: HR = 0.90, [0.65, 1.3] 3rd Q: HR = 0.83, [0.59, 1.25] 4th Q: HR = 1.00, [0.70, 1.43]	Case-cohort
(Black et al. 2014)	195	195	Aggressive PCa	At baseline	Age, BMI, SHBG, FH, smoking	White non hispanic	100 %	Stage ≥III & / or GS≥7	2nd Q: OR = 0.48, [0.26, 0.89] 3rdd Q: OR = 0.50, [0.26- 0.97] 4th Q: OR = 0.27, [0.12, 0.59]	- Logistic regression model - Nested case-control PLCO
(Schenk et al. 2016)	1025	1037	PCa incidence	At baseline	Age, race, FH, BMI, SHBG, S. Cholesterol, Physical activity, History of DM	White and black and hispanic	21%	GS≥7	**Incidence** 2nd Q: OR = 1.28, [1.00, 1.64] 3rd Q: OR = 1.10, [0.85, 1.40] 4th Q:OR = 1.12, [0.87, 1.50]	- Used logistic regression models - Nested Case-control. - 7 years follow up
			Aggressive PCa						**Aggressiveness** 2nd Q: OR = 1.71, [1.09, 2.69], 3rd Q: OR = 1.64, [1.04, 2.6] 4th Q: OR = 1.32, [0.81, 2.2]	

NR: Not Reported; E: Estradiol; T: Testosterone; E/T: Estradiol to Testosterone ratio; Q: Quartile; PCa: Prostate cancer, FH: Family history, BMI: Body Mass Index; DM: Diabetes: SHBG: Sex hormone binding globulin; PSA: Prostate Specific Antigen; OR: Odd ratio; GS: Gleason Score; S.: Serum

Summary

The associations between sex hormones and prostate cancer incidence and aggressiveness are complex. Serum testosterone is not linearly associated with prostate cancer incidence, and the stability of serum testosterone over time may be more critical. Lower serum testosterone can be associated with aggressive prostate cancer, with studies suggesting a U-shaped association between serum testosterone and aggressive prostate cancer after adjusting for other sex hormones (estradiol and SHBG). Although preclinical studies suggest a role for estradiol in prostate cancer pathogenesis, the association between estradiol and prostate cancer incidence and aggressiveness in population-based and case-control studies remains unclear. The balance between estradiol and testosterone should theoretically provide a better picture of the sex hormone milieu and its influence on prostate cancer risk than each hormone separately. However, the results of such studies have revealed inconsistent results.

The association between serum sex hormones and prostate cancer incidence and aggressiveness may be influenced by numerous factors, including the timing of sex hormone assessment relative to prostate cancer diagnosis, the methods by which sex hormones are analysed (radioimmunoassay, chemiluminescence, fluoroimmunoassay), the factors that confound sex hormones levels (including obesity, metabolic comorbidities, chronic illness, and medications (5ARIs)), and how aggressiveness is defined (using only Gleason score or a combination of Gleason score and T stage). In chapter 5 we will show that using a different definition of aggressive prostate cancer (high Gleason score ≥8, instead of high-risk, a combination

of high stage and Gleason score) markedly alters the association between sex hormones and prostate cancer incidence and aggressiveness.

5. Peri-prostatic adipose tissue: the metabolic micro-environment of prostate cancer

Statement of Authorship

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Principle Author

Name of Principle Author (Candidate)	Adel Aref		
Contribution to the Paper	Conceptualization, project design, review of literature, writing, revision of manuscript		
Overall percentage (%)	40% (contributed equally with Nassar ZD)		
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	24/06/2019

Co-Author Contributions

Name of Co-Author	Zeyad D Nassar		
Contribution to the Paper	Conceptualization, review of literature, writing, revision of manuscript & final approval of the manuscript		
Signature		Date	17/06/2019

Name of Co-Author	Dushan Miladinovic		
Contribution to the Paper	Writing, revision of manuscript and final approval of the manuscript		
Signature		Date	26/6/19

Name of Co-Author	Chui Yan Mah		
Contribution to the Paper	Writing, revision of manuscript and final approval of the manuscript		
Signature		Date	24/6/19

Name of Co-Author	Ganesh V. Raj		
Contribution to the Paper	Writing, revision of manuscript and final approval of the manuscript		
Signature		Date	09/07/2019

Name of Co-Author	Andrew J. Hoy		
Contribution to the Paper	Writing, revision of manuscript and final approval of the manuscript		
Signature		Date	25/6/19

Name of Co-Author	Lisa M. Butler		
Contribution to the Paper	Conceptualization, writing, revision of manuscript, final approval of the manuscript and acted as corresponding author		
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Review

Peri-prostatic adipose tissue: the metabolic microenvironment of prostate cancer

Zeyad D. Nassar*†‡, Adel T. Aref*†‡, Dushan Miladinovic§, Chui Yan Mah*†‡, Ganesh V. Raj¶, Andrew J. Hoy§ and Lisa M. Butler*†‡

*University of Adelaide Medical School, Adelaide, SA, Australia, †Freemasons Foundation Centre for Men's Health, Adelaide, SA, Australia, ‡South Australian Health and Medical Research Institute, Adelaide, SA, Australia, §Discipline of Physiology, School of Medical Sciences and Bosch Institute, Charles Perkins Centre, University of Sydney, Sydney, NSW, Australia, and ¶Departments of Urology and Pharmacology, UT Southwestern Medical Center at Dallas, Dallas, TX, USA

Z.D.N. and A.T.A. contributed equally to the work.

Emerging data have linked certain features of clinical prostate cancer (PCa) to obesity and, more specifically, increased adiposity. Whereas the large number of clinical studies and meta-analyses that have explored the associations between PCa and obesity have shown considerable variability, particularly in relation to prostate cancer risk, there is an accumulating weight of evidence consistently linking obesity to greater aggressiveness of disease. In probing this association mechanistically, it has been posited that peri-prostatic adipose tissue (PPAT), a significant component of the prostate microenvironment, may be a critical source of fatty acids and other mitogens and thereby influences PCa pathogenesis and progression. Notably, several recent studies have identified secreted factors from both PPAT and PCa that potentially mediate the two-way communication between these intimately linked tissues. In the present review, we summarize the available literature regarding the relationship between PPAT and PCa, including the potential biological mediators of that relationship, and explore emerging areas of interest for future research endeavours.

Keywords

adipocytes, obesity, peri-prostatic adipose tissue, prostate cancer, tumour microenvironment

Introduction

Prostate cancer (PCa) is the most commonly diagnosed cancer in men in developed countries and the second most common cancer worldwide [1]. The past 30 years have seen a progressive increase in the incidence of PCa, to an extent that cannot be explained solely by the implementation of PSA-based testing programmes. This increase in PCa incidence has largely mirrored the increase in the prevalence of obesity and metabolic syndrome [2,3]. While there are conflicting reports on the effect of obesity on PCa incidence [4], there is an increasing body of evidence demonstrating associations between obesity and more aggressive carcinoma, poor treatment outcome and higher risk of cancer-specific mortality for PCa [5,6]. A range of mechanisms have been proposed to underpin the effects of an obese environment on PCa behaviour, including increased systemic inflammation, hyperinsulinaemia, altered adipokine profiles and increased lipid availability [7,8]. Enhanced synthesis and uptake of lipids are key hallmarks of PCa and are regulated by androgen signalling (the key driver of PCa pathogenesis), reviewed in Butler et al. [9]. Taken together with observations that increased local adipose tissue amounts, specifically peri-prostatic adipose tissue (PPAT), may be associated with higher grade or aggressiveness of disease (Table 1), obesity-mediated change to the size of this lipid depot may be an important contributor to PCa pathobiology. In this review, we present the available data regarding the link between PPAT and PCa aggressiveness, drawing from both clinical studies and *in vitro* laboratory research, and consider potential mechanistic pathways by which PPAT may influence PCa.

Methodology

We searched PubMed, Medline, Scopus and Google Scholar using the following search terms: 'periprostatic', 'adipocytes', 'adipose tissue', 'prostate' and 'cancer' in various combinations. There was no limitation on the year of

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Table 1 Clinical studies investigating the association between the clinical features of peri-prostatic adipose tissue and prostate cancer.

References	Year	Method	*N*	Major findings	Analysis	OR (95%CI), *P*
[58]	2010	CT	725 patients who underwent brachytherapy	PPAT area and density were not associated with PCa aggressiveness.	Univariable logistic regression analysis.	1 (0.96–1.05), $P = 0.93$
[59]	2011	CT	932 patients who underwent brachytherapy or radiotherapy	Significant association between total PPAT area and density with high-risk PCa.	Multivariable logistic regression analysis	1.06 (1.04–1.08), $P < 0.001$
[67]	2012	TRUS	279 healthy controls 434 patients with PCa 218 patients with high-grade PCa	Increasing in PPAT thickness was a risk factor for detecting PCa and detecting high-grade PCa on biopsy. For each 1-mm increase in peri-prostatic fat thickness there was a 12% increase in odds detecting PCa and 20% in odds of detecting high-grade PCa.	Multivariable logistic regression analysis	PCa: 1.12, (1.02–1.23), $P \leq 0.02$ High grade PCa: 1.20 (1.07–1.34), $P \leq 0.002$
[63]	2014	MRI	184 patients who underwent radical retropubic prostatectomy	PPAT area and ratio (PPAT volume/ prostate volume) were associated with high-risk PCa.	Multivariable logistic regression analysis	PPAT area: 1(0.96–1.04), $P = 0.024$ PPAT ratio: 1.05(1.03–1.08), $P = 0.31$
[60]	2014	CT scan	308 patients who underwent radiotherapy	Visceral adipose tissue area and PPAT area were associated with aggressive PCa in black men but not in white men.	Multivariable logistic regression analysis	Visceral adipose tissue area: 4.23 (1.67–10.69), $P = 0.002$ PPAT area: 1.88 (0.80–4.44), $P = 0.135$
[61]	2015	MRI	190 patients who underwent MRI before prostatectomy	PPAT thickness was correlated with Gleason Score. PPAT was an independent predictive factor for high-grade PCa.	Multivariable logistic regression analysis	1.331 (1.063–1.666), $P \leq 0.013$
[62]	2016	MRI	234 patients who underwent different treatment methods	PPAT fat ratio (PPAT volume/ prostate volume) was associated with higher Gleason score.	Multivariable logistic regression analysis	3.45(1.69–7.06), $P \leq 0.001$
[56]	2017	CT scan	61 patients treated with 5ARIs for ≥12 months, and 117 patients without any exposure to 5ARIs	Weak significant correlation between the CAPRA score and the PPAT volume In multivariate linear regression analysis adjusted for 5ARI use, PPAT was not predictive of the total CAPRA score.	Multivariable logistic regression analysis	PCa: 1.55, (1.03–1.84), $P \leq 0.001$. High grade PCa: 1.46 (1.20–1.73), $P \leq 0.001$
[65]	2017	MRI	371 PCa patients; 292 with high-grade disease	PPAT thickness was an independent predictor of PCa and high-grade PCa.	Multivariable logistic regression analysis	1.398 (1.037–1.883), $P = 0.028$
[66]	2017	MRI	162 patients who underwent MRI prior to prostatectomy	PPAT fat ratio (PPAT volume/ prostate volume) was associated with PCa aggressiveness (grade and stage).	Binary logistic regression analysis	$P = 0.18$

5ARI, 5α-reductase inhibitor; CAPRA, Cancer of the Prostate Risk Assessment; OR, odds ratio; PCa, prostate cancer; PPAT, peri-prostatic adipose tissue.

publication or the publication language. The reference lists of identified publications were also searched for additional material.

Obesity and Prostate Cancer

Epidemiological Evidence Linking Obesity and Prostate Cancer

Multiple epidemiological studies have identified associations between obesity (often measured as body mass index (BMI) and increased incidence of different types of cancer, including oesophageal, gynaecological and colorectal cancers [10–17]. Obesity has also been associated with poor treatment outcome and increased cancer-specific mortality [18]. Similar relationships have been observed for PCa, although the findings are inconsistent; some studies report an association between obesity and increased PCa incidence [19–23], whilst others have shown no or only modest associations with PCa incidence [24–26]. Meta-analyses designed to examine robustly the association between obesity and PCa incidence are equally inconclusive [4–6,19,27–29].

Several studies have, however, identified significant associations between obesity and the progression of PCa, most commonly with the presence of a more aggressive carcinoma and/or higher PCa-specific mortality [25,30–33], as well as increasing the risk of biochemical recurrence [34,35]. Unlike the data for PCa incident risk, meta-analyses have shown more consistent associations between obesity and more advanced stages of PCa as well as higher disease-related mortality [4–6,19,27–29].

In a study exploring the effect of pre-diagnostic BMI on PCa incidence in the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial, BMI at the age of 50 years and at the study baseline was inversely associated with total incidence [36]. Interestingly, one meta-analysis found obesity to be associated with decreased risk of localized disease but an increased risk of a more advanced disease [37]. Several factors could be attributable for this finding including the lower PSA levels in obese men [38], the technical difficulties in examination, diagnostic procedures and treatment methods, as well as potential stage-specific interactions [39].

Proposed Mechanisms Linking Obesity and Prostate Cancer

Causative mechanisms that have been proposed as explanations for the potential link between obesity and PCa aggressiveness have largely focused on alterations in the host systemic milieu, such as increased circulating levels of insulin and other growth factors, altered inflammatory status and dyslipidaemia [7,8]. Alongside these systemic changes, it is also evident that cells of the surrounding or nearby tissue, such as stromal, endothelial, lymphocytes and adipose stem cells, contribute to a supportive tumour microenvironment [40]. Peri-tumoral adipose tissue and other adipose beds that support metastatic disease promote growth of a range of cancers through diverse mechanisms including the release of growth factors, inflammatory signalling activators, and the release of fatty acids as a source of potential usable energy [41]. Adipose tissue is a heterogeneous mix of cell types comprising resident immune cells, fibroblasts, the stem cell population termed 'pre-adipocytes' and mature adipocytes, with mature adipocytes alone able to promote tumour progression in several types of cancer [42–44]. These considerations, together with the close proximity of PPAT to the prostate, have understandably stimulated interest in this understudied adipose depot and its influence on PCa.

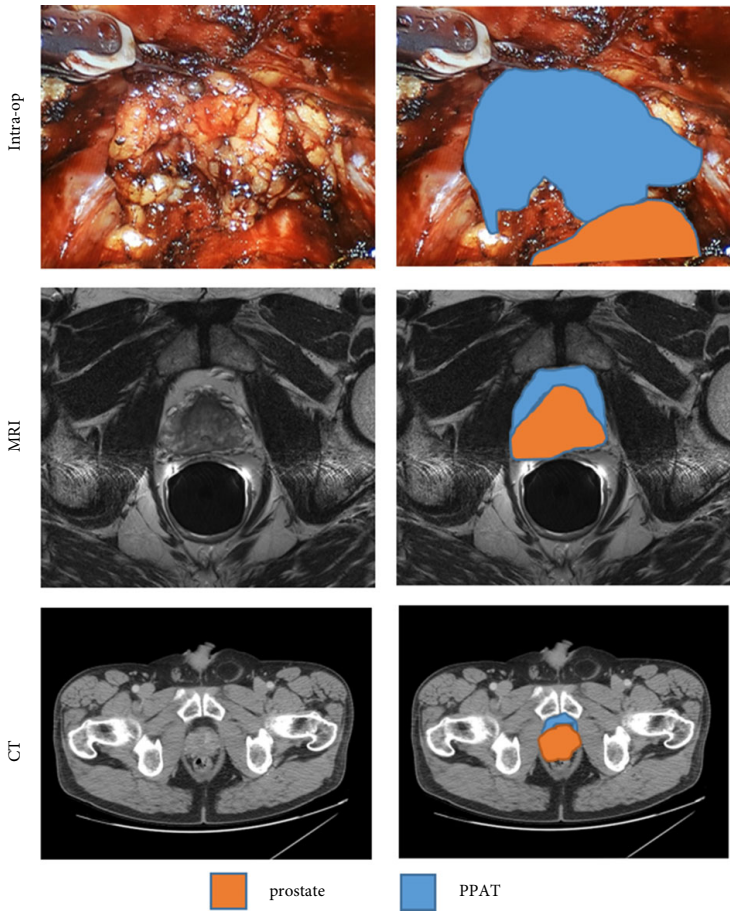
All Adipose Tissue Depots are not Equal

Adipose tissue is a metabolically active organ that can be broadly classified into visceral and subcutaneous adipose beds. Visceral adipose tissue releases more inflammatory and growth factors compared with subcutaneous adipose tissue [45], and a recent study by Lee et al. [46] reported that visceral adipose tissue, and the visceral to subcutaneous adipose tissue ratio, is an independent predictor for all-cause mortality. Moreover, in 2004 Von Hafe et al. [47] found that higher visceral fat identified by CT was associated with higher risk of PCa. To date, very few data exist on the phenotype of PPAT and how it relates to subcutaneous adipose tissue and other visceral adipose beds. Differences between the various adipose beds with respect to BMI highlight the need to analyse more accurately the associations between PCa incidence and aggressiveness with the actual differential fat content of the body using radiological methods.

Relationships Between Peri-Prostatic Adipose Tissue and Prostate Cancer Features

A range of studies have investigated the relationship between PPAT thickness and/or density and PCa clinical features, most during the past 4 years, and the impact of obesity on these measures (summarized in Table 1 and below). While there is no consistent definition of PPAT, it can be generally defined as the adipose bed that surrounds the prostatic surface (Fig. 1). Notably, the distribution of PPAT differs across the distinct surfaces of the prostate gland [48]. Whilst a fibro-muscular capsule normally separates the prostate gland from the surrounding PPAT, invasion of PCa into PPAT, resulting in the juxtaposition of PCa cells with cells of the adipose tissue, is defined as extracapsular extension and

Fig. 1 Matched intra-operative (top panel), MRI (middle panel) and CT (lower panel) images of peri-prostatic adipose tissue (PPAT) and its spatial relationship to the prostate for a representative patient. Right panels depict the prostate and PPAT as orange and blue regions, respectively.



patients with this pathological feature have poorer prognostic outcomes [49–55].

Measurements of Peri-Prostatic Adipose Tissue using CT

A common approach used to measure PPAT thickness in PCa studies is CT. Most recently, Taussky et al. [56] defined PPAT as the adipose bed located only anteriorly to the prostate gland in a CT section at the level of the intervertebral space at the L4 and L5 level. The authors reported no significant correlation between BMI and PPAT volume and density, nor between PPAT and PCa aggressiveness, as defined by Cancer of the Prostate Risk Assessment (CAPRA) score. Similar observations were reported by Tiberi et al. [57], who defined PPAT as the contour at the level of the superior border of the symphysis pubis with exclusion of the tissue medial to the levator ani muscles on both the anterior and posterior aspects of the CT image, and van Roermund et al. [58], who defined PPAT in a transverse section at the level of the caput femoris and greater trochanter of the femur. In the study by van Roermund, 31% of patients with normal BMI had a high PPAT density (>75th percentile) and only 20% of the cohort population had high-grade disease [58]. Interestingly, these authors did report that total PPAT

area and PPAT density were associated with high-grade disease, whereas BMI was not associated with high-grade disease, using the same CT technique in a separate cohort of patients including a group of patients who received intensity-modulated radiotherapy, of whom 83% had high-risk disease [59]. These studies included only white populations.

Allott et al. [60] explored the association between PCa aggressiveness and visceral and subcutaneous adipose tissue or PPAT in black vs white patients who underwent radiotherapy. The three types of adipose tissue were identified by CT scan at the level of the symphysis pubis. BMI was associated with increased risk of aggressive PCa (in terms of Gleason score ≥7) in both black and white patients, while visceral adipose tissue was associated with aggressive PCa only in black patients. Obesity, measured by BMI, was associated with PPAT area ($r = 0.2$; $P < 0.001$) and visceral adipose tissue ($r = 0.65$; $P < 0.001$), but no significant association was found between PPAT and risk of aggressive PCa in either group of patients. One important observation in their study was that, despite there being no significant difference in BMI, black patients had significantly lower amounts of visceral adipose tissue and PPAT than white patients ($P < 0.001$ for both).

Measurements of Peri-Prostatic Adipose Tissue using MRI

A number of studies have measured PPAT volume using MRI [61–64]. For example, Salji et al. [64] assessed PPAT volume by delineating the first visible facial boundary laterally, the Denonvillier's facia posteriorly, and symphysis pubis anteriorly in a small group of patients with advanced PCa before commencing androgen deprivation therapy. In that study, higher PPAT volume was significantly associated with the development of castrate-resistant PCa. Adding PPAT volume to a predictive model improved the receiver-operating characteristic (ROC) sensitivity (area under the curve 0.9 with PPAT volume vs 0.8 without PPAT volume). Notably, there was no significant association in this study between PPAT volume, body weight or waist circumference.

Likewise, Woo et al. [61] found a significant correlation between PPAT, measured by MRI, and Gleason score; however, the correlation was low ($R = 0.0228$). Interestingly, there was no association between BMI and PPAT, although 62% of the study population were non-obese. Tan et al. [62] assessed the correlation between three separate measures in MRI images and aggressive PCa in 234 men; in this case, PPAT volume was measured by identifying the area of adipose on the MRI cut from the level of the base to the apex of the prostate. The PPAT ratio was calculated by dividing PPAT volume by prostate volume. Another measurement considered was the supra-pubic adipose thickness. PPAT volume and PPAT ratio were significantly associated with higher Gleason score, but the supra-pubic adipose was not. Of note, PPAT ratio did not show significant correlation with age, PSA level or BMI. Sensitivity analysis in this study showed that age, PSA and BMI were poor determinates of high Gleason score (0.56, 0.50 and 0.51, respectively), while PPAT ratio had the higher determinate in the ROC analysis (0.64). An important limitation of that study was that the TRUS biopsy result was used as the reference standard instead of the prostatectomy pathology result. Finally, Zhang et al. [63] reported similar results in a small group of patients ($n = 184$, 30% with high-risk disease) where PPAT area was identified by MRI transverse section at the level of the femoral head and greater trochanter of the femur. In their study, there were significant associations between PPAT area and both clinical stage and Gleason score, but not for BMI. Recently, two clinical studies have also shown that PPAT measured by MRI is an independent predictor for aggressive PCa [65,66].

Other Techniques to Measure Peri-Prostatic Adipose Tissue

Alongside CT and MRI, PPAT has also been determined from images obtained for TRUS-guided biopsy procedures. Using TRUS images, Bhindi et al. [67] used the shortest perpendicular line from the pubic bone to the prostate to determine PPAT thickness, which was associated with PCa, more aggressive cancer and BMI. Again, ROC curves had an area under the curve of 0.58 for PPAT to detect PCa, and 0.59 to detect high-grade PCa, which was comparable to PSA and DRE and age as determinates.

Peri-Prostatic Adipose Tissue and Prostate Cancer Outcome

The above studies collectively demonstrate that there is an association between PPAT and PCa aggressiveness. PPAT volume also predicts time to castrate-resistant PCa [64] but its influence on other oncological outcomes of PCa are yet to be reported. Interestingly, obesity increases the risk of biochemical recurrence [34,35]; however, these studies did not explore the contribution of PPAT. Further studies are required to explore this association.

Summary: Clinical Peri-Prostatic Adipose Tissue Studies

For detecting high-grade PCa, PPAT appears to have a promising degree of sensitivity, irrespective of the method used to determine the PPAT, and could be considered for risk stratification of patients in the future. Despite this finding, information about PPAT is still not included in PCa registries [68]. The inclusion of PPAT-related data in existing

registries and its prospective collection in biobanks will help to elucidate the influence of PPAT size and composition on PCa features, treatment outcomes and risk of recurrence.

Despite the range of approaches used to define PPAT, measuring PPAT at the retropubic region seems to be adequate, and the inconsistencies between the various study findings are probably influenced more by the study population than the method by which PPAT was identified. Considering that most patients with PCa are now having MRI as part of their staging and diagnosis evaluation, identifying the PPAT via MRI images would be the preferred method, being the most accurate and least operator-dependent.

By contrast, the correlation between PPAT and BMI is complex. There may be many reasons for this. Firstly, PPAT is correlated with prostate volume, which is influenced by age and may affect the association between BMI and PPAT. Furthermore, BMI is not a robust indicator of adiposity (neither subcutaneous nor visceral), and not all men with high BMI have more visceral adipose and PPAT, and vice versa. BMI only quantifies the relationship between height and mass, and thus provides no insight into body composition or metabolic health of obese adults. There is an obvious need to identify more relevant and detailed measurements of metabolic health when considering the role of obesity in PCa. Collectively, these studies highlight the complexity of the associations between PPAT, BMI and PCa measures, some of which may be influenced by racial differences.

Potential Mechanistic Links Between Peri-Prostatic Adipose Tissue and Prostate Cancer

The epidemiological evidence of an effect of obesity on PCa progression, and the associations found between PPAT thickness and PCa aggressiveness, suggest that expansion of local adipose tissue can influence PCa behaviour. These clinical findings have spurred laboratory-based studies of the mechanistic pathways that may underlie this association. In this section, we will describe the interactions observed between adipose tissue and PCa cells (summarized in Fig. 2).

Adipocytes in the Prostatic Microenvironment

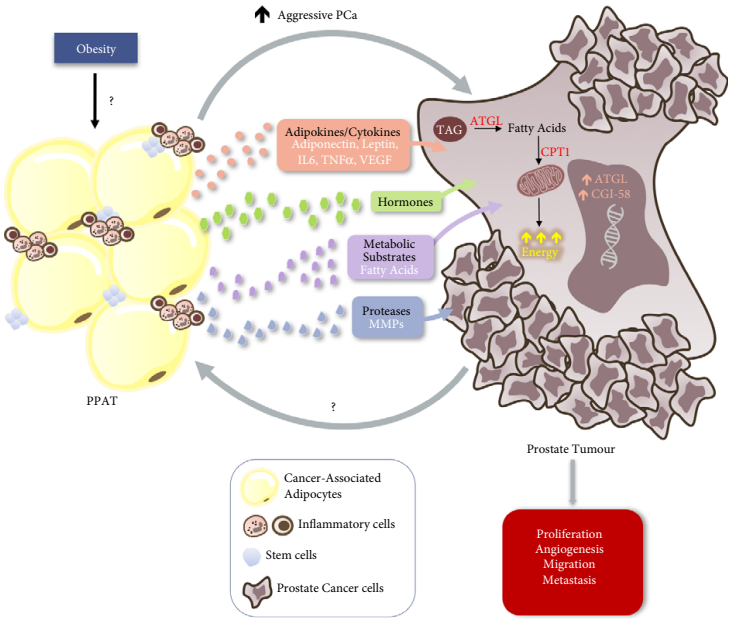
Whereas intraprostatic adipocytes are extremely rare [69,70], the extracapsular extension of PCa into the PPAT promotes tumour-adipocyte cross-talk because of the close proximity of these cell populations. This cross-talk may be further amplified by the increased number of pre-adipocytes observed in PPAT from patients with PCa compared to patients with BPH [71,72], and PPAT being richer in adipocyte precursors than other visceral adipose tissue [71]. The reciprocal interaction between adipocytes and tumour cells re-programmes adipocytes to a less differentiated status referred to as cancer-associated adipocytes, a phenotype favourable to more aggressive tumours including PCa [73–77]. Multiple lines of evidence suggest that cancer-associated adipocytes, in turn, can enhance the malignant characteristics of the cancer cells, ultimately producing a vicious self-amplifying positive feedback circle [73,78,79]. *In vitro* Boyden chamber migration assays have shown that PCa cells or PCa conditioned media (CM) can attract pre-adipocytes, and to a significantly greater extent than non-malignant cells or their CM [72,80]. Importantly, this interaction is not limited to the close proximity of the prostate gland; in an elegant study, Lin et al. [80] showed that human PCa cells injected in the right flank of athymic nude mice can recruit pre-adipocytes transplanted into the opposite flank, and this migration of the pre-adipocytes enhances tumour growth and angiogenesis. The origin of the recruited adipocytes in clinical samples still lacks solid experimental evidence; while the PPAT is a likely source, some investigators have suggested that PCa cells can differentiate into adipocyte-like cells [81], while others have implicated mobilization of adipocytes from visceral adipose tissue through local vessels or systemic circulation [71,82], although this mechanism has been questioned as being incompatible with the chemo-physical properties of adipocytes [83].

Reciprocal Interactions Between Adipose Tissue and Prostate Cancer Cells

Culturing human PPAT with PC3 cell-derived CM enhances secretion by the PPAT of adipokines (8–11 kDa secreted proteins), TNF-α, interleukin (IL)-6 and osteopontin, and increases mitochondrial DNA copy number and metalloproteinase (MMP)-9 activity [73]. Moreover, pre-adipocytes primed with PCa CM undergo neoplastic-like transformation including genetic instability, mesenchymal-to-epithelial transition, and formation of prostate-like neoplastic lesions *in vivo* [84].

Prostate cancer is, in turn, influenced by adipocyte-secreted factors that enhance its ability to proliferate, migrate and/or invade [74,79,85–89]. Punch biopsies of human prostate specimens or PPAT collected after prostatectomy revealed a strong concentration gradient of the adipokine CCL7, suggesting that the PPAT secretome passively diffuses from PPAT into the tumour tissues to enhance the directed migration of PCa cells [74]. The CM of PPAT contains higher MMP activity compared with peri-peritoneal visceral adipose tissue [79], and this MMP activity degrades extracellular matrix proteins and promotes invasion of

Fig. 2 Model of how peri-prostatic adipose tissue (PPAT) may promote prostate cancer (PCa) aggressiveness and the influence of obesity. The reciprocal interaction between adipocytes and tumour cells re-programmes adipocytes to a less differentiated status, referred to as cancer-associated adipocytes (CAAs). In turn, CAAs secrete several adipokines, cytokines, hormones, enzymes and growth factors that may boost PCa cell growth and metastasis. Fatty acids are also translocated from PPAT into the PCa cells, increasing energy production. Obesity drives inflammation within the PPAT, and modifies PPAT constituents, transcriptomic, metabolic and endocrine profiles, potentially augmenting their secretome. These effects on the PPAT, taken together with the documented systemic effects of obesity, may underpin the emerging associations between obesity and increasing PCa aggressiveness.



cancer cells into the surrounding tissues [90]. Direct adipocyte-prostate cell cross-talk has been detected in co-culture models of prostate cells with adipocytes. Mature rat epididymal adipocytes influenced the growth and differentiation of normal rat prostatic epithelium [91] or human PCa [92,93] when co-cultured in a three-dimensional collagen gel matrix. These effects were accompanied by increased expression of the cytokines VEGF and PdGF 20-fold [92], and activation of the PI3K pathway [93] in the PC3 PCa cell line. However, these studies have shown considerable variability; while PPAT CM showed a stimulatory effect on PC3 and LNCaP cell migration in one study [79], the effect was not significant in another [88]. Similarly, co-culturing rat epididymal adipocytes with PC3 cells increased PC3 proliferation in one study [92] but not in a later study [93]. These differences probably reflect the nature of the cell lines and experimental methodologies used; a critical issue discussed in detail below. Nevertheless, the functional significance of adipocyte–PCa interactions is highlighted by a study using a subcutaneous *in vivo* tumour model, in which co-injection of PCa cells with pre-adipocytes resulted in larger tumours than injection of unaccompanied PCa cells [94].

Pro-Inflammatory Effects of Adipose Tissue on Prostate Cancer Cells

Obesity can be considered a state of chronic inflammation that is characterized by increased secretion of inflammatory proteins by adipose tissues [95]. Adipose tissues produce many inflammatory and pro-mitogenic molecules, including leptin, adiponectin, IL-6, IL-8, MCP-1, VEGF, CCL5, CCL7, visfatin and TNF-α. At least some of these factors may also be produced by infiltrating immune cells such as macrophages. Strikingly, ~30% of the 100 most significantly expressed genes in adipose tissues that are correlated with body mass encode inflammation-related genes [96]; however, the interaction between inflammatory markers, insulin resistance, obesity and metabolic syndrome is complex [97–100] and the current lack of detailed metabolic phenotyping of patients with PCa makes it challenging to determine the extent to which PPAT inflammation is a consequence of obesity or metabolic syndrome.

These inflammatory markers are also associated with PCa promotion and progression both in clinical and *in vitro* studies [101–103]. Notably, IL-6 secreted by PPAT from patients with PCa was present at >375 times greater concentration than in the matched patient serum, and significantly with the disease pathological grade [86]. In a recent study, PPAT inflammation defined by the presence of crown-like structures was found to be associated with larger adipocyte size, higher circulating levels of insulin and triglycerides, and with high-grade PCa [104].

Adipose Tissue and its Constituents Modify Prostate Cancer Cell Metabolism

Adipose tissues serve as reservoirs for triglycerides, and adipocytes mobilize and locally release stored fatty acids via lipolysis to the surrounding and distant organs [105]. Lipolysis is catalysed through a sequence of lipases, initiated by adipose triglyceride lipase. Cancer cells cause metabolic changes in the adipocyte that lead to activation of lipolytic pathways and thus use adipocytes as source of energy, a condition defined as cachexia [106]. This is consistent with the increased expression of the lipolytic enzymes adipose triglyceride lipase and CGI-58 detected in high grade PCa [107]. Gazi et al. [108] have shown in a study using Fourier transform infrared spectroscopy that there is strong evidence of lipid translocation between adipocyte and PCa cells. This phenomenon has been also reported for ovarian cancer [109] and breast cancer [110], but there is little known about the role of adipocyte-derived fatty acids as metabolic substrates for PCa.

In addition to the potential for enhanced fatty acid release and transfer to PCa cells, the composition of fatty acids in the PPAT is commonly altered in PCa compared to benign prostatic tissue [111,112]. For example, Quiroga et al. [111] studied 23 patients (12 with PCa and 11 with BPH) and showed that PPAT from patients with PCa had significantly higher levels of palmitic and dihomo-gammalinolenic acids, and lower levels of arachidonic acid. Similarly, Iordanescu et al. [112] used magnetic resonance spectroscopy to show that PPAT from patients with more aggressive PCa has a higher ratio of monounsaturated/saturated fatty acids. However, this relationship was not observed with high Gleason scores (7 vs 6), which may be attributable in part to those patients with Gleason score 7 being a mixed population of 4 + 3 (only four patients) and 3 + 4 (13 patients). As fatty acids are quantitatively important sources of energy for PCa cells compared with glucose and glutamine, studies of the uptake and differential utilization of these various fatty acids by PCa cells, and their impact on PCa biology in a clinical setting of obesity, remain important gaps in our understanding and potential avenues for therapeutic intervention.

Hormonal Influences of Peri-Prostatic Adipose Tissue on Prostate Cancer Progression

Adipose tissues are endocrine organs with capacity to synthesize, secrete and metabolize steroid hormones from circulating precursors. While less is known about PPAT than other adipose beds, adipose tissue contains multiple androgens and androgen precursors, including testosterone, dihydrotestosterone, androstenedione, progesterone and dehydroepiandrosterone [113,114], which, in the case of PPAT, provide a credible local extragonadal source of androgens that may support PCa growth and metastasis. PPAT also expresses aromatase enzymes, which convert androgens to oestrogens [77], and abundant historical data support the suggestion of a promoting role for oestradiol in PCa pathogenesis and progression, and as a modifier of racial differences in PCa incidence [115]. Age-related increases in oestradiol/testosterone ratio in men predispose to PCa [116], and a high ratio has been associated with increased risk of aggressive PCa among male-to-female transsexuals [117]. In addition, oestrogen can activate both wild-type and mutated androgen receptors [118]. More detailed analyses of sex steroid production and secretion in PPAT vs other visceral and subcutaneous adipose, and the influence of PPAT size and composition on these measures, are important areas for future investigation.

Peri-Prostatic Adipose Tissue may Influence Obesity-Induced Prostate Cancer Progression

Obesity modifies the metabolic and endocrine profiles of multiple adipose tissues, resulting in increased release of growth factors, hormones, adipokines and mobilization of lipids and free fatty acids [74,119–123]. In addition, obesity increases the rate of pre-adipocyte migration from white adipose tissue, which may contribute to the obesity-induced promotion of PCa progression [82,124,125]. Given the consistent correlation between PPAT and aggressive PCa, increasing attention has focused on obesity-related changes in PPAT composition.

Venkatasubramanian et al. [126] reported that PPAT tissue composition differs from that of the subcutaneous adipose tissue in obese vs lean patients (obesity defined by BMI >30 kg/m^2). Specifically, the PPAT of obese men had increased monounsaturated/saturated lipid ratio compared to subcutaneous fat. In addition, obesity alters the PPAT gene expression profile to favour hypercellularity and reduced immune surveillance, thereby promoting a conducive environment for PCa progression [78]. PPAT in obese men is more metabolically and secretorily active than PPAT in lean men. PPAT in obese men had higher MMP-9 activity [79], and CM from these tissues induced PC3 PCa cell line and endothelial cell proliferation more than CM from tissues in

lean men [127]. PPAT from obese men exhibited increased angiogenic capacity compared with that from lean men, as evidenced by *ex vivo* measurement of T2 relaxation time [127]. Obese patients with PCa exhibited increased expression of the chemokine CXCL1, a concentration gradient of which is needed for obesity-dependent recruitment of pre-adipocytes [128].

Importance of Experimental Model Systems

Progress in understanding the biology and functional consequences of adipocyte–PCa cross-talk has been hampered by a lack of consistency in experimental conditions and model systems used, resulting in substantial variability between studies and laboratories. The choice of *in vitro* interaction models between PCa cells and adipocytes, in particular, has a significant impact on the observed effects on both cell types. For instance, adipocyte co-culture using Boydon chambers enhances RM1 PCa cell proliferation more than when RM1 cells are exposed directly to the adipocyte CM [85]. Another important factor is the use of isolated adipocytes rather than adipose tissue. The adipose tissue is a heterogeneous mixture of cells consisting of mature adipocytes, pre-adipocytes (stem cells), immune cells and fibroblasts, and thus the observed effects might be caused or modified, at least partly, by cell types other than adipocytes, or could be attributed to interactions between these different cell populations. In addition, adipose tissues are often sourced from varying anatomical depots; some studies used visceral fats [73,88,127], while others used epididymal adipose tissue [91–93]. Distinct adipose depots exhibit different adipokine [45,129–131] and gene expression [132] profiles and have different cellular composition. Further, the metabolic activity of adipose beds differs, with omental adipose tissue having increased expression of proteins involved in lipid and glucose metabolism including HSP90, HSP70, GAPDH and fatty acid binding protein 5 compared with subcutaneous adipose [133]. PPAT in particular contains higher MMP-2 and MMP-9 activity compared with adipose tissue from the median pre-peritoneal visceral region [79], and higher *in vitro* pro-angiogenic responses and viability effects on PC3 cells than for subcutaneous adipose tissue [127]. Another important source of diversity is the intrinsic heterogeneity of the patients studied, their tumour subtypes and their individual metabolic profiles. PPAT from obese and overweight men exhibits overexpression of inflammatory adipogenic, anti-lipolytic, proliferative, anti-apoptotic, and mild immunoinflammatory genes compared with that from lean men [78]. Ideally, the influence of disease stage and metabolic profiles for PPAT and human PCa tissues should be taken into account in future studies, and incorporation of patient-derived explant or xenograft models offer the opportunity to study reciprocal PCa-adipose interactions in systems that more closely recapitulate the disease heterogeneity and complexity of the clinical tissue microenvironment [134,135].

Concluding Remarks

The accumulated weight of evidence to date supports an association between PPAT quantity and increased PCa aggressiveness, although the mechanistic basis of this association remains inconclusive and has limited the development of potential interventions. Measuring PPAT area or density showed more significant correlations with PCa aggressiveness than measuring general obesity markers BMI and waist circumference [59,61,63], while the correlation of PPAT thickness with BMI or weight was non-significant or weak [56–58,61,63,67]. Moreover, no association has been demonstrated between subcutaneous adipose thickness and PCa aggressiveness [59,61]; thus, PPAT measurements may serve as an independent predictor of PCa aggressiveness rather than as a surrogate measure for body adiposity. Incorporating PPAT-related measures into a PCa risk assessment model may improve PCa prognostication and identify patients who may be in need of more aggressive treatment methods. To facilitate this, large prospective studies are required that take into consideration the assessment of obesity, subcutaneous fat, visceral fat and PPAT in determining risk of PCa incidence and aggressiveness. Ideally, these would be accompanied by *ex vivo* studies to examine functional readouts, such as the secretome, of the PPAT from these patients and provide a more robust mechanistic basis by which PPAT may influence PCa pathogenesis.

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Conflict of Interest

None declared.

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Correspondence: Andrew J. Hoy, Discipline of Physiology, School of Medical Sciences & Bosch Institute, Charles Perkins Centre, University of Sydney, NSW 2006, Australia.

e-mail: andrew.hoy@sydney.edu.au

and

Lisa M. Butler, University of Adelaide Medical School, South Australian Health and Medical Research Institute, Adelaide, SA 5000, Australia.

e-mail: lisa.butler@adelaide.edu.au

Abbreviations: BMI, body mass index; CM, conditioned media; IL, interleukin; MMP, metalloproteinase; PCa, prostate cancer; PPAT, peri-prostatic adipose tissue; ROC, receiver-operating characteristic.

Chapter 2. The inverse relationship between prostate specific antigen (PSA) and obesity

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Principle Author

Name of Principle Author (Candidate)	Adel Aref		
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Signature		Date	24/06/2019

Co-Author Contributions

Name of Co-Author	Andrew D. Vincent		
Contribution to the Paper	Conceptualization, project design, analysis and interpretation of the data, writing, review, revision of manuscript and final approval of the manuscript		
Signature		Date	25/6/19

Name of Co-Author	Michael O'Callaghan		
Contribution to the Paper	Analysis and interpretation of the data, writing, review and revision of the manuscript, and final approval of the manuscript		
Signature		Date	25/6/2019

Name of Co-Author	Sean A. Martin		
Contribution to the Paper	Analysis and interpretation of the data, writing, review and revision of the manuscript, and final approval of the manuscript		
Signature		Date	24/6/2019

Name of Co-Author	Peter D. Sutherland		
Contribution to the Paper	Analysis and interpretation of the data, writing, review and revision of the manuscript, and final approval of the manuscript.		
Signature		Date	25/6/19

Name of Co-Author	Andrew J. Hoy		
Contribution to the Paper	Analysis and interpretation of the data, writing, review and revision of the manuscript, and final approval of the manuscript.		
Signature		Date	25/6/19

Name of Co-Author	Lisa M. Butler		
Contribution to the Paper	Conceptualization, project design, analysis and interpretation of the data, writing, review, revision of manuscript, final approval of the manuscript and acted as corresponding author		
Signature		Date	24/6/19

Name of Co-Author	Gary A. Wittert		
Contribution to the Paper	Conceptualization, project design, analysis and interpretation of the data, writing, review, revision of manuscript, final approval of the manuscript and acted as corresponding author		
Signature		Date	24/6/19

RESEARCH

The inverse relationship between prostate specific antigen (PSA) and obesity

Adel T Aref[1,2,*], Andrew D Vincent[1,2,*], Michael E O'Callaghan[1,3,4,5], Sean A Martin[1,2], Peter D Sutherland[6], Andrew J Hoy[7], Lisa M Butler[1,2] and Gary A Wittert[1,2]

[1]University of Adelaide Medical School and Freemasons Foundation Centre for Men's Health, Adelaide, South Australia, Australia
[2]South Australian Health and Medical Research Institute, Adelaide, South Australia, Australia
[3]Urology Unit, SA Health, Repatriation General Hospital, Adelaide, South Australia, Australia
[4]South Australian Prostate Cancer Clinical Outcomes Collaborative (SA-PCCOC), Adelaide, South Australia, Australia
[5]Flinders Centre for Innovation in Cancer, Flinders University, Adelaide, South Australia, Australia
[6]Department of Urology, Royal Adelaide Hospital, Adelaide, South Australia, Australia
[7]Discipline of Physiology, School of Medical Sciences and Bosch Institute, Charles Perkins Centre, University of Sydney, Sydney, New South Wales, Australia

Correspondence should be addressed to L M Butler or G A Wittert: lisa.butler@adelaide.edu.au or gary.wittert@adelaide.edu.au

*(A T Aref and A D Vincent contributed equally to this work)

Abstract

Obese men have lower serum prostate-specific antigen (PSA) than comparably aged lean men, but the underlying mechanism remains unclear. The aim of this study was to determine the effect of obesity on PSA and the potential contributing mechanisms. A cohort of 1195 men aged 35 years and over at recruitment, with demographic, anthropometric (BMI, waist circumference (WC)) and serum hormone (serum testosterone, estradiol (E2)) PSA and hematology assessments obtained over two waves was assessed. Men with a history of prostate cancer or missing PSA were excluded, leaving 970 men for the final analysis. Mixed-effects regressions and mediation analyses adjusting for hormonal and volumetric factors explore the potential mechanisms relating obesity to PSA. After adjusting for age, PSA levels were lower in men with greater WC ($P=0.001$). In a multivariable model including WC, age, E2/testosterone and PlasV as predictors, no statistically significant associations were observed between with PSA and either WC ($P=0.36$) or PlasV ($P=0.49$), while strong associations were observed with both E2/testosterone ($P<0.001$) and age ($P<0.001$). In the mediation analyses with PlasV as the mediator, the average causal mediation effect (ACME) explained roughly 20% of the total effect of WC on PSA ($P=0.31$), while when E2/testosterone is a mediator, the ACME explained roughly 50% of the effect ($P<0.001$). Our findings indicate that lower PSA levels in obese men, as compared to normal weight men, can be explained both by hormonal changes (elevated E2/testosterone ratio) and hemodilution. Hormonal factors therefore represent a substantial but underappreciated mediating pathway.

Key Words
- prostate cancer
- obesity
- estradiol
- testosterone
- hemodilution
- PSA
- prostate cancer screening

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Introduction

Prostate cancer is the most common cancer affecting men in developed countries (Torre *et al.* 2015). Although the advent of prostate specific antigen (PSA) testing has resulted in earlier detection of prostate cancer, its role in decreasing prostate cancer-specific mortality is far less certain (Pron 2015). Contradictory findings of screening

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studies undertaken to date may reflect the impact of potential modifiers of PSA levels, with considerable attention focused on obesity. Men who are obese have consistently lower PSA concentrations in serum samples than non-obese men (Zhang *et al.* 2015, 2016, Bonn *et al.* 2016). The predictive power of the PSA test was not altered by BMI (kg/m^2), a relatively crude measure of obesity, in two independent studies (Banez *et al.* 2014, Vidal *et al.* 2015). Other studies have shown that the sensitivity of PSA detection is decreased by approximately 16% in obese men (Negron *et al.* 2010), leading to the proposal that an obesity-specific PSA model is required to improve the sensitivity of the PSA blood test (Hekal & Ibrahiem 2010, Liang *et al.* 2010). However, the development and implementation of such a model will be most optimally achieved with an understanding of the mechanisms underlying the relationship between obesity and serum PSA concentrations.

Currently, there are two major hypotheses to explain the reduced PSA levels in obese men (Fig. 1): the effect of hemodilution (Li *et al.* 2015, Klaassen *et al.* 2017) and low serum testosterone (Gates *et al.* 2013, Parikesit *et al.* 2016). The former is the generally accepted explanation. Men with obesity have a larger plasma volume, which dilutes the serum concentrations of tumor markers such as PSA. Obesity is associated with lower serum testosterone levels (Gates *et al.* 2013, Parikesit *et al.* 2016) and, as the prostate gland is an androgen-dependent organ, lower levels of testosterone would be expected to associate with reduced prostate gland volume and PSA secretion. As significant weight loss has been associated with increased serum testosterone and PSA, as well as decreased plasma volume (Woodard *et al.* 2012), both hormonal and hemodilution mechanisms are credible modifiers of PSA levels in a clinical setting of obesity.

A major shortcoming in the study of obesity-related changes in serum PSA, highlighted by several groups previously, is that some of the studies to date have been undertaken in cohorts of men diagnosed with prostate cancer, rather than cancer-free individuals (Banez *et al.* 2007, Bonn *et al.* 2016). The aim of this study is to assess the influence of obesity on serum PSA concentrations and to explore the underlying mechanism for these changes in a longitudinal population-based cohort of men.

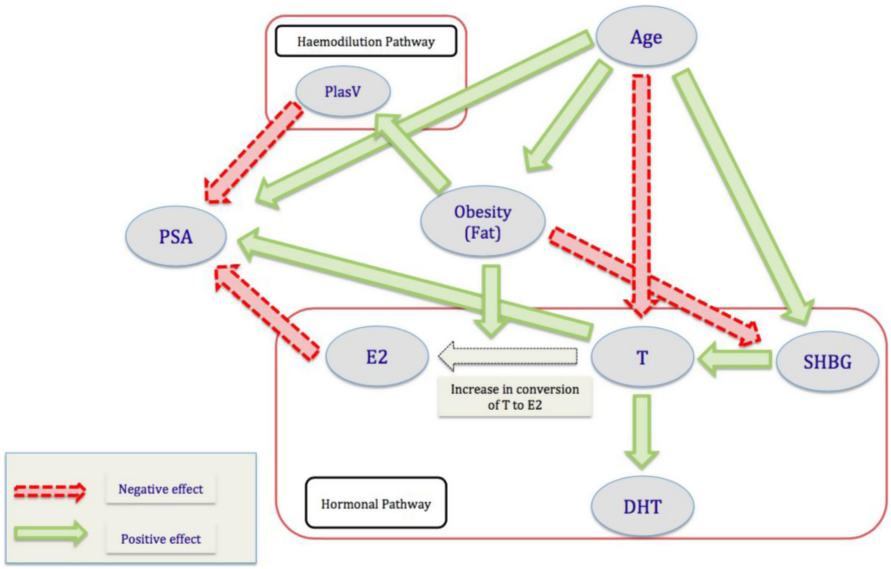

Figure 1
Hypothesized mechanistic pathways. Adiposity is associated with increases in both plasma volume and conversion of testosterone to E2. Each of these factors negatively influences serum PSA. DHT, dihydrotestosterone; E2, estradiol; PlasV, plasma volume; PSA, prostate specific antigen; SHBG, sex hormone-binding globulin

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Materials and methods

Study population and design

To study the effect of obesity on PSA as well as the underlying mechanisms, we used data from the Florey Adelaide Male Ageing Study (FAMAS) cohort. A full description of this cohort has been previously published (Martin *et al.* 2007). Briefly, 1195 urban community-dwelling men aged 35 years and over were enrolled and underwent baseline clinical assessments between 2002 and 2005. A second wave of clinical assessments was undertaken between 2007 and 2010, and 950 men returned.

There was a mean of 4.9 years between the two assessment waves. Men with prostate cancer ($n=109$ (9%)) and men with at least one PSA assessment of more than 4 ng/mL ($n=116$ (10%)), to ensure the exclusion of any undiagnosed prostate cancer cases, were excluded. For 710 men, PSA and other demographic factors were available at both assessment waves, while 260 men had only one assessment (Fig. 2). Supplementary Table 1 (see section on supplementary data given at the end of this article) presents the number of men assessed at each assessment wave and the number of non-missing data per variable and thereby the data included in the analysis cohort.

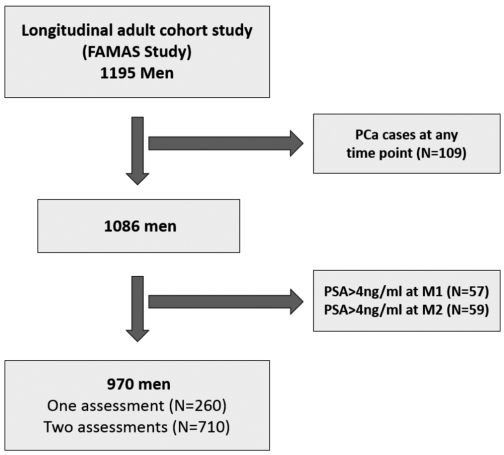

Figure 2
Study flow-chart presenting the number and reasons for inclusion/exclusion and the final analysis cohort.

Measures

Anthropometry measures (including weight, height and waist measurements as per Norton & Olds (2001)) and a fasting blood sample were taken twice, at the first baseline clinic visit and during the second wave of assessment. We included the following parameters from both assessment time points for our analysis: age at assessment (years), weight (kg), height (m), BMI (kg/m^2) and waist circumference (WC) (cm). In addition, we included the following blood investigation, which was taken after 8- to 12-h fasting (Martin *et al.* 2007); PSA (ng/mL), total testosterone (nmol/L), estradiol (E2) (pmol/L), dihydrotestosterone (DHT) (nmol/L) and sex hormone-binding globulin (SHBG) (nmol/L). Testosterone was measured using a validated stable-isotope dilution liquid chromatography–tandem mass spectrometry; E2 was measured using Immunolite I; PSA was measured using Abbott ARCHITECT. Plasma volume (PlasV) was calculated using Nadler's formula (Nadler *et al.* 1962): $\text{PlasV} = ((0.3669 \times (\text{height in meters})^3 + 0.03219 \times \text{weight in kg} + 0.6041) \times (1 - \text{hematocrit}))$. PSA-mass (μg) was calculated as the product of PSA and PlasV.

Statistical methods

Demographic summary statistics are reported as mean (s.d.) for continuous and frequency (%) for discrete variables unless otherwise stated, and compared between obesity groups (categorized by WC) using non-parametric Mann–Whitney tests for continuous variables and Fisher's exact test for discrete. Data from both assessment waves were included in the analyses of PSA. As the majority of individuals contributed two observations for both the outcome and the predictors, mixed-effect models were employed with a random intercept per individual. Non-linearity was included via restricted cubic splines with 3 degrees of freedom. After visual inspection of residual distributions, we log-transformed PSA and PSA-mass. PlasV was also log-transformed due to the assumed relation: $\log(\text{PSA}) = \log(\text{PSA-mass}) - \log(\text{PlasV})$. Being concentrations, we also log transformed the hormonal factors. To estimate obesity and hormonal associations with PSA, we constructed age-adjusted linear models.

Initially to explore the mechanistic action of obesity on PSA, we compared the magnitudes of associations of WC with adjustment for PlasV and the hormonal variables separately. Full multivariable mixed-effects linear models were constructed with age, WC, E2, testosterone and PlasV as predictors. Of note, the magnitude of effect of

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E2 and testosterone were equal but opposite, motivating the inclusion of the E2/testosterone ratio variable in all analyses. We used WC throughout due to it being more representative of abdominal fat as practically relevant methods to assess obesity. In the multivariable models missing data were imputed using multivariate imputation with chained equations (100 imputations). Sensitivity analyses for the multivariable models were performed by (i) a complete-case analysis, (ii) excluding WC, (iii) using BMI instead of WC, (iv) using the entire cohort without excluding those with PSA more than 4 ng/mL and (v) repeating the analyses using linear regressions of the cohort for each assessment wave separately. Mixed-effects coefficient of determination (R2) was calculated as per Jaeger *et al.* (2017). Finally, we considered a mechanistic model where adiposity affects PSA via either the plasma volume pathway or hormonal pathway (Fig. 1). Being controlled through a feedback regulatory axis, there was no concern that PlasV has any causal effect on hormonal levels (E2, testosterone, DHT); equally, there was no reason to believe that hormonal factors would have any causal effect on the plasma volume, nor that obesity in our cohort is a result of primary hormonal dysfunction. As such, we employed mediation analyses (Tingley *et al.* 2014) to estimate the direct effects of WC on PSA and the proportion associated with either plasma volume or the E2/testosterone ratio. These were complete case analyses adjusting for age. Linear associations are presented as estimated coefficient = β (95% CI). All statistical analyses were performed using R software (version 3.3.0, The R foundation for statistical computing, 2016).

Ethics

The FAMAS study protocol was approved by the Royal Adelaide Hospital Research Ethics committee and, where appropriate, the Aboriginal Health Research Ethics Committee of South Australia. Participants gave informed consent to be involved in the FAMAS study.

Results

The analysis cohort consisted of 970 men with a median age at accrual of 52 years (range 35–80). The mean (S.D.) baseline PSA concentration for the cohort was 1.0 (0.7) ng/mL, baseline BMI was 29 (4) kg/m^2, and baseline WC was 101 (12) cm (Table 1).

Table 1 Baseline demographic summary statistics.

			Non obese*	Obese*	Total	
			N = 577 (59%)	*N* = 393 (41%)	*N* = 970	*P*-value**
Age		Mean (S.D.)	53 (11)	55 (11)	53 (11)	0.005
		Median (range)	51 (35–80)	54 (35–79)	52 (35–80)	
Adiposity	WC (cm)	Mean (S.D.)	93 (7)	112 (9)	101 (12)	–
	BMI (kg/m^2)	Mean (S.D.)	26 (3)	32 (4)	29 (4)	<0.001
		Missing	1 (<1%)	1 (<1%)	2 (<1%)	
	PlasV (L)	Mean (S.D.)	2.8 (0.3)	3.2 (0.4)	3.0 (0.4)	<0.001
		Missing	6 (1%)	4 (1%)	10 (1%)	
PSA	PSA (ng/mL)	Mean (S.D.)	1.03 (0.70)	0.97 (0.67)	1.00 (0.69)	0.07
	PSA mass (μg)	Mean (S.D.)	2.8 (1.9)	3.0(2.1)	2.9 (2.0)	0.19
		Missing	6 (1%)	4 (1%)	10 (1%)	
Hormonal	Testosterone (nmol/L)	Mean (S.D.)	19 (7)	16 (7)	18 (7)	<0.001
		Missing	2 (<1%)	3 (<1%)	5 (<1%)	
	E2 (pmol/L)	Mean (S.D.)	89 (38)	99 (37)	93 (38)	<0.001
		Missing	5 (<1%)	5 (1%)	10 (1%)	
	DHT (nmol/L)	Mean (S.D.)	1.9 (0.8)	1.5 (0.9)	1.8 (0.9)	<0.001
		Missing	32 (5%)	46 (12%)	78 (8%)	
	E2/testosterone	Mean (S.D.)	5.1 (2.9)	7.0 (3.2)	5.9 (3.1)	<0.001
		Missing	5 (<1%)	5 (1%)	10 (1%)	
	SHBG (nmol/L)	Mean (S.D.)	37 (17)	31 (15)	35 (17)	<0.001
		Missing	2 (<1%)	0 (0%)	2 (<1%)	
Ethnicity		Caucasian	375 (65%)	254 (65%)	629 (65%)	0.005***
		Other	13 (2%)	0 (0%)	13 (1%)	
		Missing	189 (33%)	139 (35%)	328 (34%)	

*Obesity defined as WC ≥102 cm; ***P* value of Mann–Whitney tests; ****P* value of Fisher exact test.
DHT, 5α-dihydrotestosterone; E2/testosterone, estradiol-testosterone ratio; E2, estradiol; PlasV, plasma volume; PSA, prostate specific antigen; SHBG, sex hormone binding globulin; WC, waist circumference.

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Obesity

At baseline 307 men (32%) were obese (BMI ≥30 kg/m^2) of whom 32 (10%) had a WC less than 102 cm. Based on WC, 393 men (41%) were classified as obese (≥102 cm) of whom 117 (30%) had a BMI less than 30 kg/m^2. Both WC and BMI increased with age until roughly 60 years, and then decreased for older individuals (Supplementary Fig. 1).

In linear age-adjusted mixed-effect models, greater WC was associated with larger PlasV (β=0.0068, 95% CI=0.0064, 0.0073), higher E2 (β=0.0043, 95% CI=0.0026, 0.0060) and E2/testosterone (β=0.014, 95% CI=0.012, 0.016) and reduced testosterone (β=−0.097, 95% CI=−0.011, −0.008) and DHT (β=−0.010, 95% CI=−0.012, −0.008) (all P<0.001). Similar associations were observed for BMI (data not shown).

Prostate-specific antigen

Serum PSA levels were higher in older men and lower in obese men (Fig. 3). After age adjustment, negative associations with PSA were identified with PlasV (P=0.007) and both E2 (P=0.002) and E2/testosterone (P<0.001), and a positive association with testosterone (P=0.008), but not with DHT (P=0.18) (Table 2 and Supplementary Fig. 2). Of note, the magnitude of effect of E2 and testosterone was equal in opposite directions, suggesting that the magnitude of the ratio is related to PSA levels.

The magnitude of the association between PSA and obesity (in terms of either WC or BMI) was reduced when either PlasV or hormonal factors were adjusted for, with the greatest attenuation after adjustment for E2/testosterone. Adjustment for E2/testosterone and plasma volume led to attenuations of 48% and 18%, respectively (Supplementary Table 2). Notably, the negative association between E2/testosterone and PSA remained significant (both P<0.001) with minimal attenuation when adjusting for age and either BMI or WC. In multivariable mixed effects regressions, age and E2/testosterone provided the greatest explanatory value for PSA, with plasma volume providing minimal additional value (Model 2 Fig. 4A and Table 2). Repeating these analyses without WC (Fig. 4B), adjusting for SHBG or DHT or replacing WC with BMI did not affect the results (Supplementary Table 3). Including men with PSA more than 4 ng/mL or restricting to a complete-case analysis did not change the conclusions (data not shown). Repeating Model 2 (Table 2) for each assessment wave separately using linear regression model (Supplementary Fig. 3A and B) resulted in similar conclusions, albeit with a slightly stronger PlasV effect at the first assessment.

In a complete-case analysis of Model 2 a total of R^2=12.8% of the variance of PSA was explained, with age having the greatest effect (partial R^2=11.1%) followed by E2/testosterone (R^2=partial 1.3%), while plasma volume explained only 0.2% of the variance.

Mechanistic pathways

Fat mass and prostate volume increase with age in an average male (Vermeulen *et al.* 1999, Vesely *et al.* 2003). Higher levels of body fat result in both increased plasma volume (Woodard *et al.* 2012) and an increased E2/testosterone ratio (Gates *et al.* 2013). Of interest is whether hormonal and/or hemodilution (plasma volume) mediate the adiposity's effect in reducing PSA concentrations in obese men. In the mediation analyses with plasma volume as the mediator for WC on PSA (Table 3), the average causal mediation effect (ACME) was not significant (P=0.31; Fig. 4C), the point estimate suggesting roughly one-fifth of the total

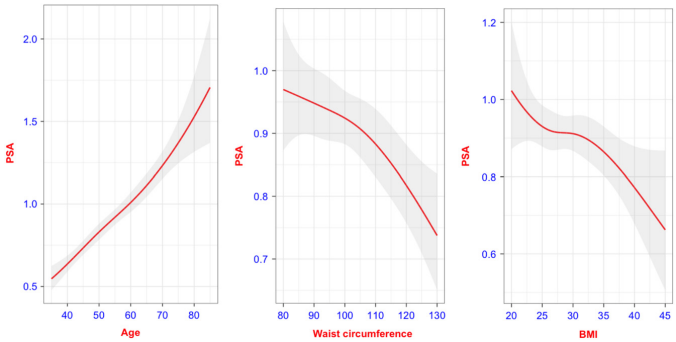

Figure 3
Non-linear mixed effect estimated PSA levels by age and adiposity. BMI, body mass index; PSA, prostate specific antigen (ng/mL); Waist circumference (cm). A full colour version of this figure is available at https://doi.org/10.1530/ERC-17-0438.

Table 2 Mixed-effect model estimates of (log transformed) PSA with age, WC, plasma volume and hormones.

		Age-adjusted associations		Model 1		Model 2	
		Coefficient (95% CI)	*P*-value	Coefficient (95% CI)	*P*-value	Coefficient (95% CI)	*P*-value
Age		0.022 (0.018, 0.025)*	<0.001	0.022 (0.019, 0.026)	<0.001	0.022 (0.019, 0.026)	<0.001
Adiposity	WC	−0.0050 (−0.0079, −0.0020)	0.001	−0.0018 (−0.0056, 0.0020)	0.36	−0.0017 (−0.0055, 0.0021)	0.36
	log(PlasV)	−0.36 (−0.62, −0.10)	0.007	−0.12 (−0.46, 0.21)	0.47	−0.12 (−0.45, 0.21)	0.49
Hormones	log(E2)	−0.13 (−0.20, −0.05)	0.002	−0.18 (−0.27, −0.09)	<0.001	–	–
	log(T)	0.11 (0.030, 0.20)	0.008	0.17 (0.07, 0.27)	0.001	–	–
	log(E2/testosterone)	−0.20 (−0.27, −0.12)	<0.001	–	–	−0.17 (−0.25, −0.10)	<0.001
	log(DHT)	0.05 (−0.03, 0.13)	0.18	–	–	–	–

PSA increases with age and after age-adjustment, increases with testosterone, and decreases with WC and plasma volume, E2 and E2/testosterone ratio. In the multivariable models including age, WC, E2, testosterone and PlasV (Model 1) and age, WC, E2/testosterone and PlasV (Model 2), there are no associations detected between PSA and either WC or plasma volume, while hormonal associations remain.
*Covariate unadjusted.
DHT, 5α-dihydrotestosterone; E2/testosterone, estradiol-testosterone ratio; E2, estradiol; PlasV, plasma volume; PSA, prostate specific antigen; WC, waist circumference.

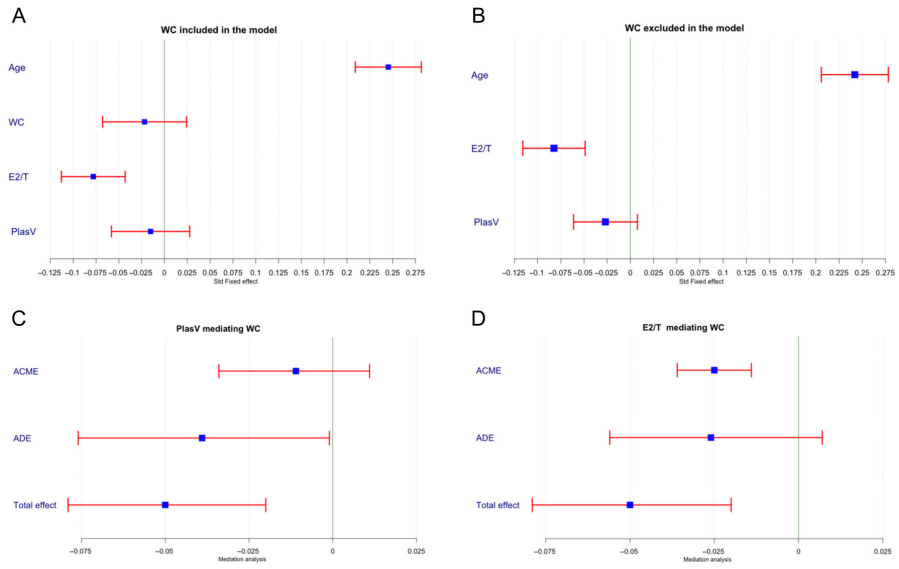

Figure 4
(A and B) Standardized mixed-effects regression coefficients for log transformed PSA concentration with (A) WC included in the model and (B) WC excluded from the model. After adjusting for age and the E2/testosterone ratio, there were no detectable associations between PSA and either WC or plasma volume. (C and D) Causal mediation estimates (including average causal mediation effects (ACME), average direct effects (ADE) and the total effects) estimating the contribution of adiposity (WC) to reduce PSA with mediation by either (C) plasma volume, or (D) E2/testosterone. E2/testosterone, estradiol-testosterone ratio; PlasV, plasma volume; WC, waist circumference. A full colour version of this figure is available at https://doi.org/10.1530/ERC-17-0438.

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Table 3 Causal mediation analyses.

	Estimate (95% CI)	*P*-value
PlasV mediating WC		
ACME	−0.0011 (−0.0034, 0.0011)	0.31
ADE	−0.0039 (−0.0076, −0.0001)	0.05
Total effect	−0.0050 (−0.0079, −0.0020)	<0.001
E2/testosterone mediating WC		
ACME	−0.0025 (−0.0036, −0.0014)	<0.001
ADE	−0.0026 (−0.0056, 0.0007)	0.12
Total effect	−0.0050 (−0.0079, −0.0020)	<0.001

The average causal mediation effect (ACME) of E2/testosterone ratio explains roughly one half of the total effect of obesity on PSA. In contrast, the ACME of the PlasV explains about one fifth of the total effect of obesity on PSA. Causal mediation analyses (including average causal mediation effects, average direct effects and the total effects) estimating the contribution of adiposity's (WC) influence on PSA mediation by plasma volume or E2/testosterone.
ACME, average causal mediation effect; ADE, average direct effect; E2/testosterone, estradiol-testosterone ratio; PlasV, plasma volume; WC, waist circumference.

effect of WC on PSA. In contrast with E2/testosterone as a mediator, the ACME suggested roughly one half of the effect ($P<0.001$; Fig. 4D). There was no evidence of mediation with DHT ($P=0.67$). Repeating the mediation models using variables and outcome from first and second assessment separately (Supplementary Fig. 3C, D, E and F) resulted in the same conclusion for E2/testosterone however similar to the mixed effects regressions, at Wave 1 there was evidence of mediation by PlasV, but not at Wave 2.

Discussion

In this study, we demonstrate that in men free of prostate cancer, serum PSA concentration is inversely associated with obesity, irrespective of the modality of assessment (either WC or BMI) and provide compelling evidence for mediation of this effect by hormonal factors.

Obesity is associated with increased plasma volume, which has been proposed to have a dilution effect, thereby decreasing serum PSA concentrations (Banez *et al.* 2007, Grubb *et al.* 2009). In a study by Banez *et al.* (2007), the decrease of PSA associated with obesity in 14,000 men with prostate cancer from three independent cohorts was attributed to haemodilution as a result of increasing the plasma volume. In that study, the non-significant change of PSA mass (PSA multiplied by the plasma volume) with obesity was used as evidence of the haemodilution effect. In agreement with this study, we observed strong associations between PSA concentration and both WC and BMI, and no equivalent significant associations with PSA mass in age-adjusted analyses (Supplementary Table 4). However, in contrast to Banez *et al.* (2007), we do not conclude that the PSA concentration–obesity association is solely due to hemodilution, but rather show that hormonal factors play a major role. We note that this conclusion is in agreement with the one-compartment PSA model (Supplementary Fig. 4). The steady-state solution indicates that PSA-mass is equal to the accumulation rate (prostate excretion) divided by the body's elimination rate multiplied by plasma volume. Hence, PSA mass, but not PSA concentration, is expected to be associated with plasma volume. In this model, the prostate excretion rate is the natural mechanism linking hormonal levels with both PSA mass and concentration. Replacing PSA with PSA mass in our multivariable regressions only changes the coefficient of PlasV, as expected, which changes from a minimal positive effect to a large negative effect as expected (Supplementary Table 4 and Fig. 5).

Using the REDUCE study cohort, Klaassen *et al.* (2017) concluded that testosterone and DHT are responsible for only 19% of the associated reduction in the PSA with obesity with the remaining effect due to hemodilution. In our study, we compared the effect of plasma volume, estradiol, testosterone, DHT and E2/testosterone in separate models (Supplementary Table 2). In our cohort, the E2/testosterone causes the greatest attenuation in the effect of obesity on PSA. Further, the hormonal changes associated with obesity (in the form of reduced testosterone, discordant E2 and thereby elevated E2/testosterone ratio) represented a substantial proportion of the decrease in the PSA associated with obesity, a result confirmed in our mediation analyses. Our findings are consistent with previous studies that show associations between PSA and hormones (Woodard *et al.* 2012, Peskoe *et al.* 2015, Usoro *et al.* 2015).

Prostate cancer detection accuracy has been improved with the use of the PSA/testosterone ratio (Gurbuz *et al.* 2012, Xu *et al.* 2018). In contrast, adjusting only for obesity in addition to PSA did not improve specificity (Oh *et al.* 2013, Banez *et al.* 2014, Vidal *et al.* 2015, Harrison *et al.* 2016). This may be due to the absence of adjustment for hormonal factors in those models. Hormonal factors play a critical role in prostate cancer development, for which obesity may be a poor surrogate.

Strong evidence exists regarding the association of obesity with elevated grade and advanced stage

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prostate cancers (Bonn *et al.* 2016, Zhong *et al.* 2016), as such it is a natural question as to whether the altered hormonal milieu, characteristic of increased obesity (reflected in the E2/testosterone ratio), contributes to more aggressive disease, potentially via direct hormonal effects on the prostate gland. It is equally possible that E2/testosterone is a surrogate for other hormonal processes influencing prostate function. Metabolic effects on the hormonal environment are very complex, and potentially influenced by other factors such as sex hormone-binding globulin (SHBG) (Moran *et al.* 2013). Adjusting for SHBG in our models however did not qualitatively change our final conclusions regarding E2/testosterone, nor were there significant associations between PSA and SHBG. The non-significant association observed between PSA and DHT may be attributed to the fact that serum DHT does not represent the true intra-prostatic DHT concentration (Cook *et al.* 2017).

A major strength of our study is that we not only examined the effect of obesity on PSA, but also directly explored two hypothesized causal mechanisms. Further, we assessed two independent measures of obesity, WC and BMI, and found little difference. We explored hormonal effects using different hormonal variables, namely testosterone, E2, DHT and E2/testosterone. Finally, we calculated the plasma volume by using hematocrit, which is considered a more accurate technique than estimation using weight and height only. Our study is limited by missing clinical data; our final cohort of 970 men may have included undiagnosed prostate cancer patients. Measures of prostate volume would have further improved our analyses, however, in its absence we assume that the observed strong age effects are in part due to the growth in prostate volume with age.

Conclusion

Observed lower PSA levels in obese men, as compared to normal weight men, can be explained by both hormonal changes (namely elevated E2/testosterone ratio) and possible hemodilution effects. As a substantial mediating pathway between obesity and PSA, hormonal factors should be considered in the development of models of obesity-dependent PSA levels.

Supplementary data

This is linked to the online version of the paper at https://doi.org/10.1530/ERC-17-0438.

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Declaration of interest

The authors have no conflicting interests to declare, other than: P Sutherland: Advisory board for Tolmar; Investigator on clinical trials for Pfizer, Bayer, Eli Lilly; Lectures fees for Tolmar, Pfizer, Bayer, Eli Lilly. G Wittert: Received research support from Bayer Schering, Eli Lilly, Resmed Foundation, Itamar, Siemens; Investigator on clinical trials for Roche, Pfizer, Astra Zeneca, Takeda, Boehringer, BMS, Amgen, Johnson and Johnson, MSD, GSK, Lawley; Lecture fees for Bayer Schering, Sanofi, Astra Zeneca, I-Nova, Roche, Abvie, Amgen, Novo Nordisk, Merck, Besins; Paid consultant to Elsevier, Lawley Pharmaceuticals; Independent Chair of the Weight Management Council of Australia; Editor-in-Chief of Obesity Research and Clinical Practice (Elsevier).

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Chapter 3. Obesity attenuates PSA based prostate cancer risk assessment

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Principle Author

Name of Principle Author (Candidate)	Adel Aref		
Contribution to the Paper	Conceptualization, project design, data collection, analysis and interpretation of the data, writing, review, revision of manuscript		
Overall percentage (%)	40% (contributed equally with Andrew D. Vincent)		
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	24/06/2019

Co-Author Contributions

Name of Co-Author	Andrew D. Vincent		
Contribution to the Paper	Conceptualization, project design, model design, analysis and interpretation of the data, writing, review, revision of manuscript and final approval of the manuscript		
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Name of Co-Author	Michael O'Callaghan		
Contribution to the Paper	Analysis and interpretation of the data, writing, review and revision of the manuscript, and final approval of the manuscript		
Signature		Date	25/6/2019

Name of Co-Author	Sean A. Martin		
Contribution to the Paper	Analysis and interpretation of the data, writing, review and revision of the manuscript, and final approval of the manuscript		
Signature		Date	24/6/2019

Name of Co-Author	Peter D. Sutherland		
Contribution to the Paper	Analysis and interpretation of the data, writing, review and revision of the manuscript, and final approval of the manuscript.		
Signature		Date	25/6/19

Name of Co-Author	Andrew J. Hoy		
Contribution to the Paper	Analysis and interpretation of the data, writing, review and revision of the manuscript, and final approval of the manuscript.		
Signature		Date	25/6/19

Name of Co-Author	Lisa M. Butler		
Contribution to the Paper	Conceptualization, project design, analysis and interpretation of the data, writing, review, revision of manuscript, final approval of the manuscript and acted as corresponding author		
Signature		Date	24/6/19

Name of Co-Author	Gary A. Wittert		
Contribution to the Paper	Conceptualization, project design, analysis and interpretation of the data, writing, review, revision of manuscript, final approval of the manuscript and acted as corresponding author		
Signature		Date	24/6/19

Title: Obesity attenuates PSA based prostate cancer risk assessment

Adel T. Aref*[1,2], Andrew D. Vincent*[1,2], Michael O'Callaghan[1,3], Sean A. Martin[1,2], Peter D. Sutherland[4], Andrew J. Hoy[5], Lisa M. Butler#[1,2] and Gary A. Wittert#[1,2]

[1]University of Adelaide Medical School and Freemasons Foundation Centre for Men's Health, Adelaide, SA 5005, Australia.

[2]South Australian Health and Medical Research Institute, Adelaide, SA 5001, Australia.

[3]Urology Unit, SA Health, Repatriation General Hospital, Adelaide, SA, Australia; South Australian Prostate Cancer Clinical Outcomes Collaborative (SA-PCCOC), Adelaide, SA, Australia; and Flinders Centre for Innovation in Cancer, Flinders University, Adelaide, South Australia

[4]Department of Urology, Royal Adelaide Hospital, Adelaide, SA 5000, Australia.

[5]Discipline of Physiology, School of Medical Sciences and Bosch Institute, Charles Perkins Centre, University of Sydney, NSW 2006, Australia.

* Co-authors, equal contribution.

Corresponding authors: Lisa M. Butler and Gary A. Wittert, University of Adelaide Medical School, South Australian Health and Medical Research Institute, Adelaide, SA 5000, Australia.

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Abstract

Background: Obesity is associated with lower levels of prostate specific antigen (PSA), which may influence interpretation of PSA test values.

Objective: To determine whether obesity attenuates risk estimates of PSA-derived screening models.

Participants: Two independent cohorts of prostate cancer (PCa)-free men were analyzed; the screening arm of the PLCO study (USA; N=23254; median age = 61 years), and the MAILES cohort (Australia; N=1120; median age = 55 years). Men who subsequently developed PCa, had a history of BPH, were missing PSA or BMI, or a baseline PSA ≥10ng/ml were excluded.

Outcome Measurements and Statistical Analysis: Quantile regressions were used to estimate the 50^{th}, 80^{th} and 95^{th} PSA-percentiles over both continuous and discrete age ranges, for each weight category. The effect of obesity on attenuating risk estimates for PCa was explored using PSA thresholds of 1, 2 or 3 ng/ml.

Results: In the PLCO cohort, obesity lead to lower 50^{th} and 80^{th} PSA-percentiles in the 55-59 and 60-65 year age-groups ($p<0.001$ in each) and a lower 95^{th} PSA-percentile in the 55-59 year age-group ($p= 0.04$). In the MAILES cohort, the effect of obesity was only detected in the 50-60 years age group ($p= 0.007, 0.003, 0.04$ for 50^{th}, 80^{th} and 95^{th} PSA-percentile respectively). Continuous-age quantile regressions in the PLCO cohort indicated that, men with severe obesity (BMI ≥ 35) were 5.2 years (95% CI =[3.3, 7.3]), 4.1 (95%CI= [0.5, 6.9]) and 4.9 (95%CI: [0.2, 9.1]) years older when attaining a PSA level of 1, 2 and 3 ng/ml respectively. In the MAILES cohort, men with obesity were 6.9 (95%CI = [0.2, 12.5]), 5.6 (95%CI = [-0.2, 11.1]) and 8.9 (95%CI=[2.9, 16.9]) years older, respectively, for the same PSA thresholds.

Conclusion: In two independent PCa-free cohorts, obesity leads to widening of PSA-derived screening intervals and potential underestimation of PCa risk.

Patient Summary

In this work, we have shown that obesity is associated with lower levels of PSA in the blood, in the test used for prostate cancer screening and diagnosis. We found that these lower levels could lead to underestimation of prostate cancer risk and potentially delay further diagnostic tests.

Conflicts of Interest: The authors have no conflicting interests to declare.

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Ethics

The PLCO protocol and participants' consents were reviewed and approved by the National Cancer Institute (NCI), the National Institutes of Health (NIH) Office of Protection from Research Risks, and the U.S. Office of Management and Budget. The MAILES study complies with the ethical standards outlined in the Australian Code for the Responsible Conduct of Research from the National Health and Medical Research Council. Ethics approval was obtained through the Royal Adelaide Hospital and The Queen Elizabeth Hospital Research Ethics Committees.

Introduction

Prostate cancer (PCa) is the most commonly diagnosed male cancer in developed countries (Fitzmaurice, C. et al. 2017). The prostate specific antigen (PSA) blood test is currently central to the initial diagnostic detection of PCa, but the interpretation of this test remains complex, with debate regarding the value of PSA screening (Pinsky, P. F. et al. 2012; Schröder et al. 2014) leading to varying testing protocols worldwide. Irrespective of the testing model employed, it is critical that key patient-specific modifiers of PSA levels are identified in order to accurately interpret the results of this widely-applied test.

There are multiple factors that influence PSA levels, the most important of which is age due to its association with prostate gland volume (Pinsky, P. F. et al. 2006; Vesely et al. 2003). Oesterling *et al* first introduced age-adjusted PSA thresholds in 1993 (Oesterling et al. 1993) and their model was subsequently validated in numerous patient cohorts (Guan et al. 2011; Luboldt, Schindler & Rübben 2007; Oesterling et al. 1995). Another factor influencing PSA is obesity, with multiple studies linking obesity to lower levels of PSA (Bonn et al. 2016; Woodard et al. 2012) and increased risk of advanced and aggressive PCa (Allott, Masko & Freedland 2013; Hu, M. B. et al. 2014; Xie et al. 2017). Recently, Chow *et al* (Chow et al. 2018) showed that obesity could lead to diagnostic bias, error in risk stratification and delay in diagnosis due to the lower levels of PSA at time of diagnosis.

Given the importance of accurate risk assessment, and the increasing prevalence of obesity, the aim of this study was to determine the effect of obesity on attenuating risk estimates of PSA-derived PCa screening models. Importantly, we performed these analyses in PCa-free men to exclude any potential cancer-related confounders.

Material and Methods

Study population

Two independent population-based cohorts were employed, the PCa screening arm of the "Prostate Lung Colon Ovarian (PLCO)" study, and the "Men Androgen Inflammation Lifestyle Environment and Stress (MAILES)" study cohort.

The prostate screening arm of the PLCO study included 38340 men with ages ranging between 55 and 75 years. The men were enrolled at 10 screening centers in the United States of America, over the period from 1993 to 2001. PSA testing was performed yearly for six years along with a digital rectal examination (DRE) every two years. Men were followed for at least 13 years for assessment of risk of PCa. Those with abnormal PSA or DRE were advised to follow up with their primary health care physicians for further investigations; however, there was no specific protocol as per the study design for further diagnostic workup. A full description of the study design is cited elsewhere (Prorok et al. 2000).

The MAILES study cohort is a population-based study established to explore associations between sex steroids, inflammation, environmental and psychological factors and risk of cardio-metabolic diseases in Australian men. The MAILES cohort consists of 2569 men from two studies, the Florey Adelaide Male Ageing Study and the North West Adelaide Health Study. Each participant in the MAILES study has received a detailed clinical assessment including anthropometry collection of a fasting venous blood sample for measurement of PSA in addition to a range of other assays that included sex steroids and markers of metabolism on two initial waves with around 4.9 years between each wave. The study has previously been described in detail (Grant et al. 2014).

Measures

In the PLCO study, baseline PSA tests were performed with a Hybritech Tandem-R assay, manufactured by Beckman-Coulter. Body Mass Index (BMI; self-reported) at study entry was recorded for each participant. In the MAILES study, PSA was measured using Abbott ARCHITECT©. The measurements including weight and height were assessed as per Norton & Olds.

The variables included in the analyses are baseline assessments of age, BMI in kg/m^2 and PSA in ng/ml. Weight groups were constructed based on the World Health Organization BMI classifications (normal weight from 18.5 to 24.9 kg/m^2, overweight 25 to 29.5 kg/m^2, obese I 30 to 34.9 kg/m^2, obese II 35 to 39.9 kg/m^2, obese III more than 40 kg/m^2). In the PLCO analyses the obese II and obese III groups were combined due to low prevalence of obese III men. For a similar reason weight groups in the MAILES cohort were defined as non-obese (BMI <30 kg/m^2) or obese (BMI ≥ 30 kg/m^2).

Statistical Methods

We report means (±SD) for continuous factors, and frequency and percentages for discrete demographic factors, unless otherwise stated. Differences in age, PSA levels and ethnicity between weight groups were assessed using linear-by-linear tests. The change in (log transformed) PSA by weight and age in each cohort was assessed using multivariable linear regressions. Quantile regression analyses were used to estimate 50^{th}, 80^{th} and 95^{th} PSA-percentiles among discrete age and weight categories. In the PLCO cohort, the age categories 55 to 59, 60 to 64, 65 to 69, and 70 to 75, and in the MAILES, 35 to 49, 50 to 59, 60 to 69 and 70 to 80 were employed. Subsequently age

was analysed as a continuous variable with nonlinearity modelled using restricted cubic splines of 2 degrees of freedom in the PLCO cohort, as this model showed the least Akaike information criterion (AIC) (Supplementary Table S1). Because the AIC for 3 models using 2, 3, or 4 degrees of freedom in the MAILES cohort analysis did not show great difference, we maintained 2 degrees of freedom for uniformity. The age-differences between men with specific PSA-quantile attaining PSA levels of 1ng/ml, 2ng/ml (the cut-off levels for detecting high risk group for screening and the frequency of screening), and 3ng/ml (the cut-off level to proceed for further investigation and biopsy) were estimated for each weight categories, with 95% confidence intervals for these age-differences were calculated using a 999 normal bootstrap. A sensitivity analysis was performed by repeating the analyses without excluding individuals with a PSA more than 10ng/ml. All statistical analyses were performed using R software (Version 3.3.0, The R foundation for statistical computing, 2016) using the "*coin*", "*quantreg"* and "*boot"* packages.

Results

The PLCO screening arm consists of 38340 men, of whom 23254 were included in the current analysis cohort. Excluded were those with PCa diagnosis either at enrolment or during follow up (n=4430), BPH (n=7937), younger than 55 years (n=3), missing either PSA (n=2399) or BMI (n=262) at baseline, or had a baseline PSA value of more than 10ng/ml (n=55) (Figure 1). The median age was 61 years (range 55-75), mean BMI was 27 kg/m^2 (±4.3), mean PSA was 1.4 ng/ml (±1.1). Obesity was associated with younger age and lower PSA (both $p<0.001$) (Table 1). The MAILES cohort consists of 2563 men, of whom 1120 were included in the analyses. Excluded were those with PCa diagnosis prior to accrual or in the subsequent follow up (n=227), BPH (n=1105), missing PSA (n=106) or BMI (n=2) at baseline, or a PSA more than 10ng/ml (n=3) (Figure 1). The median age was 55 years (range 35-80), mean BMI was 28.7 kg/m^2 (±4.7), mean PSA was 1.4 ng/ml (±1.4). Obesity was associated with lower PSA, however there was no detectable difference in age between obesity groups (Table 1).

Age-adjusted linear regressions indicated significant negative associations between mean (log-transformed) PSA and BMI in both the PLCO ($p<0.001$) and MAILES ($p<0.001$) cohorts (Supplementary Table S2). In the PLCO cohort, all obesity groups were associated with lower median (50^{th} percentile) PSA levels than normal weight men for all age groups (all $p<0.05$), except for men with severe obesity (obese II & III) aged 65-69 and 70-75 ($p= 0.22$ and $p=0.97$ respectively) (Table 2). Severe obesity was only associated with lower levels of the 80^{th} PSA-percentile in men less than 65 years of age ($p<0.001$). For the 95^{th} PSA-percentile, a reduction was only apparent in the youngest age group ($p=0.04$). For the MAILES cohort, the 50^{th}, 80^{th} and 95^{th} PSA-

percentiles decreased with obesity in the age group 50 to 59 (all $p<0.05$), with no detectable significant differences in the other age groups (Supplementary Table S3).
In the PLCO cohort, the 50^{th}, 80^{th} and 95^{th} PSA-percentile were 0.97, 1.8 and 3.5ng/ml respectively, while in the MAILES cohort, they were 0.9, 1.8 and 4.0 ng/ml respectively. These values closely approximate the PSA thresholds commonly used in PCa screening models (the 1, 2 and 3 ng/ml). Thus, we used these thresholds to explore the effect of obesity on the age at which men attained these PSA levels.

Quantile regression of continuous age indicated a 4.7-year (95%CI = [1.1, 8.2]) age difference between men with moderate obesity (obese I) and those with normal weight with median PSA equal to 1.0 ng/ml, and a 5.2 year difference (95%CI = [3.3, 7.3]) in those with severe obesity (obese II & III; Figure 2A). The same age-shift is apparent for the 80^{th} PSA-percentile (Figure 2B), as men with moderate and severe obesity were 2.0 years (95%CI = [-0.3, 4.4]) and 4.1 years (95%CI = [0.5, 6.9]) older to attain a PSA level of 2ng/ml respectively. Finally, there was a 4.9-year (95%CI = [0.2, 9.1]) difference between men with severe obesity to attain a PSA level of 3 ng/ml in comparison to men in other weight groups. These findings were also apparent in the MAILES cohort; men with obesity were 6.9 years (95%CI = [0.2, 12.4]), 5.6 (95%CI = [-0.2,11.1]) and 8.9 years (95%CI = [2.9, 16.9]) older than normal weight men to attain a PSA level of 1, 2 and 3-ng/ml respectively (Figure 3A-3C). The sensitivity analysis including the 58 men with PSA more than 10ng/ml resulted in qualitatively the same conclusions (data not shown).

Discussion

Our analysis of the 23254 men enrolled in the screening arm of the PLCO study shows that obesity can lead to a five-year delay in attaining a PSA threshold of 1 ng/ml, and a two to four-year delay in attaining 2ng/ml. Severe obesity leads to five-year age difference in attaining a PSA level of 3 ng/ml, thereby potentially delaying further diagnostic investigations. This effect was also seen in the independent MAILES cohort), differing in country of origin and decade of assessment. Together, these analyses demonstrate the potential consequences of obesity-mediated attenuation of PCa risk assessment models that rely on PSA test interpretation.

PSA testing remains the mainstay of PCa diagnosis and risk stratification, especially in high-risk populations. Theoretically, obesity may delay PCa diagnosis due to lower levels of PSA and/or the technical problems in performing DREs and biopsies (Bandini, Gandaglia & Briganti 2017; Nassar et al. 2018). Many studies have found obesity to be associated with lower overall incidence of PCa, but paradoxically with increased risk of aggressive PCa and PCa specific mortality (Discacciati, Orsini & Wolk 2012; Kelly, S. P. et al. 2017; Moller et al. 2016). This may be attributed to a delay in diagnosis that in turn misses early detection of low-grade cases. PCa specific mortality has been found to be primarily associated with pre-diagnosis obesity, rather than post-diagnosis obesity (Zhong et al. 2016), and to be confounded by the widespread adoption of PSA testing (Fowke et al. 2015). Although obesity is associated with other mechanisms that may lead to aggressive PCa biology (Allott, Masko & Freedland 2013; Ma, J et al. 2008), this does not explain why only pre-diagnosis obesity is associated with poorer PCa specific mortality.

The relationship between PSA, age and BMI is complex, as PSA increases with age and decreases with BMI, but BMI also decreases with age due to decreased muscle mass (Bonnefoy & Gilbert 2015). In our analysis, the change in PSA with age was greater in magnitude than that with obesity, which may explain why previous BMI-age-adjusted PSA models have not improved sensitivity for PCa detection (Harrison et al. 2016). Moreover, the lower age-adjusted PSA due to obesity was only clear in the severely obese group, a concern given the increasing prevalence of severe obesity in western countries (NCD Risk Factor Collaboration (NCD-RisC) 2016).

Our study provides a potential mechanism by which obesity could lead to a delay in proceeding to diagnostic investigation (based on the current clinically-recommended PSA level of 3ng/ml). This may partially explain the paradox between the associations of obesity with decreased localized PCa incidence and potentially lower overall incidence of PCa but a higher risk of advanced PCa (Allott, Masko & Freedland 2013; Discacciati, Orsini & Wolk 2012; Xie et al. 2017). The EAU-ESTRO-SIGO guidelines recommend PSA testing for men at risk, or those with PSA more than 1 ng/ml if aged less than 45 or PSA more than 2 ng/ml if aged 60 years, with a two year interval of screening (Mottet et al. 2017). According to the National Comprehensive Cancer Network guidelines 2018, for men at risk (those with age of 45 to 75 years), a two to four-year interval screening should be offered for those with baseline PSA less than 1 ng/ml, and a one to two-year interval for those with PSA levels between 1 and 3 ng/ml. For those with PSA more than 3 ng/ml, further investigations were recommended.

Notwithstanding the issues surrounding population-based screening, there exist high-risk groups of men who require more intensive screening for PCa (e.g. those with

strong positive family history of PCa), for whom a correct interpretation of the PSA test result is essential. We have shown that those men with severe obesity have approximately a five-year delay in attaining a PSA level of 1.0 ng/ml. As such, their risk could be underestimated and only a 'four or more years' PSA screening interval offered, as opposed to a one to two-year screening interval for normal weight men. Similarly, men with severe obesity took approximately four years longer to reach the PSA threshold of 2ng/ml.

Potential limitations of our study include the lack of a uniform protocol for PCa diagnosis in the PLCO study, which may underestimate the risk of PSA-derived PCa diagnosis in this cohort. In addition, PCa cases in the MAILES cohort were reported during follow up but there was no initial protocol to exclude PCa cases at baseline assessment. Thus, there may also be undiagnosed cases in this cohort. We have attempted to overcome this problem by excluding those with PSA of more than 10ng/ml, however this will not eliminate all undiagnosed PCa cases. Secondly, the MAILES cohort was too small to categorize obesity into three groups, as for the PLCO cohort. It is unclear whether the observed increased delays in the MAILES cohort are an overestimation due to small sample sizes, or a true population-specific effect. Despite the large size of the PLCO cohort, there are few elderly men in the obese group. Notwithstanding these potential limitations, our study is the first to our knowledge to report weight and age-specific PSA-quantiles in a PCa-free population. We also provide evidence for attenuation in PCa risk assessment due to obesity in two independent cohorts; this attenuation has potential to tangibly delay diagnosis of PCa. These data suggest the need for BMI- in addition to age adjusted PSA cut-offs for defining PCa risk with prospective evaluation to determine the impact on PCa outcomes.

Conclusion: In two independent PCa-free cohorts, obesity is associated with significantly lower PSA levels, potentially widening the screening interval and leading to an underestimation of PCa risk. BMI should be considered when interpreting PSA results, especially in men with severe obesity.

Tables

Table 1: Demographic summary statistics for the PLCO and MAILES cohorts.

PLCO		Normal weight	Overweight	Obese I	Obese II & Obese III	All	p-value
		N = 5968	N = 11538	N = 4437	N = 1311	N = 23254	
Age (years)	Median (range)	62 (55-75)	62 (55-74)	61 (55-74)	60 (55-74)	61 (55-75)	<0.001
BMI (kg/m^2)	Mean	23.1	27.2	31.9	38.4	27.7	--
	SD	1.5	1.4	1.4	3.6	4.3	
PSA (ng/ml)	Mean	1.36	1.32	1.24	1.11	1.30	<0.001
	SD	1.12	1.12	1.1	1.02	1.11	
Race	White Non-Hispanic	5061 (85%)	10304 (89%)	4007 (90%)	1184 (90%)	20556 (88%)	<0.001
	Asian	511 (9%)	449 (4%)	75 (2%)	15 (1%)	1050 (5%)	
	Black Non-Hispanic	250 (4%)	412 (4%)	206 (5%)	75 (6%)	943 (4%)	
	Others	145 (2%)	364 (3%)	145 (3%)	37 (3%)	691 (3%)	
	Unknown	1 (<1%)	9 (<1%)	4 (<1%)	0 (0%)	14 (<1%)	
MAILES		Non Obese		Obese		All	p-value
		N = 756		N = 364		N = 1120	
Age(Years)	Median (range)	55 (35- 80)		55 (35- 80)		55 (35- 80)	0.41
BMI (Kg/m^2)	Mean (SD)	26.2 (2.5)		34.0 (3.8)		28.7 (4.7)	--
PSA (ng/ml)	Mean (SD)	1.38 (1.38)		1.15 (1.16)		1.31 (1.32)	0.004

Difference in age, PSA and race between weight groups was assessed using linear by linear tests. PSA: Prostate specific antigen, BMI: Body mass index. World Health Organization (WHO) BMI classifications (normal weight: from 18.5 to 24.9 kg/m^2, overweight: 25 to 29.5 kg/m^2, obese I: 30 to 34.9 kg/m^2, obese II: 35 to 39.9 kg/m^2, obese III: more than 40 kg/m^2. Weight groups were defined as non-obese (BMI <30 kg/m^2) or obese (BMI ≥30 kg/m^2).

Table 2: Observed PSA percentiles and quantile regression estimated 95% confidence intervals for the PLCO cohort.

		55 to 59				60 to 64				65 to 69				70 to 75			
		N	PSA	95% CI	p-value	N	PSA	95% CI	p-value	N	PSA	95% CI	p-value	N	PSA	95% CI	p-value
50th Percentile	Normal	2443	0.91	[0.88,0.94]	--	1701	1.00	[0.95,1.10]	--	1261	1.21	[1.14,1.29]	--	563	1.36	[1.21,1.53]	--
	Overweight	4966	0.90	[0.88,0.92]	0.62	3493	1.01	[0.98,1.04]	0.76	2206	1.12	[1.07,1.17]	0.04	873	1.29	[1.2,1.38]	0.43
	Obese I	2215	0.84	[0.81,0.88]	0.003	1232	0.90	[0.85,0.95]	0.008	734	1.07	[0.99,1.16]	0.01	256	1.08	[0.88,1.33]	0.05
	Obese II&III	700	0.72	[0.68,0.77]	<0.001	352	0.74	[0.67,0.81]	<0.001	193	1.13	[1.03,1.24]	0.22	66	1.39	[1.0,1.87]	0.97
80th Percentile	Normal	2443	1.66	[1.59,1.73]	--	1701	1.92	[1.83,2.02]	--	1261	2.28	[2.16,2.41]	--	563	2.65	[2.42,2.9]	--
	Overweight	4966	1.60	[1.55,1.65]	0.16	3493	1.87	[1.80,1.94]	0.41	2206	2.17	[2.07,2.27]	0.17	873	2.44	[2.3,2.61]	0.15
	Obese I	2215	1.56	[1.49,1.63]	0.05	1232	1.78	[1.67,1.9]	0.07	734	2.15	[1.99,2.35]	0.28	256	2.24	[2.04,2.46]	0.01
	Obese II & III	700	1.40	[1.29,1.52]	<0.001	352	1.41	[1.24,1.63]	<0.001	193	2.00	[1.66,2.45]	0.23	66	2.49	[1.19,5.22]	0.87
95th Percentile	Normal	2443	3.01	[2.79,3.25]	--	1701	3.37	[3.16,3.59]	--	1261	3.96	[3.65,4.29]	--	563	4.36	[3.8,4.98]	--
	Overweight	4966	2.99	[2.84,3.15]	0.89	3493	3.46	[3.32,3.62]	0.44	2206	3.90	[3.63,4.19]	0.78	873	4.63	[4.3,4.99]	0.45
	Obese I	2215	3.07	[2.84,3.35]	0.68	1232	3.56	[3.28,3.91]	0.27	734	3.84	[3.36,4.41]	0.72	256	3.64	[3.28,4.04]	0.03
	Obese II & III	700	2.64	[2.42,2.92]	0.04	352	2.98	[2.66,3.39]	0.09	193	3.64	[2.73,5.12]	0.73	66	4.53	[2.29,9.31]	0.88

N: Sample size of each group, PSA: Prostate specific antigen.

World Health Organization (WHO) BMI classifications (normal weight: from 18.5 to 24.9 kg/m^2, overweight: 25 to 29.5 kg/m^2, obese I: 30 to 34.9 kg/m^2, obese II: 35 to 39.9 kg/m^2, obese III: more than 40 kg/m^2)

Supplementary tables

Table S1: AIC of quantile regression models using 2,3, or 4 degrees of freedom

Degree of freedom	AIC		
PLCO	**50th percentile**	**80th percentile**	**95th percentile**
Df=2	54261	58714	68716
Df=3	54266	58721	68718
Df=4	54266	58721	68719
MAILES	**50th percentile**	**80th percentile**	**95th percentile**
Df=2	2571	2776	3222
Df=3	2570	2770	3207
Df=4	2566	2772	3208

Table S2: Multivariable linear regressions of log transformed PSA with Age and BMI; demonstrate negative associations between PSA and BMI in both the PLCO and MAILES cohort.

	PLCO data		MAILES data	
	Estimate [95% CI]	p-value	Estimate [95% CI]	p-value
Age	0.019 [0.017, 0.021]	<0.001	0.025 [0.021, 0.029]	<0.001
BMI	-0.014 [-0.017, -0.012]	<0.001	-0.021 [-0.030, -0.012]	<0.001

- *PSA: Prostate specific antigen, BMI: Body mass index*

Table S3: Observed PSA percentiles and quantile regression estimated 95% confidence intervals for the MAILES cohort. The 50th, 80th and 95th PSA percentile are decreasing with obesity in the age group 50 to 60 (all p<0.05).

N: population of each group, PSA: Prostate specific antigen, P: p-value of quantile regression model.

		35 to 49				50 to 59				60 to 69				70 to 80			
		N	PSA	95% CI	P	N	PSA	95% CI	P	N	PSA	95% CI	P	N	PSA	95% CI	P
50th percentile	Non Obese	271	0.67	[0.61,0.74]	--	210	1.00	[0.89,1.13]	--	165	1.00	[0.85,1.18]	--	100	1.90	[1.51,2.39]	--
	Obese	123	0.69	[0.56,0.85]	0.80	123	0.78	[0.68,0.89]	0.007	79	1.10	[0.84,1.44]	0.54	35	1.40	[1.15,1.71]	0.04
80th percentile	Non Obese	271	1.20	[1.04,1.39]	--	210	1.80	[1.66,1.95]	--	165	2.40	[1.84,3.13]	--	100	3.86	[3.04,4.75]	--
	Obese	123	1.20	[1.08,1.34]	1.00	123	1.30	[1.06,1.59]	0.003	79	2.94	[1.96,4.58]	0.37	35	2.20	[1.15,4.21]	0.11
95th percentile	Non Obese	271	2.20	[1.75,3.02]	--	210	3.02	[2.38,4.31]	--	165	4.98	[3.85,6.49]	--	100	6.96	[5.41,11.8]	--
	Obese	123	1.78	[1.53,2.12]	0.12	123	1.99	[1.41,2.85]	0.04	79	4.81	[4.20,8.02]	0.48	35	5.76	[3.83,13.9]	0.81

Weight groups were defined as non-obese (BMI <30 kg/m^2) or obese (BMI ≥30 kg/m^2).

Figure and figures legends

Figure 1

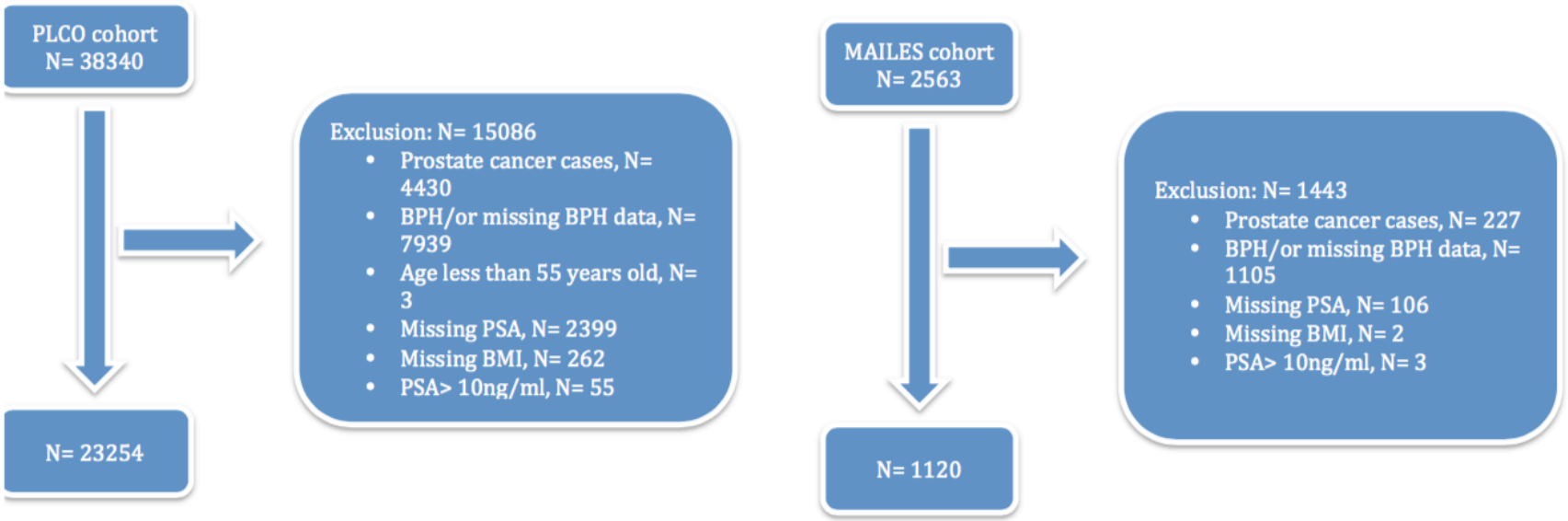

Figure 1. Flow diagram of accrual and exclusion. PLCO: Prostate Lung Colon Ovarian study; MAILES: Men Androgen Inflammation Lifestyle Environment and Stress study.

Figure 2

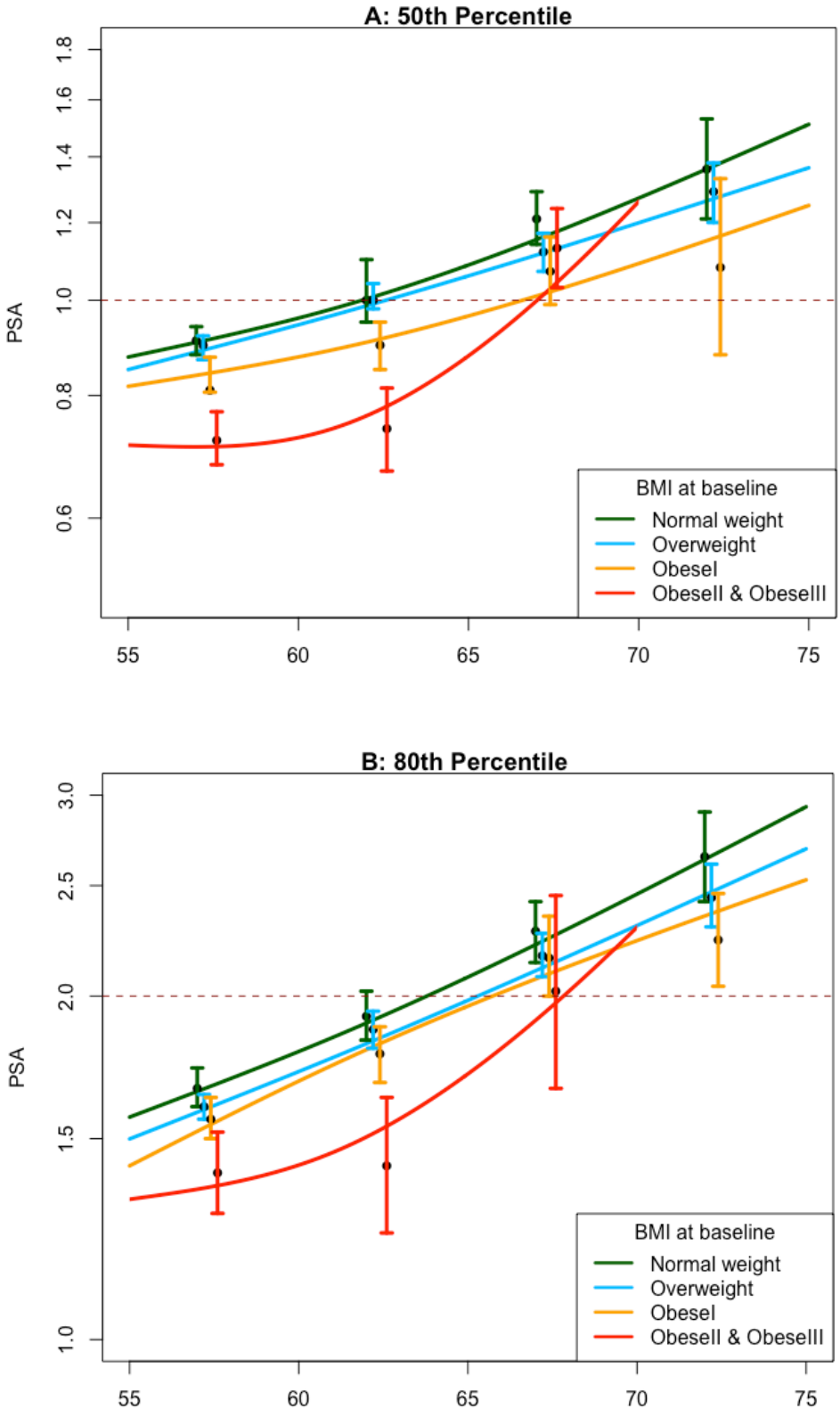
A: 50th Percentile
PSA
0.6
0.8
1.0
1.2
1.4
1.6
1.8
55
60
65
70
75
BMI at baseline
Normal weight
Overweight
Obesel
ObeselI & ObeselII
B: 80th Percentile
PSA
1.0
1.5
2.0
2.5
3.0
55
60
65
70
75
BMI at baseline
Normal weight
Overweight
Obesel
ObeselI & ObeselII

Figure 2 continue

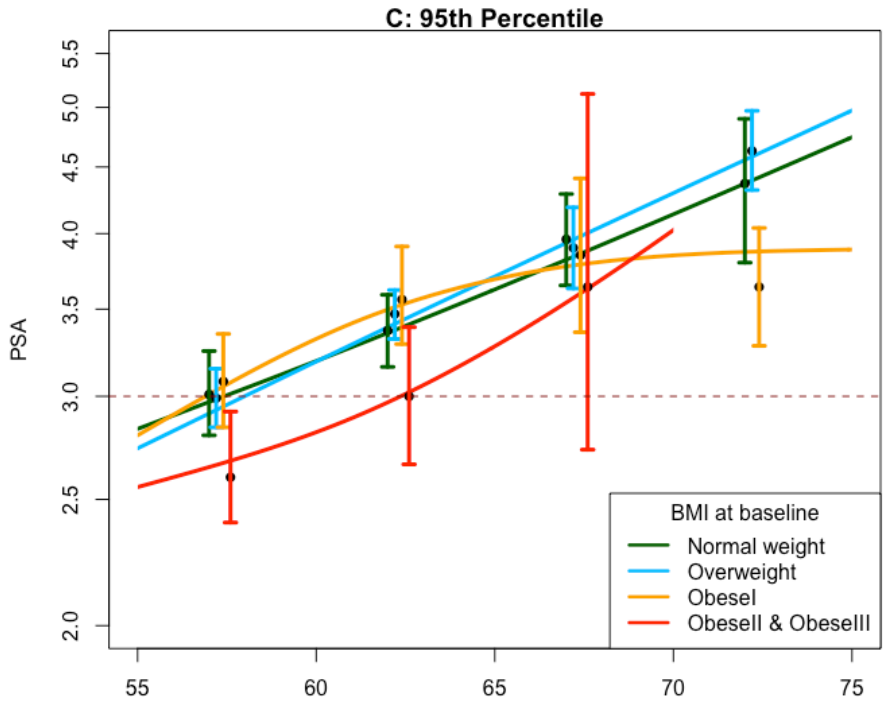

Figure 2. Quantile regression estimated PSA 50th, 80th and 95th percentiles by age for specific weight groups in the PLCO cohort. Severe obesity estimates are truncated at 70 years of age due to sample size. Horizontal dashed lines reflect the 1, 2 and 3 ng/mL PSA thresholds, respectively, and the point estimates and associated 95% confidence intervals are for discrete age categories as per Table 2. *World Health Organization (WHO) BMI classifications (normal weight: from 18.5 to 24.9 kg/m^2, overweight: 25 to 29.5 kg/m^2, obese I: 30 to 34.9 kg/m^2, obese II: 35 to 39.9 kg/m^2, obese III: more than 40 kg/m^2).*

Figure 3

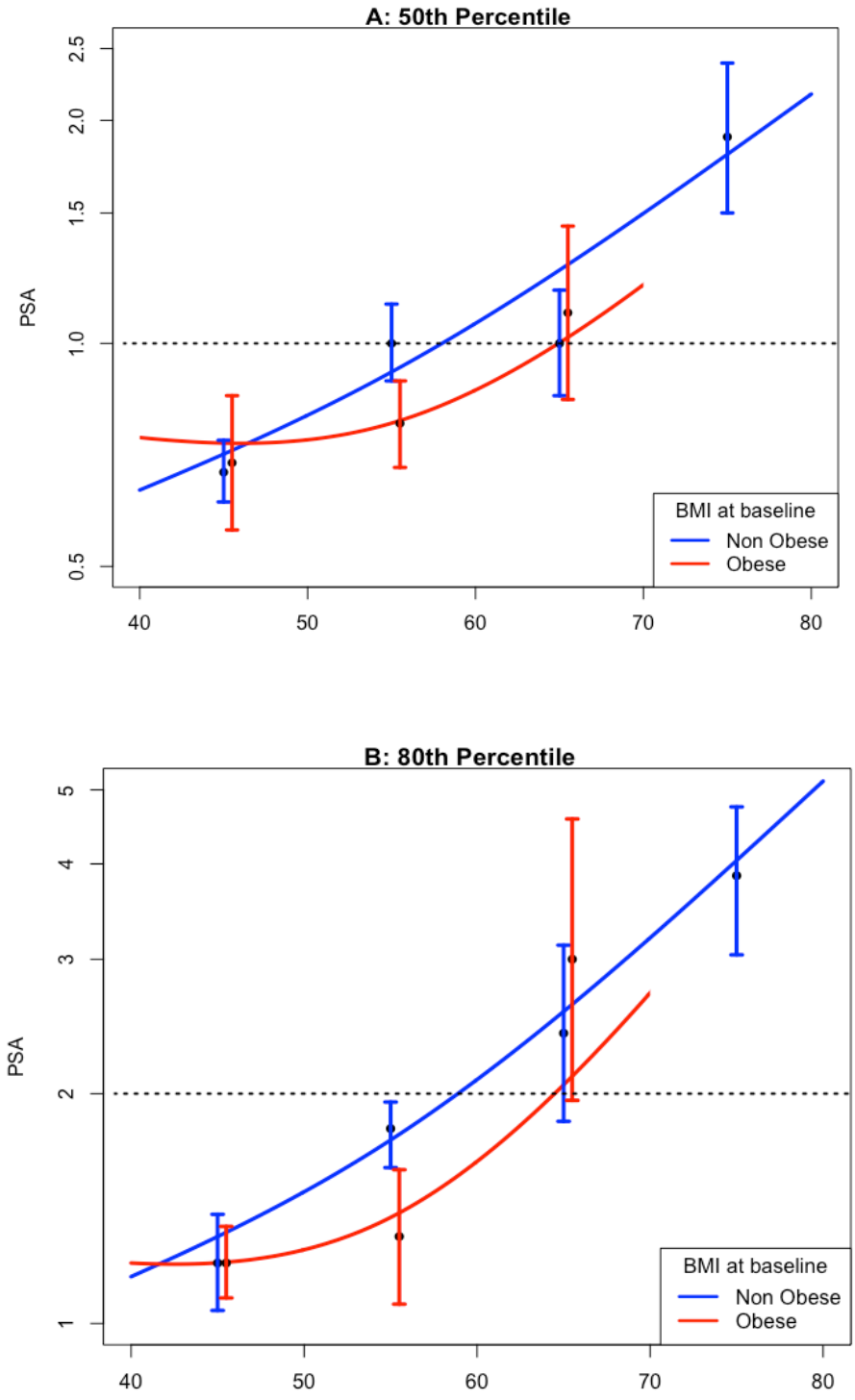
A: 50th Percentile
PSA
0.5
1.0
1.5
2.0
2.5
40
50
60
70
80
BMI at baseline
Non Obese
Obese
B: 80th Percentile
PSA
1
2
3
4
5
40
50
60
70
80
BMI at baseline
Non Obese
Obese

Figure 3 continued

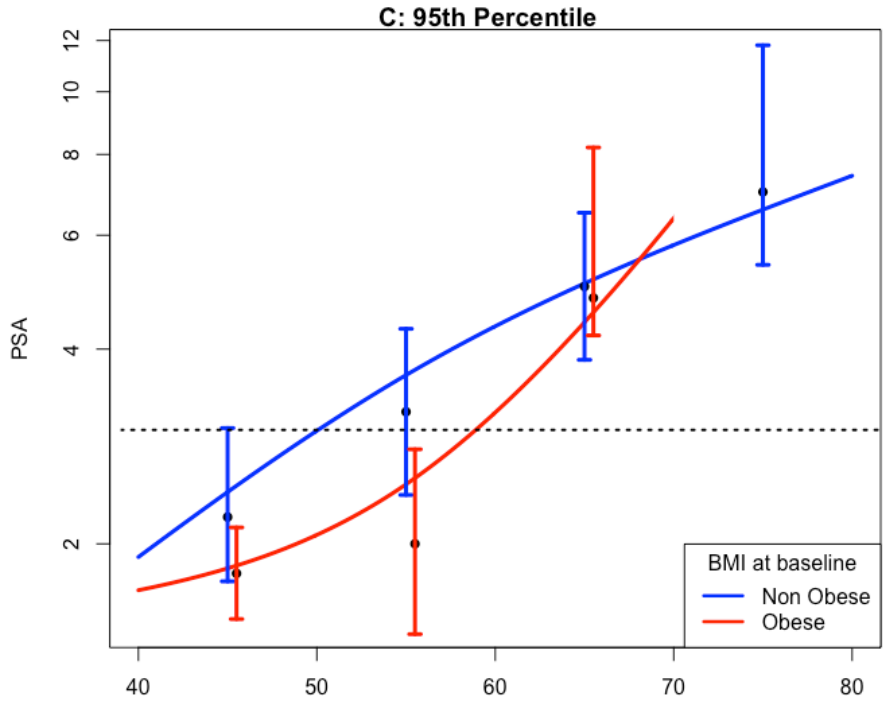

Figure 3. Quantile regression estimated PSA 50th, 80th and 95th percentiles for age for specific weight groups in the MAILES cohort. Obese estimates are truncated at 70 years of age due to sample size. Horizontal dashed lines reflect the 1, 2 and 3 ng/mL PSA thresholds, respectively, and the point estimates and associated 95% confidence intervals are for discrete age categories as per Supplementary Table S2.

Chapter 4. Prostate cancer screening may reduce prostate cancer specific mortality among metabolically healthy men

Statement of Authorship

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Publication Status	☐ Published ☐ Accepted for Publication ☐ Submitted for Publication ☒ Unpublished and Unsubmitted work written in manuscript style
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Principle Author

Name of Principle Author (Candidate)	Adel Aref		
Contribution to the Paper	Conceptualization, project design, data collection, analysis and interpretation of the data, writing, review, revision of manuscript		
Overall percentage (%)	60%		
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	14/08/2019

Co-Author Contributions

Name of Co-Author	Andrew D. Vincent		
Contribution to the Paper	Conceptualization, project design, analysis and interpretation of the data, writing, review, revision of manuscript and final approval of the manuscript		
Signature		Date	15/08/2019

Name of Co-Author	Michael O'Callaghan		
Contribution to the Paper	Analysis and interpretation of the data, writing, review and revision of the manuscript, and final approval of the manuscript		
Signature		Date	14/08/2019

Name of Co-Author	Andrew J. Hoy		
Contribution to the Paper	Analysis and interpretation of the data, writing, review and revision of the manuscript, and final approval of the manuscript.		
Signature		Date	14/08/2019

Name of Co-Author	Lisa M. Butler		
Contribution to the Paper	Conceptualization, project design, analysis and interpretation of the data, writing, review, revision of manuscript, final approval of the manuscript and acted as corresponding author		
Signature		Date	14/08/2019

Name of Co-Author	Gary A. Wittert		
Contribution to the Paper	Conceptualization, project design, analysis and interpretation of the data, writing, review, revision of manuscript, final approval of the manuscript and acted as corresponding author		
Signature		Date	14/08/2019

Title: Prostate cancer screening may reduce prostate cancer specific mortality among metabolically healthy men

Adel T. Aref[1,2], Andrew D. Vincent[1,2], Michael O'Callaghan[1,3], Andrew J. Hoy[4], Lisa M. Butler#[1,2] and Gary A. Wittert#[1,2]

[1]University of Adelaide Medical School and Freemasons Foundation Centre for Men's Health, Adelaide, SA 5005, Australia.

[2]South Australian Health and Medical Research Institute, Adelaide, SA 5001, Australia.

[3]Urology Unit, SA Health, Flinders Medical Centre, Adelaide, SA, Australia; South Australian Prostate Cancer Clinical Outcomes Collaborative (SA-PCCOC), Adelaide, SA, Australia; and Flinders Centre for Innovation in Cancer, Flinders University, Adelaide, South Australia

[4]Discipline of Physiology, School of Medical Sciences and Bosch Institute, Charles Perkins Centre, University of Sydney, NSW 2006, Australia.

Corresponding authors: Lisa M. Butler and Gary A. Wittert, University of Adelaide Medical School, South Australian Health and Medical Research Institute, Adelaide, SA 5000, Australia.

Short title: Metabolic syndrome and Prostate cancer screening

Abstract word count:

Text word count:

Key words: Prostate cancer screening, Metabolic syndrome, Obesity, prostate cancer specific survival, prostate cancer, Diabetes, Hypertension.

Abstract

Background

The current prostate cancer (PCa) screening recommendations are for an individualized approach. As PCa and metabolic comorbidities are highly prevalent diseases among the elderly, it has been suggested that PCa screening benefit may be masked because of a competing-risk effect.

Aim of the study

To explore whether there is evidence for a reduction in prostate cancer-specific mortality (PCSM) due to screening among men with no metabolic syndrome related conditions.

Study population & definitions

Men from the Prostate, Lung, Colon, Ovary (PLCO) screening study with data regarding history of having hypertension, diabetes, and/or obesity were included (analysis cohort; n=72120).

Methods

Time dependent Cox proportional hazard and Fine and Gray models with PCSM as outcome were used to assess interactions between PCa screening and the presence of metabolic syndrome related factors (obesity, diabetes and/or hypertension). These factors were assessed at baseline and again at (median) nine years post-randomization. Non-PCa mortality events were censored at date of death. Two summations of metabolic status were considered: (i) men were considered metabolically unhealthy if they had any one of the metabolic syndrome conditions (binary predictor), and (ii) the number (0-3) of the three separate conditions as a continuous linear predictor.

Results

The mean age at randomization was 63 (±5.3), at baseline 23% were obese, 10% had diabetes and 36% were hypertensive. In the time dependent Cox proportional hazard models, there was evidence for an interaction between the presence of any metabolic syndrome related conditions and screening (p=0.01), but not when the number of conditions was used (p=0.13). In the first model, screening reduced PCSM in metabolically healthy men (HR=0.75, 95%CI=[0.61, 0.92], p=0.01), but not in metabolically unhealthy men (HR=1.07, 95%CI=[0.89, 1.28], p=0.48). Although the interaction was weaker in the second model, there was still evidence that screening reduced PCSM in men without metabolic conditions (HR=0.82, 95%CI=[0.68, 0.99], p=0.04). Similar results were observed in Fine and Gray models.

Conclusion

PCa screening appears more effective among men with no metabolic syndrome related conditions in reducing prostate cancer specific mortality.

Introduction

The efficacy of screening in reducing prostate cancer specific mortality (PCSM) is an area of current debate. The U.S. Preventive Services Task Force (USPSTF) changed its recommendation recently to grade C (advocating for an individualized approach to screening) (Grossman et al. 2018). Thus identifying factors that could improve the outcome of PCa screening is essential. The 17 year follow-up mortality data report for PCa in the Prostate, Lung, Colon, and Ovary Screening trial (PLCO) showed no detectable reduction of PCSM with PCa screening, despite reporting a reduction in the percentage of high Gleason score (≥8) cases (Pinsky, P. F. et al. 2018).

As PCa is a highly prevalent disease among the elderly, an age group with elevated rates of comorbidity (mainly metabolic and cardiovascular related), it was suggested that PCa screening effect is not detected simply because men are dying from other causes, the competing risk hypothesis (Matthes et al. 2018). An alternative hypothesis is that the presence of metabolic related comorbidities may lead to more aggressive PCa and thus increase risk of PCSM (Xiang et al. 2013), or that metabolically healthy men are offered more effective treatment (Aizer et al. 2014).

An analysis of the 10 year follow-up data from the PLCO study detected an interaction between comorbidities and PCa screening in which men with no or minimal comorbidities benefited more from screening, with a reduction in their PCSM risk (Crawford et al. 2011). However in the 13-year mortality report of the PLCO no interaction between comorbidities and PCa screening was detected (Andriole, G. L. et al. 2012). It is unclear whether this discrepancy is due to the 10-year result being a spurious association or due to the difference between the two analyses regarding the type of comorbidities included. If there is an interaction,

whether some or all of metabolic syndrome comorbidities should be considered is an unanswered question.

Metabolic syndrome is a cluster of metabolic conditions including central obesity, diabetes, hypertension and dyslipidaemia, collectively the most prevalent cluster of comorbidities in Western countries (Saklayen 2018). Metabolic syndrome increases the risk of the development of numerous of metabolic and non-metabolic conditions including cardiovascular disease as well as numerous cancers (O'Neill & O'Driscoll 2015). Obesity and diabetes are also associated with lower PSA levels and lower incidence of low-grade PCa (Aref et al. 2018; Banez et al. 2007; Bonn et al. 2016; Dankner et al. 2016; Sarma et al. 2015). One explanation for these negative associations is a delay in PCa diagnosis due to the reduced PSA levels (Chow et al. 2018; Dankner et al. 2016). This may also partially explain the positive association between obesity and advanced stage PCa (Fang et al. 2018; Xie et al. 2017) and PCSM (Cao, Y & Ma 2011; Zhong et al. 2016). Thus hypothetically having one or more of these conditions may attenuate the efficacy of PCa screening.

The aim of this study is to explore whether there is evidence for a reduction in PCSM due to screening among metabolically healthy (non-diabetic, non-hypertensive, and non-obese) men using the extended 17-year follow up data of the PLCO study.

Material and Methods

Study population

Our study cohort is the PLCO screening and control arm consisting of 76635 men aged between 49 and 75 years. Over the period 1993 to 2001 men were enrolled at 10 screening centers across the United States of America. PSA testing was performed annually for six years along with a digital rectal examination (DRE) annually for four years. Men were followed for a median of 17 years for assessment of PCSM. Those with abnormal PSA or DRE were advised to follow up with their primary health care physicians for further investigations. During this 17-year period 8334 men were diagnosed with PCa. A full description of the study design is reported Prorok, PC *et al.* (Prorok et al. 2000).

Definition of metabolic syndrome factors

The baseline questionnaire of the PLCO study included current BMI, history of hypertension and history of diabetes. Unfortunately dyslipidemia was not recorded at baseline, as such we were not able to use the exact definition of metabolic syndrome as per the Adult Treatment Panel III from the National Cholesterol Education Program (NCEP-ATPIII) ('Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report' 2002), but rather we used the occurrence of one or more of the following conditions: hypertension, diabetes and/or obesity. A follow up questionnaire (median) nine years post baseline reassessed the presence and/or development of any of the previously mentioned three conditions.

Analysis Cohort

Out of the 76635 men included in the screening and control arms of the PLCO we excluded men who were missing baseline data regarding race, BMI, hypertension, diabetes and/or follow-up mortality data. The remaining 72120 men were included in the analysis cohort (Figure 1). Out of the analysis cohort, 42280 men have been re-assessed with a follow up questionnaire after median of nine years (Figure 1).

Statistical methodology

Unless otherwise stated means (SD) and frequencies (percentages) are reported for demographic variables as appropriate. Time dependent Cox proportional hazards models with PCSM as outcome were used to assess interactions between screening and the presence of metabolic syndrome related factors. Two time points for the assessment of metabolic conditions were identified, at baseline and at the time of the follow up questionnaire. Four models were constructed, model 0; with no interaction, model 1; includes all the metabolic factors as three separate factors (diabetes, hypertension and obesity), model 2; includes metabolic syndrome related factors as one factor (having one or more of the following conditions, diabetes, hypertension and/or obesity- yes versus no) and model 3; which includes metabolic syndrome as a sum (0-3) of diabetes (0-1), hypertension (0-1) and obesity (0-1). In these models we assessed the interaction between screening with (i) all three conditions simultaneously (i.e. three pairwise interactions with screening); (ii) having any of diabetes and/or hypertension and/or obesity (i.e. a single interaction with this composite measure); and for completeness (iii) the number of metabolic syndrome factors as a linear score (0-3). A sensitivity analysis confirming the findings of the time dependent models consisted of (non-time dependent) multivariable Cox

proportional hazards models for PCSM that assessed the interaction between screening and presence of metabolic syndrome conditions either solely at baseline or at the 9-year follow-up assessment. In the time-dependent models and in the baseline-only (non-time dependent) model survival duration was from date of randomization until date of last contact. In the analysis of using only 9-year follow-up data, the survival duration was from date of the follow-up assessment until date of last contact. All models were adjusted for age at randomization (continuous linear variable) and race (black non-Hispanic versus other). Men who were alive at time of last contact or died due to non-PCa causes were censored at date of death. Proportional hazard assumptions were assessed by examining Martingale residuals of time varying coefficients (Grambsch & Therneau 1994). The time dependent model was repeated using a multivariable Fine and Gray regression with the same definitions for survival duration, but with death by non-PCa causes considered as a competing risk instead of being censored. Cumulative incidence of PCSM with/without screening was presented for men without any of the three metabolic factors vs. men with at least one, both for metabolic assessments at baseline and at follow-up.

To assess the effect of metabolic syndrome and its component factors on PCa incidence and other cause mortality (dying from causes other than PCa time dependent multivariable Cox proportional hazard regression models were constructed. Similar to the PCSM models above these models adjusted for age and race, and the at-risk duration was from date of randomization until date last contact. With the event of interest being PCa incidence or other cause mortality respectively.

In men who were diagnosed with PCa, age and race adjusted generalized linear (logistic and linear) regressions were used to explore the conditional (on PCa diagnosis) risk of developing (i) high Gleason score (≥8), (ii) high T stage (≥T3), (iii)

high risk PCa (Gleason score ≥8 and/or Stage III or IV and/or PSA at diagnosis >20), and (iv) the mean difference in log transformed PSA levels at time of diagnosis with metabolic syndrome conditions assessed at baseline.

Mantel-Haenszel chi-squared test was used to compare distribution of treatment across the two arms of study and metabolic health status in each of high risk and low risk PCa groups.

All analyses were performed using R software (version 3.5.1, The R foundation for statistical computing, 2018) using the '*survival', 'survminer'* and *'riskRegression'* packages.

Results

Demographics: The mean age at randomization was 63 (±5.3) years, at baseline 23% were obese, 10% were diabetic and 36% were hypertensive (Table 1), with 51% having one or more of the three metabolic syndrome comorbidities (Supplementary table S1). In men who did not report any of the three metabolic syndrome related conditions at baseline, 18% developed at least one condition by the time of follow-up (Supplementary table S2). High-risk PCa cases were more likely to receive radical prostatectomy as primary treatment among screened metabolically healthy men but more likely to receive radiation therapy among screened metabolically non-healthy men (Supplementary table S3)

Effect of screening: There was no evidence that screening reduced PCSM in the entire cohort (HR=0.91, 95%CI=[0.79, 1.04], p=0.17; Model 0 in Table 2 & S4, and Figure S1A) or in men with a follow up assessment (HR=0.87, 95%CI=[0.70, 1.11], p=0.26; Model 0 in Table S5 and Figure S1B). There was also no evidence for interactions between the three metabolic syndrome factors and screening in the time dependent model or in the baseline sub-cohort (Model 1 in Table 2 & Supplementary

table S5). However there was evidence for an interaction between screening and having one or more of the metabolic syndrome related factors in the time dependent model (p=0.01, Model 2 in Table 2), the time dependent Fine and Gray model (p=0.03, Model 2 in Supplementary table S6) and in men with a follow-up assessment (p=0.02, Model 2 in Supplementary table S5), and weak evidence in analysis only using baseline data (p=0.06, Model 2 in Supplementary table S4). These interactions were attenuated when metabolic syndrome was implemented as a continuous linear number of factors present (0-3) in all four analyses (Model 3 in tables 2, S4, S5 and S6). In metabolically healthy men (men without any of the three metabolic syndrome factors) screening appeared effective in reducing PCSM (HR=0.75, 95%CI=[0.61, 0.92], p=0.01; Model 2 in Table 2; Figure 2A). While screening appeared to have no effect in men with one or more of these three factors at baseline (HR=1.07, 95%CI=[0.89, 1.28], p=0.48; Figure 2B). Similar improvements in PCSM due to screening were observed in metabolically healthy men in the other three analyses (Model 2 in tables S4, S5 and S6) and in time dependent model using continuous linear count of factors (Model 3 in tables 2). With regards to the interactions with each metabolic syndrome factor separately, there was only weak evidence for interactions in the follow-up assessment analyses (diabetes p=0.04; hypertension p=0.06; Model 1 in Supplementary table S5). There was no evidence of a failure of the proportional hazards assumption for any of the interaction models.

Associations with PCa incidence (The delay hypothesis): As expected, screening was associated with increase in PCa incidence (HR=1.06, 95%CI = [1.03, 1.10], p<0.001). Having diabetes at baseline was associated with a reduction in PCa incidence (HR=0.87, 95%CI=[0.82, 0.92], p<0.001; Supplementary table S7), and similarly for being obese (HR=0.94, 95%CI=[0.90, 0.98], p=0.004). There was no

evidence of an association between hypertension and incidence (HR=1.01, 95%CI=[0.97, 1.05], p=0.59).

Associations with other cause mortality (Competing risk hypothesis): There was no effect of screening on other cause mortality (HR= 0.99, 95%CI= [0.96, 1.01], p= 0.18). As expected each metabolic syndrome related conditions were associated with an increased risk of other cause mortality either separately or as having one or more (HR= 1.16, 1.48, 1.05 and 1.29 with p value <0.001 for hypertension, diabetes, obesity, or having one or more of them respectively) (Supplementary table S7).

Associations with PCa characteristics at diagnosis (Aggressive PCa hypothesis): Among men who developed PCa both diabetes (HR=1.4, 95%CI=[1.1, 1.8], p=0.003) and obesity (HR=1.3, 95%CI=[1.1, 1.5], p=0.01) were associated with the presence of high Gleason tumours, and high-risk PCa (diabetes HR=1.5, 95%CI=[1.2, 1.8], p<0.001; obesity HR=1.2, 95%CI=[1.0, 1.4], p=0.02) while hypertension was associated with lower risk of high T stage (≥T3) and lower PSA at diagnosis (Table 3).

Effect of PCa treatment modality: In a time dependent cox model adjusted for screening, age, race, treatment modality (radical prostatectomy versus others) and having one or more of the metabolic syndrome related factors there was no interaction between screening and treatment modality (p= 0.37 for the interaction), however, the interaction between screening and having one or more of the metabolic syndrome related factors was maintained (p= 0.01 for interaction) and screening among metabolically healthy men reduced risk of PCSM (HR= 0.71, 95%CI= [0.58, 0.88], p=0.002). No interaction was detected in the same model between treatment and the metabolic health status (p= 0.61 for the interaction).

Discussion

Using the 17-year mortality data for the PLCO study we have shown that for men free of all three metabolic related conditions (diabetes, hypertension and obesity), a reduction in PCSM was observed in the screening arm, which was not observed in men with one or more of the metabolic syndrome related conditions. We have excluded that this observation could be due to the difference in treatment modalities across the analysis cohort.

If PCa screening decision is based on individualized approach, then identifying the group of men who may benefit from screening is paramount. PCa is a disease of the elderly, a group in which metabolic syndrome related conditions are prevalent. Such conditions are a competing risk as men may die from non-PCa causes while PCa remains undiagnosed (Grossmann, M. & Wittert 2012). Furthermore metabolic syndrome factors have been associated with more aggressive PCa at diagnosis, and reduced PCa specific survival thereafter due to poor treatment outcomes (Gacci et al. 2017; Xiang et al. 2013). Thus metabolic syndrome may attenuate the effect of screening both by reducing the duration for which men are at risk of PCSM, and by reducing the benefits of early detection in men with PCa.

Our analyses showed that having one or more metabolic syndrome related conditions is associated with a lower cumulative incidence of PCa and a substantial increase in other cause mortality, both of which support the competing risk hypothesis. In addition, obesity and diabetes were associated with lower risk of PCa incidence but increased risk of having high-grade and high-risk disease. This may support the diagnosis bias hypothesis (delay in diagnosis due to lower PSA levels) thereby reducing the efficacy of screening. However, there was not detectable effect

of either diabetes or obesity on high T-stage (≥T3), which may make the diagnosis bias hypothesis questionable.

Using the 10-year follow-up data of the PLCO study Crawford *el al*. showed that men who have no or minimal comorbidities benefit from screening in terms of reducing risk of PCSM versus those who have one or more significant comorbidities related to cardiovascular conditions or cancer (Crawford et al. 2011). However there is concern regarding how significant comorbidities were defined, the inclusion of obesity and hypertension in this group increased the percentage of the unhealthy group to 64% (in comparison to 51% in our cohort analysis using only metabolic related conditions) (Andriole, G. L. et al. 2012), and thus this finding should be interpreted with caution (Bach & Vickers 2011). The 13-year follow-up report of the PLCO did not detect an interaction between the presence of comorbidities and PCa screening on PCSM (Andriole, G. L. et al. 2012). In this report a modified Charlson comorbidity score (0 versus ≥1) was used based on the PLCO baseline questionnaire and it included myocardial infarction, stroke, diabetes, cancer, liver and pulmonary disease. In our analysis, an interaction was detected between screening and cardiovascular comorbidities (stroke, ischemic heart disease, and heart failure) either alone or with addition of hypertension, obesity and diabetes in the time dependent model and in the follow up cohort, but not in the baseline cohort (data not shown).

Our study features notable strengths compared to existing analyses of comorbidities and PCa screening in PLCO. We have taken advantage of the extended median 17-year follow-up mortality data now available, which includes a total of 627 PCSM events, more than double the number observed in the 13-year follow-up report. By using only a limited number of comorbidities, our analysis have a clinical utility in terms of being able to easily identify such a group of men in clinical practice who are

free from these three conditions, rather than free from a long list of comorbidities. This does not rule out that the presence of other types of comorbidities may attenuate screening efficacy. Our results are robust to modelling assumptions: time-dependent versus non-time dependent Cox models and Cox versus Fine & Gray competing risks regression models. We have also explored different possible explanations for our observation.

Our analyses have the following limitations; this was an unplanned post hoc subgroup analysis of the PLCO data. Second there was no data available regarding medication usage for diabetes and hypertension, or whether these conditions were controlled for or not. Third, there was no data available at baseline about history of having hyperlipidaemia, which prevents us from using NCEP-ATPIII's definition of metabolic syndrome.

Notwithstanding these issues, our results support those of Crawford *el al* (Crawford et al. 2011) in terms of detecting an interaction between presence of comorbidities and PCa screening. We have also tried to overcome a criticism of their study by limiting the comorbidities to only metabolic syndrome related conditions to have a reasonable sized healthy and unhealthy study cohort (Andriole, G. L. et al. 2012). However, identifying the type of comorbidities that may attenuate screening remains an area for future research.

Conclusion

The presence of metabolic syndrome appears to attenuate the effect of PCa screening, with PCa screening possibly being effective among healthy men with no metabolic syndrome related conditions. If confirmed in other cohorts or in a prospective study, then this result has major implications for PCa screening guidelines.

Conflicts of Interest:

The authors have no conflicting interests to declare, other than:

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Ethics:
The PLCO protocol and participants' consents were reviewed and approved by the National Cancer Institute (NCI), the National Institutes of Health (NIH) Office of Protection from Research Risks, and the U.S. Office of Management and Budget.

Tables

Table 1: Demographics at baseline assessment.

		Control	Screening	Total
		N = 35338	N = 36782	N = 72120
Age	mean (SD)	62.7 (5.3)	62.7 (5.3)	62.7 (5.3)
Race	white non-Hispanic	31275 (89%)	32511 (88%)	63786 (88%)
	black non-Hispanic	1591 (5%)	1659 (5%)	3250 (5%)
	Asian	1405 (4%)	1501 (4%)	2906 (4%)
	other	1067 (3%)	1111 (3%)	2178 (3%)
BMI	mean (SD)	27.5 (4.1)	27.6 (4.2)	27.6 (4.2)
Obesity	no	27182 (77%)	28107 (76%)	55289 (77%)
	yes	8156 (23%)	8675 (24%)	16831 (23%)
Diabetes	no	31953 (90%)	33241 (90%)	65194 (90%)
	yes	3385 (10%)	3541 (10%)	6926 (10%)
Hypertension	no	22408 (63%)	23493 (64%)	45901 (64%)
	yes	12930 (37%)	13289 (36%)	26219 (36%)
Prostate cancer sub-cohort (Those who diagnosed with prostate cancer during the 18 years of follow up)				
		Control	Screening	Total
		N = 3910 (47%)	N = 4424 (53%)	N = 8334
Obesity	no	3108 (79%)	3504 (79%)	6612 (79%)
	yes	802 (21%)	920 (21%)	1722 (21%)
Diabetes	no	3631 (93%)	4115 (93%)	7746 (93%)
	yes	279 (7%)	309 (7%)	588 (7%)
Hypertension	no	2538 (65%)	2823 (64%)	5361 (64%)
	yes	1372 (35%)	1601 (36%)	2973 (36%)
Metabolic Syndrome	no	2067 (53%)	2241 (51%)	4308 (52%)
	yes	1843 (47%)	2183 (49%)	4026 (48%)
High Gleason Score (≥8)	no	529 (14%)	476 (11%)	1005 (12%)
	yes	3331 (85%)	3876 (88%)	7207 (86%)
	Missing	50 (1%)	72 (2%)	122 (1%)
High T stage (≥T3)	no	93 (2%)	75 (2%)	168 (2%)
	yes	3817 (98%)	4349 (98%)	8166 (98%)
PSA at diagnosis	Mean (SD)	2615 (67%)	3619 (82%)	6234 (75%)
	0 -10	2615 (67%)	3619 (82%)	6234 (75%)
	10 - 20	524 (13%)	557 (13%)	1081 (13%)
	> 20	261 (7%)	215 (5%)	476 (6%)
	Missing	510 (13%)	33 (<1%)	543 (7%)
High Risk	no	2729 (70%)	3687 (83%)	6416 (77%)
	yes	724 (19%)	660 (15%)	1384 (17%)
	Missing	457 (12%)	77 (2%)	534 (6%)

Table 2: Models with all coefficients for the association between screening and metabolic factors (hypertension, diabetes, obesity), either separate or as "having one or more" with interactions for the effect of screening on prostate cancer specific mortality

	Time dependent Cox regressions							
	Model 0		Model 1		Model 2		Model 3	
	HR [95% CI]	P value	HR [95% CI]	P value	HR [95% CI]	P value	HR [95% CI]	P value
Age at randomization	1.04 [1.03, 1.06]	<0.001	1.04 [1.03, 1.06]	<0.001	1.04 [1.03, 1.05]	<0.001	1.04 [1.03, 1.05]	<0.001
Race (black non-Hispanic vs. others)	2.07 [1.58, 2.71]	<0.001	2.07 [1.58, 2.71]	<0.001	2.03 [1.55, 2.65]	<0.001	2.03 [1.55, 2.65]	<0.001
Screening (screening vs. control)	0.91 [0.79, 1.04]	0.17	0.82 [0.68, 0.99]	0.04	0.75 [0.61, 0.92]	0.01	0.82 [0.68, 0.99]	0.04
Hypertension (yes vs. no)	0.81 [0.70, 0.94]	0.004	0.72 [0.59, 0.88]	0.002	------	------	------	------
Diabetes (yes vs. no)	0.80 [0.64, 1.00]	0.05	0.65 [0.47, 0.93]	0.02	------	------	------	------
Obesity (yes vs. no)	1.24 [1.06, 1.46]	0.009	1.34 [1.07, 1.67]	0.01	------	------	------	------
Metabolic syndrome related factors (yes vs. no)	------	------	------	------	0.74 [0.61, 0.89]	0.002	0.88 [0.78, 0.99]	0.03
Screening x Hypertension	------	------	1.27 [0.96, 1.68]	0.10	------	------	------	------
Screening x Diabetes	------	------	1.44 [0.92, 2.26]	0.11	------	------	------	------
Screening x Obesity	------	------	0.86 [0.63, 1.19]	0.37	------	------	------	------
Screening x Metabolic syndrome related factors	------	------	------	------	1.43 [1.09, 1.88]	0.01	1.14 [0.96, 1.34]	0.13

- Model 0 – Model including diabetes, hypertension and obesity without interactions with screening.
- Model 1 – Model including pairwise interactions between screening and each factor: diabetes, hypertension and obesity.
- Model 2 – Model including an interaction between screening and having one or more of the metabolic syndrome related factors: diabetes, hypertension and/or obesity (yes for any of them vs. no).
- Model 3 – Model including an interaction between screening and the sum (0-3) of diabetes (0-1), hypertension (0-1) and obesity (0-1).
- Each metabolic factor is implemented as a time dependant variable with assessments at baseline and at (median) nine years follow-up.

Table 3: Associations with prostate cancer characteristics at diagnosis

	Logistic regression						Linear regression	
	High Gleason score (≥8)		High T stage (≥T3)		High Risk		PSA (log) at diagnosis	
	OR [95% CI]	P value	OR [95% CI]	P value	OR [95% CI]	P value	ß [95% CI]	P value
Hypertension	0.89 [0.77, 1.03]	0.11	0.71 [0.50,0.99]	0.05	0.88 [0.78, 1.0]	0.06	-0.04 [-0.10, -0.004]	0.03
Diabetes	1.4 [1.1, 1.8]	0.01	1.4 [0.8, 2.4]	0.19	1.5 [1.2, 1.8]	<0.001	0.04 [-0.03, 0.11]	0.29
Obesity	1.20 [1.11, 1.50]	0.01	0.81 [0.52, 1.20]	0.32	1.20 [1.0, 1.40]	0.03	0.003 [-0.04, 0.05]	0.91
Metabolic syndrome related factors	1.0 [0.91, 1.20]	0.53	0.82 [0.60, 1.10]	0.21	1.0 [0.91, 1.20]	0.71	-0.02 [-0.06, 0.01]	0.21
Metabolic syndrome related factors as continuous (0-3)	1.09 [1.00, 1.19]	0.05	0.84 [0.67, 1.04]	0.12	1.08 [0.99, 1.16]	0.06	-0.013 [-0.04, 0.01]	0.27

- Multivariable models with the three metabolic conditions at baseline adjusted for screening, age at randomization and race.
- High Risk: Stage ≥T3 and/or Gleason score ≥8 and /or PSA at diagnosis >20 ng/ml
- Metabolic syndrome related factors as continuous factor, as a sum (0-3) of diabetes (0-1), hypertension (0-1) and obesity (0-1).

Supplementary table S1: Additional demographic data at baseline assessment

		Control	Screening	Total
		N = 35338	N = 36782	N = 72120
Metabolic syndrome related factors (Diabetes &/or hypertension &/or obesity)	No	17509 (50%)	18126 (49%)	35635 (49%)
	Yes	17829 (50%)	18656 (51%)	36485 (51%)
Prostate cancer	No diagnosis	31428 (89%)	32358 (88%)	63786 (88%)
	Diagnosis	3910 (11%)	4424 (12%)	8334 (12%)
Median Follow up	Mean (±SD)	12.6 (±4.8)	12.5 (±4.7)	12.6 (±4.7)
Status at last contact	Alive	22583 (64%)	23819 (65%)	46402 (64%)
	Died of other causes	12436 (35%)	12655 (34%)	25091 (35%)
	Died due to PCa	319 (<1%)	308 (<1%)	627 (<1%)

Supplementary table S2: Demographics at the 9-year follow-up assessment

		Control	Screening	Total	Not assessed at follow-up
		N = 20045	N = 22235	N = 42280	N= 24923
Age at randomization	Mean (±SD)	62.1 (±5.1)	62.0 (±5.0)	62.1 (±5.1)	63.0 (±5.6)
Hypertension	No	9282 (46%)	10241 (46%)	19523 (46%)	15077 (60%)
	Yes	10763 (54%)	11994 (54%)	22757 (54%)	9846 (40%)
Diabetes	No	16691 (83%)	18494 (83%)	35185 (83%)	21656 (87%)
	Yes	3354 (17%)	3741 (17%)	7095 (17%)	3267 (13%)
Obesity	No	14376 (72%)	15766 (71%)	30142 (71%)	18663 (75%)
	Yes	5669 (28%)	6469 (29%)	12138 (29%)	6260 (25%)
Hypertension and /or diabetes and /or obesity	No	6897 (34%)	7433 (33%)	14330 (34%)	11231 (45%)
	Yes	13148 (66%)	14802 (67%)	27950 (66%)	13692 (55)%)
No hypertension, diabetes or obesity at baseline, but developed any at the 9-year follow-up	No	16567 (83%)	18280 (82%)	34847 (82%)	--------
	Yes	3478 (17%)	3955 (18%)	7433 (18%)	--------
Prostate cancer	No diagnosis	17609 (88%)	19439 (87%)	37048 (88%)	22469 (90%)
	Diagnosis	2436 (12%)	2796 (13%)	5232 (12%)	2454 (10%)
Time from randomization to follow-up	Mean (±SD)	9.4 (±1.9)	9.4(±1.9)	9.4(±1.9)	--------
Time from follow up till last contact	Mean (±SD)	7.9 (±2.4)	8.0 (±2.4)	8.0 (±2.4)	--------
Status at last contact	Alive	15497 (77%)	17179 (77%)	32676 (77%)	10243 (41%)
	Died of other causes	4419 (22%)	4930 (22%)	9349 (22%)	14356 (58%)
	Died due to PCa	129 (<1%)	126 (<1%)	255 (<1%)	324 (1%)

Supplementary table S3: Distribution of treatment modalities by screening arm across low and high risk prostate cancer cases in metabolically healthy and unhealthy men.

	Metabolically healthy N= 4308				Metabolically unhealthy N= 4026			
	Low risk N= 3336 (77%)		High risk N= 696 (16%)		Low risk N= 3080 (77%)		High risk N= 688 (17%)	
	Control N=1443 (43%)	Screening N= 1893 (57%)	Control N=384 (55%)	Screening N=312 (45%)	Control N=1286 (42%)	Screening N=1794 (58%)	Control N=340 (49%)	Screening N=348 (51%)
Radiation therapy alone	331 (23%)	424 (22%)	29 (7.6%)	21 (6.7%)	336 (26%)	437 (24%)	26 (8%)	35 (10%)
Radiation with hormonal	272 (19%)	278 (15%)	127 (33%)	102 (33%)	259 (20%)	314 (18%)	137 (40%)	117 (34%)
Radical prostatectomy	561 (17%)	833 (44%)	125 (33%)	106 (34%)	441 (34%)	660 (37%)	75 (22%)	85 (24%)
Hormonal treatment only	54 (1.7%)	63 (3.3%)	79 (21%)	63 (20%)	74 (6%)	87 (5%)	76 (22%)	78 (22%)
Other treatment	189 (6%)	251 (13%)	15 (3.9%)	15 (4.8%)	131 (10%)	250 (14%)	15 (4%)	19 (5%)
Other ablative	28 (0.9%)	34 (1.8%)	8 (2%)	5 (1.6%)	36 (3%)	36 (2%)	11 (3%)	13 (4%)
Missed	8 (<1%)	10 (<1%)	1 (<1%)	0 (0%)	9 (<1%)	10 (<1%)	0 (0%)	1 (<1%)

Using Mantel-Haenszel chi-squared test, among low risk group, there no difference between treatments options across screening arms and metabolic status (p= 0.13). Among high-risk group, there was a difference between treatments options across screening arms and metabolic status (p= 0.03).

Supplementary table S4: Models with all coefficients for the association between screening and metabolic factors (hypertension, diabetes, obesity), either separate or as "having one or more" with interactions for the effect of screening on prostate cancer specific mortality using data from baseline cohort.

	Cox Proportional Hazard model							
	Model 0		Model1		Model 2		Model 3 (Linear model)	
	HR [95% CI]	P value	HR [95% CI]	P value	HR [95% CI]	P value	HR [95% CI]	P value
Age at randomization	1.13 [1.11, 1.15]	<0.001	1.13 [1.11, 1.15]	<0.001	1.13 [1.11, 1.14]	<0.001	1.13 [1.11, 1.14]	<0.001
Race (black non-Hispanic vs. others)	2.24 [1.67, 3.00]	<0.001	2.24 [1.67, 3.00]	<0.001	2.19 [1.64, 2.93]	<0.001	2.18 [1.63, 2.91]	<0.001
Screening (screening vs. control)	0.91 [0.78, 1.07]	0.26	0.86 [0.70, 1.06]	0.16	0.78 [0.63, 0.98]	0.03	0.87 [0.71, 1.07]	0.18
Hypertension (yes vs. no)	0.91 [0.77, 1.08]	0.30	0.84 [0.66, 1.06]	0.14	-------	-------	-------	-------
Diabetes (yes vs. no)	0.77 [0.56, 1.05]	0.09	0.78 [0.50, 1.21]	0.27	-------	-------	-------	-------
Obesity (yes vs. no)	1.42 [1.18, 1.7]	<0.001	1.42 [1.09, 1.84]	0.009	-------	-------	-------	-------
Metabolic syndrome	-------	-------	-------	-------	0.90 [0.72, 1.12]	0.36	1.00 [0.87, 1.16]	0.99
Screening x Hypertension	-------	-------	1.20 [0.861, 1.68]	0.28	-------	-------	-------	-------
Screening x Diabetes	-------	-------	0.96 [0.52, 1.79]	0.90	-------	-------	-------	-------
Screening x Obesity	-------	-------	1.00 [0.69, 1.44]	0.98	-------	-------	-------	-------
Screening x Metabolic syndrome	-------	-------	-------	-------	1.36 [0.99, 1.86]	0.06	1.08 [0.881, 1.32]	0.46

- Model 0 – Model including diabetes, hypertension and obesity without interactions with screening.
- Model 1 – Model including pairwise interactions between screening and each factor: diabetes, hypertension and obesity.
- Model 2 – Model including an interaction between screening and having one or more of the metabolic syndrome related factors: diabetes, hypertension and/or obesity (yes vs. no).
- Model 3 – Model including an interaction between screening and the sum (0-3) of diabetes (0-1), hypertension (0-1) and obesity (0-1).
- Cohort of men with baseline information about metabolic syndrome related conditions, from time of randomization until time of last contact

Supplementary table S5 Models with all coefficients for the association between screening and metabolic factors (hypertension, diabetes, obesity), either separate or as "having one or more" with interactions for the effect of screening on prostate cancer specific mortality using data from follow up cohort

	Cox Proportional Hazard model							
	Model 0		Model 1		Model 2		Model 3	
	HR [95% CI]	P value	HR [95% CI]	P value	HR [95% CI]	P value	HR [95% CI]	P value
Age at randomization	1.14 [1.11, 1.17]	<0.001	1.14 [1.11, 1.17]	<0.001	1.14 [1.11, 1.16]	<0.001	1.14 [1.11, 1.16]	<0.001
Race (black non-Hispanic vs. others)	1.75 [0.95, 3.21]	0.07	1.74 [0.95, 3.2]	0.07	1.79 [0.98, 3.28]	0.06	1.74 [0.95, 3.20]	0.07
Screening (screening vs. control)	0.87 [0.70, 1.11]	0.26	0.64 [0.43, 0.94]	0.02	0.57 [0.37, 0.88]	0.01	0.65 [0.45, 0.95]	0.03
Hypertension (yes vs. no)	0.96 [0.74, 1.23]	0.72	0.75 [0.53, 1.07]	0.12	-------	-------	-------	-------
Diabetes (yes vs. no)	1.21 [0.88, 1.67]	0.23	0.83 [0.50, 1.39]	0.49	-------	-------	-------	-------
Obesity (yes vs. no)	1.25 [0.95, 1.66]	0.11	1.46 [0.99, 2.16]	0.06	-------	-------	-------	-------
Metabolic syndrome	-------	-------	-------	-------	0.79 [0.56, 1.13]	0.19	0.97 [0.80, 1.19]	0.79
Screening x Hypertension	-------	-------	1.62 [0.98, 2.70]	0.06	-------	-------	-------	-------
Screening x Diabetes	-------	-------	1.97 [1.02, 3.80]	0.04	-------	-------	-------	-------
Screening x Obesity	-------	-------	0.74 [0.43, 1.29]	0.29	-------	-------	-------	-------
Screening x Metabolic syndrome	-------	-------	-------	-------	1.88 [1.10, 3.21]	0.02	1.32 [1.00, 1.74]	0.05

- Model 0 – Model including diabetes, hypertension and obesity without interactions with screening.
- Model 1 – Model including pairwise interactions between screening and each factor: diabetes, hypertension and obesity.
- Model 2 – Model including an interaction between screening and having one or more of the metabolic syndrome related factors: diabetes, hypertension and/or obesity (yes vs. no).
- Model 3 – Model including an interaction between screening and the sum (0-3) of diabetes (0-1), hypertension (0-1) and obesity (0-1).
- Cohort of men with baseline and follow up information about metabolic syndrome related conditions, from time of follow up questionnaire until time of last contact

Supplementary table S6: Models with all coefficients for the association between screening and metabolic factors (hypertension, diabetes, obesity), either separate or as "having one or more" with interactions for the effect of screening on prostate cancer specific mortality in a time dependent Fine and Gray model

	Time dependent models							
	Model 0		Model 1		Model 2		Model 3	
	HR [95% CI]	P value	HR [95% CI]	P value	HR [95% CI]	P value	HR [95% CI]	P value
Age at randomization	1.06 [1.05, 1.07]	<0.001	1.06 [1.05, 1.07]	<0.001	1.05 [1.04, 1.06]	<0.001	1.05 [1.04, 1.06]	<0.001
Race (black non-Hispanic vs. others)	2.27 [1.67, 3.08]	<0.001	2.26 [1.67, 3.07]	<0.001	2.19 [1.61, 2.96]	<0.001	2.19 [1.62, 2.98]	<0.001
Screening (screening vs. control)	0.93 [0.80, 1.09]	0.38	0.86 [0.69, 1.06]	0.16	0.77 [0.61, 0.97]	0.03	0.87 [0.70, 1.07]	0.19
Hypertension (yes vs. no)	0.78 [0.66, 0.92]	<0.001	0.66 [0.53, 0.84]	<0.001	-------	-------	-------	-------
Diabetes (yes vs. no)	0.74 [0.57, 0.95]	0.02	0.63 [0.43, 0.92]	<0.001	-------	-------	-------	-------
Obesity (yes vs. no)	1.29 [1.08, 1.55]	0.01	1.51 [1.17, 1.93]	<0.001	-------	-------	-------	-------
Metabolic syndrome	-------	-------	-------	-------	0.72 [0.58, 0.89]	<0.001	0.87 [0.76, 1.00]	0.05
Screening x Hypertension	-------	-------	1.37 [0.99, 1.88]	0.05	-------	-------	-------	-------
Screening x Diabetes	-------	-------	1.35 [0.81, 2.25]	0.24	-------	-------	-------	-------
Screening x Obesity	-------	-------	0.74 [0.51, 1.05]	0.09	-------	-------	-------	-------
Screening x Metabolic syndrome	-------	-------	-------	-------	1.42 [1.04, 1.94]	0.03	1.10 [0.91, 1.32]	0.33

- Model 0 – Model including diabetes, hypertension and obesity without interactions with screening.
- Model 1 – Model including pairwise interactions between screening and each factor: diabetes, hypertension and obesity.
- Model 2 – Model including an interaction between screening and having one or more of the metabolic syndrome related factors: diabetes, hypertension and/or obesity (yes vs. no).
- Model 3 – Model including an interaction between screening and the sum (0-3) of diabetes (0-1), hypertension (0-1) and obesity (0-1).
- Time dependent Fine and Gray competing risk model
- Each variable is dealt with as a time dependant variable with two time points; at time of baseline questionnaire, and at time of follow up questionnaire

Supplementary table S7: Effect of metabolic condition at baseline on prostate cancer incidence, and mortality

	Time dependent Cox models							
	PCa incidence				Other cause mortality			
	HR [95% CI]	P value	HR [95% CI]	P value	HR [95% CI]	P value	HR [95% CI]	P value
Age at randomization	0.98 [0.97, 0.98]	<0.001	0.98 [0.97, 0.98]	<0.001	1.04 [1.03, 1.04]	<0.001	1.04 [1.04, 1.04]	<0.001
Race (black non-Hispanic vs. others)	1.22 [1.12, 1.32]	<0.001	1.21 [1.12, 1.31]	<0.001	1.26 [1.19, 1.32]	<0.001	1.3 [1.24, 1.36]	<0.001
Screening (screening vs. control)	1.06 [1.03, 1.10]	<0.001	1.06 [1.03, 1.1]	<0.001	0.99 [0.96, 1.01]	0.18	0.99 [0.96, 1.01]	0.18
Hypertension (yes vs. no)	1.01 [0.97, 1.05]	0.59	------	------	1.16 [1.13, 1.19]	<0.001	------	------
Diabetes (yes vs. no)	0.87 [0.82, 0.92]	<0.001	------	------	1.48 [1.44, 1.53]	<0.001	------	------
Obesity (yes vs. no)	0.94 [0.90, 0.98]	0.004	------	------	1.05 [1.02, 1.08]	<0.001	------	------
Metabolic syndrome	------	------	0.96 [0.92, 0.99]	0.01	------	------	1.29 [1.26, 1.32]	<0.001

- Time dependent Cox regression models with time dependent variables, each variable is dealt with as a time dependant variable with two time points; at time of baseline questionnaire, and at time of follow up questionnaire

Figures

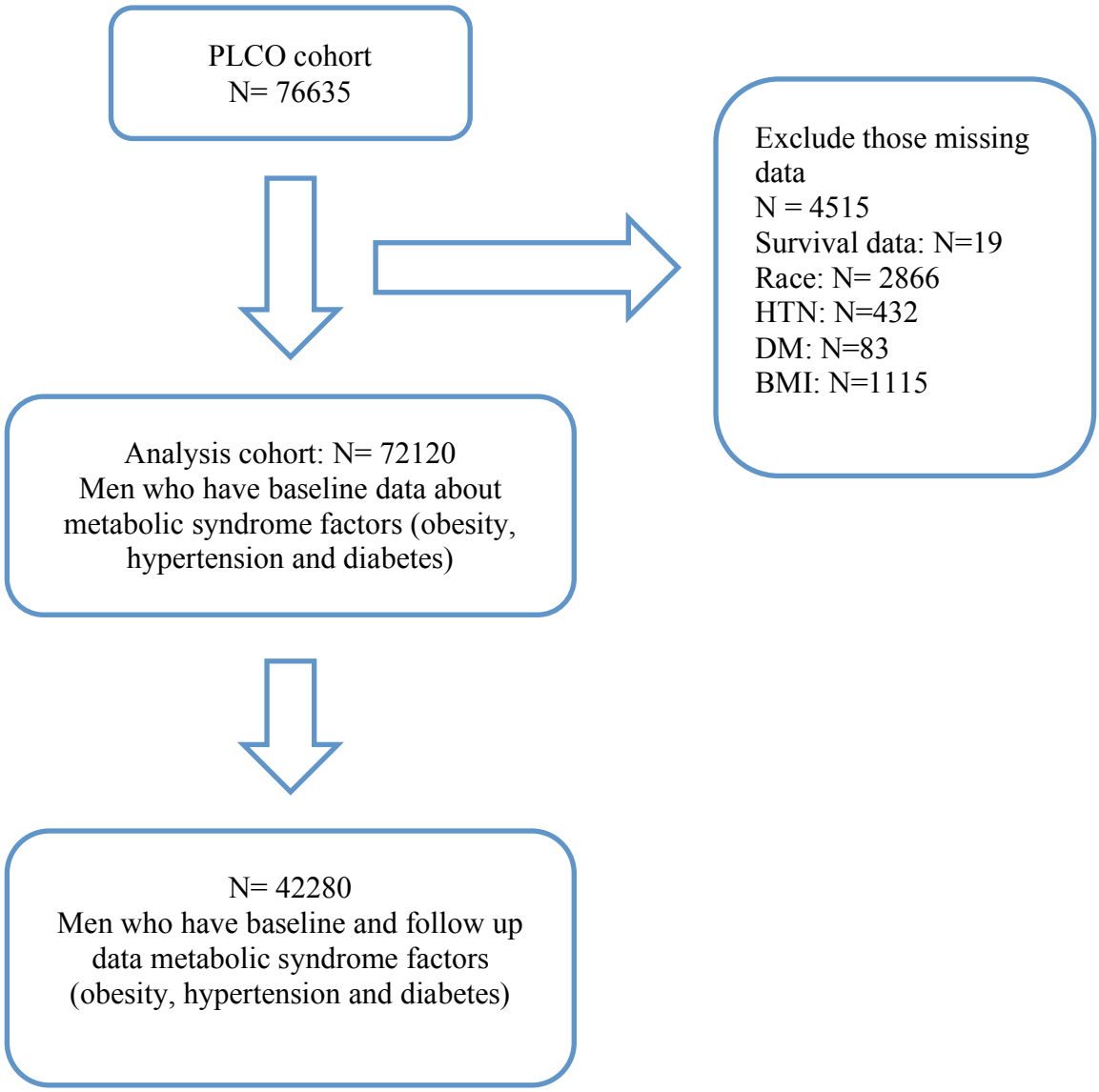

Figure 1: Participant flow diagram.
HTN: Hypertension; DM: Diabetes; BMI: Body mass index

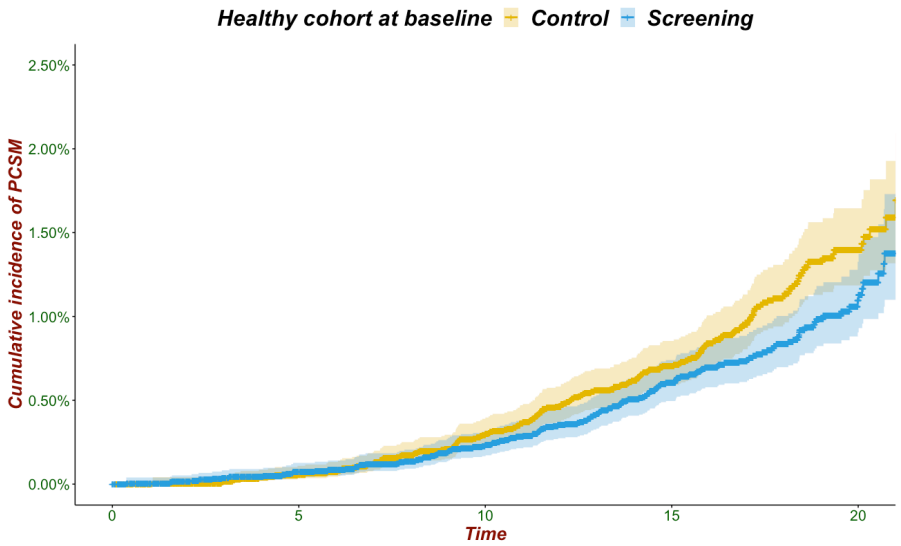

Fig: 2A

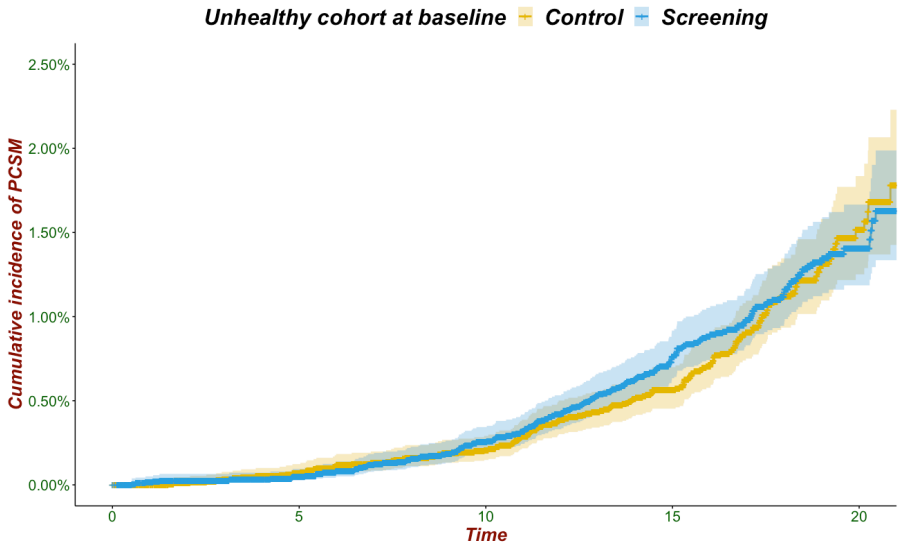

Fig: 2B

Figure 2: Difference in cumulative incidence of PCSM due to screening in (A) metabolically healthy men (no diabetes, hypertension or obesity at baseline), and (B) metabolically unhealthy men (one or more of the three metabolic conditions at baseline), using covariate unadjusted Cox proportional hazard regressions with survival from date of randomization until date of last contact

Supplementary figures

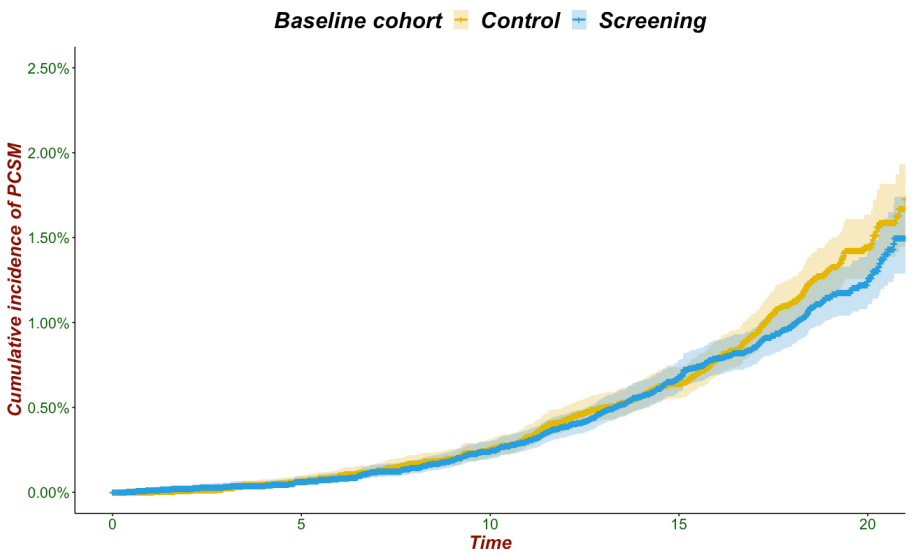

Fig: S1A

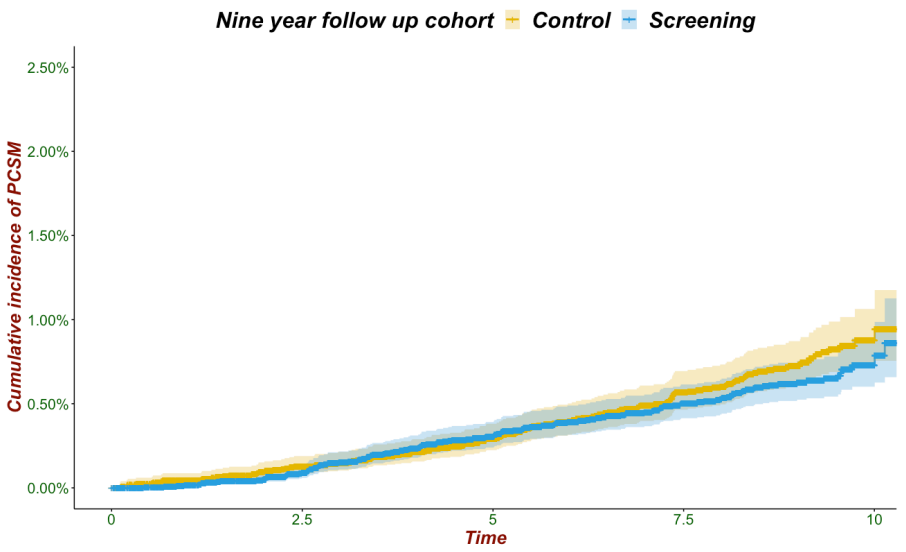

Fig: S1B

Figure S1: Difference in cumulative incidence of PCSM due to screening in (A) entire cohort, (B) men with nine-year follow-up, using covariate unadjusted Cox proportional hazard regressions. In (A) survival is from date of randomization until date of last contact, and in (B) survival is from date of follow-up assessment until date of last contact.

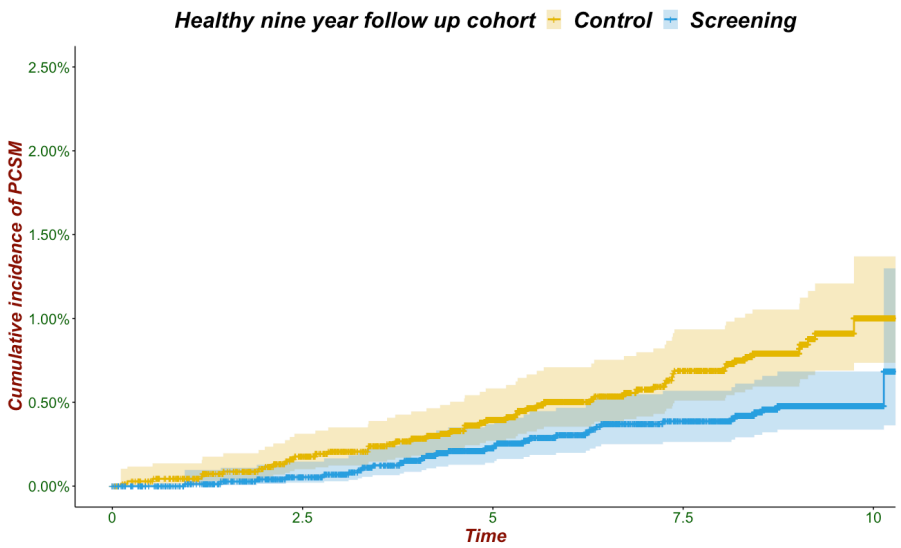

Fig: S2A

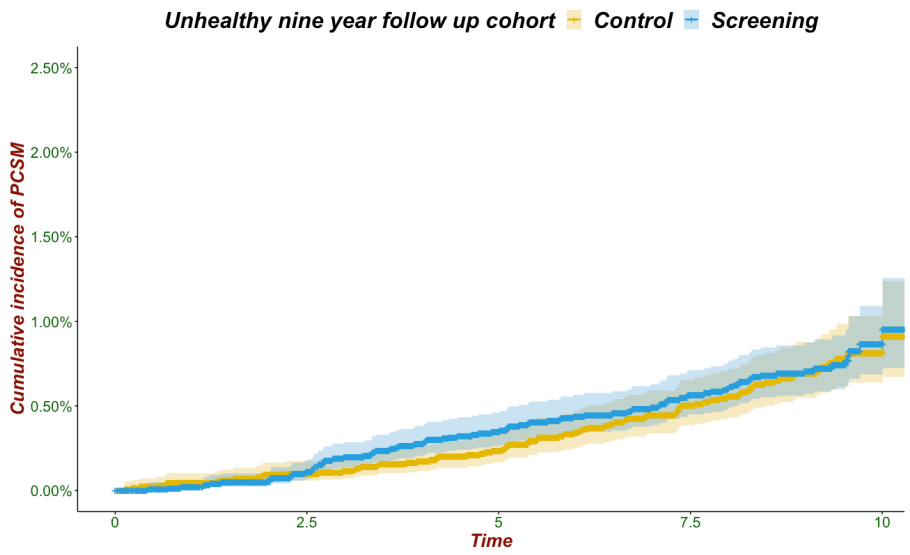

Fig: S2B

Figure S2: Difference in cumulative incidence of PCSM due to screening in men with a nine-year follow-up assessment in (A) metabolically healthy men (no diabetes, hypertension or obesity at follow-up) and (B) metabolically unhealthy men (one or more of the three metabolic conditions at follow-up) using covariate unadjusted Cox proportional hazard regressions with survival from date of follow-up assessment until d of last contact.

Chapter 5. Associations between sex hormones, prostate cancer incidence and disease characteristics at diagnosis

Statement of Authorship

Title of Paper	Associations between sex hormones, prostate cancer incidence and disease characteristics at diagnosis
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Publication Details	

Principle Author

Name of Principle Author (Candidate)	Adel Aref		
Contribution to the Paper	Conceptualization, project design, data collection, analysis and interpretation of the data, writing, review, revision of manuscript		
Overall percentage (%)	60%		
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	24/06/2019

Co-Author Contributions

Name of Co-Author	Andrew D. Vincent		
Contribution to the Paper	Conceptualization, project design, analysis and interpretation of the data, writing, review, revision of manuscript and final approval of the manuscript		
Signature		Date	25/6/19

Name of Co-Author	Michael O'Callaghan		
Contribution to the Paper	Analysis and interpretation of the data, writing, review and revision of the manuscript, and final approval of the manuscript		
Signature		Date	25/6/2019

Name of Co-Author	Andrew J. Hoy		
Contribution to the Paper	Analysis and interpretation of the data, writing, review and revision of the manuscript, and final approval of the manuscript.		
Signature		Date	25/6/19

Name of Co-Author	Lisa M. Butler		
Contribution to the Paper	Conceptualization, project design, analysis and interpretation of the data, writing, review, revision of manuscript, final approval of the manuscript and acted as corresponding author		
Signature		Date	24/6/19

Name of Co-Author	Gary A. Wittert		
Contribution to the Paper	Conceptualization, project design, analysis and interpretation of the data, writing, review, revision of manuscript, final approval of the manuscript and acted as corresponding author		
Signature		Date	24/6/19

TITLE: Associations between sex hormones, prostate cancer incidence and disease characteristics at diagnosis

Adel T. Aref[1,2], Andrew D. Vincent[1,2], Michael O'Callaghan[1,3], Andrew J. Hoy[4], Lisa M. Butler#[1,2] and Gary A. Wittert#[1,2]

[1]University of Adelaide Medical School and Freemasons Foundation Centre for Men's Health, Adelaide, SA 5005, Australia.

[2]South Australian Health and Medical Research Institute, Adelaide, SA 5001, Australia.

[3]Urology Unit, SA Health, Flinders Medical Centre, Adelaide, SA, Australia; South Australian Prostate Cancer Clinical Outcomes Collaborative (SA-PCCOC), Adelaide, SA, Australia; and Flinders Centre for Innovation in Cancer, Flinders University, Adelaide, South Australia

[4]Discipline of Physiology, School of Medical Sciences and Bosch Institute, Charles Perkins Centre, University of Sydney, NSW 2006, Australia.

Corresponding authors: Lisa M. Butler and Gary A. Wittert, University of Adelaide Medical School, South Australian Health and Medical Research Institute, Adelaide, SA 5000, Australia.

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Abstract word count: 238

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Abstract:

Introduction: Prostate cancer (PCa) is an androgen-dependent cancer, however the relationship between sex hormones and the incidence and aggressiveness of PCa remains poorly understood.

Methods: A nested case-control sub-cohort from the PLCO (Prostate, Lung, Colorectal and Ovarian) study that has hormonal data available (N= 371) was used to examine associations between sex hormones and PCa incidence and tumour characteristics at diagnosis in terms of Gleason score (GS), T stage and PSA at diagnosis. Estradiol (E2), testosterone (T), and sex-hormone binding globulin (SHBG) data were available from baseline blood samples. The change in sex hormone levels with body mass index (BMI) was assessed using age-adjusted linear regression models. Conditional logistic regressions were employed to assess associations between hormone levels and PCa incidence. Binomial logistic regressions were employed to assess hormonal associations with high GS (GS>7), high T stage (≥T) and PSA at diagnosis in the PCa cohort (N= 180) in age-adjusted and multivariable models.

Results: Higher BMI was associated with lower T ($p<0.001$), lower SHBG ($p=<0.001$) and higher E2/T ($p=<0.001$). After covariate adjustment, BMI was not associated with PCa incidence ($p= 0.55$), or with high GS ($p=0.7$). Men with higher E2/T ratios had a lower risk of incident PCa (OR=0.43; 95%CI=[0.22, 0.85], $p=0.02$), and greater risk of high GS at diagnosis (OR=13.0; 95%CI=[2.9, 74], $p=0.002$). Higher testosterone was associated with reduced risk of high GS (OR=0.08; 95%CI=[0.01, 0.5], $p=0.01$).

Conclusion: E2/T is positively associated with BMI, an increased risk of high GS at diagnosis, and negatively associated with PCa incidence. No associations are detected between sex hormones, T stage or PSA at diagnosis.

Introduction:

Prostate cancer (PCa) is an androgen dependent cancer, however our understanding of the role of serum sex hormones on PCa incidence and aggressiveness is incomplete. This is compounded in a setting of obesity and its associated metabolic alterations, which is typified by increased conversion of testosterone (T) to estradiol (E2), thereby altering the ratio between E2 and T in obese men relative to non-obese men (Wu, A et al. 2018). There is accumulating evidence, associating obesity with aggressive PCa (Zhong et al. 2016), however the contribution of obesity-related hormonal changes (Gates et al. 2013; Gautier et al. 2013) remains elusive.

The serum E2/T ratio is a good reflection of the net sex hormonal milieu in men. Previous studies that have explored the association between serum E2/T and risk of PCa aggressiveness have reported contrasting results ranging from positive associations between higher E2/T and PCa aggressiveness (Schenk et al. 2016) to inverse associations (Black et al. 2014; Salonia et al. 2012; Tsai et al. 2006), with others unable to detect significant associations (Daniels et al. 2010; Severi et al. 2006; Sher et al. 2009). One factor that may contribute to these inconsistent findings is variability in the definition of aggressive PCa. Some studies use only Gleason score (GS) (Daniels et al. 2010; Schenk et al. 2016; Sher et al. 2009), while others use a combination of clinical stage and GS (Black et al. 2014; Salonia et al. 2012; Severi et al. 2006; Tsai et al. 2006).

Aggressive PCa is commonly defined by grouping the following disease characteristics: GS, tumor stage (T stage), prostate specific antigen at diagnosis (PSA Dx), presence of involved lymph nodes (N) and the presence of metastasis (M) (https://www.nccn.org/professionals/physician_gls/pdf/prostate.pdf, reviewed on 22nd

of October 2018). Despite risk scores providing clinical value for patient prognosis, they combine factors relating to distinct pathways (pathology versus tumor development versus tumor propensity for metastasis, with tumor development potentially confounded by delays in diagnosis). Thus using combined risk scores may explain inconsistent findings when examining associations between sex hormones and PCa outcomes. For instance, sex hormones are affected by obesity(Aref et al. 2018), at the same time obesity has been reported to be negatively associated with total incidence, tumor stage and localized stage, but positively associated with higher GS and PCa specific mortality (Amling et al. 2004; Burton et al. 2013; Kelly, Scott P et al. 2016; Pischon et al. 2008; Zhong et al. 2016).

The aim of our study is to examine associations between sex hormones and PCa incidence and characteristics at diagnosis in terms of Gleason score (GS), T stage and PSA at diagnosis.

Material and Methods

Study population

Our study population is nested case-control subgroup of the PLCO PCa screening arm, which have sex hormones assessment. The PLCO study includes 38340 men with ages ranging between 49 and 75 years. Over the period from 1993 to 2001 men were enrolled at 10 screening centers in the United States of America. PSA testing was performed annually for six years along with a digital rectal examination (DRE) every two years. Men were followed for at least 13 years for assessment of risk of PCa. 4430 men were diagnosed with PCa during follow up. A full description of the study design is cited elsewhere (Prorok et al. 2000). A nested case-control sub-cohort

of 727 PCa cases and 889 controls (PCa free) from the PLCO study (matched on age at randomization, fiscal year of first screen and study year of diagnosis/reference) were chosen and had androgen hormone assessments (Weiss et al. 2008). Out of this group, another sub-cohort (total hormonal sub-cohort N= 371, PCa free N = 191, PCa N = 180) was chosen and had their stored serum sample analyzed for estradiol. This sub-cohort is described in detail in (Black et al. 2014).

The hormonal sub-cohort was selected to re-explore the association between sex hormonal milieu and each of PCa incidence and prostate cancer criteria in terms of Gleason score (GS), T stage and PSA at diagnosis (Figure 1).

Measures

Estradiol (E2), testosterone (T), and sex hormone binding globulin (SHBG) were collected from baseline blood samples. E2 was quantified using stable isotope dilution LC/MS-MS, T was measured using direct RIA (Immunotech; CV= 14%), and SHBG was measured by a sandwich immunoradiometric assay. Full details are cited in (Black et al. 2014; Weiss et al. 2008).

Statistical Methods

We report means (±SD) and frequency (percentages) for demographic factors as appropriate, unless otherwise stated. The associations between E2, T, E2/T, and SHBG with body mass index (BMI) were assessed using age adjusted linear regression models. In all analyses these hormone measures were log transformed. Spearman correlation coefficients were used to assess correlations between hormonal variables in the hormonal sub-cohort. Except for data on PSA before randomization which were missing 10%, no other variables have missing data more than one percent,

thus naïve imputation using mean for continuous variables or most abundant for factorial variables was used to impute for missing variables in the hormonal sub-cohort.

Conditional logistic regression (matched on age at randomization, fiscal year of first screen and study year of diagnosis/reference) was employed to assess the association between the hormonal variables and PCa incidence in the hormonal sub-cohort. Binomial logistic regression models were employed to assess the associations between hormonal variables and both high GS (GS>7) and high T stage (≥T2c). A linear regression model was employed to assess the association between hormonal variables and log transformed PSA at diagnosis. The association between the hormonal variables and prostate cancer characteristics was initially assessed using age-adjusted regressions models, then in multivariable models with two sets of covariates. The first model adjusted for age at baseline, BMI at study entry and SHBG, the second model extended the first model to include history of having diabetes, history of having hypertension, positive family history of PCa, and smoking duration, education (high school or less versus more than high school), marital status (married versus single) and having PSA test before randomization (one or less versus more than one). The multivariable SHBG models were adjusted for estradiol and testosterone in addition to the previously mentioned covariates. To account for non-linearity, each model was repeated with the hormonal variables divided into quartiles (using the lowest quartile as the reference group).

All statistical analyses were performed using R software (Version 3.5.1, The R foundation for statistical computing, 2018) using the '*survival'* package.

Results

Hormonal associations with BMI: The mean age of our analysis cohort was 63 (±3.5) and mean BMI was 28 (±4). Of those who have PCa, 34% were stage III or IV, 3% had T stage of T3 or higher at diagnosis and 14% had a Gleason score of 8 or more (Table 1). Using an age-adjusted linear regression model, higher BMI was associated with lower T ($p<0.001$), lower SHBG ($p=<0.001$) and higher E2/T ($p=<0.001$). The association with E2 ($p=0.13$) was not significant (Supplementary Table S1). The correlation between E2 and E2/T and SHBG was low ($r= -0.38$ and $r= 0.11$ respectively), however as expected T and SHBG showed higher correlation ($r= 0.72$) (Supplementary Table S2).

Prostate cancer incidence: Using a conditional logistic regression model, E2/T was associated with a reduced PCa incidence in both age adjusted and multivariable models (age adjusted OR=0.62, 95%CI=[0.4, 0.97], p= 0.04; full multivariable OR=0.43, 95% CI=[0.22, 0.85], p=0.02), while higher T was associated with increased PCa incidence in the multivariable model (OR=2.1, 95%CI=[1.1, 4.0], p= 0.04; Table 2). After covariate adjustment, higher BMI at baseline was not associated with PCa incidence.

Prostate cancer at diagnosis: When restricting to men diagnosed with PCa in the hormonal cohort (N= 180), E2/T was positively associated with high GS (≥8) both in age adjusted and multivariable models (age adjusted OR=2.4; 95% CI=[1.0, 6.0], p= 0.05, full multivariable model OR=13.0; 95% CI=[2.9, 74], p = 0.002). We detected an inverse association between T and high GS in full multivariable model (OR=0.08, 95% CI=[0.01, 0.5], p= 0.01) (Table 3). A positive association between SHBG and

high GS was detected in the full multivariable model (OR=15, 95% CI=[2.4, 120], p= 0.006) (Table 3)

No association was detected between any of the sex hormones and T stage at diagnosis except for weak inverse association with higher E2/T ratio in the age-adjusted model (Table 4). No association was detected between any of the sex hormones and the level of PSA at diagnosis expect for a weak inverse association with higher T in age adjusted model (Table 5).

Non-linear analyses: Using E2/T quantiles indicated a decreased risk of PCa incidence with the 4^{th} quantile (Supplementary Table S3), while the association with higher GS was near significant in the age-adjusted model (p=0.07) and significant for the age, BMI and SHBG adjusted and the full multivariable models (both p=0.01). After adjusting for other sex hormones (E2 and T), there was a positive association between SHBG and higher GS in the multivariable models (both p= 0.01) (Supplementary Table S4). No associations were detected between any of the sex hormones quantiles and higher T stage (Supplementary table S4). A weak negative association was detected between SHBG and PSA at diagnosis in age-adjusted model (Supplementary table S4).

The complete case sensitivity analyses using same models after excluding men with missing data showed qualitatively similar results (data not shown).

Discussion

Our analyses have shown that higher serum E2/T, reduced testosterone and higher SHBG are associated with lower risk of PCa incidence; and a higher risk of high GS (>7). In the full multivariable model, no other associations were detected between any of the sex hormones and the T stage or PSA at diagnosis.

There was no association detected between obesity and either PCa incidence or high GS in this nested case control cohort. However, in a previous publication which analysed the entire PLCO prostate cancer cohort, a negative association between BMI and prostate cancer incidence and a positive association with fatal PCa were reported (Kelly, Scott P et al. 2016). Of note, in our analyses, the point estimates for the association between obesity and PCa incidence as well as T stage were in the negative direction, while that with high GS was in the positive direction, albeit none reached a statistical significance, which is following the association direction of the whole cohort.

In our analysis, E2/T ratio increased with obesity, which is consistent with previous data that support the increase in the E2/T ratio with obesity(Fejes et al. 2006; Parikesit et al. 2016). Obesity and the associated lower PSA values may lead to delayed diagnosis and thus a decrease in the PCa incidence rate (Bandini, Gandaglia & Briganti 2017; Chow et al. 2018). We have shown in a previous study that the lower PSA in obese men is partially due to the association with E2/T (Aref et al. 2018). Alternatively the inverse association between E2/T and PCa may be due to a protective effect induced by a hormonal milieu with elevated E2 and reduced T.

The increase in E2/T observed in obese men may be one of the mediating mechanisms for previously reported obesity and PCa aggressiveness associations(Zhong et al.

2016), in particular, high GS. Alternatively it is possible that these associations may be a consequence of disease progression due to delayed diagnoses. It is currently unknown whether tumour GS can progress or whether it is established with initial tumour formation (Penney et al. 2013; Porten et al. 2011). Previous studies have shown an effect of environmental and lifestyle-related factors on attenuating PCa risk for those with similar genetic predispositions (Lichtenstein et al. 2000; Loeb et al. 2015). In addition, high fat diet mouse models support the role of obesity influencing the development of high-grade (higher GS) tumours (Cho et al. 2015; Hu, Meng-Bo et al. 2018). The 18-year follow up data from the PLCO showed a reduction in the incidence of tumours with high GS in the screening arm, suggestive of GS progression over time (Pinsky, P. F. et al. 2018).

The development of PCa and the aggressiveness of disease progression depend on both the balance and interaction with androgenic hormones (Rahman, Hofland & Foster 2016). Preclinical studies have shown that estradiol has an important role in both prostate cancer pathogenesis and progression. Transgenic mice model with aromatase enzyme overexpression (AROM+) have shown that an increase in the E2/T ratio due to aromatase over expression was associated with increased fat deposition and is correlated with Gleason grade and increased proliferation and invasion (Ellem et al. 2009). Estradiol can activate both wild-type androgen receptor and (T877A) mutated androgen receptor in LNCaP cells (Susa et al. 2015). The increase in the E2/T ratio was also found to induce an oxidative/nitrosative damages to the DNA of the prostatic epithelial cells (Tam, Leav & Ho 2007). Studies on NBL rat models showed that the combination of low dose testosterone with estradiol administration leads to the development of prostate cancer in 100% of the experiment population and that estrogen treatment leads to DNA damage in the NBL rate prostate prior to cancer

development (Bosland 2013). Mice studies showed that higher levels of estrogens during embryonic life is associated with intraepithelial neoplasia and tumor formation during adult life (Prins & Ho 2010), this is consistent with the hypothesis that the increase risk of prostate cancer incidence and aggressiveness among African-American men may be due to the exposure to higher levels of estrogens during the embryonic life (Henderson et al. 1988). The differences in sex hormones have been suggested to underlie race-specific differences in PCa aggressiveness (Gapstur et al. 2002; Rohrmann et al. 2007). Previous studies have also suggested that the association between PCa and advanced age (Vermeulen et al. 2002), obesity (Allott, Masko & Freedland 2013), and hormonal management in transsexual male to female individuals (Gooren & Morgentaler 2014) can be explained at least in part by the imbalance between E2 and T (higher E2/T). In addition, lower serum T has been associated with higher GS (Schatzl et al. 2001) and advanced stage PCa (Massengill et al. 2003). The prolonged use of 5-alpha-reductase inhibitors (5ARIs) was found to be associated with increased risk of high GS tumours, despite the decrease in the incidence of PCa, possibly due to both, hormonal perturbation (Lebdai, Bigot & Azzouzi 2010; Scailteux et al. 2018) and delay in diagnosis (Sarkar et al. 2019).

A study by Black et al. (Black et al. 2014), using the same nested case-control cohort within the PLCO study (Prorok et al. 2000) assessed the association between sex hormones and aggressive PCa. In this study, cases were defined as those with aggressive PCa (stage III or IV or GS≥7) and controls were defined as men free from PCa for the duration of follow up. By design all PCa cases selected for hormonal assessment were aggressive according to this definition. The study found E2/T to be negatively associated with aggressive PCa. Our analyses however suggest that this

association may have been driven largely by the association between E2/T and PCa incidence rather than disease characteristics (e.g. high GS).

Our analyses of obesity and sex hormones with PCa incidence and tumour aggressiveness characteristics indicate associations in opposing directions. In particular, E2/T was associated with reduced incidence but elevated GS and weak negative association with T stage. This highlights a potential problem with combining incidence and aggressiveness in time-to-event analyses. Further, risk stratification scores combining T-stage, GS and PSA, while crucial in the clinical setting and for treatment decision-making, are not ideal for association studies when different components are affected differently by metabolic factors. In our analysis, we were unable to detect any association between sex hormones and either T stage or PSA at diagnosis (potentially due to smaller sample size). Thus defining aggressive PCa using the combination of GS, T stage and PSA may not be ideal for association studies like ours, as the weak association between sex hormones and T stage and PSA may attenuate the results.

A major limitation of our study was that our analysis cohort is small, with only 14% having a GS greater than 7. Despite this we were still able to detect associations with three out of five hormonal factors (Table 3). Secondly the E2 and T assessments for this cohort did not use the same analytical techniques, and thereby may give different absolute values. However it was shown by Black *et al* that the results between these two techniques are strongly correlated (Black et al. 2014). Thirdly primary and secondary Gleason scores were unavailable for these samples. Differences between men with GS of 7 comprising (4+3) versus (3+4) may explain some differences between our results and others (Daniels et al. 2010; Sher et al. 2009) when including GS 7 in the definition of aggressiveness. Fourthly, we do not have data on other

ethnic groups who may exhibit different hormonal associations or of baseline medication that may alter the sex hormones metabolism. Finally, it is unclear how PCa screening may have affected our results, with all our samples being from the PCa screening arm of the PLCO study.

We have shown that in men with elevated E2/T, PCa incidence is reduced, but at diagnosis GS scores are higher than in men with lower levels of E2/T. However defining the impact of the serum sex hormones on PCa incidence and aggressiveness is confounded by multiple factors including how aggressiveness is defined, combining GS, T stage and PSA together which may attenuate the association detected, age, race, obesity and the duration of an altered hormonal milieu. Further studies on larger cohorts are required for better understanding of how sex hormones influence PCa incidence and aggressiveness, and the impact of obesity on these relationships.

Conclusions

E2/T is positively associated with BMI, and with an increased risk of higher GS (≥8), while it is negatively associated with PCa incidence. This suggests that E2/T may be a mediating factor for incidence and aggressiveness associations with obesity. Further mechanistic studies to confirm this finding are warranted.

Conflicts of Interest:

The authors have no conflicting interests to declare, other than:

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Ethics:

The PLCO protocol and participants' consents were reviewed and approved by the National Cancer Institute (NCI), the National Institutes of Health (NIH) Office of Protection from Research Risks, and the U.S. Office of Management and Budget.

Tables

Table 1: Baseline demographic summary of the hormonal Sub-cohort

		No PCa	PCa	Total
		N = 191	**N = 180**	**N = 371**
Age At Randomization (years)	Mean (±SD)	63 (±3.6)	63 (±3.4)	63 (±3.5)
Baseline BMI (Kg/m^2)	Mean (±SD)	28(±4.1)	27(±3.7)	28(±3.9)
Baseline PSA (ng/ml)	Mean (±SD)	1.4 (±1.3)	4.5(±6.3)	2.9(±4.8)
Diabetes	No	174 (91%)	174 (97%)	348 (94%)
	Yes	15 (8%)	6 (3%)	21 (6%)
	Missing	2 (1%)	0 (0%)	2 (<1%)
Hypertension	No	135 (71%)	130 (72%)	265 (71%)
	Yes	55 (29%)	50 (28%)	105 (28%)
	Missing	1 (<1%)	0 (0%)	1 (<1%)
E2 (pg/ml)	Mean (±SD)	5.3(±2.2)	5.0(±1.4)	5.1(±1.9)
Testosterone (ng/ml)	Mean (±SD)	5.0(±2.4)	5.2(±2.4)	5.1(±2.4)
SHBG (nmol/L)	Mean (±SD)	49(±23)	47(±22)	48(±23)
E2/T	Mean (±SD)	1.3(±1.2)	1.1(±0.6)	1.2(±1.0)
Having PSA Before Randomization	PSA More Than Once	24 (13%)	27 (15%)	51 (14%)
	PSA One Or Never	146 (76%)	137 (76%)	283 (76%)
	Missing	21 (11%)	16 (9%)	37 (10%)
Family History Of Pca	Negative	178 (93%)	156 (87%)	334 (90%)
	Positive	12 (6%)	24 (13%)	36 (10%)
	Missing	1 (<1%)	0 (0%)	1 (<1%)
Smoking Duration years	Mean (±SD)	19(±17)	12(±15)	16(±16)
	Missing	2 (1%)	2 (1%)	4 (1%)
Education	High School Or Less	44 (23%)	46 (26%)	90 (24%)
	More Than High School	147 (77%)	134 (74%)	281 (76%)
Marital Status	Married	168 (88%)	160 (89%)	328 (88%)
	Single	23 (12%)	19 (11%)	42 (11%)
	Missing	…	1 (<1%)	1 (<1%)
Gleason score	Low GS (<8)	…	155 (86%)	…
	High GS (≥8)	…	25 (14%)	…
T stage	Low T stage (<T2c)	…	162 (90%)	…
	High T stage (≥T2c)	…	18 (10%)	…
PSA at diagnosis	Mean (±SD)	…	12.3 (63)	…
	PSA < 20 ng/ml		172 (96%)	…
	PSA ≥ 20 ng/ml		8 (4%)	…

BMI: Body mass index, PSA: Prostate specific antigen, T: Testosterone, E2: Estradiol, SHBG: Sex hormone binding globulin, E2/T: Estradiol to Testosterone ratio, T/SHBG: Testosterone to Sex hormone binding globulin ratio, GS: Gleason score

Table 2: Association between obesity / hormonal factors and PCa incidence in the hormonal sub-cohort (nested case-control)

Sub-cohort with hormonal data						
	Incidence of PCa					
	Conditional Logistic					
	Age adjusted		Multivariable			
			Model 1[#]		Model 2[##]	
	OR [95% CI]	**p-value**	**OR [95% CI]**	**p-value**	**OR [95% CI]**	**p-value**
BMI	0.94 [0.71, 1.2]	0.68	0.91 [0.67, 1.2]	0.52	0.91 [0.65, 1.3]	0.55
E2	0.56 [0.27, 1.2]	0.12	0.55 [0.27, 1.1]	0.11	0.47 [0.21, 1.1]	0.07
T	1.3 [0.84, 2.1]	0.22	2.1 [1.0, 4.0]	0.04*	1.40 [0.73, 2.9]	0.29
E2/T	0.62 [0.40, 0.97]	0.04*	0.36 [0.19, 0.68]	0.002*	0.43 [0.22, 0.85]	0.02*
SHBG	0.84 [0.52, 1.4]	0.50	0.38 [0.18, 0.81]	0.01*	0.47 [0.21, 1.0]	0.06

BMI: Body mass index, PSA: Prostate specific antigen, T: Testosterone, E2: Estradiol, SHBG: Sex hormone binding globulin, E2/T: Estradiol to Testosterone ratio, GS: Gleason score), OR: odd ratio, CI: confidence interval

Model 1: Adjusted for BMI at baseline (except in BMI model), age at baseline and SHBG (expect in SHBG model which is adjusted for E2 and T). ##Model 2: Adjusted for factors in Model 1 and HTN, DM, education, marital status, positive family history, smoking duration and having PSA assessment before randomization.

Table 3: Association between BMI and hormonal factors with high GS (>7) in the PCa group of the hormonal sub-cohort.

PCa group Sub-cohort with hormonal data						
Association with high GS≥8						
Logistic regression model						
	Age adjusted		Multivariable			
			Model 1[#]		Model 2[##]	
	OR [95% CI]	**p-value**	**OR [95% CI]**	**p**	**OR [95% CI]**	**p-value**
BMI	1.1 [0.6, 1.9]	0.72	1.1 [0.6, 2.0]	0.70	1.1 [0.6, 2.0]	0.75
E2	2.0 [0.4, 9.6]	0.41	2.0 [0.4, 10]	0.41	2.9 [0.5, 16.0]	0.22
T	0.4 [0.1, 1.1]	0.07	0.07 [0.01, 0.4]	0.01*	0.08 [0.01, 0.5]	0.01*
E2/T	2.4 [1.0, 6.0]	0.05*	11.0 [2.5, 52]	0.002*	13.0 [2.9, 74.0]	0.002*
SHBG	1.0 [0.41, 2.7]	0.94	12 [2.2, 81]	0.006*	15 [2.4, 120]	0.006*

BMI: Body mass index, PSA: Prostate specific antigen, T: Testosterone, E2: Estradiol, SHBG: Sex hormone binding globulin, E2/T: Estradiol to Testosterone ratio, GS: Gleason score), OR: odd ratio, CI: confidence interval

Model 1: Adjusted for BMI at baseline (except in BMI model), age at baseline and SHBG (expect in SHBG model). ##Model 2: Adjusted for factors in Model 1 and HTN, DM, education, marital status, positive family history, smoking duration and having PSA assessment before randomization. Both SHBG models were adjusted for estradiol and testosterone.

Table 4: Association between BMI and hormonal factors with high T stage in the PCa group of the hormonal sub-cohort.

PCa group Sub-cohort with hormonal data						
Association with high T stage (≥T2c)						
Logistic regression model						
	Age adjusted		Multivariable			
			Model 1#		Model 2##	
	OR [95% CI]	p-value	OR [95% CI]	p	OR [95% CI]	p-value
BMI	0.51 [0.22, 1.08]	0.10	0.54 [0.23, 1.20]	0.14	0.45 [0.17, 1.02]	0.08
E2	0.26 [0.04, 1.72]	0.17	0.34 [0.04, 2.38]	0.29	0.16 [0.01, 1.37]	0.11
T	2.2 [0.7, 7.1]	0.17	2.3 [0.4, 13]	0.34	1.4 [0.2, 8.7]	0.73
E2/T	0.35 [0.11, 0.98]	0.05*	0.23 [0.04, 1.20]	0.08	0.23 [0.04, 1.30]	0.10
SHBG	1.8 [0.6, 5.6]	0.30	0.5 [0.08, 3.1]	0.46	0.7 [0.1, 5.3]	0.74

BMI: Body mass index, PSA: Prostate specific antigen, T: Testosterone, E2: Estradiol, SHBG: Sex hormone binding globulin, E2/T: Estradiol to Testosterone ratio, GS: Gleason score), OR: odd ratio, CI: confidence interval

Model 1: Adjusted for BMI at baseline (except in BMI model), age at baseline and SHBG (expect in SHBG model which is adjusted for E2 and T). ##Model 2: Adjusted for factors in Model 1 and HTN, DM, education, marital status, positive family history, smoking duration and having PSA assessment before randomization.

Table 5: Association between BMI and hormonal factors with PSA at diagnosis in the PCa group of the hormonal sub-cohort.

PCa group Sub-cohort with hormonal data						
Association with log transformed PSA at time of diagnosis						
Linear regression model						
	Age adjusted		Multivariable			
			Model 1#		Model 2##	
	ß [95% CI]	p-value	ß [95% CI]	p	ß [95% CI]	p-value
BMI	0.04 [-0.13, 0.2]	0.67	0.01 [-0.16, 0.17]	0.93	0 [-0.17, 0.18]	0.96
E2	-0.21 [-0.65, 0.24]	0.36	-0.28 [-0.73, 0.17]	0.22	-0.35 [-0.82, 0.12]	0.14
T	-0.27 [-0.53, 0]	0.05*	-0.21 [-0.62, 0.21]	0.33	-0.23 [-0.66, 0.21]	0.30
E2/T	0.15 [-0.09, 0.39]	0.22	-0.02 [-0.39, 0.34]	0.89	-0.06 [-0.44, 0.33]	0.77
SHBG	-0.23 [-0.48, 0.03]	0.09	-0.14 [-0.57, 0.29]	0.52	-0.14 [-0.58, 0.31]	0.55

BMI: Body mass index, PSA: Prostate specific antigen, T: Testosterone, E2: Estradiol, SHBG: Sex hormone binding globulin, E2/T: Estradiol to Testosterone ratio, GS: Gleason score), OR: odd ratio, CI: confidence interval

Model 1: Adjusted for BMI at baseline (except in BMI model), age at baseline and SHBG (expect in SHBG model which is adjusted for E2 and T). ##Model 2: Adjusted for factors in Model 1 and HTN, DM, education, marital status, positive family history, smoking duration and having PSA assessment before randomization.

Supplementary table S1: Associations of sex hormones with obesity in the hormonal sub-cohort.

Age adjusted linear regression model		
	Hormonal Sub-cohort	
Log transformed	**Estimate [95%CI]**	**p-value**
T	-0.18 [-0.25, -0.12]	<0.001*
E2	0.03 [-0.01, 0.07]	0.13
E2/T	0.21 [0.15, 0.28]	<0.001*
SHBG	-0.17 [-0.23, -0.12]	<0.001*

BMI: Body mass index, PSA: Prostate specific antigen, T: Testosterone, E2: Estradiol, SHBG: Sex hormone binding globulin, E2/T: Estradiol to Testosterone ratio, GS: Gleason score

Supplementary table S2: Correlation between hormonal variables

	E2	T	E/T ratio	SHBG
E2	1.00	0.22	0.21	0.11
T	<0.001	1.00	-0.52	0.72
E/T ratio	<0.001	<0.001	1.00	-0.38
SHBG	0.04	<0.001	<0.001	1.00

BMI: Body mass index, PSA: Prostate specific antigen, T: Testosterone, E2: Estradiol, SHBG: Sex hormone binding globulin, E2/T: Estradiol to Testosterone ratio, T/SHBG: Testosterone to Sex hormone binding globulin ratio, GS: Gleason score.

Supplementary table S3: Effect of hormonal factors quartiles on total incidence and association with high GS (>7)

	Whole hormonal sub-cohort					
	Total PCa Incidence Conditional logistic					
	Age adjusted		Model 1#		Model 2##	
	OR [95% CI]	p	OR [95% CI]	p	OR [95% CI]	p
E2 Q1	1	1	1	1	1	1
E2 Q2	1.4 [0.73, 2.7]	0.31	1.4 [0.74, 2.8]	0.29	1.3 [0.61, 2.6]	0.52
E2 Q3	1.1 [0.59, 1.9]	0.85	1.1 [0.6, 2.0]	0.80	0.84 [0.43, 1.6]	0.61
E2 Q4	0.74 [0.41, 1.4]	0.33	0.74 [0.4, 1.4]	0.33	0.67 [0.34, 1.3]	0.26
T Q1	1	1	1	1	1	1
T Q2	1.5 [0.76, 3.1]	0.23	2.1 [0.97, 4.5]	0.06	1.8 [0.79, 4.3]	0.16
T Q3	1.2 [0.65, 2.1]	0.58	1.7 [0.84, 3.4]	0.14	1.3 [0.59, 2.8]	0.54
T Q4	1.5 [0.8, 2.8]	0.22	2.8 [1.1, 6.7]	0.03*	1.8 [0.67, 4.6]	0.25
E2/T Q1	1	1	1	1	1	1
E2/T Q2	0.77 [0.43, 1.4]	0.38	0.66 [0.36, 1.2]	0.19	0.71 [0.36, 1.4]	0.32
E2/T Q3	0.99 [0.53, 1.9]	0.97	0.70 [0.33, 1.5]	0.35	0.77 [0.34, 1.7]	0.52
E2/T Q4	0.51 [0.27, 0.97]	0.04*	0.33 [0.15, 0.75]	0.01*	0.41 [0.17, 0.99]	0.05*
SHBG Q1	1	1	1	1	1	1
SHBG Q2	0.87 [0.49, 1.6]	0.64	0.66 [0.35, 1.2]	0.19	0.78 [0.39, 1.6]	0.49
SHBG Q3	1.1 [0.58, 2.0]	0.80	0.63 [0.29, 1.4]	0.25	0.86 [0.36, 2.0]	0.73
SHBG Q4	0.95 [0.51, 1.8]	0.87	0.45 [0.18, 1.1]	0.08	0.49 [0.19, 1.3]	0.15

BMI: Body mass index, PSA: Prostate specific antigen, T: Testosterone, E2: Estradiol, SHBG: Sex hormone binding globulin, E2/T: Estradiol to Testosterone ratio, Q: Quartiles (1to 4), OR: odd ratio, CI: confidence interval

Model 1: Adjusted for BMI at baseline (except in BMI model), age at baseline and SHBG (expect in SHBG model which is adjusted for E2 and T). ##Model 2: Adjusted for factors in Model 1 and HTN, DM, education, marital status, positive family history, smoking duration and having PSA assessment before randomization

Supplementary table S4: Effect of hormonal factors quartiles on PCa aggression criteria, high GS, high T stage and PSA at diagnosis

	PCa group in hormonal sub-cohort																	
	Association with high GS Logistic regression model						Association with high T stage (≥T2c) Logistic regression model						Association with PSA at diagnosis Linear regression model					
	Age adjusted		Model 1[#]		Model 2[##]		Age adjusted		Model 1[#]		Model 2[##]		Age adjusted		Model 1[#]		Model 2[##]	
	OR [95% CI]	p	OR [95% CI]	p	OR [95% CI]	p	OR [95% CI]	p	OR [95% CI]	p	OR [95%CI]	p	ß [95% CI]	p	ß [95% CI]	p	ß [95% CI]	p
E2 Q1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
E2 Q2	0.8 [0.2, 2.8]	0.75	0.8 [0.23, 2.8]	0.73	0.8 [0.2, 2.9]	0.72	1.4 [0.4, 5.0]	0.57	1.5 [0.5, 5.4]	0.53	1.5 [0.4, 6.1]	0.53	-0.2 [-0.5, 0.1]	0.27	-0.2 [-0.5, 0.2]	0.32	-0.2 [-0.5, 0.2]	0.28
E2 Q3	0.7 [0.2, 2.7]	0.65	.7 [0.2, 2.6]	0.62	0.9 [0.2, 3.4]	0.86	0.6 [0.1, 2.4]	0.45	0.7 [0.1, 2.9]	0.58	0.5 [0.08, 2.5]	0.39	-0.1 [-0.4, 0.3]	0.60	-0.09 [-0.4, 0.3]	0.61	-0.1 [-0.5, 0.2]	0.46
E2 Q4	1.4 [0.5, 4.8]	0.53	1.5 [0.5, 5.0]	0.53	1.9 [0.5, 6.8]	0.33	0.4 [0.05, 20.0]	0.29	0.5 [0.06, 2.5]	0.41	0.3 [0.04, 1.9]	0.24	-0.1 [-0.5, 0.2]	0.45	-0.2 [-0.5, 0.2]	0.32	-0.2 [-0.6, 0.1]	0.21
T Q1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
T Q2	1.2 [0.4, 3.6]	0.80	0.8 [0.2, 3.0]	0.76	0.9 [0.2, 3.6]	0.91	1.5 [0.2, 12]	0.68	1.4 [0.2, 12.0]	0.76	1.4 [0.2, 14.0]	0.73	-0.2 [-0.6, 0.1]	0.16	-0.2 [-0.6, 0.2]	0.37	-0.2 [-0.6, 0.2]	0.33
T Q3	0.7 [0.2, 2.2]	0.50	0.4 [0.08, 1.8]	0.24	0.5 [0.1, 2.1]	0.33	2.8 [0.6, 21.0]	0.23	2.9 [0.5, 26.]	0.28	2.6 [0.4, 25.0]	0.35	-0.4 [-0.8, -0.06]	0.02*	-0.3 [-0.7, 0.09]	0.13	-0.4 [-0.8, 0.05]	0.08
T Q4	0.5 [0.1, 1.8]	0.30	0.2 [0.04, 1.3]	0.11	0.3 [0.04,1.5]	0.15	3.5 [0.8, 24.0]	0.12	4.3 [0.6, 46.0]	0.19	4.3 [0.5, 54.0]	0.22	-0.2 [-0.6, 0.09]	0.16	-0.08 [-0.5, 0.4]	0.75	-0.08 [-0.6, 0.4]	0.73
E2/T Q1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
E2/T Q2	1.2 [0.3, 4.6]	0.79	2.0 [0.5, 9.0]	0.33	2.4 [0.5, 12.0]	0.27	1.6 [0.5, 5.3]	0.46	1.4 [0.4, 5.2]	0.65	1.3 [0.31, 6.0]	0.70	-0.1 [-0.5, 0.2]	0.45	-0.2 [-0.5, 0.2]	0.28	-0.2 [-0.6, 0.1]	0.23
E2/T Q3	1.3 [0.4, 4.9]	0.67	3.0 [0.7, 14.0]	0.15	3.9 [0.8, 20.0]	0.09	0.9 [0.2, 3.1]	0.83	0.7 [0.1, 3.3]	0.64	0.6 [0.09, 3.5]	0.57	-0.05 [-0.4, 0.3]	0.77	-0.2 [-0.5, 0.2]	0.42	-0.2 [-0.6, 0.2]	0.30
E2/T Q4	3.1 [0.95, 11]	0.07	13.0 [2.1, 87.0]	0.01*	16 [2.4,140.0]	0.01*	NA	NA	NA	NA	NA	0.99	0.3 [-0.08, 0.6]	0.13	0.09 [-0.4, 0.6]	0.73	0.05 [-0.5, 0.6]	0.84
SHBG Q1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
SHBG Q2	0.38 [0.1, 1.4]	0.18	1.2 [0.21, 5.6]	0.86	2.4 [0.5, 12.0]	0.27	1.8 [0.5, 7.3]	0.41	0.9 [0.2, 4.2]	0.90	1.0 [0.2, 5.1]	0.98	-0.3 [-0.7, -0.01]	0.04*	-0.3 [-0.7, 0.03]	0.08	-0.3 [-0.7, 0.05]	0.09
SHBG Q3	0.7 [0.2, 2.2]	0.52	4.1 [0.8, 22.0]	0.09	3.9 [0.8, 20.0]	0.09	0.7 [0.1, 3.5]	0.69	0.2 [0.03, 1.3]	0.09	0.3 [0.04,2.0]	0.23	-0.3 [-0.58, 0.06]	0.10	-0.2 [-0.6, 0.2]	0.29	-0.2 [-0.6, 0.2]	0.35
SHBG Q4	1.1 [0.4, 3.3]	0.85	18 [2.4, 170.0]	0.01*	16 [2.4, 140]	0.01*	1.4 [0.4, 6.1]	0.62	0.2 [0.02,1.5]	0.12	0.2 [0.02,2.1]	0.19	-0.4 [-0.7,-0.03]	0.03*	-0.3 [-0.8, 0.2]	0.25	-0.3 [-0.8, 0.2]	0.28

BMI: Body mass index, PSA: Prostate specific antigen, T: Testosterone, E2: Estradiol, SHBG: Sex hormone binding globulin, E2/T: Estradiol to Testosterone ratio, Q: Quartiles (1to 4), OR: odd ratio, CI: confidence interval, GS: Gleason score# Model 1: Adjusted for BMI at baseline (except in BMI model), age at baseline and SHBG (expect in SHBG model which is adjusted for E2 and T). ##Model 2: Adjusted for factors in Model 1 and HTN, DM, education, marital status, positive family history, smoking duration and having PSA assessment before randomization

Figure and figure legends

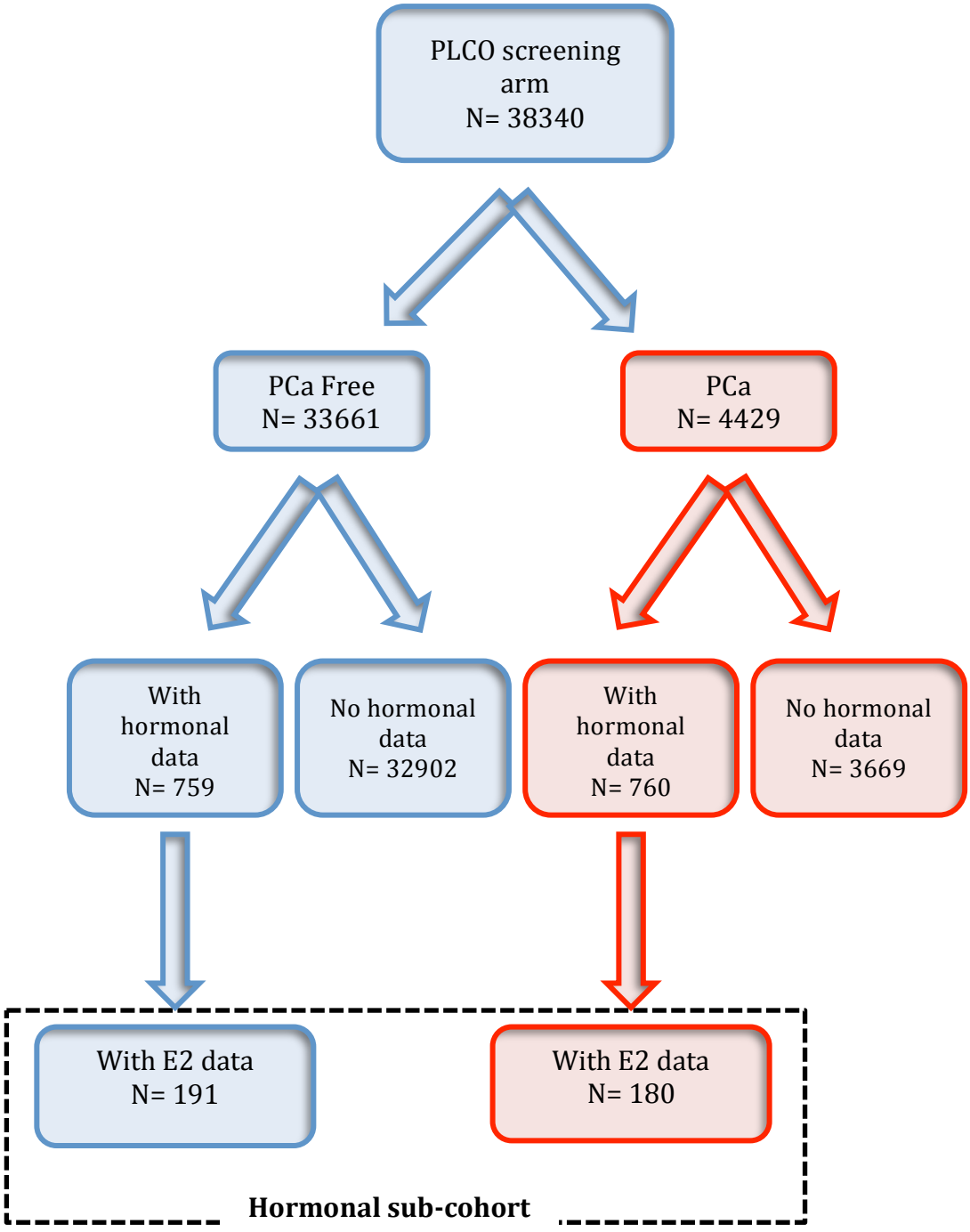

Figure 1: study flow-chart presenting the number and reasons for inclusion/exclusion and the final analysis cohort.

Chapter 6. Discussion and future directions

This project has documented the influence of metabolic factors (namely obesity, hypertension, and diabetes) on prostate cancer screening, prostate cancer diagnosis, and prostate cancer aggressiveness. It has shown: the effect of obesity on PSA levels and screening models; how changes in sex hormones among men with obesity may affect PSA levels; how the presence of metabolic factors may attenuate the efficacy of prostate cancer screening; and how sex hormones are associated with aggressive prostate cancer. It has also shown that the way aggressive prostate cancer is defined may affect the results of association studies, especially those involving metabolic factors or hormonal factors.

As discussed in sections one to four of Chapter 1 (literature review), prostate cancer is highly influenced by metabolic status, and it can be considered, at least in part, as a metabolic disease. There is strong preclinical evidence that shows prostate cancer cell survival and progression is dependent on cross-talk between lipid/fatty acid synthesis and androgen pathway signalling, both of which are intimately linked to metabolism. From an epidemiological perspective, racial and geographical differences in prostate cancer incidence and mortality may be partially explained by differences in metabolic status, risk of developing obesity and other metabolic related conditions (metabolic syndrome) as well as interactions between metabolic status and the sex hormone milieu.

Obesity *per se* is an independent risk factor for prostate cancer aggressiveness. This was shown in numerous previous studies and meta-analyses. However, when defining obesity using only subcutaneous fat, the association with aggressive prostate cancer appears weaker. This has raised a question regarding the association between visceral fat and risk of aggressive prostate cancer. In our published review article (Chapter 1, **section 5**) we have shown that an increase in visceral fat (peri-prostatic fat

in particular) is positively associated with aggressive prostate cancer and the risk of prostate cancer progression. We have posited in this review that peri-prostatic fat measurement could increase the sensitivity of prostate cancer risk stratification models. On a cellular level, we have shown in the review the evidence of cross-talk between the peri-prostate adipose cells and prostate cancer cells through the release of adipokines, tumour necrotic factors (TNF-α), interleukins and metalloproteinase activity among others. The volume of peri-prostatic fat might partially explain the positive association between obesity and aggressive prostate cancer. This is through the effect of obesity on the peri-prostatic fat, which showed to have a different cellular composition in obese men compared to lean men and to be more metabolically and secretory active.

The association between obesity and aggressive prostate cancer is not only explained by biological mechanisms (through the effect of chronic inflammation condition, TNF, interleukins, peri-prostatic fat, etc.) but also through the effect of obesity on the sex hormone milieu and a possible effect on screening and time to diagnosis. Obesity was found to be negatively associated with the serum levels of the prostate specific antigen (PSA) protein. There has been considerable debate around how obesity influences PSA levels in the blood, with the majority of previous studies supporting the haemodilution mechanism (the increase in the plasma volume with obesity and the resulting dilution of the PSA concentration in the blood). Fewer studies have compared the effect of obesity on serum testosterone with the haemodilution effect and concluded that the haemodilution effect predominates.

In chapter 2, in published research using a South Australian population-based cohort of prostate cancer-free men, we have shown that the lower levels of PSA in men with obesity is explained by both the haemodilution effect as well as by the

effect of obesity on the sex hormone milieu. We have shown for the first time that the increase in the estradiol to testosterone ratio with obesity can explain approximately 50% of the effect of obesity on PSA levels. The results of this research are important for the following reasons. First, the use of estradiol to testosterone ratio as a surrogate for the changes in sex hormones among men with obesity appeared to be more representative than using either of these hormones separately (as it summarises the net effect on the sex hormones milieu). Second, we have highlighted the potential importance of the estradiol to testosterone ratio in reducing the PSA levels among men with obesity. This opens new research questions in the future regarding the role of the estradiol to testosterone ratio with respect to a resultant delay in prostate cancer diagnosis, the need to adjust the PSA levels to the estradiol to testosterone ratio (instead of adjusting it to the testosterone levels which has been suggested by some researchers in the past), and the links between the estradiol to testosterone ratio and prostate cancer aggressiveness.

Lower levels of PSA among men with obesity have been suggested to lead to a delay in prostate cancer diagnosis (diagnosis bias). Despite the existence of multiple models indicating that lower PSA levels could lead to longer time to prostate cancer diagnosis and an increased risk of aggressive prostate cancer at the time of diagnosis, the consideration of obesity when interpreting PSA levels is still not included in current prostate cancer screening guidelines.

In **chapter 3** (submitted for publication) we have shown that the lower PSA levels in men with severe obesity has led to around seven years delay to reach a threshold level of 1ng/ml, around five years delay to reach 2ng/ml and around four years delay to reach 3ng/ml. These findings have strong potential to be clinically significant. Those PSA threshold levels mentioned earlier are used in current prostate

cancer screening recommendations to base the future schedule of screening and even the need for any future screening. Besides, the PSA level of 3 ng/ml is commonly used as a trigger for further diagnostic procedures (imaging and biopsies) for prostate cancer diagnosis. Thus it is hypothetically possible that the lower PSA levels among men with severe obesity could delay prostate cancer diagnosis and thus contribute to the association between obesity and more advanced prostate cancer.

A failure to consider the effect of obesity (especially among men with severe obesity) on PSA levels during conventional screening protocols could lead to an incorrect interpretation and sub-optimal medical advice. Whether this would lead to a delay in prostate cancer diagnosis and thus explain the poorer prognosis of prostate cancer among men with severe obesity still needs to be explored in future research studies. Future randomised controlled trials that include the effect of obesity on PSA and screening models would answer this question.

Although prostate cancer is a significant health problem among Western countries in general and in Australia in particular, there is considerable debate surrounding the overall benefit of prostate cancer screening. In the literature review (Chapter 1, section 3), the available evidence regarding the efficacy of prostate cancer screening was discussed and, in particular, the drawbacks of each of the five main randomised clinical studies that have examined the efficacy of prostate cancer screening. The available evidence shows a very modest effect of prostate cancer screening in reducing prostate cancer-related death; at the cost of increased diagnosis of early prostate cancer and increases in biopsy related complications, as well as the negative impact of biopsy, over-diagnosis and treatment of indolent cases on quality of life. Consequently, whether to screen or not remains an open question. However, the results of available clinical studies should be considered with caution due to

limitations in study design: low compliance, variation in the intensity of screening and the rate of contamination (PSA screening) in the control arm. Apart from the variation in study design, there has been a suggestion about the role of having chronic comorbidities on the efficacy of prostate cancer screening through a competing risk effect. However, to date, there is not enough data to support this hypothesis.

In **chapter 4** (prepared for publication), we have shown for the first time that having metabolic syndrome-related factors could attenuate the efficacy of prostate cancer screening. We have shown that men who were metabolically healthy at the start of the screening, as well as men who maintained metabolically healthy status for an average of nine years, benefited from prostate cancer screening in terms of reducing the risk of prostate cancer-related death. In this work, we have also shown that the competing risk effect can partially explain this effect. Men with one or more factors of the metabolic syndrome (obesity, diabetes or hypertension) have a higher risk of dying from non-prostate cancer causes, and thus they mostly die before being diagnosed with prostate cancer or before dying of prostate cancer if they have been diagnosed. We have also shown that the effect of having metabolic syndrome factors (mainly diabetes and obesity) on screening efficacy can be explained by their inverse association with the PSA levels (leading to potential delay in diagnosis) and their positive association with aggressive prostate cancer (mainly high grade prostate cancer) and thus their presence may mask the efficacy of prostate cancer screening.

There is evidence regarding the effect of metabolic factors (obesity, diabetes, and hypertension) on the sex hormone milieu and vice versa. Whether the hormonal changes can explain the effect of metabolic syndrome factors on prostate cancer aggressiveness is still an area of debate requiring further research. The current body of research consists of inconsistent associations between the changes in sex hormone

milieu and prostate cancer incidence and aggressiveness. Several factors can attribute to this inconsistency, mainly the way of measuring sex hormones, neglecting the variation of sex hormones over time and the effect of extremely high or low levels of sex hormones. Also, how aggressive prostate cancer is defined has been suggested as another reason. Although combining the tumour size (Stage), grade (Gleason score) and PSA at time of diagnosis is of clinical value in clinical risk stratification models, and is used for predicting prostate cancer prognosis, it may not be the best approach for association studies which includes factors that may affect time to prostate cancer diagnosis. A good example is obesity and diabetes, both of which are associated with lower PSA levels and thus a potential delay in diagnosis and inverse association with the tumour stage. However, they have positive associations with the tumour grade (Gleason score). Thus combining these tumour aggressiveness factors may mask overall associations with certain metabolic factors.

In **chapter 5** (submitted for publication), we have shown that redefining aggressive prostate cancer can lead to different conclusions. In particular, if only tumour grade is used as a surrogate for prostate cancer aggressiveness instead of a combination of the stage, grade, and PSA, a different conclusion can be reached. We have shown that the association between sex hormones (mainly the estradiol to testosterone ratio) and tumour grade is not consistent as for tumour stage or PSA levels. In this work, we have shown that higher estradiol to testosterone ratio is associated with increased risk of having aggressive prostate cancer when defined by Gleason score only, but not when defined by tumour stage or a combination of Gleason score and stage.

Future research direction and projects

The work done for this project opens the opportunity for distinct future research questions to improve the efficacy of the screening models, determine more effective risk reduction modalities, and provide a better understanding of how metabolic factors and the sex hormones influence prostate cancer progression. Based on the work presented in this thesis, the following represent future directions and possible future projects:

Obesity and PSA levels:

In our analysis of the association between obesity and PSA levels and the role of sex hormones in reducing PSA levels, we were limited by the small size of our study cohort, and the fact that our cohort featured prostate cancer-free men. Besides, the clinical impact of reduced PSA levels in men with obesity needs to be examined in more detail. These points can be covered in the following projects:

i. The role of sex hormones (in comparison to the haemodilution mechanism) in reducing PSA levels among men with moderate and severe obesity needs to be validated in different cohorts, with larger sample sizes.

ii. The association between obesity and PSA and the role of sex hormones in that association needs to be compared between prostate cancer-free cohorts and a prostate cancer cohort to understand if both mechanisms have the same contribution in the presence/absence of malignancy.

iii. The effect of obesity on PSA and whether this leads to a clinically-meaningful delay in prostate cancer diagnosis needs to be explored in different cohorts to confirm this finding. A promising option would be

to use the South Australian Prostate Cancer registry and examine whether men with moderate/severe obesity are diagnosed later than those with a normal BMI.

iv. Explore the utility of PSA levels adjusted for the estradiol to testosterone ratio in prostate cancer diagnosis and whether the E/T adjusted PSA levels have better predictive power than PSA alone.

v. One challenging way to explore the effect of obesity on PSA and whether this affects screening efficacy is to explore this in a prospective three-arm randomised control clinical study, in which men are randomised to either no screening, screening, and biopsy based on the current recommended PSA levels, or screening and biopsy-based on adjusted PSA levels (Obesity adjusted PSA or E/T adjusted PSA levels). One drawback of such design is the need for a large sample size, the very long duration of follow up required (~15 to 20 years) and the cost. Considering this drawback, alternative approaches should be considered including micro-simulation modelling of screening outcome based on BMI values. While not within the scope of this work, the prospect of modelling the effect of more specifically targeted screening groups would assist in understanding the potential success of implementing targeted screening based on BMI.

Obesity and prostate cancer

An outstanding issue to be resolved is the best way to assess obesity and its relationship to prostate cancer. It is also necessary to discern the respective roles of

visceral versus subcutaneous obesity. The following project may answer these research questions:

i. To confirm the conclusion of our review, we need to assess if defining obesity using different modalities leads to different associations with aggressive prostate cancer and whether visceral fat (mainly peri-prostatic fat) can explain the association between obesity and aggressive prostate cancer. Using data from prostate cancer cohorts and registries, this can be done through a retrospective assessment of obesity using different modalities including recalled BMI and waist circumference at the age of 50 and at time of diagnosis and comparing this with the subcutaneous and visceral fat measurement at the time of diagnosis using the MRI images. This will allow examining the impact of using different modalities to assess obesity, the association with aggressive prostate cancer at the time of diagnosis and whether visceral peri-prostatic fat is a key mediator of the association between obesity and aggressive prostate cancer. Of note, although recalled data are not as accurate as collected data from registries, multiple studies have shown that they are robust and reliable (Dahl et al. 2010; Dahl & Reynolds 2013; Munoz et al. 1996; Tamakoshi et al. 2003).

Metabolic syndrome and prostate cancer

The associations between metabolic syndrome and prostate cancer are complex and are influenced by different factors. The most important factors are the duration of having metabolic syndrome, the degree of control of metabolic syndrome

with medication, and the changes in sex hormones that are associated with metabolic syndrome. The following research projects may uncover the roles of these factors in the association of metabolic syndrome with prostate cancer:

ii. A retrospective analysis of a prostate cancer cohort (or men from a prostate cancer registry) that has collected information about the presence of metabolic syndrome, duration of metabolic syndrome and medications used for treatment or control of metabolic syndrome components may clarify the effect of duration and medication on the association between metabolic syndrome and risk of aggressive prostate cancer.

iii. As mentioned previously, metabolic syndrome is a risk factor for cardiovascular and all cause-related mortality. One of the main side effects of hormonal treatment in advanced prostate cancer is the development of metabolic syndrome (a metabolic complication of androgen deprivation therapy). A retrospective analysis of the effects of taking metformin and statin (medications used for treatment of metabolic syndrome and that decrease risk of cardiovascular-related complications) before and at time of starting the androgen deprivation therapy on; a) risk of developing metabolic syndrome after initiation of androgen deprivation therapy, and b) on time to prostate cancer progression would clarify the effects of those two common medications on controlling the androgen deprivation side effects and prostate cancer progression.

Metabolic syndrome and prostate cancer screening

We have shown the effect of having metabolic syndrome-related conditions on the efficacy of prostate cancer screening; however, these results need to be validated:

i. The effect of MS on PSA screening needs to be validated in another screening and prostate cancer cohorts (including the ERSPC study cohort, the South Australian prostate cancer registry, among others). This could lead to practice change and may lead to improving the efficacy of prostate cancer screening through identifying the sub-group/s of men who benefit the most from screening.

ii. Future work investigating the cost and benefit of more specific testing algorithms for men in different risk groups would add more insight about the absolute benefit of prostate cancer screening. This would include health economic evaluation of prostate cancer screening among different risk groups with identification of the best strategy to implement for each group.

Sex hormones and prostate cancer

The association between sex hormones and prostate cancer aggressiveness and whether the changes in sex hormones can explain the association between obesity/ metabolic syndrome and aggressive prostate cancer needs to be explored in more detail. This can be done through the following projects:

i. The association between sex hormones (estradiol to testosterone ratio) and risk of aggressive prostate cancer needs to be confirmed and validated in another cohort with larger sample size. Using a different way to define aggressive prostate cancer (including Gleason score only, T stage only or combination of Gleason score and tumour stage) will address our hypothesis that the definition of aggressive prostate cancer used may influence the association between sex hormones and risk of aggressive prostate cancer.

ii. It remains unclear whether changes in the sex hormone milieu can explain the association between metabolic syndrome (and obesity specifically) and aggressive prostate cancer. An analysis that examines the association between a) obesity, b) metabolic syndrome components and risk of aggressive prostate cancer after adjusting for the sex hormones levels might clarify this association. Besides, it would be useful to identify a phenotype profile that includes metabolic syndrome components and sex hormone levels that may be risk factors for developing prostate cancer and/or aggressive prostate cancer. This analysis can be done using a retrospective cohort with available bio-specimens. A potential candidate cohort is the Australian Prostate Cancer BioResource cohort, which the Lipids and Prostate Cancer

Research Group is part of, and the MAILES cohort, which includes prostate cancer-free men.

Of course, there is the opportunity to integrate other methodological approaches to address these and additional questions. Some examples include, i) genetic profiling for risk of prostate cancer and aggressive prostate cancer based on metabolic and hormonal profiling (phenotype), ii) a Mendelian randomisation study design to identify the causal relationship between metabolic factors and prostate cancer, and iii) adaptive design studies that explore the best approach to control metabolic side effects with androgen deprivation therapy and how this can reduce metabolic comorbidities and if this can reduce time to prostate cancer progression.

The results of this project, as well as the potential future projects, will lead to a complete understanding of the association between metabolic factors, especially obesity, and prostate cancer. It will provide greater insight into underlying mechanisms through which metabolic factors affect prostate cancer pathogenesis and aggressiveness. This will allow for the identification of risk reduction modalities and interventions that may reduce the risk of aggressive prostate cancer and improve prostate cancer outcomes. The direct objective of these proposed studies is to ultimately improve clinical practice, mainly in the area of prostate cancer screening, by identifying the effect of considering obesity when interpreting PSA results and by identifying sub-group of men who benefit the most from prostate cancer screening.

Appendix 1. Prostate cancer staging and definition of aggressiveness

AJCC 8th Edition (Buyyounouski et al. 2017)

Primary Tumor (T)

TX	**Primary tumor cannot be assessed**
T0	**No evidence of primary tumor**
T1	**Clinically unapparent tumor neither palpable nor visible by imaging**
T1a	**Tumor incidental histologic finding in 5% or less of tissue resected**
T1b	**Tumor incidental histologic finding in more than 5% of tissue resected**
T1c	**Tumor identified by needle biopsy (for example, because of elevated PSA**
T2	**Tumor confined within prostate[1]**
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 prostate capsule**
T3a	**Extra capsular extension (unilateral or bilateral)**
T3b	**Tumor invades seminal vesicle(s)**
T4	**Tumor is fixed or invades adjacent structures other than seminal vesicles, such as external sphincter, rectum, bladder, levator muscles, and/or pelvic wall**

Regional Lymph Nodes (N)

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

Distant Metastasis (M)

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

Grading and Gleason Score

ISUP Grade Group	Gleason Score	Gleason Pattern
1	≤6	≤3+3
2	7	3+4
3	7	4+3
4	8	4+4, 3+5, 5+3
5	9 or 10	4+5, 5+4, 5+5

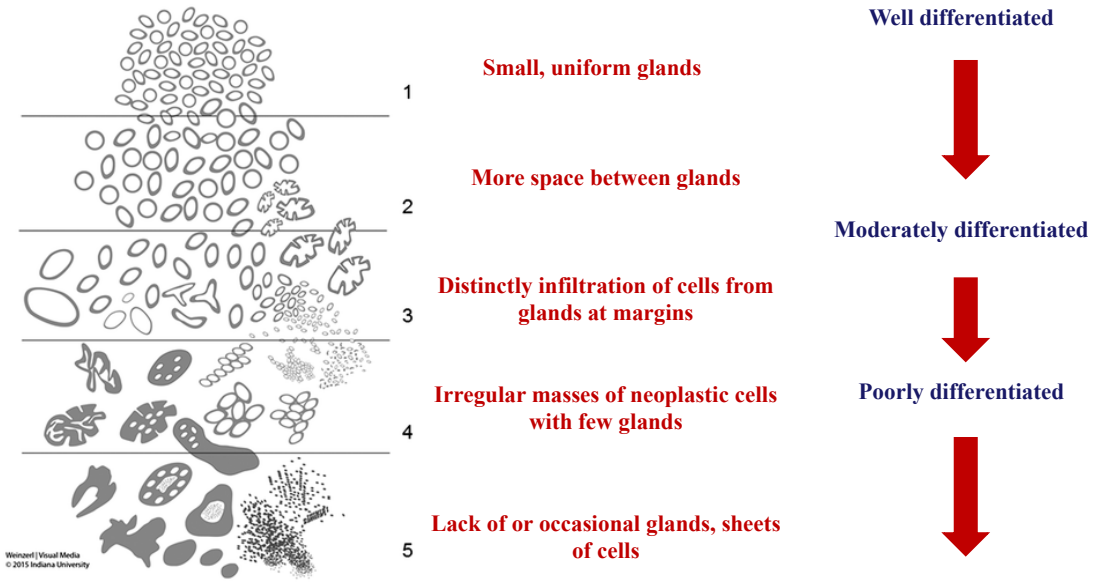

Figure 1-Appendix: Gleason score, Figure adapted with permission from Shah, R. B., and M. Zhou (Shah & Zhou 2016)

Prostate Cancer staging (AJCC 8th Edition)

Stage	T	N	M	PSA	Grade
I	cT1a-c, cT2a	N0	M0	<10 ng/mL	1
I	pT2	N0	M0	<10 ng/mL	1
IIA	cT1a-c, cT2a	N0	M0	≥10, <20 ng/mL	1
IIA	pT2	N0	M0	≥10, <20 ng/mL	1
IIA	cT2b-c	N0	M0	<20 ng/mL	1
IIB	T1-2	N0	M0	<20 ng/mL	2
IIC	T1-2	N0	M0	<20 ng/mL	3
IIC	T1-2	N0	M0	<20 ng/mL	4
IIIA	T1-2	N0	M0	≥20 ng/mL	1-4
IIIB	T3-4	N0	M0	Any	1-4
IIIC	Any T	N0	M0	Any	5
IVA	Any T	N1	M0	Any	Any
IVB	Any T	Any	M1	Any	Any

NCCN 2019 Version 2 Risk grouping

Risk group	Clinical/pathologic features		
Very low	• T1c AND • Grade Group 1 AND • PSA <10 ng/mL AND • Fewer than 3 prostate biopsy fragments/cores positive, • ≤50% cancer in each fragment/core AND • PSA density <0.15 ng/mL/g		
Low	• T1-T2a AND • Grade Group 1 AND • PSA <10 ng/mL		
Intermediate	Has no high- or very-high-risk features and has one or more intermediate risk factors (IRF): • T2b-T2c • Grade Group 2 or 3 • PSA 10–20 ng/mL	**Favourable intermediate**	• 1 IRF and • Grade Group 1 or 2 and • <50% biopsy cores positive
		Unfavourable intermediate	• 2 or 3 IRFs and/or • Grade Group 3 and/or • >50% biopsy cores positive
High	• T3a OR • Grade Group 4 or Grade Group 5 OR • PSA >20 ng/mL		
Very high	• T3b-T4 OR • Primary Gleason pattern 5 OR • >4 cores with Grade Group 4 or 5		

https://www.nccn.org/professionals/physician_gls/pdf/prostate_blocks.pdf

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