THE EFFECTS OF SERUM FROM OBESE PATIENTS AND ADIPOCYTE-DERIVED CYTOKINES ON GROWTH OF PROSTATE CANCER CELLS IN VITRO

by

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A Thesis Submitted in Conformity with the Requirements for Degree of Master of Science Graduate Department of Institute of Medical Science University of Toronto

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Master of Science

Institute of Medical Science
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Abstract

Obesity has been related to a greater incidence of more aggressive, advanced stage prostate cancer. It is expected that serum adipokines related to obesity will promote a more aggressive phenotype in PC cells in vitro. Patient serum (n = 80) was obtained for analysis and divided into four patient groups based on obesity and prostate cancer status. Characteristics of serum-treated PC cells in vitro were measured. In a separate set of analyses, LNCaP and PC3 cells were treated with adiponectin and resistin in vitro, and cell characteristics were analyzed. Serum from obese PC patients induces greater amounts of cell migration and lower amounts of cell proliferation and invasion in vitro. Exogenous treatment of adiponectin on PC cells in vitro does not affect cell migration or invasion. However, adiponectin modulates cytosolic protein levels of soluble β-catenin and GSK-3β, indicating that its mechanism of action may be through the Wnt signalling pathway.
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List of Abbreviations

AS: active surveillance
ADT: androgen deprivation therapy
Akt: protein kinase B, a serine/threonine-specific protein kinase
P-Akt: phosphorylated protein kinase B
AMPK: adenosine monophosphate-activated protein kinase
AUA: American Urological Association
BC: beta-catenin
PBC: phosphorylated beta-catenin
Bcl-2: B-cell lymphoma 2
BCR: biochemical recurrence
BMI: body mass index
BPH: benign prostatic hyperplasia
BT: brachytherapy
CC3: cleaved caspase-3
CI: confidence interval
CRPC: castrate resistant prostate cancer
CVD: cardiovascular disease
DRE: digital rectal examination
EBRT: external beam radiation therapy
ELISA: enzyme-linked immunosorbent assay
ER: estrogen receptor
FGC: fast growing colony
GSK-3β: glycogen synthase kinase 3 beta
P-GSK-3β: phosphorylated glycogen synthase kinase 3 beta
H6D: histidine to aspartic acid substitution-6
HDL: high-density lipoprotein
HGF: hepatocyte growth factor
HR: hazard ratio
IL-6: interleukin-6
IGF: insulin-like growth factor
IGFR: insulin-like growth factor receptor
JNK: c-Jun N-terminal kinase
MCP-1: monocyte chemotactic protein-1
MetS: metabolic syndrome
MRI: magnetic resonance imaging
NAG-1: non-steroidal anti-inflammatory drug-activated gene-1
NFκB: nuclear factor kappa B kinase
NGF: nerve growth factor
NOX: mono-nitrogen oxides
OD: optical densities
OR: odds ratio
P21: cyclin-dependent kinase inhibitor p21
P27: cyclin-dependent kinase inhibitor p27
P53: tumor protein 53
PAI-1: plasminogen activator inhibitor-1
PARP: poly ADP ribose polymerase
PC: prostate cancer
PCNA: proliferating cell nuclear antigen
PI3K: phosphatidylinositol 3-kinase
PSA: prostate-specific antigen
PPAR: peroxisome proliferator-activated receptor
PR: progesterone receptor
PTEN: phosphatase and tensin homolog
RCT: randomized clinical trial
RE: risk estimate
ROC: receiver operating characteristic
RR: relative risk
RP: radical prostatectomy
RT: radiation therapy
SNP: single nucleotide polymorphism
SHBG: sex-hormone-binding globulin
STAT3: signal transducer and activator of transcription 3
T: testosterone
T2D: type 2 diabetes
TG: triglyceride
TGF-β: transforming growth factor beta
TNM: tumor staging: tumor, lymph nodes, metastasis
TRAMP: transgenic adenocarcinoma of mouse prostate
TRUS: transrectal ultrasound
TNF-α: tumor necrosis factor alpha
uPA: urokinase-type plasminogen activator
VEGF: vascular endothelial growth factor
WHR: waist-to-hip ratio
WW: watchful waiting
CHAPTER ONE

1. Literature Review

1.1 Prostate Cancer Overview

Cancer is the leading and second leading cause of death in developed and developing countries respectively (Jemal, et al. 2011). PC is the second most frequently diagnosed cancer and the sixth leading cause of cancer deaths in males worldwide (Jemal, et al. 2011). It is estimated that PC will account for 28% (238 590) of all newly diagnosed cancers in American men in 2013 (Siegel, Naishadham and Jemal 2013). Approximately 580 350 Americans will die from cancer this year, with 29 720 of those deaths being attributed to PC (Siegel, Naishadham and Jemal 2013). In 2008, 72% (899 000) of cases and 53% (258 000) of PC deaths occurred in developed countries, including Europe, North America, Austria/New Zealand, and Japan, representing <20% of the world population (Center, et al. 2012). PC incidence and mortality rates varied 24- and 10-fold worldwide respectively, with the highest incidence rates occurring in North America, Western Europe, Australia/New Zealand, and the Caribbean, and the lowest rates occurring in South Central Asia, Northern Africa, and Eastern Asia (Center, et al. 2012).

The variation in PC incidence rates internationally has been partly attributed to differences in diagnostic practices and the implementation of PSA testing in most of the developed countries in the mid-1980s (Center, et al. 2012). PSA screening results in the detection of some biologically indolent tumors. However, a 120-fold
difference in PC incidence rates worldwide was observed before PSA screening was introduced (Zaridze, Boyle and Smans 1984), indicating that other factors may be contributing to the variations. Global disparities in cancer incidence and mortality are likely due to non-modifiable risk factors, such as genetics and aging, and modifiable factors, such as smoking, infectious agents, diet and physical activity (Kamangar, Dores and Anderson 2006). Environmental factors, such as diet (Gonzalez and Riboli 2010), physical activity (Orsini, et al. 2009), and obesity (Buschemeyer and Freedland 2007) have been shown to influence tumor development (Khan, Afaq and Mukhtar 2010) and may affect PC incidence/mortality rates internationally.

The established prognostic factors reported and used for planning patient management for PC are TNM stage, surgical margin status, serum PSA, and Gleason grade (Humphrey 2004). The most frequently used tests to detect localized PC are elevated PSA and irregular DRE (Thompson, et al. 2007). Detection of PC requires confirmation with a TRUS-guided biopsy, where several small cores of tissue are removed from the prostate, examined by a pathologist, and assigned a Gleason score (Thompson, et al. 2007). Gleason grading is a 5-tier system, where 1 represents a well-differentiated pattern and 5 a poorly differentiated pattern (Iczkowski and Lucia 2011). The most prevalent pattern (1-5) is the primary grade, the second most prevalent pattern is the secondary grade, and the sum is the Gleason score (2-10) (Iczkowski and Lucia 2011). Clinical practice guidelines indicate that localized PC (T1 to T2 disease with no regional lymph node or distant metastasis, T1 to T2N0-
NxM0) can be managed or treated with WW or AS, EBRT, BT or RP (Cooperberg, et al. 2013).

PSA is a glycoprotein expressed by both normal and cancerous prostate tissue (Adhyam and Gupta 2012). Serum PSA levels are used to determine the extent of PC, the response to treatment, and to screen for PC (Adhyam and Gupta 2012). Screening techniques are designed to reduce morbidity and mortality by identifying PC more frequently and earlier (Ilic, et al. 2013). However, the medical community remains divided as to whether PSA testing is able to detect clinically relevant disease. The AUA recommends against PSA screening in men under 40 yr; does not recommend routine screening in men between 40 to 54 yr at average risk; advises only undergoing PSA screening in shared decision-making with patients 55 to 69 yr; and does not recommend routine PSA screening in men over 70 yr or any man with less than a 10 to 15 yr life expectancy (Carter, et al. 2013). A Cochrane meta-analysis including five RCTs failed to show decreases in PC-specific mortality with PC screening (Ilic, et al. 2013). The PC incidence and PC-specific mortality in men 9 yr after the discontinuation of PSA-based screening caught up and matched non-screened controls (Grenabo Bergdahl, et al. 2013). Furthermore, screening is associated with minor harms, including bleeding, bruising, and short-term anxiety; and major harms, including overdiagnosis and overtreatment, infection, blood loss requiring transfusion, pneumonia, erectile dysfunction, and incontinence (Ilic, et al. 2013).

Most PC cases are detected with PSA screening (Dahabreh, et al. 2012), which identifies disease 6 to 13 yrs before it presents clinically (Lu-Yao, et al. 2009).
moderately differentiated disease is managed conservatively, patients are more likely to die of causes other than PC (Lu-Yao, et al. 2009). Many tumors of the prostate are estimated to have a protracted natural history and thus pose minimal threat during the patient’s lifetime (Dall-Era, et al. 2012). The treatment options available for localized PC—RP, RT, and ADT—though offered with curative intent, probably confer minimal clinical benefit due to the slow progression of the disease (Dahabreh, et al. 2012). Treatments for localized PC result in significant adverse events, including impotence, urinary or bowel dysfunction, among other complications (Dahabreh, et al. 2012). The majority of men with newly diagnosed PC undergo aggressive treatment regardless of the risk, raising concerns of overdiagnosis and overtreatment (Dall-Era, et al. 2012). Thus, alternative management strategies for early-stage, low-risk PC include AS and WW, observational strategies that involve forgoing immediate therapy (Dahabreh, et al. 2012).

Forty percent of diagnosed PC patients who choose definitive therapy will undergo RP, and 20-30% will experience disease recurrence, often in the form of BCR (Simmons, Stephenson and Klein 2007). BCR is defined as any detectable PSA levels following RP, or a PSA rise following a period of no PSA detection (Simmons, Stephenson and Klein 2007). Since not all PSA-producing cells are confined to the prostate, RP does not always entirely clear PSA expression from the body (Roberts and Han 2009). Tumor cells tend to invade tissue locally surrounding the prostate, into pelvic lymph nodes, or metastasize to distant sites throughout the body, such as bone or lung tissue (Roberts and Han 2009). BCR indicates the presence of prostatic
epithelial tissue, assumed to represent cancer (Simmons, Stephenson and Klein 2007), and typically precedes metastatic progression (Roberts and Han 2009). Patients suspected of metastatic disease are offered hormonal therapy, often castration via surgically (orchiectomy) or medically (luteinizing hormone releasing hormone agonists/antagonists) (Fong, Hare and Jarkowski 2012). Hormone therapy, or ADT, provides control temporarily, followed by disease progression to CRPC, characterized by increasing tumor volume (via radiography) or progressively rising PSA, despite castrate levels of T (<50 ng/dL) (Fong, Hare and Jarkowski 2012). Patients with CRPC who have detectable macroscopic metastatic disease can be offered systemic chemotherapy (e.g. docetaxel and prednisone) (Saad and Hotte 2010). Recently approved therapeutic options for CRPC include hormonal therapy (Abiraterone and MDV3100), immunotherapy (Sipuleucel-T), cytotoxic therapy (Cabazitaxel), targeted therapy (Cabozantinib), and bone-directed therapy (Alpharadin and Denosumab) (Galsky, et al. 2012).

1.2 Impact of Obesity on Human Health

In 2008, one in every three adults was overweight (BMI 25 ≤ 30), and 1 in every 9 was obese (BMI ≥30) worldwide (Stevens, et al. 2012). The increase in the prevalence of obesity has accelerated in the 2000s compared to the 1980s and 1990s (Stevens, et al. 2012). The global age-standardized prevalence of obesity nearly doubled from 6.4% (95% CI: 5.7-7.2%) in 1980 to 12.0% (95% CI: 11.5-12.5%) in 2008 (Stevens, et al. 2012). A gender disparity exists in the prevalence of overweight and obesity globally (Kanter and Caballero 2012). Examining 105
different countries, it was found that all income groups had a greater overall prevalence of female obesity compared with male obesity (Kanter and Caballero 2012). However, among the high-income groups there were more overweight males than females (Kanter and Caballero 2012). Forecasts predict a 33% and a 130% increase in the prevalence of obesity and severe obesity respectively in the next 20 yrs, with 51% of the population being obese by 2030 (Finkelstein, et al. 2012). On the one hand, globalization has reduced poverty and increased nutrition, and on the other has created factors that have fueled the obesity epidemic. Global trade along with increases in income and urbanization have created obesogenic environments, promoting deleterious nutritional transitions and technology-driven reductions in physical activity, resulting in positive energy balance, with subsequent weight-gain and obesity (Malik, Willett and Hu 2013).

Abdominal obesity is related to a constellation of signs that constitute the MetS. According to the Adult Treatment Panel III of the National Cholesterol Education Program, MetS has the following characteristics: 1) abdominal obesity with a waist circumference > 102 cm for men, > 88 cm for women; 2) triglycerides ≥ 150 mg dL⁻¹; 3) HDL cholesterol levels < 40 mg dL⁻¹ for men, < 50 mg dL⁻¹ for women; 4) blood pressure ≥130/≥85 mmHg; and 5) fasting glucose ≥ 110 mg dL⁻¹ (Johnson, et al. 2013). Age is an important factor for MetS, with the prevalence among Canadian adults being only 17% for individuals 18-39 yrs, compared with 39% for individuals 70-79 yrs (Riediger and Clara 2011).

The constellation of symptoms, along with associated characteristics, that make up the MetS—such as insulin resistance, abnormal fatty acid metabolism,
increased oxidative stress, endothelial dysfunction, and cytokine dysregulation—
increase the likelihood of CVD complications (Nikolopoulou and Kadoglou 2012),
including ischemic heart disease (Nordestgaard, et al. 2012), angina (Wolk, et al.
2003), and myocardial infarction (Abdulla, et al. 2008). BMI and waist
circumference are established clinical measurements used to estimate CVD risk
(Rheaume, Leblanc and Poirier 2011). Elevated BMI is associated with increased
prevalence and serves as an independent risk factor for CVD (Rheaume, Leblanc and
Poirier 2011).

A major comorbidity of obesity is T2D (Shamseddeen, et al. 2011). Obesity is
associated with low-grade, chronic inflammation that may be causally involved in
the development of insulin resistance (Zeyda and Stulnig 2009). Adipocytes release
cytokines (termed adipokines) that contribute to peripheral insulin resistance,
including adiponectin, resistin, TNF-α, and IL-6 (Keller 2006). A complex interplay
exists between genetics, epigenetics, and the environment in the susceptibility to
T2D and obesity (including heritable epigenetics, environment-derived epigenetics,
gene-environment interactions, and metastable epialleles) (Drong, Lindgren and
McCarthy 2012).

Various other health complications and chronic diseases have been
associated with elevated BMI and/or obesity, including, but not limited to, stroke
(Ryu, et al. 2011), multiple sclerosis (Hedstrom, Olsson and Alfredsson 2012),
depression (Morris, et al. 2012), osteoarthritis (Zhang, et al. 2013), fatty liver
disease (Wang, et al. 2013), obstructive sleep apnea (Degache, et al. 2013), asthma
(Dandona, et al. 2013), erectile dysfunction (Janiszewski, Janssen and Ross 2009),
urinary incontinence (Khullar, et al. 2013), chronic renal failure (Ejerblad, et al. 2006), polycystic ovarian syndrome (Zeng, et al. 2013), and infertility (Esmaeilzadeh, et al. 2013). In an analysis of 97 studies, with a pooled sample size of over 2.88 million with more than 270 000 deaths, obesity was associated with greater all-cause mortality (HR, relative to normal weight, 1.18, 95% CI: 1.12-1.25) (Flegal, et al. 2013).

1.3 Impact of Obesity on Cancer

1.3.1 Epidemiology of Obesity and Cancer

Obesity has been associated with risk of death from cancer. A 2001 meta-analysis on epidemiological data for countries in the European Union concluded that excess body mass accounts for 5% of all cancers combined, 3% and 6% in men and women respectively, or 27 000 and 45 000 annually in men and women respectively (Bergstrom, Pisani, et al. 2001). The cancer sites that had the strongest relationship with BMI were endometrium, kidney, colon, breast, and gallbladder (Bergstrom, Pisani, et al. 2001). In a prospective analysis of cancer incidence in more than 900 000 US adults followed over a 16-yr period, it was determined that the heaviest individuals (≥40 BMI) had death rates from all cancers 52% (RR of 1.52, 95% CI: 1.13-2.05) and 62% (RR of 1.62, 95% CI: 1.40-1.87) higher for men and women respectively compared to men and women of normal weight (Calle, et al. 2003). BMI was associated with higher RR of death due to cancer of the esophagus, colon and rectum, liver, gallbladder, pancreas, kidney, non-Hodgkin's lymphoma, and multiple
myeloma (Calle, et al. 2003). The authors of the study concluded that overweight and obesity may account for as high as 14% and 20% of cancer-related deaths in men and women respectively >50 yrs of age (Calle, et al. 2003). Another study examined incident cancer across 30 European countries and concluded that excess BMI (>25) contributed to 2.5% and 4.1% in men and women respectively, or over 70 000 new cancer cases in 2002 (Renehan, Soerjomataram, et al. 2010). Of the incident cases, 65% were endometrial, post-menopausal breast, and colorectal cancer (Renehan, Soerjomataram, et al. 2010). A meta-analysis in 2008 analyzed 141 articles (with a total of 282 137 incident cases) and found that a 5-unit increase in BMI was associated with esophageal, thyroid, colon, and renal cancer in men, and endometrial, gallbladder, esophageal, and renal cancer in women (Renehan, Tyson, et al. 2008). Another meta-analysis in 2009 analyzed 18 co-morbidities in 89 articles related to overweight and obesity, and found that overweight was associated with colorectal, kidney, breast, ovarian, endometrial, and pancreatic cancer (Guh, Zhang, et al., The incidence of co-morbidities related to obesity and overweight: a systematic review and meta-analysis 2009). Combining data from cross-sectional surveys and meta-analyses from China and East Asia in 2005, it was determined that 0.32% of cancer deaths and 0.65% of cancer cases were attributable to overweight (BMI 25-30) and obesity (BMI ≥ 30) combined (Wang, et al. 2012).
1.3.2 Meta-analyses on the Relationship Between Obesity and Cancer

**Table 1** summarizes published meta-analyses on the association between obesity and different cancer sites. Section 1.3 considers obesity and cancer in general, whereas section 1.4 considers PC specifically.

**Table 1 – Meta-analyses summarizing published data on association between obesity and various types of cancer.**

<table>
<thead>
<tr>
<th>Cancer Site</th>
<th>Reference</th>
<th># of Studies</th>
<th>Summary Result</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>(Connolly, et al. 2002)</td>
<td>19</td>
<td>RE were 1.80 (95% CI: 1.29-2.50) for case-control studies and 1.27 (95% CI: 1.07-1.51) for cohort studies; 1.79 (95% CI: 1.22-2.62) for pre-menopausal women and 1.50 (95% CI: 1.10-2.04) for post-menopausal women; 1.62 (95% CI: 1.28-2.04) for all studies combined.</td>
<td>WHR was associated with increased risk of breast cancer.</td>
</tr>
<tr>
<td></td>
<td>(Vrieling, et al. 2010)</td>
<td>11</td>
<td>Highest versus lowest categories of adult weight gain, risk increased for ER and PR positive and ER positive tumors combined (RE: 2.013, 95% CI: 1.62-2.45). Data was heterogeneous (p=0.002) between results for pre- and postmenopausal women combined and postmenopausal women alone.</td>
<td>Association between weight gain and postmenopausal breast cancer risk was heterogeneous according to ER/PR status. Association only found between weight gain and higher risk of ER+PR+ postmenopausal breast cancer risk.</td>
</tr>
<tr>
<td></td>
<td>(Yang, et al. 2011)</td>
<td>34</td>
<td>BMI ≥30 in women ≤50yr old was more frequent in ER-/PR- than in ER+/PR+ tumors (p&lt;0.001). BMI ≥30 in women ≥50yr old was less frequent in PR- than in PR+ tumors (p&lt;0.001).</td>
<td>BMI in younger women was strongly, positively associated with hormone receptor positive tumors.</td>
</tr>
<tr>
<td></td>
<td>(Key, Appleby, et al. 2003)</td>
<td>8</td>
<td>Breast cancer risk positively associated with BMI (p=0.002); association reduced with adjustment for serum free estradiol, RR from 1.19 (95% CI: 1.05-1.34) to 1.02 (95% CI: 0.89-1.17). Adjusting for other estrogens also reduced positive association between BMI and breast cancer risk (total estradiol, non-sex hormone-binding globulin-bound estradiol, estrone, and estrone sulfate).</td>
<td>The association between increased breast cancer risk and increased BMI in postmenopausal women was largely explained by alterations in estrogen levels.</td>
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<td>(Niraula, et al. 2012)</td>
<td>21</td>
<td>Hazard ratios for breast cancer specific survival were 1.36 (95% CI: 1.20-1.54) for ER/PR+ cancers and 1.46 (95% CI: 0.98-2.19) for ER/PR- cancers; 1.18 (95% CI 0.82-1.70) for premenopausal women, and 1.38 (95% CI: 1.11-1.71) for post-menopausal women.</td>
<td>The association between obesity and breast cancer specific survival does not appear to differ with hormone receptor status.</td>
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<td>(Harvie, Hooper and Howell 2003)</td>
<td>8</td>
<td>39% lower risk of breast cancer in postmenopausal women with the smallest waist, and 24% lower risk with smallest WHR. No</td>
<td>Waist and WHR were associated with breast cancer risk in post-menopausal</td>
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<tr>
<td>Study</td>
<td>Year</td>
<td>Evidence</td>
<td>Details</td>
<td>Conclusion</td>
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<tr>
<td>(Cheraghi, et al. 2012)</td>
<td>50</td>
<td>Positive correlation</td>
<td>BMI and breast cancer risk in postmenopausal women: OR=1.15 (95% CI: 1.07-1.24), risk ratio=1.16 (95% CI: 1.08-1.25), and rate ratio=0.98 (95% CI: 0.88-1.09). No correlation between BMI and breast cancer risk in premenopausal women.</td>
<td>Elevated BMI is a risk factor for breast cancer in postmenopausal women.</td>
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<td>(Protani, Coory and Martin 2010)</td>
<td>43</td>
<td>Obese women had worse breast cancer specific survival when BMI (HR=1.33, 95% CI: 1.21-1.47) or WHR (HR=1.31, 95% CI: 1.08-1.58) was used as a measure for obesity.</td>
<td>Survival among obese women, compared to non-obese women, with breast cancer was worse.</td>
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<td>(Druene-Pecollo, et al. 2012)</td>
<td>13</td>
<td>Elevated BMI was associated with second primary cancer incidence in women with breast cancer, including contralateral breast (RR=1.37, 95% CI: 1.24-1.58), endometrial (RR=1.96, 95% CI: 1.43-2.70), and colorectal second primary cancers (RR=1.89, 95% CI: 1.28-2.79).</td>
<td>Obesity was associated with increased risk of contralateral breast, breast, endometrial, and colorectal second primary cancers.</td>
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<td>(Maruthur, et al. 2009)</td>
<td>17</td>
<td>The OR for mammography in the past 2 yrs were 1.01 (95% CI: 0.95-1.08), 0.93 (95% CI: 0.83-1.05), 0.90 (95% CI: 0.78-1.04), and 0.79 (95% CI: 0.68-0.92) for women with BMI 25-29.9, 30-34.9, 35-39.9, and ≥40 respectively.</td>
<td>Women with BMI ≥40 were less likely to report recent mammography.</td>
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<td>(Pierobon and Frankenfeld 2013)</td>
<td>11</td>
<td>There was a significant association between triple-negative breast cancer and obesity in case-case (OR=1.20, 95% CI: 1.03-1.40), and case-control analysis (OR=1.24, 95% CI: 1.06-1.46). Relationship only found in premenopausal group (OR=1.43, 95% CI: 1.23-1.65).</td>
<td>Obesity was significantly associated with triple-negative breast cancer in premenopausal women.</td>
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<td>(Amadou, et al. 2013)</td>
<td>30</td>
<td>Each 5-unit increase in BMI was inversely associated with risk of premenopausal breast cancer (RR=0.95, 95% CI: 0.94-0.97) only in African (RR=0.95, 95% CI: 0.91-0.98) and Caucasians (RR=0.93, 95% CI: 0.91-0.95). The association was positive among Asian women. Each 0.1 unit increase in WHR was positively associated with premenopausal breast cancer (RR=1.08, 95% CI: 1.01-1.16), with largest effect in Asian women.</td>
<td>Increase in WHR was associated with increased risk of premenopausal breast cancer. The relationship between BMI and premenopausal breast cancer varied according to ethnicity.</td>
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<td>(Liu and Liu 2011)</td>
<td>26</td>
<td>Obesity-related polymorphisms (LEP G2540A, LEPR Q223R, LEPR Lys109Arg and PON1 Q192R) failed to show association with breast cancer risk. PON1 L55M was significantly associated with breast cancer risk (OR=2.16, 95% CI: 1.76-2.66). LEPR Q223R polymorphism was significantly associated with breast cancer risk only in East Asians (OR=0.50, 95% CI: 0.36-0.70), but not in Caucasians or Africans.</td>
<td>PON1 L55M polymorphism was associated with increased breast cancer risk. LEPR Q223R polymorphism was associated with increased breast cancer risk in East Asians.</td>
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<tr>
<td>Colorectal</td>
<td>56</td>
<td>Those with a BMI ≥23, 23-24.9, 25.0-27.4, 27.5-29.9, and ≥30.0 were associated with 14%, 19%, 24%, and 41% increased risk of colorectal cancer. Each 5-unit BMI increase</td>
<td>BMI was positively associated with risk of colorectal cancer.</td>
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</table>

**Relationship found in premenopausal women.** However the relationship is mostly explained by correlation between waist and WHR with BMI.
was associated with 18% increased risk. Asians and premenopausal women had increased risk of colorectal cancer with low BMIs. Obesity was more strongly associated with colon than rectal cancer; men than women; self-reported BMI than directly measured BMI; and for studies that accounted for physical activity.

(Matsuo, et al. 2012) 8 In Japanese populations, 1-unit increase in BMI was positively associated with colorectal cancer risk in males (HR=1.03, 95% CI: 1.02-1.04) and females (HR=1.02, 95% CI: 1.00-1.03). The relationship was stronger for colon cancer. Increased BMI was positively associated with colorectal cancer in Japanese males and females.

(Ben, et al. 2012) 36 A 5-unit increase in BMI was positively associated with colorectal adenoma risk (RR=1.19, 95% CI: 1.13-1.26). The association was independent of race, geographic location, study design, sex, adenoma progression, and confounders. The association was stronger for colon than rectal adenoma. Increased BMI was positively associated with colon adenoma risk.

(Larsson and Wolk, Obesity and colon and rectal cancer risk: a meta-analysis of prospective studies 2007) 30 A 5-unit increase in BMI was associated with increased colon cancer risk in men (RR=1.30, 95% CI: 1.25-1.35) and women (RR=1.12, 95% CI: 1.07-1.18). The association was stronger in men (P<0.001). Rectal cancer was only associated with BMI in men (RR=1.12, 95% CI: 1.09-1.16). Increased WHR was associated with increased colon cancer in men (RR=1.43, 95% CI: 1.19-1.71) and women (RR=1.20, 95% CI: 1.08-1.33). Obesity was positively associated with colon cancer risk in men and women, and with rectal cancer risk in men.

(Dai, Xu and Niu 2007) 15 Obesity was positively associated with colorectal cancer risk in men (RR=1.37, 95% CI: 1.21-1.56) but not women. Colon and rectal cancer risk was positively associated with obesity in women (and men) when comparing highest with lowest quantiles of BMI. WHR was positively associated with colon cancer in men (RR=1.91, 95% CI: 1.46-2.49) and women (RR=1.49, 95% CI: 1.23-1.81); and rectal cancer in men only (RR=1.93, 95% CI: 1.19-3.13). Obesity was positively associated with colon cancer, and WHR was a more sensitive index than overall obesity according to BMI.

(Ma, et al. 2013) 54 Obesity was positively associated with colorectal cancer (RR=1.33, 95% CI: 1.25-1.42). Waist circumference was positively associated with colorectal cancer (RR=1.46, 95% CI: 1.33-1.60). Increased BMI and waist circumference were positively associated with colorectal cancer risk.

(Harriss, et al. 2009) 29 Increased BMI was associated with risk of colon (RR=1.24, 95% CI: 1.20-1.28) and rectal (RR=1.09, 95% CI: 1.05-1.14) cancer in men, and colon cancer (RR=1.09, 95% CI: 1.04-1.12) in women. The relationship was stronger in men than women. Obesity was associated with colon and rectal cancer risk in men and colon cancer risk in women.

(Robsham, et al. 2013) 30 Increased BMI was associated with distal colon cancer risk (RR=1.59, 95% CI: 1.34-1.89), along with proximal colon (RR=1.24, 95% CI: 1.08-1.42) and rectum (RR=1.23, 95% CI: 1.02-1.48) cancer risk. Obesity was associated with increased risk of distal colon cancer risk, and proximal colon and rectal cancer risk.

Endometrium (Esposito, et al. 2013) 6 MetS was associated with increased risk of MetS was associated with
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<th>Year</th>
<th>Study Description</th>
<th>Effect Measures</th>
<th>Summary</th>
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<td>2013</td>
<td>endometrial cancer (RR=1.89, 95% CI: 1.34-2.67). The risk estimates were 2.21 (P&lt;0.001) for higher values of BMI or waist circumference.</td>
<td>Increased risk of endometrial cancer. Obesity was the strongest factor of the syndrome associated with endometrial cancer.</td>
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<td>Esophageal adenocarcinoma (Turati, et al. 2013)</td>
<td>BMI 25-30 (RR=1.71, 95% CI: 1.50-1.96) and ≥30 (RR=2.34, 95% CI: 1.95-2.81) was associated with increased risk of esophageal and gastric cardia adenocarcinoma. The association was stronger for esophageal adenocarcinoma than gastric cardia adenocarcinoma.</td>
<td>Obesity was associated with increased risk of esophageal and gastric cardia adenocarcinoma. The relationship with obesity was stronger for esophageal adenocarcinoma.</td>
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<td>(Kubo and Corley 2006)</td>
<td>BMI ≥25 was associated with increased risk of esophageal adenocarcinoma in males (OR=2.2, 95% CI: 1.7-2.7) and females (OR=2.0, 95% CI: 1.4-1.9). Increased BMI was associated with risk of cardio adenocarcinoma (OR=1.5, 95% CI: 1.3-1.8).</td>
<td>Obesity was associated with increased risk of esophageal and cardia adenocarcinoma.</td>
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<td>Gallbladder (Larsson and Wolk, Obesity and the risk of gallbladder cancer: a meta-analysis 2007)</td>
<td>Overweight (RR=1.15, 95% CI: 1.01-1.30) and obesity (RR=1.66, 95% CI: 1.47-1.88) were associated with increased risk of gallbladder cancer respectively. The relationship was stronger for women than men.</td>
<td>Overweight and obesity were associated with increased risk of gallbladder cancer.</td>
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<td>Gastric (Chen, Liu, et al. 2013)</td>
<td>BMI 25-30 (RR=1.01, 95% CI: 0.96-1.07) and ≥30 (RR=1.06, 95% CI: 0.99-1.12) both were not associated with risk of gastric cancer. BMI 25-30 (RR=1.21, 95% CI: 1.03-1.42) ≥30 (RR=1.82, 95% CI: 1.32-2.49) was positively associated with gastric cardia cancer risk.</td>
<td>Increased BMI was associated with risk of gastric cardia cancer.</td>
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<td>(Yang, et al. 2009)</td>
<td>BMI ≥25 was associated with increased risk of gastric cancer (OR=1.22, 95% CI: 1.06-1.41). BMI ≥25 was associated with increased risk of gastric cardia cancer (OR=1.55, 95% CI: 1.31-1.84) and gastric cancer among non-Asians (OR=1.24, 95% CI: 1.14-1.36). However, there was no association between non-cardia gastric cancer and BMI.</td>
<td>Increased BMI was associated with risk of cardia gastric cancer but not non-cardia gastric cancer.</td>
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<td>Leukemia (Castillo, et al. 2012)</td>
<td>Obesity was associated with incidence (RR=1.26, 95% CI: 1.17-37) and mortality (RR=1.29, 95% CI: 1.11-1.49) of leukemia. Obesity was also associated with increased incidence of acute myeloid leukemia (RR=1.53, 95% CI: 1.26-1.85), chronic lymphocytic leukemia (RR=1.17, 95% CI: 1.08-1.27), chronic myeloid leukemia (RR=1.16, 95% CI: 1.04-1.30), and acute lymphoblastic leukemia (RR=1.62, 95% CI: 1.12-2.32).</td>
<td>Obesity, but not overweight, were associated with increased risk of leukemia.</td>
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<tr>
<td>(Larsson and Wolk, Overweight and obesity and incidence of leukemia: a meta-analysis of cohort studies 2008)</td>
<td>BMI 25-30 (RR=1.14, 95% CI: 1.03-1.25) and ≥30 (RR=1.39, 95% CI: 1.25-1.54) were associated with increased risk of leukemia. Obesity was associated with chronic lymphocytic leukemia (RR=1.25, 95% CI: 1.11-1.41), acute lymphocytic leukemia (RR=1.65, 95% CI: 1.16-2.35), acute myeloid leukemia (RR=1.52, 95% CI: 1.19-1.95), and chronic myeloid leukemia (RR=1.26, 95% CI: 1.09-1.46).</td>
<td>Overweight and obesity were associated with increased risk of leukemia.</td>
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<td>Condition</td>
<td>Reference</td>
<td>Study Details</td>
<td>Findings</td>
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<td>Liver</td>
<td>(Wang, Wang, et al. 2012)</td>
<td>BMI, when compared with reference of lowest median value, was positively</td>
<td>Obesity was associated with increased risk of primary liver cancer. Those with hepatitis C virus or cirrhosis are at increased risk of primary liver cancer.</td>
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<td>associated with liver cancer at BMI 25 (RR=1.02, 95% CI: 1.02-1.03), 30 (RR=1.35, 95% CI: 1.24-1.47), and 35 (RR=2.22, 95% CI: 1.74-2.83).</td>
<td>BMI was positively associated with risk of liver cancer.</td>
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<td>(Rui, et al. 2012)</td>
<td>BMI, when compared with reference of lowest median value, was positively</td>
<td>BMI was positively associated with risk of liver cancer.</td>
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<td>associated with liver cancer at BMI 25 (RR=1.48, 95% CI: 1.31-1.67) and ≥30 (RR=1.83, 95% CI: 1.59-2.11). Obese males had a higher risk for primary liver cancer than obese females.</td>
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<td></td>
<td>(Larsson and Wolk, 2012)</td>
<td>BMI, when compared with reference of lowest median value, was positively</td>
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<td>associated with liver cancer at BMI 25-30 (RR=1.17, 95% CI: 1.02-1.34) and ≥30 (RR=1.89, 95% CI: 1.51-2.36).</td>
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<td>Lung</td>
<td>(Yang, et al. 2013)</td>
<td>BMI was inversely associated with incidence of lung cancer for BMI ≥25 (RR=0.79, 95% CI: 0.73-0.85). The inverse association was strengthened when analyzing only smokers, current (RR=0.63, 95% CI: 0.57-0.70) and former (RR=0.73, 95% CI: 0.58-0.91).</td>
<td>Obesity was inversely associated with incidence of lung cancer, and thus protective. The relationship was stronger for current and former smokers.</td>
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<td>Malignant Melanoma</td>
<td>(Sergentanis, et al. 2013)</td>
<td>BMI was positively associated with risk of malignant melanoma in males, at BMI 25-30 (RR=1.31, 95% CI: 1.18-1.45) and ≥30 (RR=1.31, 95% CI: 1.19-1.44). There was no significant association in women. Sunlight exposure was a confounding factor in obese males.</td>
<td>BMI was positively associated with risk of malignant melanoma in males.</td>
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<tr>
<td>Multiple Myeloma</td>
<td>(Wallin and Larsson 2011)</td>
<td>BMI was positively associated with incidence of multiple myeloma, at BMI 25-30 (RR=1.12, 95% CI: 1.07-1.18) and ≥30 (RR=1.21, 95% CI: 1.08-1.35); and mortality at BMI 25-30 (RR=1.15, 95% CI: 1.04-1.27) and ≥30 (RR=1.54, 95% CI: 1.35-1.76).</td>
<td>BMI was positively associated with incidence and mortality of malignant myeloma.</td>
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<td>(Larsson and Wolk, Body mass index and risk of multiple myeloma: a meta-analysis 2007)</td>
<td>BMI was positively associated with risk of multiple myeloma in cohort studies at BMI 25-30 (RR=1.12, 95% CI: 1.07-1.18) and ≥30 (RR=1.27, 95% CI: 1.15-1.41); and case-control studies at BMI 25-30 (RR=1.43, 95% CI: 1.23-1.68) and ≥30 (RR=1.82, 95% CI: 1.47-2.26).</td>
<td>BMI was positively associated with risk of multiple myeloma in cohort and case-control studies.</td>
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<tr>
<td>Non-Hodgkin Lymphoma</td>
<td>(Larsson and Wolk, Body mass index and risk of non-Hodgkin’s and Hodgkin’s)</td>
<td>BMI was positively associated with non-Hodgkin’s lymphoma incidence (RR=1.07, 95% CI: 1.04-1.10) and mortality (RR=1.14, 95% CI: 1.04-1.26). There was a nonlinear association between BMI and Hodgkin’s lymphoma. BMI</td>
<td>BMI was positively associated with incidence of non-Hodgkin’s lymphoma and Hodgkin’s lymphoma, and mortality of non-Hodgkin’s lymphoma.</td>
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<td>Study</td>
<td>Results</td>
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<td><strong>Pancreas</strong></td>
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<td>(Willett, et al. 2008)</td>
<td>BMI ≥40 was positively associated with B-cell non-Hodgkin's lymphoma</td>
<td>Increased BMI serves as an independent risk factor for pancreatic cancer.</td>
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<td>(OR=1.80, 95% CI: 1.24-2.62). BMI 25-29.9 and 30-39.9 were not</td>
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<td>associated with non-Hodgkin's lymphoma.</td>
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<td>(Larsson and Wolk, Obesity and risk of</td>
<td>BMI was positively associated with risk of non-Hodgkin's lymphoma at</td>
<td>BMI was positively associated with risk of non-Hodgkin's lymphoma,</td>
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<tr>
<td>non-Hodgkin's lymphoma: a meta-analysis</td>
<td>BMI 25-30 (RR=1.07, 95% CI: 1.07-1.14) and ≥30 (RR=1.20, 95% CI:</td>
<td>particularly risk of diffuse large B-cell lymphoma.</td>
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<td>of prospective studies 2011)</td>
<td>1.07-1.34). Based on subtype analysis, BMI was particularly</td>
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<td>positively associated with risk of diffuse large B-cell lymphoma.</td>
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<td>(Genkinger, et al. 2011)</td>
<td>Obesity was associated with an increased risk of pancreatic cancer</td>
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<td>(RR=1.19, 95% CI: 1.14-1.75), but not for BMI 25-30.</td>
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<td>(Berrington de Gonzalez, Sweetland and</td>
<td>Obesity was associated with increased mortality from ovarian cancer</td>
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<td>Spencer 2003)</td>
<td>(HR=1.60, 95% CI: 1.10-2.34).</td>
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<td>(Protani, Nagle and Webb 2012)</td>
<td>Obesity was associated with poorer survival from ovarian cancer (HR=</td>
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<td>1.17, 95% CI: 1.03-1.34). The association was stronger for BMI</td>
<td>There was a large amount of inter-study variation.</td>
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<td>≥30 compared with BMI ≥25.</td>
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<td>(Olsen, et al. 2007)</td>
<td>Overweight (OR=1.20, 95% CI: 1.0-1.3) and obesity (OR=1.30, 95% CI:</td>
<td>Obesity was associated with increased risk of ovarian cancer.</td>
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<td>1.1-1.5) were associated with greater risk of ovarian cancer. The</td>
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<td>association was stronger for case-control studies compared to cohort</td>
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<td>studies.</td>
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<tr>
<td>(Collaborative Group on Epidemiological</td>
<td>A 5-unit increase in BMI was associated with greater risk of ovarian</td>
<td>Increased BMI is associated with risk of ovarian cancer among women</td>
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<td>Studies of Ovarian Cancer 2012)</td>
<td>cancer in women who have never used hormone therapy (RR=1.10, 95% CI:</td>
<td>who have never used hormone therapy.</td>
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<td>1.07-1.13) and a lower risk for women who have used hormone</td>
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<td>therapy (RR=0.95, 95% CI: 0.92-0.99).</td>
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<td><strong>Ovarian</strong></td>
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<td>(Yang, et al. 2011)</td>
<td>Obesity was associated with increased mortality from ovarian cancer</td>
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<td>(HR=1.60, 95% CI: 1.10-2.34).</td>
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<td>(Berrington de Gonzalez, Sweetland and</td>
<td>Per-unit increase in BMI was associated with a greater risk of</td>
<td>Obesity was associated with greater risk of pancreatic cancer.</td>
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<td>Spencer 2003)</td>
<td>pancreatic cancer (RR=1.02, 95% CI: 1.01-1.03). The association was</td>
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<td>stronger for obese individuals compared to non-obese individuals.</td>
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<td>(Genkinger, et al. 2011)</td>
<td>BMI ≥30 was associated with greater risk of pancreatic cancer</td>
<td>BMI and WHR were positively associated with risk of pancreatic cancer.</td>
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<td>(RR=1.47, 95% CI: 1.23-1.75). A positive association was observed for</td>
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<td>increased BMI in early adulthood. A greater risk was observed for</td>
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<td>individuals who gained BMI ≥10 between BMI at baseline and younger</td>
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<td>ages compared to those who remained stable. WHR was</td>
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<td>positively associated with pancreatic cancer risk (RR=1.35, 95% CI:</td>
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<td>1.03-1.78).</td>
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<td>(Aune, et al. 2012)</td>
<td>A 5-unit increase in BMI was associated with greater risk of</td>
<td>Increased BMI, waist circumference, and WHR were associated with risk</td>
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<td>pancreatic cancer (RR=1.10, 95% CI: 1.07-1.14). A 10-cm increase in</td>
<td>of pancreatic cancer.</td>
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<td>waist circumference (RR=1.11, 95% CI: 1.05-1.18), and a 0.1-unit</td>
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<td>increment in WHR (RR=1.19, 95% CI: 1.09-1.31) was associated with</td>
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<td>greater risk of pancreatic cancer</td>
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<td>(Jiao, et al. 2010)</td>
<td>A 5-unit increase in BMI was associated with an increased risk of</td>
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<td>pancreatic cancer</td>
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<td>Study</td>
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<td>Thyroid</td>
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<td>Overweight was associated with increased risk of thyroid cancer (RR=1.13, 95% CI: 1.04-1.22). Obesity and overweight combined were associated with increased risk of thyroid cancer (RR=1.18, 95% CI: 1.11-1.25). The association was not found among Asians.</td>
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<td>Prostate</td>
<td>(Discacciati, Orsini and Wolk 2012) 25</td>
<td>For localized PC, BMI was inversely associated with PC risk (RR=0.94, 95% CI: 0.91-0.97). For advanced PC, BMI was directly associated with PC risk (RR=1.09, 95% CI: 1.02-1.16). Obesity had a dual effect on PC, with a decreased risk for localized PC and an increased risk for advanced PC.</td>
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<td>(Cao and Ma 2011) 28</td>
<td>In six population-based cohorts, a 5-unit increase in BMI was associated with a higher risk of PC-specific mortality (RR=1.15, 95% CI: 1.06-1.25). In six post-diagnosis survival studies, a 5-unit increase in BMI was associated with higher PC-specific mortality (RR=1.20, 95% CI: 0.99-1.46). In 16 studies that followed patients after primary treatment, a 5-unit increase in BMI was associated with a higher risk of biochemical recurrence (RR=1.21, 95% CI: 1.11-1.31). Increased BMI was associated with risk of PC-specific mortality and biochemical recurrence.</td>
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<td>(Maclnnis and English 2006) 56</td>
<td>BMI was positively associated with risk of PC (RR=1.05, 95% CI: 1.01-1.08). For studies that reported results by stage of disease, BMI was positively associated with advanced PC (RR=1.12, 95% CI: 1.01-1.23). Increased BMI is weakly associated with an increased risk of PC, particularly advanced stage tumors.</td>
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<td>Renal</td>
<td>(Choi, et al. 2013) 20</td>
<td>The 5-yr cancer-specific survival from renal cell cancer increased from 76.1% to 92.7% in the highest BMI category. Obesity was associated with higher overall survival (HR=0.45, 95% CI: 0.29-0.68) and cancer-specific survival (HR=0.47, 95% CI: 0.29-0.77). Increased BMI was associated with greater survival among patients with renal cell cancer.</td>
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<td>(Ildaphonse, George and Mathew 2009) 27</td>
<td>A 1-unit increase in BMI was associated with an increased risk in renal cancer (RR=1.05, 95% CI: 1.04-1.06) in cohort studies in men. BMI was positively associated with risk of renal cancer in men.</td>
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<td>(Mathew, George and Ildaphonse 2009) 28</td>
<td>A 1-unit increase in BMI was associated with an increased risk in renal cancer (RR=1.06, 95% CI: 1.05-1.07) in cohort studies in women. BMI was positively associated with risk of renal cancer in women.</td>
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<td>(Bergstrom, Hsieh, et al. 2001) 36</td>
<td>A 1-unit increase in BMI was associated with an increased risk in renal cancer (RR=1.07, 95% CI: 1.05-1.09). The association was not modified by sex. BMI was positively associated with risk of renal cell cancer in men and women.</td>
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1.3.3 Pathophysiology of Obesity and Cancer

The relationship between obesity and cancer is complex and multifaceted, with many interacting biological and non-biological mechanisms, along with numerous confounding factors. At present, the mechanisms linking obesity with cancer are better understood for cancers with an endocrine component (Ceschi, et al. 2007). The primary biological mechanisms linking obesity with cancer include the concomitant conditions of the MetS, alterations resulting from dysfunctional adipose tissue, sex hormones and androgens, insulin/IGF/C-peptide, adipokines (adiponectin/leptin, PAI-1, etc.), vascular perturbations, VEGF, immune cell-related inflammation, inflammatory mediators, PPARs, oxidative stress and antioxidants, and changes in energy metabolism.

Obesity is associated with the MetS, constituted by abdominal obesity, hypertension, dyslipidemia, and hyperglycemia, each of which has been implicated in the etiology of cancer. Dyslipidemia has been associated with the incidence of lung cancer, Non-Hodgkin lymphoma, PC, and pre- and postmenopausal breast cancer (Braun, Bitton-Worms and LeRoith 2011). Diabetes has been associated with increased incidence of pancreatic, hepatic, colorectal, breast, urinary tract, and endometrial cancers; and, paradoxically, with decreased incidence of PC (Shikata, Ninomiya and Kiyohara 2013). C-peptide (a surrogate of insulin secretion) has been independently associated with increased risk of post-menopausal breast cancer, colorectal cancer, and endometrial cancer (Renehan, Roberts and Dive, Obesity and cancer: pathophysiological and biological mechanisms 2008).
Obesity results in dysfunctional adipose tissue. As adipocytes increase in number (hyperplasia) and size (hypertrophy), oxidative stress and hypoxia are triggered, which increases the rate of apoptosis and inflammation (Park, Euhus and Scherer 2011). Dysfunctional adipose tissue results in greater lipid accumulation in intra-abdominal (visceral) adipose depots, compared to subcutaneous depots, highlighting the importance of fat distribution versus overall fat mass (as indicated by BMI) (Park, Euhus and Scherer 2011). Adipocytes undergo a transformation from being differentiated during the early stages of tumor development to fibroblast-like preadipocytes and/or dedifferentiated during the later stages of tumor progression (Park, Euhus and Scherer 2011). Tumor-associated fibroblasts may promote tumor progression/metastasis through signaling molecules, including TGFβ1 and stromal cell-derived factor-1 (Park, Euhus and Scherer 2011).

Chronic inflammation increases cancer risk (Grivennikov, Greten and Karin 2010). Immune cells affect cancer cells through the production of cytokines, chemokines, growth factors, prostaglandins, and reactive oxygen and nitrogen species (Grivennikov, Greten and Karin 2010). Inflammation affects all the stages of tumorigenesis, including initiation, promotion, and metastasis (Grivennikov, Greten and Karin 2010). Obesity is associated with a chronic low-grade inflammatory state characterized by macrophage infiltration into adipose tissue, and adipocyte-macrophage transdifferentiation, both of which elevate tissue levels of inflammatory cytokines (Harvey, Lashinger and Hursting 2011). Adipocyte enlargement creates ineffective oxygen diffusion, resulting in hypoxia, inflammation, and increased macrophage infiltration, contributing to the subsequent development of insulin
resistance (Harvey, Lashinger and Hursting 2011). Elevated visceral adipose tissue deposition, typical in obesity, in close proximity to the portal vein allows excess free fatty acids and inflammatory mediators to pool directly into the liver, further affecting systemic inflammation and metabolism (Harvey, Lashinger and Hursting 2011).

Obesity alters the production and bioavailability of endogenous sex steroids, and is associated with elevated levels of serum estradiol and estrone, and decreased T (Prieto-Hontoria, et al. 2011). Estradiol is increased as a result of increased adipose tissue mass and increased aromatase activity, which converts androgens to estradiol (Prieto-Hontoria, et al. 2011). Changes in sex steroid levels affects the risk of endometrial, postmenopausal breast, and PC.

IGF-1 and IGF-1R, necessary components of normal cell growth and development, have been shown to be overexpressed in cancer cells (Braun, Bitton-Worms and LeRoith 2011). Alterations in the insulin-IGF-1 axis may stimulate the production of adipocyte-derived VEGF, which supports cell survival and migration (Parekh, Chandran and Bandera, Obesity in cancer survival 2012). VEGF is produced by adipocytes and tumor cells, has angiogenic, mitogenic, and vascular permeability-enhancing activities, and is increased in obesity (Hursting and Hursting 2012). Blood vessel formation necessary for metastasis is aided by VEGF production, which is triggered by tumor cell nutrient and oxygen needs (Hursting and Hursting 2012).

Dysfunctional adipose tissue results in abnormal alterations in serum adipokine levels (such as adiponectin, leptin and PAI-1). The two major protein products produced from adipocytes are adiponectin and leptin, both of which have
been implicated in cancer development/progression. Adiponectin has significant anti-inflammatory and insulin-sensitizing effects (van Kruisdijk, van der Wall and Visseren 2009). Clinical studies have indicated that plasma concentrations of adiponectin may be inversely associated with the risk of various cancers (van Kruisdijk, van der Wall and Visseren 2009). Leptin plays a role in regulating energy balance, by decreasing appetite and increasing metabolism (van Kruisdijk, van der Wall and Visseren 2009). Whereas adiponectin levels are decreased in obesity, conversely, leptin levels are increased, and have been positively associated with cancer development/progression. PAI-1 is a serine protease inhibitor produced by adipocytes in visceral adipose depots, and affects adipocyte differentiation and insulin signaling (van Kruisdijk, van der Wall and Visseren 2009). Through its effects on uPA, vitronectin, and integrins, PAI-1 is involved in tumor growth, invasion, metastasis, and angiogenesis (van Kruisdijk, van der Wall and Visseren 2009).

Other obesity-related factors implicated in the development/progression of cancer include dysregulation of hormones such as glucocorticoid, ghrelin, obestatin, visfatin, PAI-1, and PPARs (Parekh, Chandran and Bandera, Obesity in cancer survival 2012). PPAR-γ is a ligand-inducible transcription factor, primarily produced in adipose tissue, important in adipocyte differentiation, and cell proliferation and survival (van Kruisdijk, van der Wall and Visseren 2009). PPAR-γ exhibits antitumor effects, and is downregulated by TNF-α in obesity, contributing to dysfunctional adipose tissue (van Kruisdijk, van der Wall and Visseren 2009).
Confounding factors may exist obfuscating the relationship between obesity and cancer. For instance, gastric acid reflux is more common in obese patients, and is also implicated as a risk factor for esophageal adenocarcinoma (van Kruijsdijk, van der Wall and Visseren 2009). Obesity is associated with hypertension, which is a risk factor for the development of renal cell carcinoma (Renehan, Roberts and Dive, Obesity and cancer: pathophysiological and biological mechanisms 2008). Obesity is also associated with increased uptake of iodine by the thyroid, which may affect thyroid carcinoma (Renehan, Roberts and Dive, Obesity and cancer: pathophysiological and biological mechanisms 2008). Vitamin D levels decrease with increasing BMI, presumably since vitamin D is lipophilic, and can be stored in adipose tissue (Lagunova, et al. 2010). It was estimated that low vitamin D levels might explain at least 20% of the cancer risk attributable to elevated BMI (Lagunova, et al. 2010).

It may be objected that obesity is unlikely to cause cancer through such a plurality of mechanisms. However, the etiology of cancer differs according to the various organ sites (Wolin, Carson and Colditz 2010). Androgens may be the driving factors in breast and endometrial cancer; insulin in colon and PC; and inflammation in other tumors (Wolin, Carson and Colditz 2010). Thus, it is plausible that the pathophysiological relationship between obesity and cancer is complex and multisystemic (Wolin, Carson and Colditz 2010).
1.4 Impact of Obesity on Prostate Cancer

1.4.1 Epidemiology of Obesity and Prostate Cancer

Reflecting the complexity in the relationship between obesity and PC, clinical trials and meta-analyses present conflicting data. A 2001 meta-analysis analyzing epidemiological data across Europe on the proportion of various cancers attributable to overweight concluded that elevated BMI was associated with PC with only borderline statistical significance (RR=1.01, fixed effects 95% CI: 1.00-1.02) (Bergstrom, Pisani, et al. 2001). However, a 2008 Lancet meta-analysis, which reviewed 20 types of cancer, failed to find a significant relationship between obesity and PC risk (RR=1.03, 95% CI: 1.00-1.07, p=0.11) (Renehan, Tyson, et al. 2008). An analysis across 30 European countries found that excess BMI was not associated with incident PC (Population Attributable Risk=1.95, 95% CI: 0.09-3.81) (Renehan, Soerjomataram, et al. 2010). A large meta-analysis, which reviewed 89 studies, analyzed the association between BMI and various cancers and co-morbidities, and found that obesity was not associated with risk of PC (RR=1.05, 95% CI: 0.85-1.30) (Guh, Zhang, et al., The incidence of co-morbidities related to obesity and overweight: a systematic review and meta-analysis 2009).

Meta-analyses, which reviewed studies specific to PC, found more compelling results. The first identified meta-analysis, in 2006, to examine obesity specifically in relation to PC found a weak association for overall risk (RR=1.05, 95% CI: 1.01-1.08, per 5-unit increment in BMI), and a stronger association for advanced PC risk (RR=1.12, 95% CI: 1.01-1.23, per 5-unit increment in BMI) (MacInnis and English 2006). A subsequent meta-analysis found that among six population-based cohorts,
BMI was associated with PC-specific mortality (RR=1.15, 95% CI: 1.06-1.25, with a 5-unit increase in BMI; and RR=1.20, 95% CI: 0.99-1.46, with a 5-unit increase in BMI for six post-diagnosis survival studies) (Cao and Ma 2011). In 16 studies, which followed patients after primary treatment, BMI was associated with risk of BCR (RR=1.21, 95% CI: 1.11-1.31) (Cao and Ma 2011).

The influence of obesity on PC appears to be more complex than simply a direct positive effect of adiposity volume potentiating tumor aggressiveness. Recent evidence indicates a dual effect of body adiposity on PC development. A meta-analysis in 2012 identified 12 studies on localized PC and 13 on advanced PC and found an inverse association between BMI and localized disease (RR=0.94, 95% CI: 0.91-0.97, for every 5-unit increase in BMI) and a direct association between BMI and advanced disease risk (RR=1.09, 95% CI: 1.02-1.16, for every 5-unit increase in BMI) (Discacciati, Orsini and Wolk 2012). In the following section, a number of explanatory hypotheses will be discussed to make sense of the apparent paradox, including detection biases, variations in treatment outcomes, associated comorbidities, and various biological mechanisms. A summary of the impact of obesity on PC is shown in Figure 1.
Obesity results in detection biases due to difficult DREs, larger prostates, and PSA hemodilution, all of which result in lower positive biopsies and fewer detected cancers. Technical difficulties in obese patients undergoing RP lead to more complications and worse recovery. Obese patients have an increased risk of BCR, PC-specific, and all-cause mortality, following surgical and non-surgical treatments for PC.

**1.4.2 Detection Biases with Obesity and Prostate Cancer**

Among prospective studies, which are less prone to biases plaguing retrospective and case-control studies, evidence indicates that obesity increases the risk of fatal PC (Golabek, et al. 2013). For instance, in a 12-yr follow-up of 6 673 men diagnosed with PC, high BMI was associated with increased risk of PC-specific mortality (RR=1.36, 95% CI: 1.08-1.71, top vs. bottom quintile of BMI) (Haggstrom, et al. 2012). However, evidence is more conflicting for an association between obesity and PC incidence. A study in 2012, with a mean 15-yr follow-up of 16 514 men, determined that BMI was only associated with aggressive PC risk (HR=1.27,
95% CI: 1.08-1.49, per 5-unit increase in BMI), and not total PC risk (Bassett, et al. 2012). Another study in 2008, with a mean 8.5-yr follow-up of 129,502 men, determined that obesity (BMI ≥ 30) was not associated with overall PC risk (RR=0.97, 95% CI: 0.85-1.10) for either localized (RR=0.91, 95% CI: 0.74-1.11) or advanced disease (RR=1.09, 95% CI: 0.83-1.44), low-grade tumors (RR=0.87, 95% CI: 0.70-1.08) or high-grade tumors (RR=1.08, 95% CI: 0.83-1.41). The conflicting data on PC incidence rates may in part be due to detection biases in obese patients.

Several factors exist that may make detecting PC in obese patients more challenging. For instance, DREs are more difficult to perform in obese men (Freedland and Platz, Obesity and prostate cancer: making sense out of apparently conflicting data 2007). Obesity is also associated with prostate enlargement (Parsons, et al. 2006). In a study of 8,122 participants from the REduction by DUtasteride of prostate Cancer Events (REDUCE) trial, which examined the association between obesity and enhanced prostate volume growth measured by serial TRUS, men with BMI ≥ 30 had enhanced prostate volume growth on placebo and attenuated prostate volume reduction on dutasteride (Muller, et al. 2013). In an analysis of 787 subjects undergoing prostate biopsy, obese men had fewer abnormal DRE findings, and larger prostate volumes (Freedland, Terris, et al. 2005). It is more difficult to detect cancer via biopsy in a prostate with a larger volume. Elevated BMI has been associated with lower odds of PC detection via biopsy (Lee, Hong, et al. 2010).

Obesity appears to impact serum PSA levels. Among 2,779 healthy subjects, mean PSA was 1.01 ng/mL for normal weight men and 0.69 ng/mL for obese men.
(Baillargeon, Pollock, et al. 2005). In a study of 3152 participants, there was a lower likelihood of having serum PSA levels ≥4.0 ng/mL with increased BMI (Culp and Porter 2009). Taking blood volume into account, it was determined that elevated BMI was associated with increased plasma volume, and decreased PSA concentration, but not with total PSA mass (Grubb, et al. 2009). Thus, the relationship between obesity and PSA appears to be explained by a hemodilution effect (Banez, Hamilton, et al. 2007). An analysis of three cross-sectional surveys determined that obesity was inversely associated with T concentrations, obese men were less likely to have PSA levels that reached biopsy threshold (>4 ng/mL), and biopsy rates were lower in obese men (Parekh, Lin, et al. 2010). Obesity-associated decreases in T, along with hemodilution, cause PSA concentrations to be lower, resulting in a lower likelihood of being referred for biopsy, thus artificially lowering incidence rates of PC (Parekh, Lin, et al. 2010). Some studies, however, indicate that BMI may not affect PSA accuracy for predicting PC (Oh, et al. 2013).

1.4.3 Non-Surgical Treatment Outcomes for Prostate Cancer in Obese Patients

1.4.3.1 Radiation Therapy

While obese men are more likely to receive non-surgical treatments, including BT, EBRT, and ADT (Davies, et al. 2008), obesity has been associated with increased rates of complications following primary radiation treatment for PC (Dieperink, et al. 2012). For instance, obese patients are at a higher risk for urethral
striction treatment, including bladder neck contracture, after primary treatment for localized PC (Elliott, et al. 2007).

Studies reporting on the association between obesity and RT outcomes are not consistent when comparing different RT modalities. Of three BT studies identified, obesity was not associated with BT outcomes, including time to BCR. A retrospective analysis of 1 530 subjects with localized PC who were treated with BT determined that BMI had no affect on BCR, cancer-specific survival, or overall survival (van Roermund, Hinnen, et al. 2010). A study of 686 men who underwent BT found that BMI was not associated with BCR (Merrick, Butler, et al. 2005). And lastly, in a study of 1 093 men treated with BT for T1b-T3a PC, BMI was not found to be associated with BCR or overall survival (Merrick, Galbreath, et al. 2007).

On the other hand, evidence indicates that obesity may be associated with EBRT outcomes. A retrospective analysis of 1 868 subjects with localized PC who were treated with EBRT determined that BMI was an independent predictor of BCR (Stroup, et al. 2007). In a study of 706 men treated with EBRT it was determined that BMI was associated with reduced time to BCR and cancer-specific survival (Palma, et al. 2007). However, a retrospective analysis of 564 subjects treated with EBRT determined that BMI had no affect on BCR, PC-specific survival, or overall survival (Geinitz, et al. 2011). The disparity in results on the association between obesity and EBRT may in part be explained by technical errors. Greater setup error has been reported for EBRT in morbidly obese men (Millender, et al. 2004). Furthermore, obese patients are more likely to have greater prostate shift during EBRT (Wong, et al. 2009).
1.4.3.2 Androgen Deprivation Therapy

The impact of obesity on ADT is unclear and understudied. ADT has been associated with numerous side effects, including osteoporosis, sarcopenia, lipid alterations, insulin resistance, diabetes, cardiovascular morbidity, and obesity (Isbarn, Boccon-Gibod, et al. 2009). ADT may be associated with an increased risk of incident diabetes and cardiovascular disease (Keating, O’Malley and Smith, Diabetes and cardiovascular disease during androgen deprivation therapy for prostate cancer 2006). Elevated BMI has been associated with increased risk of developing new-onset diabetes mellitus in men who received ADT (Derweesh, et al. 2007). However, in a retrospective cohort of 24 038, obesity did not modify the association between ADT and second primary cancer risk (Wallner, et al. 2013). Moreover, obesity did not modify the risk of myocardial infarction associated with ADT (Keating, O’Malley and Freedland, et al. 2013).

ADT has been shown to have undesirable effects on body composition. A cross-sectional study of 58 subjects determined that men on ADT had higher BMI, lower total and free T levels, elevated triglycerides, and higher prevalence of MetS (specifically abdominal obesity and hyperglycemia) (Braga-Basaria, et al. 2006). ADT has been shown to decrease whole-body and regional lean mass and increase whole-body and regional fat mass in men with PC (Galvao, et al. 2008). A meta-analysis of 16 studies was conducted to determine the effect of ADT on body composition and found that ADT increased BMI and body weight, increased percent body fat by 7.7%, and decreased percent lean body mass by 2.8% (Haseen, et al. 2010).
It is well established that ADT leads to accumulation of body fat, but how body fat influences ADT outcomes is less obvious. In a retrospective analysis of 287 men, higher BMI was associated with greater risk of progression to castration-resistant PC, increased risk of metastases, and elevated PC-specific mortality (Keto, et al. 2012). A retrospective study of 82 men treated with ADT determined that MetS was associated with shorter time to PSA progression (Flanagan, et al. 2011). However, prospective studies need to be conducted in order to determine causality.

1.4.3.3 Active Surveillance

Limited information exists on the effect of obesity on AS outcomes in PC patients. A study that examined 398 subjects after RP determined that obesity was associated with BCR (Kane, et al. 2010). Another study of 230 subjects after RP who were eligible for AS determined that BMI was the only factor significantly associated with risk of upstaged disease (pathologic stage >pT2) (Ploussard, et al. 2012). However, the study failed to report other factors of the MetS important for PC progression (such as triglycerides and fasting plasma insulin); prostate volume was not considered for classification; total and free T were not reported; and time between biopsy and surgery was not reported (Caliskan 2012). Obese patients may be more eligible for AS since they often present with comorbidities making them poorer candidates for primary treatment (Allott, Masko and Freedland 2013).
1.4.4 Surgical Treatment Outcomes for Prostate Cancer in Obese Patients

Many studies have examined the relationship between obesity and RP. A number of studies have either shown a positive or no association between BMI/obesity and biochemical failure. For instance, in a study of 1,877 men who underwent RP, BMI was associated with adverse pathologic findings and BCR (Magheli, et al. 2008). In a study of 1,106 men who underwent RP, BMI was associated with BCR (Freedland, Aronson, et al. 2004). A retrospective study of 143 men who underwent retropubic RP analyzed obesity in terms of fat thickness in the anterior, posterior, and total anteroposterior abdominal diameters via endorectal coil MRI, and determined that percent visceral fat thickness was larger in obese men with biochemical failure (Mucksavage, et al. 2012). On the other hand, to highlight several examples, BMI was not correlated with BCR in two Dutch populations (Paaskesen and Borre 2008, van Roermund, Kok, et al. 2009), and a Japanese population (Narita, et al. 2013). However, another study in a Japanese population indicated that obesity, following RP, was associated with disease recurrence, operative time, and blood loss during surgery (Komaru, et al. 2010). In a Korean population, following RP, BMI was not associated with BCR-free survival (Lee, Lee, et al. 2011). BMI was weakly associated with disease progression following RP (Mallah, et al. 2005). In a study of 964 patients who underwent RP, BMI failed to predict biochemical failure rate (Motamedinia, et al. 2008).

Various surgical methods offer different advantages for obese patients. Studies have indicated that, when compared with retropubic RP, robot-assisted laparoscopic RP was more effective and safer for obese patients (Bee, et al. 2012),
whereas radical perineal prostatectomy showed no advantages (Fitzsimons, et al. 2007). Obese patients undergoing both laparoscopic RP (Campeggi, et al. 2012) and robotic assisted RP (Castle, et al. 2008) have been shown to have longer operative time, greater blood loss, and higher positive surgical margins. BMI failed to be associated with the rate and location of positive surgical margins in robot-assisted laparoscopic RP (Zilberman, et al. 2012). In a study of 1 538 men treated with RP, BMI was not associated with rate of extracapsular extension, seminal vesicle invasion, lymph node invasion, or positive surgical margins (Isbarn, Jeldres, et al. 2009). Furthermore, open retropubic RP in obese patients, compared to robotic-assisted and laparoscopic, may incur greater costs due to increased operating room service and anesthesia costs (Bolenz, et al. 2010).

Obese patients represent a technical challenge when performing RP, since access to the pelvic organs is obstructed by excess adiposity (Freedland, Grubb, et al. 2005). For instance, it has been shown that BMI is associated with capsular incision during RP (Freedland, Grubb, et al. 2005). Obesity has been associated with anastomotic strictures (Sandhu, et al. 2011). Obese patients undergoing robotic laparoscopic RP have been shown to have poorer baseline urinary function, sexual function, more complications, required more time to return to baseline activities and urinary function, and required more time to achieve pad-free urinary continence (Ahlering, et al. 2005). Following retropubic RP, obesity has been associated with urinary incontinence and vesicourethral strictures (van Roermund, van Basten, et al. 2009). Obesity has been associated with worse preoperative hormonal/vitality health-related quality of life, along with delayed recovery of
bowl function and health-related quality of life following RP (Montgomery, et al. 2006). However, another investigation determined that increased BMI did not affect health-related quality of life following RP (Freedland, Haffner, et al. 2005). Despite obesity-related complications, surgical outcomes seem to have improved over the years as surgeons operate more frequently on obese patients (Lindner, et al. 2010).

Obesity has been shown to not alter the area under ROC curves for PSA to predict pathological Gleason sum, positive surgical margins, extracapsular extension, seminal vesicle invasion, or biochemical failure (Banez, Sun, et al. 2009). In another population of 215 patients, preoperative PSA velocity was only associated with higher relapse rates following RP in non-obese patients but not in obese patients (King, et al. 2007). It has been suggested that PSA screening is less effective in detecting cancer in obese men, leading to more aggressive tumors at diagnosis (Freedland, Sun, et al. 2008). Obese patients with PSA-detected PC treated with RP were at greater risk of biochemical progression compared to obese men diagnosed with DRE (Freedland, Sun, et al. 2008). A retrospective analysis of 1038 RP patients examined the relationship between BMI and BCR with and without PSA nadir, and found that BMI was associated with increased BCR even after adjusting for PSA nadir, suggesting that obesity promoted faster growing tumors (Ho, et al. 2012). PSA-based screening may have strengthened the association between obesity and aggressive PC among RP patients (Freedland, Isaacs, et al. 2005).

A number of confounding factors exist with obesity that may help explain how obesity impacts surgical outcomes. For instance, obese men with diabetes have been shown to have higher metastasis risk following RP, suggesting that diabetes
may be an important factor to consider in the relationship between obesity and RP outcomes (Wu, et al. 2013). Impaired glucose regulation (Wright, et al. 2013), and hypertension (Asmar, et al. 2013), commonly related to obesity, may explain some of the relationship between obesity and BCR. The relationship between obesity and biochemical failure may be in part explained by AR polymorphisms more frequently present in obese patients (Zeigler-Johnson, et al. 2012). Furthermore, in populations of subjects undergoing RP, black men had the highest rates of obesity, suggesting that race may be a confounding factor (Freedland, Aronson, et al. 2004).

1.4.5 Pathophysiology of Obesity and Prostate Cancer

The best documented biological mechanisms linking obesity and PC to date include insulin/IGF signaling, sex hormones, and adipokine signaling. However, several shortfalls exist for each of these proposed mechanisms, thus prompting investigators to pursue novel explanatory mechanisms. An analysis of genes associated with elevated BMI from patients undergoing RP demonstrated important novel mechanisms in the obesity-PC relationship, including genes associated with lipid metabolism, and cholesterol homeostasis, specifically the SCD1 gene (a stearoyl-CoA desaturase) (Sharad, et al. 2011).

1.4.5.1 Insulin/IGF Axis

IGF-1 is a peptide hormone that circulates in the blood bound to IGF binding protein-3, primarily produced by the liver, and stimulates mitosis and inhibits apoptosis (Key, Diet, insulin-like growth factor-1 and cancer risk. 2011). Various studies have demonstrated that high circulating IGF-1 levels are positively
associated with PC risk (Key, Diet, insulin-like growth factor-1 and cancer risk. 2011), specifically in obese men (Weiss, et al. 2007). ADT has been linked to the development of insulin resistance, which is associated with alterations in the IGF axis and up-regulation of IGF-1, which in turn are associated with CRPC (Aggarwal, Ryan and Chan 2013).

In addition to IGF-1, insulin acts as a growth hormone stimulating cell proliferation and differentiation, possibly by enhancing epidermal growth factor and IGFs (Nandeesha 2009). Though data is conflicting, some studies indicate that insulin production is associated with PC by enhancing tumor growth via insulin receptors (Cox, et al. 2009) found on epithelial cells of the prostate (Nandeesha 2009). Insulin receptor isoform A to B ratio has been shown to be higher, and insulin receptor substrate 1 to 2 ratio lower, in PC tissue compared to benign prostate tissue (Heni, et al. 2012).

1.4.5.2 Sex Hormones

The dual effect of obesity on PC development may in part be explained by changes in androgen levels. It has long been recognized that obese men have lower levels of SHBG, and free and total T (Seidell, et al. 1990). Weight loss via gastric bypass surgery has been shown to increase free and total T (Hammoud, et al. 2009). In patients undergoing RP, low preoperative free T levels were associated with higher tumor stage, higher Gleason score, positive lymph node status, and advanced disease (Schnoeller, et al. 2013). Furthermore, the quantity of visceral adipose tissue is proportional to the amount of aromatase, an intracellular enzyme that converts T
to estradiol (G. P. Williams 2010). Increased intracellular prostatic estradiol stimulates prostatic ER-α and rapid mitogenic non-genomic transmembrane G protein-coupled ER pathways, which directly induce prostatic basal cell hyperplasia, cellular proliferation, and metaplastic change (G. Williams 2012).

1.4.5.3 Adipokines

Adipocytes, along with supporting stromal cells and infiltrated macrophages, secrete a variety of hormone-like biologically active polypeptides, termed adipokines (Baillargeon and Rose, Obesity, adipokines, and prostate cancer (review) 2006). The most prominent adipokines involved in PC etiology include adiponectin, leptin, IL-6, and VEGF. Other adipokines implicated in tumor biology include resistin, visfatin, osteopontin, MCP-1, HGF, NGF, and TNF-α.

Adiponectin is a protein primarily produced by white adipose tissue (Brochu-Gaudreau, et al. 2010). In a study of 1 298 subjects, serum adiponectin concentrations were positively associated with lower risk of high grade or fatal PC (Li, et al. 2010). An investigation of haplotype tagging SNPs of adiponectin (ADIPOQ) and adiponectin receptor 1 (ADIPOR1) in 906 subjects found that various ADIPOQ/ADIPOR1 SNPs were associated with PC risk (Kaklamani, et al. 2011). Adiponectin receptors have been found in prostate epithelial tissue (Mistry, Digby and Chen, et al. 2006). Various studies indicate that adiponectin signals through JNK/STAT3 (Miyazaki, et al. 2005), AMPK/Akt/mTOR (Barb, et al. 2007), and p53/bcl-2 (Mistry, Digby and Desai, et al. 2008) in PC tissue.
Resistin is an adipokine that was originally proposed to link obesity with diabetes (Kim, et al. 2011). PC3 and DU145 PC cells, and high-grade PC tissue, were found to express resistin mRNA (Kim, et al. 2011). Resistin was also shown to stimulate PC cell proliferation via the Akt signaling pathway (Kim, et al. 2011). The adipokine visfatin (pre-B cell enhancing factor / nicotinamide phosphoribosyltransferase [NAMPT]) is correlated with visceral adiposity and has been shown to be expressed in PC3 and LNCaP cells and stimulate PC cell proliferation via the ERK-1/2 and p38 signaling pathway (Patel, et al. 2010). Visfatin expression increases during prostate neoplasia (Wang, et al. 2011). Inhibition of visfatin suppressed cell growth, colony formation, and cell invasion of xenografted PC cells in mice (Wang, et al. 2011). Lastly, the neurotrophin NGF has been implicated in PC cell growth and differentiation, exhibiting an antiproliferative effect which is progressively lost during PC progression attributable to a decline in the expression of the NGF receptor p75\textsuperscript{NGFR} (Arrighi, et al. 2010).

1.5 Adiponectin and Prostate Cancer

Adiponectin/Acrp30 is a 244-amino acid primarily produced by adipocytes (Maeda, et al. 1996) and is implicated in systemic insulin sensitivity (Pajvani, et al. 2003). Adiponectin is found in circulation as a low molecular weight (dimer or trimer) and high molecular weight complex (Pajvani, et al. 2003). Adiponectin exhibits various properties including insulin-sensitizing, anti-inflammatory, antiatherogenic, proapoptotic, and antiproliferative (Dalamaga, Diakopoulos and Mantzoros 2012). Serum adiponectin levels are inversely associated with T2D, CVD,
and prostate as well as other cancers, including colorectal, breast, endometrial, gastric, esophageal, pancreatic, prostate, hematological, and lung malignancies (Dalamaga, Diakopoulos and Mantzoros 2012).

1.5.1 In Vitro Studies

In a study using androgen insensitive (DU145 (Stone, et al. 1978) and PC3 (Kaighn, et al. 1979)) and androgen sensitive (LNCaP-FGC (Horoszewicz, Leong and Chu, et al. 1980)) PC cells, it was shown using a tetrazolium bromide assay that high molecular weight, non-proteolytic f- (full length) adiponectin inhibits PC cell growth at subphysiological concentrations (Bub, Miyazaki and Iwamoto 2006). Adiponectin receptors AdipoR1 and AdipoR2 are expressed in LNCaP and PC3 cells, as well as in normal prostate tissue (Mistry, Digby and Chen, et al. 2006).

Furthermore, adiponectin has been shown to interact with other cytokines. Leptin and f-adiponectin in PC3 cells decreased cell proliferation (Mistry, Digby and Desai, et al. 2008). Additionally, f-adiponectin reversed leptin-induced inhibition of p53 expression in LNCaP cells (Mistry, Digby and Desai, et al. 2008).

Opposing effects of adiponectin have been observed in PC cells in vitro. For instance, adiponectin has been shown to stimulate AMPK phosphorylation in LNCaP cells (which are PTEN deficient), while simultaneously activating mTOR via PI3/Akt activation (Barb, et al. 2007). These data indicate that adiponectin activation of the PI3K/Akt/mTOR pathway could in some cases stimulate tumorigenesis (Barb, et al. 2007). Furthermore, adiponectin has been reported to stimulate PC3 and DU145 migration via α5β1 integrin and AdipoR1 (Tang and Lu 2009). Adiponectin-induced migration was in part mediated by p38, AMPK, and NF-κB (Tang and Lu 2009).

1.5.2 In Vivo Studies

Limited animal model studies have been reported examining the effect of adiponectin in relation to PC. A study on caloric restriction using TRAMP mice housed at 27°C (versus 21°C) pair fed (with similar caloric intake), had greater weight, fat, serum leptin, lower adiponectin, and lower prostatic adenocarcinoma (Huffman, et al. 2007). In a study where TRAMP mice were fed ad lib, intermittent (where mice were the oldest at tumor detection), and chronic caloric restriction, serum adiponectin levels were lowest, and leptin:adiponectin ratio the highest, for chronic caloric restriction compared to the other groups (Bonorden, et al. 2009). NAG-1, along with a C to G SNP at position 6 (H6D) of the NAG-1, is associated with
lower human PC incidence (Wang, et al. 2012). Mice injected with DU145 cells expressing H6D NAG1 or wild-type NAG-1 demonstrated that H6D expression was associated with a reduction in adiponectin, leptin and IGF-1 serum levels (Wang, et al. 2012). Soy phytochemical concentrate and tea treatments in mice—the combination of which has been shown to reduce PC in humans—resulted in lower serum adiponectin in male, but not female mice (Zhou, Li and Pan 2007). Lastly, castration in mice has been shown to result in increased serum adiponectin levels (Inoue, et al. 2010).

1.5.3 Human Studies

A number of clinical studies have been published examining adiponectin in relation to PC. Evidence indicates that serum adiponectin levels are inversely related to PC risk and high-grade disease. For instance, a prospective study of 654 cases and 644 controls examined prediagnostic serum adiponectin with risk of incident PC, and found that adiponectin levels positively correlated with decreased risk of high grade (metastatic or lethal disease) PC, and PC-specific mortality (HR=0.39, 95% CI: 0.17-0.85) in men with BMI ≥25 (Li, et al. 2010). In a study of 25 BPH and 43 PC patients with organ-confined or locally advanced disease, serum adiponectin levels were similar between BPH and pT2, but were elevated in pT3 compared to pT2 (Housa, et al. 2008). A study of 236 men treated with RP measured preoperative adiponectin serum levels and determined that adiponectin was inversely associated with BMI, not associated with cancer stage or grade, positively associated with high stage PC (in normal weight men only), and inversely associated
with high grade PC (in overweight and obese men only), indicating that the relationship between adiponectin and PC aggressiveness may depend upon body adiposity (Freedland, Sokoll, et al. 2005). A nested case-control study of 311 men with ≥T3, N1, or M1 PC and 413 men with T≤2 and NX-0 and M0 found that adiponectin was inversely associated with PC stage (in overweight and obese men only) (Burton, et al. 2013). Serum collected from 30 PC patients, 41 benign prostatic obstruction patients, and 36 controls, displayed lower adiponectin levels in PC versus benign prostatic obstruction patients, and in advanced disease versus organ-confined (Goktas, et al. 2005). In a study of 539 patients, with 199 who had undergone RP, elevated BMI, but not adiponectin, was associated with increased odds of Gleason Score 7 or higher, whereas decreased adiponectin was associated with higher rate of upgrading in patients (Sher, et al. 2008). A case-control study of 75 PC cases, 75 BPH cases, and 150 controls, found that PC patients had lower adiponectin levels, and weaker expression of AdipoR1 and R2 compared to healthy prostate tissue (Michalakis, et al. 2007). A study of 150 men divided into PC, BPH or control, examining lipoprotein profiles, showed that PC patients had elevated TG/HDL-cholesterol, decreased HDL-cholesterol, and decreased adiponectin, compared to controls (Grosman, et al. 2010). Furthermore, BPH has been associated with higher adiponectin levels in physically active men (Schenk, et al. 2009).

However, not all studies have demonstrated a relationship between adiponectin and PC risk/grade. For instance, in a study of 70 men, adiponectin failed to correlate with presence or aggressiveness of PC (Lopez Fontana, et al. 2011). In a nested case-control study of 125 incident PC cases and 125 controls, BMI and
Adiponectin were not associated with incident PC or high grade disease (Baillargeon, Platz, et al. 2006).

Evidence suggests that adiponectin may be related to PSA levels. In a cross-sectional study of 219 Arab men, serum triglycerides and WHR were positively, and adiponectin negatively associated with total PSA, indicating that insulin resistance and visceral adiposity are related to prostate volume markers (Alokail, et al. 2011). However, in a study of 121 African-American and 121 Caucasian men, BMI was inversely correlated with adiponectin levels, and PSA failed to correlate with adiponectin categories (Fowke, Matthews, et al. 2008).

ADT is known to have effects on adipose deposition and markers for obesity, including adiponectin. A prospective study of 25 nondiabetic men with locally advanced or recurrent PC treated with ADT had increased fat body mass, decreased insulin sensitivity index, and increased adiponectin (by 37% ± 7.2%) (Smith, Lee and Fallon, et al. 2008). Twenty-six men with recurrent or locally advanced PC treated with ADT for 12 months had increased body fat, decreased lean body mass, increased adiponectin levels, and unchanged resistin and C-reactive protein levels (Smith, Lee and McGovern, et al. 2008).

Some studies have indicated that adiponectin genes are related to PC risk/grade. An analysis of 465 PC cases and 441 controls demonstrated that five ADIPOQ SNPs and one ADIPOR1 SNP were associated with PC risk (Kaklamani, et al. 2011). An analysis of 29 SNPs in a nested case-control study of 1286 cases and 1267 controls, determined that four ADIPOQ SNPs were associated with overall PC risk (but not grade or stage) (Dhillon, et al. 2011). However, in a study of 1053 PC
cases and 1,053 controls followed over 11 yrs, ADIPOQ was not associated with PC (Moore, et al. 2009). Additionally, a study of 131 African American men with PC and 344 controls analyzed 10 SNPs in ADIPOQ and ADIPOR1 and found no association between any of the SNPs and PC risk (Beebe-Dimmer, et al. 2010).
CHAPTER TWO

2. Hypothesis, Objectives, and Rationale

2.1 Rationale

Taking into account all the various confounding factors in the relationship between obesity and PC, including detection biases (related to more difficult DREs, PSA hemodilution, and increased prostate volumes), mechanical complications (that is, physical obstructions and difficulties during RP and RT), related health problems (such as the conditions of the MetS), and other confounders (such as ethnicity, socioeconomic status, dietary intake, etc.), evidence indicates that there remains plausible biological mechanisms whereby increased (visceral) adiposity may negatively impact PC progression (particularly with respect to advanced disease and mortality). It appears highly probable that an accumulation of dysfunctional adiposity negatively impacts PC progression via paracrine (through periprostatic fat deposition) and endocrine pathways. However, the majority of clinical research has investigated the endocrine pathways relating obesity to PC, most likely due to practicality in study design and availability of patient serum. The following research is conducted under the a priori assumption that obesity, at least in part, is affecting PC progression via endocrine pathways (through serum-born pathological alterations in adipokine and cytokine levels).

In vitro experimentation allows for an analysis strictly of the biological mechanisms, without any of the confounding factors that traditionally plague
clinical studies. By treating PC cells *in vitro* with human serum, we can directly assess the effect of blood-born, obesity-related factors on PC progression.

### 2.2 Hypothesis

It is hypothesized that adipokines and factors that circulate in the blood related to obesity will promote a more aggressive phenotype in PC cells *in vitro*. Two independent studies were conducted. The first was a clinical study utilizing serum from obese and non-obese PC patients and control subjects. The hypothesis is that serum from obese PC patients will stimulate PC cell proliferation, invasion, and migration *in vitro*, given obesity’s impact on high grade and aggressive disease.

The second study was an *in vitro* investigation examining the effect of adipokines on the growth of PC cells. The hypothesis is that treatment of PC cells *in vitro* with human adiponectin will result in reductions in proliferation, migration, and invasion, given adiponectin’s inverse relationship with visceral and total adiposity, and numerous obesity-related diseases in clinical studies. Additionally, combination of adiponectin with resistin will result in an amelioration of adiponectin's effects on PC cell proliferation, migration, and invasion.

### 2.3 Objectives

The overall objective of this research is to determine if obesity impacts PC progression at the biological level, and to elucidate the molecular mechanisms that mediate this relationship. To address hypothesis one, samples of serum from obese
and non-obese PC and non-PC patients will be obtained and added to LNCaP and PC-3 cells \textit{in vitro}. Following serum-supplementation, cell proliferation, migration, and invasion will be measured. To address hypothesis two, human resistin and adiponectin will be added separately and in combination to various PC cell lines \textit{in vitro}. Following addition of the adipokines, cell proliferation, migration, and invasion will be measured.
CHAPTER THREE

3. The Effects of Serum from Prostate Cancer Patients with Elevated Body Mass Index on Prostate Cancer Cells In Vitro

3.1 Abstract

**Background:** Obesity has been related to a greater incidence of more aggressive, advanced stage PC. Elevated BMI may create a growth-enhancing tumor microenvironment that may either contribute to local tumor growth, or tumor migration and metastasis. **Hypothesis:** Serum from obese PC patients will stimulate PC cell proliferation, invasion, and migration in vitro, given obesity's impact on high grade and aggressive disease. **Material and Methods:** Patient serum (n = 80) and covariate data collected from the Genito-Urinary BioBank at Princess Margaret Hospital between 2008 and 2012 was obtained for analysis. Four patient groups (n = 20/group) included normal weight (≤25 BMI; ≥10 PSA; ≥6 GS) and overweight/obese (≥28 BMI; ≥11 PSA; ≥6 GS) men with PC, and normal weight (≤24 BMI; ≤3 PSA) and obese (≥30 BMI; ≤3 PSA) men without PC. Serum cytokine levels of MCP-1, resistin, IL-6, TNF-α, HGF and NGF were measured using ELISA. Cell proliferation of serum-treated LNCaP cells was measured using a non-radioactive cell proliferation assay (MTS). Cell invasion and migration in serum-treated PC3 cells was measured using wound-healing assay and migration chamber assay.

**Results:** Serum TNF-α levels were correlated with LNCaP cell proliferation in vitro in non-obese PC (p = 0.003) and non-obese control groups (p = 0.049). When correcting for age, serum HGF was significantly (p = 0.019) higher in obese (mean =
patients compared to non-obese patients (669.6 ± 379.3), and markedly higher (p < 0.001) in PC patients (880.7 ± 523.7) compared to non-PC patients (480.8 ± 283.3). Proliferation of LNCaP cells in vitro cultured with patient serum-supplemented media was seen to be significantly (p=0.050) greater in non-obese (mean=1.26 ±0.20) vs. obese patients (mean=1.16 ±0.19). In terms of mean migration score (PC3 cells in vitro with patient serum-supplemented media), there were significant differences among the four groups (p<0.001): obese PC (mean=4.18 ±0.83), non-obese PC (mean=2.87 ±0.91), obese control (mean=3.55 ±0.69), and non-obese control (mean=3.86 ±0.99) patients. The greatest difference in PC3 migration was observed among the PC patient groups, between obese and non-obese PC patients, with serum from obese PC patients inducing significantly greater amounts of cell migration (p<0.001, post-hoc analysis). Significant differences were detected (p<0.001) in terms of the ability for serum from patients to induce PC3 cell invasion among the four groups: non-obese PC (mean=19.1 ±5.9), non-obese control (mean=17.1 ±4.2), obese PC (mean=8.8 ±4.8), and obese control (mean=7.6 ±4.7) patients. Obese patients, including both PC and non-PC patients, had significantly (p<0.001) lower amounts of PC3 cell invasion (mean=8.2 ±4.5) compared to non-obese patients (mean=18.1 ±5.0). Conclusion: Serum from obese PC patients induced greater amounts of cell migration in vitro. However, unexpectedly serum from obese PC patients induced lower amounts of cell proliferation and invasion (with a more pronounced effect on the latter). The unexpected disparity in these results may be attributed to insufficient patient sample size, warranting the need for
future investigations regarding the impact of obesity-related, endocrine factors on PC progression.

3.2 Introduction

Overweight and obesity have been associated with numerous co-morbidities, including T2D, CVD, and various cancers (Guh, Zhang, et al., The incidence of co-morbidities related to obesity and overweight: a systematic review and meta-analysis 2009). A 5 kg/m² increase in BMI has been shown to be associated with an increased incidence of esophageal, thyroid, colon, renal, endometrial, and gallbladder cancer (Renehan, Tyson, et al. 2008). A more complicated relationship emerges when considering the incidence of PC in obese men. A recent meta-analysis indicates that BMI is inversely associated with the risk of localized PC and directly associated with the risk of advanced PC (Discacciati, Orsini and Wolk 2012). Another meta-analysis indicated that increased BMI was associated with an elevated risk of PC-specific mortality and BCR following primary treatment (Cao and Ma 2011). A number of factors may be operating to obfuscate the relationship between obesity and PC, primarily regarding detection biases. Obese men have hemodilution-related depressed PSA serum levels (Oh, et al. 2013). However, PSA as a predictor of PC may not be significantly altered by BMI (Banez, Albisinni, et al. 2012). The predictive value of an abnormal DRE in PC detection seems to be modified by obesity (Chu, et al. 2011). Moreover, obesity-driven prostate enlargement may reduce prostate biopsy efficiency and the ability to differentiate benign disease from PC (Fowke, Motley, et al. 2013). The paradoxical relationship between obesity and
PC may in part be explained by biological mechanisms, such as obesity-related decreases in T levels, coupled with aromatase up-regulation (G. Williams 2012), and increased incidence of diabetes in obese men (since there exists an inverse association between diabetes and PC) (Pierce 2012).

In light of the aforementioned factors complicating the explication of the obesity-PC relationship, it appears important to examine the direct impact of obesity on PC cell biology. The present study utilized serum from obese and non-obese PC patients for the treatment of several PC cell lines in vitro in an effort to characterize the direct biological effects of obesity on PC. By studying the interaction between obesity-related serum-born factors on PC cells in vitro, extraneous, detection-bias-related confounding factors present in clinical studies can be minimized. The hypothesis is that serum from obese PC patients will have abnormal levels of one or several of various adipokines (HGF, IL-6, MCP-1, TNF-α, NGF, resistin) in comparison to non-obese PC patients. In addition, human PC cells (LNCaP and PC3) treated with serum from obese PC patients will exhibit elevated levels of proliferation, migration and/or invasion in vitro, in comparison to non-obese PC patients. The primary finding is that serum from obese PC patients induced a greater amount of cell migration in vitro. However, unexpectedly serum from obese PC patients induced a lower amount of cell proliferation and invasion.
3.3 Methods

Patient Serum Samples

A retrospective cohort selected from patients who consented to participate in the Princess Margaret Hospital Genitourinary BioBank between 2008 and 2012 was the source of the serum samples for this study. This study has received approval by the University Health Network and Sunnybrook Research Ethics Board. Serum from 80 patients was collected according to four groups (20/group): 1) normal weight (≤25 BMI; ≥10 PSA; ≥6 GS) and 2) overweight/obese (≥28 BMI; ≥11 PSA; ≥6 GS) men with PC, and 3) normal weight (≤24 BMI; ≤3 PSA) and 4) obese (≥30 BMI; ≤3 PSA) men without PC. The patient cohort was predominantly of European descent (n=63, 79%), followed by Asian (n=4, 5%), African/Caribbean (n=2, 3%), and other (n=8, 10%). Three patients did not have their ethnicity recorded. None of the patients were diabetic, or receiving hormone therapy, Avodart (dutasteride) and Proscar (finasteride), or Metformin at the time of blood draw.

Cytokine Measurement

The following cytokines were assayed using Milliplex kits, obtained from Millipore (Billerica, MA, USA): IL-6, TNF-α, MCP-1, HGF, NGF, and resistin. Cytokine analysis was conducted at the microarray facility at Princess Margaret Hospital.

Cell Culture

Human PC cell lines LNCaP and PC3 were obtained from the American Type Culture Collection (Rockville, MA, USA), and cultured at 37 °C in a 5% CO₂ incubator. LNCaP cells were maintained in RPMI 1640 medium (Invitrogen, Burlington, Ontario,
Canada) supplemented with 10% fetal bovine serum (Sigma, St Louis, MO, USA), 0.3 mg ml\(^{-1}\) L-glutamine and 100 IU ml\(^{-1}\) penicillin and 100 μg ml\(^{-1}\) streptomycin (Invitrogen, Burlington, Ontario, Canada). PC3 cells were maintained in Dulbecco’s minimal essential medium/F12 (Invitrogen, Burlington, Ontario, Canada) with 10% fetal bovine serum, 0.3 mg ml\(^{-1}\) L-glutamine and 100 IU ml\(^{-1}\) penicillin and 100 μg ml\(^{-1}\) streptomycin (Invitrogen, Burlington, Ontario, Canada).

**Cell Proliferation Assay**

LNCaP (1 × 10\(^4\) cells/well) and PC3 (1 × 10\(^4\) cells/well) cells were seeded in 96-well plates and allowed to grow in FBS (10%) for 48 h at 37 °C prior to patient serum treatment. After the attachment, cells were incubated with patient serum (10%) for 48 h at 37 °C (at which time the effects on cell proliferation were most pronounced). After the treatment period, CellTiter96® AQueous Non-Radioactive Cell Proliferation Assay (MTS), obtained from Promega (Madison, WI, USA), was used to determine cell proliferation by adding 20 μl MTS reagent for 2 h at 37 °C. OD at 490 nm was measured by a plate reader. Patient serum was treated in triplicate wells and each experiment was repeated three times.

**Matrigel Invasion Assay**

The effect of patient serum on PC3 cell invasion was determined using BD BioCoat™ Matrigel™ Invasion Chamber 8.0 Micron, obtained from BD Biosciences (Mississauga, ON, Canada). PC3 (1 × 10\(^5\) cells/well) cells were seeded into the upper chamber/insert, using 12-well plates, and cultured for 24 h at 37 °C. Patient serum (5%) was added into the bottom wells as a chemoattractant. After 24 h, the non-
migrating cells were removed from the upper chamber with a cotton swab. The inserts were fixed with methanol and stained with 0.1% crystal violet solution. The number of migrated cells was quantified by counting the stained cells (cells per four fields) with a microscope. Patient serum was treated in duplicate wells and each experiment was repeated three times.

**Wound-Healing (Scratch) Assay**

Cell migration was assessed in PC3 cells using a wound-healing assay. PC3 cells were cultured in a 24-well plate for 24 h at 1.0 x 10^6 cells/well to reach 100% confluence. A vertical scratch across the well was made with a 10 μl pipette tip, followed by two washes with PBS. PC3 cell media supplemented with 5% patient serum was then added to each well for 24 h. Microscopy images were taken at 0 and 24 h to visually assess cell migration. Each patient serum sample was added in duplicate wells, and each experiment was repeated twice.

**Statistics**

All analyses were conducted using SPSS version 20. Differences in cytokine levels between groups were analyzed by one-way analysis of variance, and multiple comparisons were corrected for post-hoc with Bonferroni correction. Univariate analysis of variance was used to analyze differences between groups in terms of proliferation, migration, and invasion. Age was corrected for post-hoc. Associations were considered significant at p ≤ 0.05.
3.4 Results

Description of Study Participants

Table 2 – Clinicobiologic/pathologic characteristics of patient cohort (n=80).

Clinicobiologic characteristics of the four patient groups, along with serum cytokine levels. Significant differences are noted in the right column.

<table>
<thead>
<tr>
<th></th>
<th>Non-Obese PC (n=20) Mean (SD)</th>
<th>Obese PC (n=20) Mean (SD)</th>
<th>Non-Obese Control (n=20) Mean (SD)</th>
<th>Obese Control (n=20) Mean (SD)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>64.4 (8.8)</td>
<td>62.0 (8.7)</td>
<td>55.7 (9.1)</td>
<td>54.0 (6.0)</td>
<td>&lt;0.01</td>
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<tr>
<td>BMI</td>
<td>22.3 (1.9)</td>
<td>33.7 (7.9)</td>
<td>21.8 (2.0)</td>
<td>32.0 (2.0)</td>
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</tr>
<tr>
<td>PSA</td>
<td>24.8 (19.9)</td>
<td>32.1 (26.9)</td>
<td>2.1 (0.7)</td>
<td>1.9 (0.9)</td>
<td></td>
</tr>
<tr>
<td>MCP-1</td>
<td>320.4 (116.7)</td>
<td>291.1 (136.6)</td>
<td>344.2 (174.3)</td>
<td>332.2 (141.4)</td>
<td>0.68</td>
</tr>
<tr>
<td>Resistin</td>
<td>11958.0 (4776.3)</td>
<td>15041.7 (6800.1)</td>
<td>12988.5 (5343.5)</td>
<td>12184.9 (3156.0)</td>
<td>0.23</td>
</tr>
<tr>
<td>IL-6</td>
<td>3.7 (2.7)</td>
<td>2.8 (1.9)</td>
<td>5.9 (7.7)</td>
<td>3.1 (1.5)</td>
<td>0.11</td>
</tr>
<tr>
<td>TNF-α</td>
<td>5.4 (3.7)</td>
<td>5.2 (2.4)</td>
<td>4.8 (3.2)</td>
<td>7.2 (9.2)</td>
<td>0.51</td>
</tr>
<tr>
<td>HGF</td>
<td>897.6 (363.3)</td>
<td>863.8 (656.0)</td>
<td>441.7 (231.8)</td>
<td>519.9 (328.4)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>NGF</td>
<td>7.3 (7.3)</td>
<td>4.8 (2.3)</td>
<td>8.9 (17.2)</td>
<td>5.2 (3.3)</td>
<td>0.51</td>
</tr>
</tbody>
</table>

One-way ANOVA with post-hoc Bonferroni correction was used to calculate differences between groups.
Patient serum was obtained from the Princess Margaret Hospital Genitourinary BioBank.

Clinicobiologic and pathologic characteristics of the patient cohort are found in Table 2. The average BMI was 33.7 ± 7.9 and 32.0 ± 2.0 (for obese PC and obese control groups respectively) and 22.3 ± 1.9 and 21.8 ± 2.0 (for non-obese PC and non-obese control groups respectively). The PC patient groups (non-obese PC group mean = 64.4 ± 8.8 yrs; obese PC group mean = 62.0 ± 8.7 yrs) were significantly older than the control groups (non-obese control group mean = 55.7 ± 9.1 yrs; obese control group mean = 54.0 ± 6.0 yrs). The average serum levels of the cytokines MCP-1, resistin, IL-6, TNF-α, and NGF were not significantly different between the
four groups. The average serum levels of HGF were significantly higher in the PC groups (non-obese PC group mean = 897.6 ± 363.3; obese PC group mean = 863.8 ± 656.0) compared to the control groups (non-obese control group mean = 441.7 ± 231.8; obese control group mean = 519.9 ± 328.4).

**Serum Cytokine Levels**

Table 3 – Correlation between serum cytokine levels and LNCaP cell proliferation, corrected for age.

<table>
<thead>
<tr>
<th></th>
<th>Non-obese PC group</th>
<th>Obese PC group</th>
<th>Non-obese control group</th>
<th>Obese control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCP-1</td>
<td>0.190</td>
<td>0.930</td>
<td>0.722</td>
<td>0.510</td>
</tr>
<tr>
<td>Resistin</td>
<td>0.441</td>
<td>0.357</td>
<td>0.306</td>
<td>0.395</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.565</td>
<td>0.863</td>
<td>0.681</td>
<td>0.892</td>
</tr>
<tr>
<td>TNF-α</td>
<td><strong>0.003</strong></td>
<td>0.694</td>
<td><strong>0.049</strong></td>
<td>0.413</td>
</tr>
<tr>
<td>HGF</td>
<td>0.330</td>
<td>0.108</td>
<td>0.384</td>
<td>0.466</td>
</tr>
<tr>
<td>NGF</td>
<td>0.861</td>
<td>0.607</td>
<td>0.149</td>
<td>0.623</td>
</tr>
</tbody>
</table>

Numbers represent p values for partial correlation between absorbance at 490 nm (cell proliferation value) and serum cytokine levels, corrected for age (yrs).

A correlation analysis between the various serum cytokine levels and the individual patient group LNCaP cell proliferation levels *in vitro*, corrected for age, is shown in Table 3. The cytokines MCP-1, resistin, IL-6, HGF, and NGF failed to show any correlation with cell proliferation *in vitro*. Serum TNF-α levels showed a significant correlation with cell proliferation *in vitro* in non-obese PC (p = 0.003) and non-obese control groups (p = 0.049), but not with obese PC (p = 0.694) and obese control groups (p = 0.413).
Table 4 – Correlation between serum cytokine levels and BMI, corrected for age.

Serum cytokine levels between the four patient groups; with p values representing strength of correlation between cytokine level and patient BMI.

<table>
<thead>
<tr>
<th></th>
<th>Non-obese PC group</th>
<th>Obese PC group</th>
<th>Non-obese control group</th>
<th>Obese control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCP-1</td>
<td>0.142</td>
<td>0.093</td>
<td>0.247</td>
<td>0.084</td>
</tr>
<tr>
<td>Resistin</td>
<td><strong>0.021</strong></td>
<td>0.665</td>
<td>0.836</td>
<td>0.762</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.219</td>
<td>0.863</td>
<td>0.723</td>
<td>0.924</td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.273</td>
<td>0.944</td>
<td>0.760</td>
<td>0.355</td>
</tr>
<tr>
<td>HGF</td>
<td>0.583</td>
<td>0.513</td>
<td>0.123</td>
<td>0.638</td>
</tr>
<tr>
<td>NGF</td>
<td>0.395</td>
<td>0.565</td>
<td>0.607</td>
<td>0.232</td>
</tr>
</tbody>
</table>

Numbers represent p values for partial correlation between patient BMI and serum cytokine levels, corrected for age (yrs).

Serum cytokine levels were analyzed as to whether a correlation existed within each of the four patient groups with BMI, corrected for age, in Table 4. The cytokines MCP-1, IL-6, TNF-α, HGF, and NGF failed to show any correlation with BMI within each of the four patient groups. However, resistin showed a significant correlation with BMI within the non-obese PC group (p = 0.021), but not within any of the other three groups.

Table 5 – Univariate analysis of variance of serum cytokine levels between either obese vs. non-obese or PC vs. control.

Differences of serum cytokine levels between obese and non-obese (including both PC and non-PC) and PC and non-PC (including both obese and non-obese) patients.

<table>
<thead>
<tr>
<th></th>
<th>Obese</th>
<th>Non-obese</th>
<th>p</th>
<th>PC</th>
<th>Non-PC</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCP-1</td>
<td>311.7 ± 138.8</td>
<td>332.3 ± 146.9</td>
<td>0.544</td>
<td>305.8 ± 126.3</td>
<td>338.2 ± 156.7</td>
<td>0.553</td>
</tr>
<tr>
<td>Resistin</td>
<td>13613.3 ± 5429.0</td>
<td>12473.2 ± 5029.6</td>
<td>0.390</td>
<td>13499.8 ± 6006.7</td>
<td>12586.6 ± 4350.7</td>
<td>0.631</td>
</tr>
<tr>
<td>IL-6</td>
<td>2.8 ± 1.8</td>
<td>4.3 ± 5.6</td>
<td>0.283</td>
<td>3.1 ± 2.4</td>
<td>4.0 ± 5.4</td>
<td>0.578</td>
</tr>
<tr>
<td>TNF-α</td>
<td>6.2 ± 6.7</td>
<td>5.1 ± 3.4</td>
<td>0.677</td>
<td>5.2 ± 3.1</td>
<td>6.0 ± 6.9</td>
<td>0.728</td>
</tr>
<tr>
<td>HGF</td>
<td>691.9 ± 540.8</td>
<td>669.6 ± 379.3</td>
<td><strong>0.019</strong></td>
<td>880.7 ± 523.7</td>
<td>480.8 ± 283.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NGF</td>
<td>4.8 ± 3.0</td>
<td>8.2 ± 13.1</td>
<td>0.091</td>
<td>6.0 ± 5.6</td>
<td>6.9 ± 12.4</td>
<td>0.402</td>
</tr>
</tbody>
</table>

Values represent mean ± standard deviation, corrected for age.
An univariate analysis of variance, corrected for age, shown in Table 5, was used to assess whether the various serum cytokine levels significantly differed between either obese vs. non-obese or PC vs. non-PC patients. The cytokines MCP-1, resistin, IL-6, TNF-α, and NGF failed to show significant differences between either obese vs. non-obese or PC vs. non-PC patients, when corrected for age. When corrected for age, serum HGF was significantly (p = 0.019) higher in obese (mean = 691.9 ± 540.8) patients compared to non-obese patients (669.6 ± 379.3), and markedly higher (p < 0.001) in PC patients (880.7 ± 523.7) compared to non-PC patients (480.8 ± 283.3) (Figure 1). Mean serum NGF levels in obese patients were nearly half that of non-obese patients, but failed to reach significance due to large variations in non-obese patients.
Figure 2 – Serum HGF levels for obese vs. non-obese and PC vs. non-PC patients, corrected for age.

A. Non-cancer patients, including obese and non-obese, had lower serum levels of HGF compared to PC patients (p<0.01). B. Non-obese patients, including PC and non-PC, had slightly lower serum levels of HGF compared with obese patients (p=0.019).

Univariate analysis of variance, corrected for age post-hoc, was used to determined differences between groups.

Values in bars represent group mean.

*P≤0.05, **P≤0.001.
**Effects of Patient Serum on PC Cell Proliferation.**

After a 24 h treatment period of media supplemented with 10% patient serum (n=80, 20/group), analysis revealed marginal differences in terms of LNCaP cell proliferation *in vitro* between the four groups: non-obese PC (mean=1.28 ±0.20), non-obese control (mean=1.23 ±0.20), obese PC (mean=1.23 ±0.18), and obese control (mean=1.10 ±0.19) (Figure 2). Pre-hoc statistical analysis revealed significant differences between the four groups (p=0.045). However, upon post-hoc analysis, the most pronounced difference among the four groups, between non-obese PC and obese control patients, failed to remain significant (p=0.083).

Proliferation of LNCaP cells *in vitro* was significantly (p=0.050) greater in non-obese (mean=1.26 ±0.20) vs. obese patients (mean=1.16 ±0.19). Cell proliferation failed to be significantly different (p=0.101) between PC (mean=1.26 ±0.19) and non-PC (mean=1.16 ±0.21) patients, although the mean values were higher in PC patients.

A similar protocol for proliferation assays was repeated using PC3 cells with a subset of the patient population randomly selected (n=20, 5/group) (Figure 3). No differences in cell proliferation were detected among the four groups (p=0.352): non-obese PC (mean=0.96 ±0.03), non-obese control (mean=0.93 ±0.04), obese PC (mean=0.92 ±0.06), and obese control (mean=0.90 ±0.05) patients. No differences in cell proliferation were detected (p=0.189) between PC (mean=0.91 ±0.05) and non-PC (mean=0.94 ±0.04) patients. Lastly, no differences in cell proliferation were detected (p=0.444) between obese (mean=0.94 ±0.05) and non-obese (mean=0.91 ±0.05) patients.
Figure 3 – LNCaP cell proliferation *in vitro* represented as mean absorbance at 490nm.

Serum from 80 patients (20 per group) was used to treat LNCaP cells cultured *in vitro* for 24 h. The cell media was supplemented with 10% patient serum. Absorbance at 490nm served as an index for cell proliferation using MTS assay. A. Represents *in vitro* LNCaP cell proliferation with patient serum-supplemented media according to the four groups indicated. The groups were not significantly different (p=0.083). B. Serum from non-obese patients, including PC and non-PC, marginally induced greater *in vitro* LNCaP cell proliferation compared to obese patients (p=0.050). C. *In vitro* LNCaP cell proliferation did not significantly differ between PC and non-PC patients (p=0.101). Univariate analysis of variance, corrected for age post-hoc, was used to determined differences between groups. Values in bars represent group mean. *P≤0.05.
Figure 4 – PC3 cell proliferation in vitro represented as mean absorbance at 490nm.

Serum from 20 patients (5 per group) was used to treat PC3 cells cultured in vitro for 24 h. The cell media was supplemented with 10\% patient serum. Absorbance at 490nm served as an index for cell proliferation using MTS assay. A. Represents in vitro PC3 cell proliferation with patient serum-supplemented media according to the four groups indicated. There were no significant differences between the four groups in terms of in vitro PC3 cell proliferation (p=0.352). In vitro PC3 cell proliferation did not significantly differ between B. obese and non-obese (p=0.444), and C. PC and non-PC patients (p=0.189). Univariate analysis of variance, corrected for age post-hoc, was used to determined differences between groups. Values in bars represent group mean. Significance defined at P≤0.05.

Effects of Patient Serum on PC3 cell Migration.

Due to a large amount of variation along the wound border and in wound thickness, a general rating scale was devised by us in order to grade the microscopy images of cell migration, and arrive at an average score (between 1-5) from the duplicates and repeats (Table 6). In terms of mean migration score (PC3 cells in vitro), there were significant differences among the four groups (p<0.001): obese PC
(mean=4.18 ±0.83), non-obese PC (mean=2.87 ±0.91), obese control (mean=3.55 ±0.69), and non-obese control (mean=3.86 ±0.99) patients (n=80, 20/group) (Table 7). The greatest difference in PC3 migration was among the PC groups, between obese and non-obese patients, with serum from obese PC patients inducing significantly greater amounts of cell migration (p<0.001, post-hoc analysis) (Figure 4). Additionally, serum from non-obese PC patients induced significantly less PC3 cell migration compared with serum from non-obese control patients (p=0.005, post-hoc analysis).

Serum from obese patients, including both PC and non-PC patients, may have induced greater PC3 cell proliferation in vitro compared to non-obese patients, though the relationship was not statistically significant (p=0.075). There was no difference in the ability of serum from PC vs. non-PC patients to induce PC3 cell proliferation in vitro (p=0.784). All statistical analyses were controlled for age differences among the groups. The wound healing assay protocol was repeated with LNCaP cells in vitro. However no definite results were obtained due to the low migratory capacity of these cells.
### Table 6 – Rating scale for cell migration.

Each microscopy image was assigned a rating of 1-5, and the mean rating between experimental repeats and among groups was determined to assess differences in cell migration.

<table>
<thead>
<tr>
<th>Rating</th>
<th>Description</th>
<th>Image</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No migration; 0% confluent.</td>
<td>![Image 1]</td>
</tr>
<tr>
<td>2</td>
<td>Minimal migration with few cells dispersed across wound; 10-25% confluent.</td>
<td>![Image 2]</td>
</tr>
<tr>
<td>3</td>
<td>Cell migration filled approximately half of wound; 50% confluent.</td>
<td>![Image 3]</td>
</tr>
<tr>
<td>4</td>
<td>Wound approximately 75-90% confluent with cells.</td>
<td>![Image 4]</td>
</tr>
<tr>
<td>5</td>
<td>Wound 100% confluent with cells.</td>
<td>![Image 5]</td>
</tr>
</tbody>
</table>
**Table 7 – Representative images of wound healing assay.**

PC3 cells were treated with patient serum (5%) for 24 h, following microscopy imaging to visually assess degree of migration. All serum from patients was added to duplicate wells, and all experiments were repeated twice. Below are representative images across the four groups.

<table>
<thead>
<tr>
<th></th>
<th>0 h</th>
<th>24 h</th>
<th>Average Rating</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Obese PC patient</strong></td>
<td><img src="image1.jpg" alt="Image" /></td>
<td><img src="image2.jpg" alt="Image" /></td>
<td>4.2</td>
</tr>
<tr>
<td><strong>Non-obese PC patient</strong></td>
<td><img src="image3.jpg" alt="Image" /></td>
<td><img src="image4.jpg" alt="Image" /></td>
<td>2.9</td>
</tr>
<tr>
<td><strong>Obese control patient</strong></td>
<td><img src="image5.jpg" alt="Image" /></td>
<td><img src="image6.jpg" alt="Image" /></td>
<td>3.5</td>
</tr>
<tr>
<td><strong>Non-obese control patient</strong></td>
<td><img src="image7.jpg" alt="Image" /></td>
<td><img src="image8.jpg" alt="Image" /></td>
<td>3.9</td>
</tr>
</tbody>
</table>
Figure 5 – PC3 in vitro cell migration for 24 h, corrected for age.

At 0 h a wound made with a 10 μl pipette tip was made across a 100% confluent plate of PC3 cells. Media was supplemented with 5% patient serum (n=80) and imaged following a 24 h migration period. **A.** Represents in vitro PC3 cell migration with patient serum-supplemented media according to the four groups indicated. Serum from non-obese PC patients induced the least and from obese PC patients induced the greatest amount of PC3 cell migration in vitro (p<0.001). **B.** Serum from obese patients may have induced greater PC3 cell migration in vitro compared to serum from non-obese patients (p=0.075). **C.** There was no difference in the affect on PC3 cell migration in vitro with serum from PC vs. non-PC patients (p=0.784). Univariate analysis of variance, corrected for age post-hoc, was used to determined differences between groups. Values in bars represent group mean.

*P≤0.05, **P≤0.001.
The Effects of Patient Serum on PC3 Cell Invasion.

Representative microscopy images of the invaded cells following a 24 h period of PC3 cell invasion *in vitro* are shown in Table 8. Significant differences were detected (p<0.001) in terms of the ability for serum from patients to induce PC3 cell invasion among the four groups: non-obese PC (mean=19.1 ±5.9), non-obese control (mean=17.1 ±4.2), obese PC (mean=8.8 ±4.8), and obese control (mean=7.6 ±4.7) patients (Figure 5). Obese patients, including both PC and non-PC patients, demonstrated a significantly (p<0.001) lower capacity for PC3 cell invasion (mean=8.2 ±4.5) compared to non-obese patients (mean=18.1 ±5.0). However, no significant differences (p=0.583) in invasive capacity were found between PC patients (mean=14.0 ±7.4), including both obese and non-obese, compared to non-PC patients (mean=12.4 ±6.5), though the mean was higher for PC patients.
PC3 cells were treated with patient serum (5%) for 24 h, following microscopy imaging to count number of invaded cells. All patient serum was added to duplicate wells, and all experiments were repeated twice. Below are representative images across the four groups.

<table>
<thead>
<tr>
<th></th>
<th>24 h</th>
<th>Average number of invaded cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-obese PC patient</td>
<td></td>
<td>22.0</td>
</tr>
<tr>
<td>Non-obese control patient</td>
<td></td>
<td>15.1</td>
</tr>
<tr>
<td>Obese PC patient</td>
<td></td>
<td>9.8</td>
</tr>
<tr>
<td>Obese control patient</td>
<td></td>
<td>5.9</td>
</tr>
</tbody>
</table>
Figure 6 – PC3 in vitro cell invasion for 24 h, corrected for age.

PC3 cells were allowed to undergo invasion for 24 h in media supplemented with 5% patient serum (n=12). A. Represents the average number of invaded PC3 cells in vitro following a 24 h period of migration, separated into the four groups indicated. Significant differences were found among the groups (p<0.001), with the greatest to least number of invaded cells in the following order: 1) non-obese PC, 2) non-obese control, 3) obese PC, and 4) obese control patients. B. Obese patients, including PC and non-PC patients, had fewer numbers of invaded cells (p<0.001) compared to non-obese patients. C. PC patients, including obese and non-obese patients, may have had greater numbers of invaded cells compared to non-PC patients, however the relationship failed to reach statistical significance (p=0.583).

Univariate analysis of variance, corrected for age post-hoc, was used to determined differences between groups. Values in bars represent group mean. *P≤0.05, **P≤0.001.
3.5 Discussion

Obesity is believed to promote more aggressive disease in PC patients, and a number of biological mechanisms have been proposed to explain the relationship, many of which implicate blood-born factors. Therefore, this study used serum from obese and non-obese PC patients to treat PC cells in vitro, and to assess the effects on cell proliferation, migration, and invasion. As stated in the results, proliferation of LNCaP cells in vitro cultured with media supplemented with patient serum was significantly (p=0.050) greater in non-obese compared to obese patients (Figure 2). In terms of mean migration score (PC3 cells in vitro with media supplemented with patient serum), there were significant differences among the four groups (p<0.001): obese PC patients had the highest migration, followed by non-obese control, obese control, and non-obese PC patients (Figure 4). Significant differences were detected (p<0.001) in terms of the ability for serum from patients to induce PC3 cell invasion among the four groups: non-obese PC patients had the highest invasion, followed by non-obese control, obese PC, and obese control patients (Figure 5). Therefore, serum from obese PC patients reduces proliferation and invasion, and increases migration in vitro of two PC cell lines derived from metastatic disease.

A meta-analysis in 2006 that examined obesity in relation to PC found a weak association for overall risk (RR=1.05, 95% CI: 1.01-1.08, per 5-unit increment in BMI), and a stronger association for advanced disease (RR=1.12, 95% CI: 1.01-1.23, per 5-unit increment in BMI) (MacInnis and English 2006). A subsequent meta-analysis found that among six population-based cohorts, BMI was associated with PC-specific mortality (RR=1.15, 95% CI: 1.06-.125, with a 5-unit increase in BMI;
and RR=1.20, 95% CI: 0.99-1.46, with a 5-unit increase in BMI for six post-diagnosis survival studies) (Cao and Ma 2011). In 16 studies, which followed patients after primary treatment, BMI was associated with risk of BCR (RR=1.21, 95% CI: 1.11-1.31) (Cao and Ma 2011). The influence of obesity on PC appears to be more complex than merely a direct positive effect of adiposity volume potentiating tumor aggressiveness. Recent evidence indicates a dual effect of body adiposity on PC development. A meta-analysis in 2012 identified 12 studies on localized PC and 13 on advanced PC and found an inverse association between BMI and localized disease (RR=0.94, 95% CI: 0.91-0.97, for every 5-unit increase in BMI) and a direct association between BMI and advanced disease (RR=1.09, 95% CI: 1.02-1.16, for every 5-unit increase in BMI) (Discacciati, Orsini and Wolk 2012). There may be several explanations for this paradox between localized vs. advanced PC and obesity, including the presence of detection biases, variations in treatment outcomes, associated comorbidities, and various biological mechanisms.

There were no significant differences in terms of the serum levels of the cytokines MCP-1, resistin, IL-6, TNF-α, and NGF (Table 2). The patient population in this study was relatively small (n=80) and thus may not have had enough power to detect the subtle differences of these cytokines in healthy vs. pathological subjects. The serum levels of HGF were significantly higher in PC compared to control patients. Within the PC groups, HGF was higher in the non-obese PC group compared to the obese PC group. The results of this study relating to HGF are in accordance with previous published data. Hepatocyte growth factor and its receptor Met tyrosine kinase are overexpressed in PC cells and increase in expression
throughout the stages of PC progression (Varkaris, et al. 2011). Pharmacological antagonists of the HGF/Met pathway have been shown to be effective in treating PC (Cecchi, Rabe and Bottaro 2012). No studies were found which investigated the interaction between HGF and body adiposity with PC progression or outcomes. However, HGF may interact with the alterations in obesity-related prostate volume (Nishimura, et al. 2008), explaining why HGF serum levels were different between PC obese and non-obese patients in the present study. Resistin showed a correlation with BMI within the non-obese PC group, but not within any of the other three groups. Serum resistin levels have previously been reported to correlate with obesity and T2D (Gharibeh, et al. 2010). Serum TNF-α levels showed a correlation with LNCaP cell proliferation in vitro in non-obese PC and non-obese control groups, but not with obese PC and obese control groups. A paradoxical role for TNF-α in PC biology exists, where TNF-α has been shown to stimulate tumor angiogenesis, development of CRPC, and metastasis on the one hand, and on the other, inhibit neovascularization, induce apoptosis, and stimulate antitumor immunity (Tse, Scott and Russell 2012). The present study indicates TNF-α’s effect on PC cells is modified by body adiposity.

Proliferation of LNCaP cells in vitro was significantly (p=0.050) greater in non-obese (mean=1.26 ±0.20) vs. obese patients (mean=1.16 ±0.19). Cell proliferation failed to demonstrate a level of significance (p=0.101) between PC (mean=1.26 ±0.19) and non-PC (mean=1.16 ±0.21) patients, though the mean was higher in PC patients. No significant differences were found in terms of PC3 cell proliferation in vitro supplemented with patient serum from the four groups,
indicating that AR may mediate the effect of obesity on PC cell proliferation, since an effect was only observed in LNCaP cells (an androgen sensitive cell line).

The fact that non-obese patient serum caused greater PC cell proliferation in vitro is an unexpected finding given that obesity appears to promote aggressive disease in clinical studies, and the LNCaP cell line represents metastatic disease (Horoszewicz, Leong and Kawinski, et al. 1983). We would expect obese PC patient serum to induce greater proliferation in a metastatic cell line in vitro. Several published animal studies with a similar design, found dissimilar results compared to the present study. In one study in 2007, it was shown that obese Zucker rat serum, compared to lean serum, added to LNCaP cells in vitro had a greater mitogenic effect and induced greater cell proliferation (Lamarre, et al. 2007). In another study in 2012, it was shown that serum from C57BL/6 mice with diet-induced obesity, compared to lean serum, added to LNCaP and PacMetUT1 cells in vitro increased cell proliferation (Price, et al. 2012). The disparity between the present results and previous data may be explained by obesity-related hemodilution minimizing the amount of cancer-promoting, serum-born factors, lessening proliferative potential. Increased periprostatic adipose tissue may enhance PC progression locally, enhancing metastatic potential via paracrine as opposed to endocrine signaling.

In terms of mean migration score, there were significant differences among the four groups, with the highest to lowest scores being: obese PC, non-obese control, obese control, and non-obese PC patients. The greatest difference in PC3 migration was among the PC groups, between obese and non-obese patients, with serum from obese PC patients inducing significantly greater amounts of cell
migration. As would be expected, obese PC patient serum induced the greatest amount of cell migration in vitro, indicating that obesity may promote metastatic disease. In a previous study, cited above, that used C57BL/6 mouse serum from obese and non-obese mice to treat LNCaP and PacMetUT1 cells in vitro, it was found that LNCaP cells exposed to obese serum had increased migration, invasion and MMP-9 activity (Price, et al. 2012).

Obese patients, including both PC and non-PC patients, had significantly (p<0.001) lower capacity for PC3 cell invasion (mean=8.2 ±4.5) compared to non-obese patients (mean=18.1 ±5.0). However, no significant differences (p=0.583) were found between PC patients (mean=14.0 ±7.4), including both obese and non-obese, compared to non-PC patients (mean=12.4 ±6.5), though the mean was higher for PC patients. There was an unexpected pronounced effect in serum from obese patients inducing lower PC cell invasion in vitro compared to non-obese patients. Again, given obesity’s effect on promoting more aggressive disease and increasing PC-related mortality in clinical studies, it is expected that serum from obese patients will induce greater PC cell invasion in vitro. The disparity between the migration and invasion results in the present study may be attributed to the fact that only a small subset of the patient cohort was used for the invasion experiments. Furthermore, the 1-5 migration rating scale was devised in order to accommodate the large amount of observed variation in width along the wound on the cell culture plate. A more objective marker and sensitive analysis for cell migration could better accurately determine the effect of serum from obese patients on PC cells in vitro.
Additionally, the use of other cell lines, such as DU145, may have rendered different results.

The three primary biological mechanisms linking obesity and PC include insulin/IGF signaling, sex hormones, and adipokine signaling. Various studies have demonstrated that high circulating IGF-1 levels are positively associated with PC risk (Key, Diet, insulin-like growth factor-1 and cancer risk. 2011), specifically in obese men (Weiss, et al. 2007). The dual effect of obesity on PC development may in part be explained by changes in androgen levels. It has long been recognized that obese men have lower levels of SHBG, and free and total T (Seidell, et al. 1990). Furthermore, the quantity of visceral adipose tissue is proportional to the amount of aromatase, an intracellular enzyme that converts T to estradiol (G. P. Williams 2010). Various adipokines, principally adiponectin and leptin, have been implicated in obesity-related cancers, including PC (Paz-Filho, et al. 2011).

The primary strength of this study is that in vitro experimentation allows for an analysis strictly of the biological mechanisms, without any of the confounding factors that traditionally plague clinical studies. By treating PC cells in vitro with human serum, we can directly assess the effect of blood-born, obesity-related factors on PC progression and promotion. On the other hand, there were several limitations with this study. We were unable to balance the study population in terms of age. The PC patient groups were older than the control patient groups, meaning that any effects seen in the PC groups may be exaggerated due to age-related biological effects rather than cancer or obesity-related effects. Univariate analysis of variance was used to control for covariates such as age. However such statistical
corrections can only go so far in correcting for the pervasive effects age has on pathophysiology. Furthermore, BMI serves as a crude measure of body adiposity, and fails to appreciate the difference between subcutaneous and visceral depots. Disease complications appear to be associated specifically with increased visceral adiposity, and thus a more accurate measure of obesity, in terms of its relation to diseases such as PC, would have been WHR, waist circumference, or periprostatic fat thickness (via MRI imaging).

Several of the cytokines reported in this study, such as HGF, TNF-α, and resistin, presented promising results and warrant the need for further investigations into their cell signaling pathways in PC biology. We found that serum from obese PC patients induced greater amounts of cell migration in vitro. However, unexpectedly serum from obese PC patients was associated with lower amounts of cell proliferation and invasion (with a more pronounced effect on the latter). The unexpected disparity in these results may be further clarified with a larger sample size within each group. Future investigations should explore the impact of obese serum on PC cell characteristics in different cell lines, using larger patient populations, and utilizing genetic analyses.
CHAPTER FOUR

4.1 Study Progression: From Human Serum to Adipokine Signaling

From the data obtained in chapter three, it was determined that endocrine-related factors were mediating a significant effect on PC cells in vitro. Serum from obese PC patients significantly altered PC cell proliferation, migration, and invasion. Then next step was then to elucidate the biological mechanisms whereby obesity impacts PC cells. One of the prominent mechanisms that link obesity to PC is adipokine signaling. Adipose tissue functions as a metabolically active endocrine organ and secretes a variety of hormones and adipokines. Adipokines exert their effects on PC cells through both endocrine and autocrine/paracrine pathways, especially when extracapsular extension and invasion of the retropubic fat pad occurs (Mistry, Digby and Desai, et al. 2008).

The study in chapter four was undertaken to assess if adipokines mediate a significant effect on PC cell proliferation, migration, invasion, and signaling molecules. Since the serum adipokine levels in chapter three showed large variation between patients, an in vitro study then was needed that controlled for the concentration of adipokines added to PC cells.
4.2 The Effect of Adiponectin and Resistin on Prostate Cancer Cell Proliferation, Migration, and Invasion *In Vitro*

4.2.1 Abstract

**Background:** Obesity has been associated with high grade PC and BCR. Obesity affects PC progression and outcomes through a number of mechanisms, including dysfunctional IGF/insulin signaling, sex hormone production, and adipokine (adipocyte-derived cytokines) signaling. The most prominent protein synthesized by adipocytes is adiponectin, which has been shown to have insulin-sensitizing, anti-inflammatory, and pro-apoptotic effects. Evidence implicates adiponectin signaling in PC biology, and clinical data has shown inverse correlations between serum adiponectin levels and high-grade PC outcomes. Another prominent adipokine, resistin, was shown to be produced by high-grade PC tissue, and when added to PC cells *in vitro* stimulated cell proliferation. **Hypothesis:** Treatment of PC cells *in vitro* with human adiponectin will result in reductions in proliferation, migration, and invasion. Additionally, combination of adiponectin with resistin will result in an amelioration of adiponectin’s effects on PC cell proliferation, migration, and invasion. **Materials and Methods:** Various adipokines, including adiponectin, resistin, visfatin, and NGF, were treated on PC cells *in vitro* (LNCaP, PC3, PC3-AR2, and DU145) and cell proliferation was measured using a non-radioactive cell proliferation assay (MTS). LNCaP and PC3 cells were treated with adiponectin and resistin *in vitro*. Cell proliferation, migration (wound healing assay), invasion (matrigel invasion assay), and protein expression (western blot) were analyzed.
Results: Visfatin significantly decreased LNCaP and PC3 cell proliferation at 48 h of treatment (p<0.001); NGF significantly decreased LNCaP and PC3 cell proliferation by the end of 24 h of treatment (p<0.001 and p=0.034 respectively); and resistin significantly decreased LNCaP and PC3 cell proliferation at 24 h of treatment (p<0.001 for both). Adiponectin decreased LNCaP and PC3-AR2 cell proliferation at 24 and 48 h of treatment (p<0.001 and p=0.010 for 24 h, and p<0.001 for both at 48 h), and increased DU145 cell proliferation at 48 h of treatment (p=0.017). In a combination treatment using LNCaP cells, adiponectin alone decreased cell proliferation, and resistin with adiponectin caused the effect to disappear (p<0.001). There were no significant differences in terms of average migration score among the four treatment groups of adiponectin and resistin alone and in combination compared to control (p=0.063). No significant differences were found in terms of average number of invaded PC3 cells after 24 h among the different adiponectin and resistin treatment groups compared to control (i.e. adiponectin at 1.0 and 10.0 μg/ml, resistin at 5.0 μg/ml, and adiponectin 10.0 and resistin 5.0 μg/ml combination) (p=0.196). Treatment of LNCaP cells with adiponectin significantly decreased the protein expression of GSK-3β (p=0.042), and increased the protein expression of P-GSK-3β (p=0.057). Adiponectin and resistin combination treatment increased BC protein expression (p=0.044 post-hoc). Conclusion: A number of different cytokines derived from human adipocytes, including NGF, visfatin, resistin, and adiponectin modify PC cell proliferation in vitro. Exogenous treatment of PC cells with adiponectin and resistin separately and in combination does affect cell migration or invasion in vitro. However, adiponectin modulates cytosolic protein
levels of soluble BC and GSK-3β, indicating that its mechanism of action may be through the Wnt signalling pathway.

4.2.2 Introduction

PC is the second most frequently diagnosed cancer and the sixth leading cause of cancer deaths in males worldwide (Jemal, et al. 2011). Overweight and obesity have been associated with risk of death from cancer. A meta-analysis in 2008 analyzed 141 published articles and the overall consensus was that a 5-unit increase in BMI was associated with esophageal, thyroid, colon, and renal cancer in men, and endometrial, gallbladder, esophageal, and renal cancer in women (Renehan, Tyson, et al. 2008). However, the relationship between obesity and PC appears to be more complex. BMI has been shown to be associated with PC-specific mortality and risk of BCR (Cao and Ma 2011). A meta-analysis in 2012 identified 12 studies on localized PC and 13 on advanced PC and found an inverse association between BMI and localized disease (RR=0.94, 95% CI: 0.91-0.97, for every 5-unit increase in BMI) and a direct association between BMI and advanced disease (RR=1.09, 95% CI: 1.02-1.16, for every 5-unit increase in BMI) (Discacciati, Orsini and Wolk 2012).

The three primary biological mechanisms linking obesity and PC include insulin/IGF signaling, sex hormones, and adipokine signaling. Adipocytes, along with supporting stromal cells and infiltrating macrophages, secrete a variety of hormone-like biologically active polypeptides, termed adipokines (Baillargeon and Rose, Obesity, adipokines, and prostate cancer (review) 2006). The most prominent
Adipokines involved in PC etiology include adiponectin, leptin, IL-6, and VEGF. Other adipokines implicated in tumor biology include resistin, visfatin, osteopontin, MCP-1, HGF, NGF, and TNF-α.

Resistin is an adipokine that was originally proposed to link obesity with diabetes (Kim, et al. 2011). PC3 and DU145 PC cells, and high-grade PC tissue, were found to express resistin mRNA (Kim, et al. 2011). Resistin was also shown to stimulate PC cell proliferation via the Akt signaling pathway (Kim, et al. 2011).

Visfatin (pre-B cell enhancing factor / nicotinamide phosphoribosyltransferase [NAMPT]) is correlated with visceral adiposity and has been shown to be expressed in PC3 and LNCaP cells and stimulate PC cell proliferation via the ERK-1/2 and p38 signaling pathway (Patel, et al. 2010). Visfatin expression increases during prostate neoplasia (Wang, et al. 2011). Inhibition of visfatin suppressed cell growth, colony formation, and cell invasion of xenografted PC cells in mice (Wang, et al. 2011).

Lastly, the neurotrophin NGF has been implicated in PC cell growth and differentiation, exhibiting an antiproliferative effect that is progressively lost during PC progression, attributed to a decline in the expression of the NGF receptor p75NGFR (Arrighi, et al. 2010).

Adiponectin/Acrp30 is a 244-amino acid primarily produced by adipocytes (Maeda, et al. 1996) and is implicated in systemic insulin sensitivity (Pajvani, et al. 2003). In circulation, adiponectin is present as a low molecular weight (dimer or trimer) and high molecular weight complex (Pajvani, et al. 2003). Adiponectin exhibits various properties including insulin-sensitizing, anti-inflammatory, antiatherogenic, proapoptotic, and antiproliferative effects (Dalamaga, Diakopoulos
and Mantzoros 2012). Serum adiponectin levels are inversely associated with T2D, CVD, and various types of cancers, including colorectal, breast, endometrial, gastric, esophageal, pancreatic, prostate, hematological malignancies, and lung (Dalamaga, Diakopoulous and Mantzoros 2012).

A prospective study of 654 cases and 644 controls examined prediagnostic serum adiponectin with risk of incident PC, and found that adiponectin levels positively correlated with decreased risk of high grade (metastatic or lethal disease) PC, and PC-specific mortality (HR=0.39, 95% CI: 0.17-0.85) in men with BMI ≥25 (Li, et al. 2010). In a study using androgen insensitive (DU145 (Stone, et al. 1978) and PC3 (Kaighn, et al. 1979)) and androgen sensitive (LNCaP-FGC (Horoszewicz, Leong and Chu, et al. 1980)) PC cells, it was shown using a tetrazolium bromide assay that high molecular weight f- (full length) adiponectin inhibits PC cell growth at subphysiological concentrations (Bub, Miyazaki and Iwamoto 2006). Adiponectin receptors AdipoR1 and AdipoR2 are expressed in LNCaP and PC3 cells, as well as in normal prostate tissue (Mistry, Digby and Chen, et al. 2006).

The pleiotropic effects of adiponectin are mediated through a variety of cell signaling pathways. F-adiponectin constitutively activated JNK and c-Jun in DU145, PC3 and LNCaP-FGC cells (Miyazaki, et al. 2005). Non-proteolytic f-adiponectin and proteolytic g- (globular) adiponectin inhibited constitutive STAT3 activation in DU145 cells (Miyazaki, et al. 2005). Treatment of DU145 and 22Rv1 (Sramkoski, et al. 1999) cells with adiponectin increased anti-oxidative defense mechanisms and inhibited oxidative stress (Lu, et al. 2012). Furthermore, adiponectin has been shown to interact with other cytokines. Leptin and f-adiponectin in PC3 cells

Contradictory effects of adiponectin have been observed in PC cells in vitro. For instance, adiponectin has been shown to stimulate AMPK phosphorylation in LNCaP cells (which are PTEN deficient), while simultaneously activating mTOR via PI3/Akt activation (Barb, et al. 2007). These data indicate that adiponectin activation of the PI3K/Akt/mTOR pathway could in some cases stimulate tumorigenesis (Barb, et al. 2007). Furthermore, adiponectin has been reported to stimulate PC3 and DU145 migration via α5β1 integrin and AdipoR1 (Tang and Lu 2009). Adiponectin-induced migration was in part mediated by p38, AMPK, and NF-κB (Tang and Lu 2009).

In the present study, we have used various adipokines and tested their effect on different PC cell lines in vitro. Adiponectin and resistin were used alone and in combination to assess PC cell migration, invasion, and protein expression in vitro. The adipokines NGF, visfatin, resistin, and adiponectin modulated PC cell proliferation in vitro. Adiponectin and resistin alone and in combination did not affect PC cell migration or invasion in vitro. Adiponectin modulated protein expression of BC and GSK-3β, components of the Wnt signalling pathway.
4.2.3 Methods

Chemicals

Recombinant human adiponectin/Acrp30 was obtained from R&D Systems® (Cedarlane®, Burlington, ON, Canada). Recombinant human resistin was obtained from AdipoGen® (Cedarlane®, Burlington, ON, Canada). Recombinant human visfatin and recombinant human beta nerve growth factor were both obtained from Cedarlane® (Burlington, ON, Canada). Compounds were reconstituted in PBS at 50 μg/ml. Control cultures received PBS alone.

Cell Culture

Human PC cell lines LNCaP, PC3, and DU145 were obtained from the American Type Culture Collection (Rockville, MA, USA), and cultured at 37 °C in a 5% CO₂ incubator. PC3-AR2 cells are PC3 cells transfected with full-length functional AR. PC3-AR2 and LNCaP cells express functional AR, whereas PC3 and DU145 cells do not. LNCaP cells were maintained in RPMI 1640 medium (Invitrogen, Burlington, Ontario, Canada) supplemented with 10% fetal bovine serum (Sigma, St Louis, MO, USA), 0.3 mg ml⁻¹ L-glutamine and 100 IU ml⁻¹ penicillin and 100 μg ml⁻¹ streptomycin (Invitrogen, Burlington, ON, Canada). PC3 and DU145 cells were maintained in Dulbecco’s minimal essential medium/F12 (Invitrogen, Burlington, ON, Canada) with 10% fetal bovine serum, 0.3 mg ml⁻¹ L-glutamine and 100 IU ml⁻¹ penicillin and 100 μg ml⁻¹ streptomycin (Invitrogen, Burlington, ON, Canada). PC3-AR2 cells were maintained in RPMI 1640 medium supplemented with 5% fetal bovine serum, 0.3 mg ml⁻¹ L-glutamine, 100 IU ml⁻¹ penicillin and 100 μg ml⁻¹
streptomycin, Fungizone (250 μg ml⁻¹ amphotericin B and 250 μg ml⁻¹ deoxycholate) (Invitrogen, Burlington, ON, Canada) and 100 μg ml⁻¹ hygromycin B (Invitrogen, Burlington, ON, Canada).

**Cell Proliferation Assay**

LNCaP (1 × 10⁴ cells/well) and PC3 (1 × 10⁴ cells/well) cells were seeded in 96-well plates and allowed to grow in FBS (10%) for 48 h at 37 °C prior to adipokine treatment. After the cell growth period, cells were incubated with adipokine for 24, 48, or 72 h at 37 °C. After the treatment period, CellTiter96® AQueous Non-Radioactive Cell Proliferation Assay (MTS), obtained from Promega (Madison, WI, USA) was used to determine cell proliferation by adding 20 μl MTS reagent for 2 h at 37 °C. OD at 490 nm were measured by a plate reader. Treatments were added in triplicate wells and each experiment was repeated three times.

**Western Blot Analyses**

LNCaP cells were exposed to adiponectin and/or resistin (A1=1.0 μg/ml; A10=10.0 μg/ml; R=5.0μg/ml; AR=10.0μg/ml adiponectin and 5.0μg/ml resistin) for 24 h before cell lysis (NP-40 lysis buffer with inhibitors (leupeptin/pepsatin, aprotinin and phenylmethanesulfonylfluoride), SDS, deoxycholate and EDTA). Protein levels were quantified using the Bradford protein assay (Biorad, Hercules, CA, USA) before loading into gradient SDS gels for electrophoresis. Following overnight transfer, membranes were probed to assess protein expression related to 1) receptor expression levels (AR), 2) downstream proteins of AR, (including PSA), 3)
proliferation and apoptosis markers (PCNA, PARP, CC3), 4) cell cycle markers (p21, p27, CyclinD1, and 5) cell signaling molecules (Akt, P-Akt, BC, P-BC, GSK-3β, P-GSK-3β). Primary antibodies, Akt, P-Akt, BC, PBC, GSK-3β, P-GSK-3β, β-Actin, and PARP were obtained from Cell Signaling (Danvers, MA, USA). Primary antibodies, AR, PSA, CC3, PCNA, p21, p27, and cyclin D1 were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Secondary antibodies, anti-rabbit and anti-mouse were obtained from Promega (Madison, WI, USA). Secondary antibody, anti-goat, was obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Protein expression levels, relative to β-actin, were determined using image quantification software from ImageJ (NIH, Bethesda, MA, USA). Western blot experiments were repeated twice. Average protein expression levels were calculated for each group.

**Matrigel Invasion Assay**

The effect of patient serum on PC3 cell invasion was determined using BD BioCoat™ Matrigel™ Invasion Chamber 8.0 Micron, BD Biosciences, (Mississauga, ON, Canada). PC3 (1 × 10⁵ /well) cells were seeded in 12-well plates for 24 h at 37 °C into the upper chamber/insert, and adipokines at various concentrations were added to non-serum containing media into the bottom wells as a chemoattractant. After 24 h, the non-migrating cells were removed from the upper chamber with a cotton swab. The inserts were fixed with methanol and stained with 0.1% crystal violet solution. The number of migrated cells was quantified by counting the stained cells (cells per four fields) with a microscope. Treatments were done in duplicate wells and each experiment was repeated three times.
Wound-Healing (Scratch) Assay

Cell migration was assessed in PC3 cells using a wound-healing assay. PC3 cells were cultured in a 24-well plate for 24 h at 1.0 x 10⁶ cells/well to reach 100% confluence. A vertical scratch across the well was made with a 10 μl pipette tip, followed by two washes with PBS. PC3 cell media with adipokines at various concentrations were then added to each well for 24 h. Microscopy images were taken at 0 and 24 h to visually assess cell migration. Each treatment sample was added in duplicate wells, and each experiment was repeated twice.

Statistics

All analyses were conducted using SPSS version 20. Analysis of between-group variations was conducted using Student’s t testing. Analysis between more than two groups was assessed using one-way analysis of variance, with multiple comparisons corrected post-hoc with Bonferroni correction. Associations were considered significant at p ≤ 0.05.
4.2.4 Results

The Effects of Adipokines on PC Cell Proliferation.

Various adipokines, including resistin, adiponectin, NGF, and visfatin, were initially screened for their impact on PC cell proliferation *in vitro*.

**Visfatin**

**Figure 7** – Cell proliferation analysis following treatment of LNCaP and PC3 cells with visfatin.

LNCaP and PC3 cells were cultured in media supplemented with visfatin (at the above indicated concentrations) for 24 and 48 h. Cell proliferation was assessed with MTS proliferation assay. All experiments were done in triplicate. One-way ANOVA with post-hoc Bonferroni correction was used to determine differences between treatment groups.

*P*≤0.05, **P**≤0.001.
LNCaP and PC3 cells were treated with visfatin at 25, 50, 100, 200, and 400 ng/ml for 24 and 48 h (Figure 6). The effects of visfatin on PC cell proliferation were small and more pronounced at 48 h of treatment. Treatment of LNCaP cells with visfatin at 24 h failed to elicit any significant changes in cell proliferation (p=0.487). At 48 h of treatment, visfatin significantly (p<0.001) decreased LNCaP cell proliferation at a concentration of 100, 200, and 400 ng/ml (p<0.001, p<0.001, and p=0.007 respectively, with post-hoc analysis). Visfatin appeared to marginally increase PC3 cell proliferation at 24 h (p=0.011). However, the relationship failed to remain significant with post-hoc analysis (p=0.104 at 400 ng/ml). Visfatin decreased PC3 cell proliferation at 48 h at all concentrations (p<0.001 at all concentrations, post-hoc, except at 100 ng/ml p=0.004 post-hoc).
NGF

Figure 8 – Cell proliferation analysis following treatment of LNCaP, PC3, and DU145 cells with NGF.

LNCaP (for 24 h), PC3 (for 24 and 48 h), and DU145 cells (for 24 and 48 h) were cultured in media supplemented with NGF (at the above indicated concentrations). Cell proliferation was assessed with MTS proliferation assay. All experiments were done in triplicate. One-way ANOVA with post-hoc Bonferroni correction was used to determine differences between treatment groups.

*P ≤ 0.05, **P ≤ 0.001.
NGF was added to LNCaP cells for 24 h, PC3 cells for 24 and 48 h, and DU145 cells for 24 and 48 h at concentrations of 10, 25, 50, and 100 ng/ml (Figure 7).

Treatment of LNCaP cells with NGF significantly decreased cell proliferation at 24 h (p<0.001) at a concentration of 50 and 100 ng/ml (p=0.025 and p<0.001 respectively, post-hoc). NGF treatment at 100 ng/ml decreased PC3 cell proliferation at 24 h (p=0.034 post-hoc). Treatment of PC3 cells with NGF at 48 h appeared to decrease cell proliferation (p=0.003), but the highest concentration, 100 ng/ml, failed to remain significant with post-hoc analysis (p=0.07). Treatment of DU145 cells with NGF appeared to decrease cell proliferation at 24 h (p=0.028), but failed to remain significant after post-hoc analysis. Treatment of DU145 cells with NGF at 48 h did not affect cell proliferation (p=0.553).
**Resistin**

LNCaP and PC3 cells were treated with resistin for 24 and 48 h at 10, 50, 100, 200, and 500 ng/ml (Figure 8). Resistin had more pronounced effects on cell proliferation in both cell lines at 24 h than 48 h. Treatment of LNCaP cells with resistin for 24 h decreased cell proliferation at 100, 200, and 500 ng/ml (p<0.001 for all three concentrations, post-hoc). Changes in LNCaP cell proliferation at 48 h
were lost (nearly significant only with pre-hoc analysis, p=0.053). Resistin significantly decreased PC3 cell proliferation at 24 h (p<0.001 for all concentrations post-hoc, except at 10 ng/ml p=0.013 post-hoc). The effects on PC3 cell proliferation were mostly lost at 48 h, with treatment at 200 ng/ml only remaining significant (p=0.048, post-hoc) in decreasing proliferation.

**Adiponectin**

![Graphs showing the effects of adiponectin on LNCaP, PC3, and PC3-AR2 cells at 24 h and 48 h.](image-url)
LNCaP, PC3, PC3-AR2, and DU145 cells were cultured in media supplemented with adiponectin (at the above indicated concentrations) for 24 and 48 h. Cell proliferation was assessed with MTS proliferation assay. All experiments were done in triplicate. One-way ANOVA with post-hoc Bonferroni correction was used to determine differences between treatment groups. *P ≤ 0.05, **P ≤ 0.001.

LNCaP, PC3, PC3-AR2, and DU145 cells were treated with adiponectin for 24 and 48 h at 0.01, 0.1, 0.5, and 1.0 μg/ml (i.e. at subphysiological doses (Bub, Miyazaki and Iwamoto 2006)) (Figure 9). Adiponectin treatment resulted in significant decreases in cell proliferation in all four cell lines. Adiponectin treatment most significantly decreased cell proliferation in LNCaP cells (p<0.001 for all concentrations except 0.01 μg/ml, post-hoc, at 24 h). Treatment of LNCaP cells with adiponectin at 48 h had a pronounced effect in decreasing proliferation at all concentrations (p<0.001 for all concentrations post-hoc, except at 0.01 μg/ml p=0.024 post-hoc). Treatment of PC3 cells with adiponectin appeared to decrease cell proliferation (p=0.018), however no changes were significant after post-hoc analysis. Similarly at 48 h, adiponectin treatment appeared to increase PC3 cell proliferation (p=0.031), however no changes were significant after post-hoc analysis. Treatment of PC3-AR2
cells with adiponectin at 24 h decreased cell proliferation at the highest concentration 1.0 μg/ml (p=0.010 post-hoc). Treatment of PC3-AR2 cells with adiponectin at 48 h had a pronounced effect in decreasing cell proliferation at all concentrations (p<0.001 post hoc for all concentrations). Adiponectin treatment decreased DU145 cell proliferation at 24 h for 1.0 μg/ml (p<0.001 post hoc) and increased proliferation at 48 for 0.01 and 0.5 μg/ml treatments (p=0.041 and p=0.017 post hoc, respectively). Treated of LNCaP cells with adiponectin at 48 h at a physiologically relevant dose of 15.0 μg/ml decreased cell proliferation (p<0.001) (data not shown).
Adiponectin and Resistin

**Figure 11** – Cell proliferation analysis following treatment of LNCaP cells with adiponectin and resistin.

LNCaP cells were cultured in media supplemented with adiponectin and resistin separately and in combination (at the above indicated concentrations) for 24, 48, and 72 h. Cell proliferation was assessed with MTS proliferation assay. All experiments were done in triplicate. One-way ANOVA with post-hoc Bonferroni correction was used to determine differences between treatment groups.

*P≤0.05, **P≤0.001.

LNCaP cells were treated with adiponectin and resistin in combination at 500 ng/ml (resistin), 1.0 μg/ml (adiponectin), or 500 ng/ml (resistin) plus 1.0 μg/ml (adiponectin) together, for 24, 48, and 72 h with LNCaP cells (Figure 10). Resistin treatment alone appeared to have no effect on LNCaP cell proliferation; adiponectin treatment decreased cell proliferation (p<0.001 post hoc); and combination
treatment again had no significant effect on cell proliferation. Treatment of LNCaP cells with resistin alone, and adiponectin and resistin in combination at 48 h significantly decreased cell proliferation (p=0.026 and p=0.042 post hoc, respectively). However, adiponectin failed to affect LNCaP cell proliferation at 48 h (p=0.112). Treatment of LNCaP cells with resistin at 72 h had an opposite effect compared to 48 h treatment, and increased cell proliferation alone and in combination with adiponectin (p<0.001 for both resistin alone and resistin in combination, post-hoc). Adiponectin failed to effect LNCaP cell proliferation at 72 h (p=0.075 post-hoc).

**The Effects of Adiponectin and Resistin on PC3 cell Migration.**

Due to a large amount of variation along the wound border and in wound thickness, a general rating scale was devised in order to grade the microscopy images of cell migration, and arrive at an average score (between 1-5) from the duplicates and repeats (Table 9).
**Table 9 – Rating scale for cell migration.**

Each microscopy image was assigned a rating of 1-5, and the mean rating between experimental repeats and among groups was determined to assess differences in cell migration.

<table>
<thead>
<tr>
<th>Rating</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No migration (0%)</td>
</tr>
<tr>
<td>2</td>
<td>Minimal migration with few cells dispersed across wound (&lt;25%)</td>
</tr>
<tr>
<td>3</td>
<td>Cell migration filled approximately half of wound (~50%)</td>
</tr>
<tr>
<td>4</td>
<td>Wound mostly confluent with cells (&gt;75%)</td>
</tr>
<tr>
<td>5</td>
<td>Wound fully confluent with cells (~100%)</td>
</tr>
</tbody>
</table>
Table 10 – Representative images of wound healing assay.

PC3 cells were treated with adiponectin and/or resistin for 24 h, following microscopy imaging to visually assess degree of migration. All treatments were added in duplicate wells, and all experiments were repeated twice. Below are representative images across the five groups.

<table>
<thead>
<tr>
<th></th>
<th>0 h</th>
<th>24 h</th>
<th>Average Rating</th>
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<td><img src="image2" alt="Image" /></td>
<td>3.6</td>
</tr>
<tr>
<td>A1.0</td>
<td><img src="image3" alt="Image" /></td>
<td><img src="image4" alt="Image" /></td>
<td>3.5</td>
</tr>
<tr>
<td>A10.0</td>
<td><img src="image5" alt="Image" /></td>
<td><img src="image6" alt="Image" /></td>
<td>4.0</td>
</tr>
<tr>
<td>R5.0</td>
<td><img src="image7" alt="Image" /></td>
<td><img src="image8" alt="Image" /></td>
<td>2.8</td>
</tr>
<tr>
<td>AR</td>
<td><img src="image9" alt="Image" /></td>
<td><img src="image10" alt="Image" /></td>
<td>3.5</td>
</tr>
</tbody>
</table>
Figure 12 – Migration analysis of PC3 cells following 24 h treatment with adiponectin and resistin.

At 0 h a wound made with a 10 μl pipette tip was made across a 100% confluent plate of PC3 cells. Media was supplemented with adiponectin and resistin (in the above indicated concentrations) and imaged following a 24 h migration period. Experiments were done in duplicate. One-way ANOVA with post-hoc Bonferroni correction was used to determine differences between treatment groups. Significance defined at P≤0.05.

PC3 cells were treated with adiponectin and resistin alone and in combination for 24 h to assess their individual and combined effects on cell migration (Figure 11). There were no significant differences in terms of average migration score among the four treatment groups compared to control (i.e. adiponectin at 1.0 and 10.0 μg/ml, resistin at 5.0 μg/ml, and adiponectin 10.0 and resistin 5.0 μg/ml combination) (p=0.063) (Table 10). Resistin treatment alone at 5.0 μg/ml appeared to decrease PC3 migration, however the effect was not significant (p=0.393 post-hoc).
The Effects of Adiponectin and Resistin on PC3 Cell Invasion.

Table 11 – Representative images of invasion assay with PC3 cells for 24 h.

PC3 cells were treated with adiponectin and/or resistin for 24 h, following microscopy imaging to count number of invaded cells. All treatments were added to duplicate wells, and all experiments were repeated twice. Below are representative images across the five groups.

<table>
<thead>
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<th>Average number of invaded cells</th>
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<tr>
<td>AR</td>
<td>![Image]</td>
<td>59.3</td>
</tr>
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</table>
Figure 13 – Invasion analysis of PC3 cells following 24 h treatment with adiponectin and resistin.

PC3 cells were allowed to undergo invasion for 24 h in media supplemented with adiponectin and resistin (in the above indicated concentrations) and imaged following a 24 h invasion period. Experiments were done in duplicate. One-way ANOVA with post-hoc Bonferroni correction was used to determine differences between treatment groups. Significance defined at P≤0.05.

Representative microscopy images of the invaded cells following a 24 h period of PC3 cell invasion in vitro are shown in Table 11. No significant differences were found in terms of average number of invaded PC3 cells after 24 h among the different adiponectin and resistin treatment groups compared to control (i.e. adiponectin at 1.0 and 10.0 μg/ml, resistin at 5.0 μg/ml, and adiponectin 10.0 and resistin 5.0 μg/ml combination) (p=0.196) (Figure 12). Adiponectin and resistin treatments alone appeared to increase PC3 invasion, however due to large variability no differences were significant.
Effect of Adiponectin using LNCaP cells on Expression of Protein Markers

Western blot analysis was used to assess the changes in expression of various proteins with adiponectin treatment of LNCaP cells for 24 h at 1.0 and 10.0 μg/ml.

![Western blot analysis of AR expression](image1)

**Figure 14** – AR expression from adiponectin treatment on LNCaP cells for 24 h

Western blot analysis of AR protein expression following adiponectin treatment (at 1.0 and 10.0 μg/ml) for 24 h. Bottom figure represents densitometry analysis of AR standardized against β-Actin as percent change from control (set as 100%). (Graph results represent average between experimental repeats).

![Western blot analysis of PSA expression](image2)

**Figure 15** – PSA expression from adiponectin treatment on LNCaP cells for 24 h

Western blot analysis of PSA protein expression following adiponectin treatment (at 1.0 and 10.0 μg/ml) for 24 h. Bottom figure represents densitometry analysis of PSA standardized against β-Actin as percent change from control (set as 100%). (Graph results represent average between experimental repeats).

There were borderline significant differences in the protein expression of AR among the treatment groups (1.0 and 10.0 μg/ml) (p=0.055), which however no longer remained significant with post-hoc analysis (p=0.073 and p=0.496 for 1.0 and 10.0...
μg/ml respectively, post-hoc) (Figure 13). Adiponectin did not significantly affect the expression of PSA protein levels in LNCaP cells (p=0.134) (Figure 14).

**Figure 16** – P21 expression from adiponectin treatment on LNCaP cells for 24 h

Western blot analysis of p21 protein expression following adiponectin treatment (at 1.0 and 10.0 μg/ml) for 24 h. Bottom figure represents densitometry analysis of p21 standardized against β-Actin as percent change from control (set as 100%). (Graph results represent single experimental repeat).

**Figure 17** – P27 expression from adiponectin treatment on LNCaP cells for 24 h

Western blot analysis of p27 protein expression following adiponectin treatment (at 1.0 and 10.0 μg/ml) for 24 h. Bottom figure represents densitometry analysis of p27 standardized against β-Actin as percent change from control (set as 100%). (Graph results represent single experimental repeat).

Since only one experimental repeat was done for both p21 and p27 western blots, statistical analyses were unable to be conducted. There appeared to be a two-fold increase in p21 protein expression with adiponectin treatment of LNCaP cells, especially at 1.0 μg/ml (mean change = 200% and 179% for 1.0 and 10.0 μg/ml adiponectin respectively) (Figure 15). P27 protein expression levels following
adiponectin treatment did not change (mean change = 96% for both 1.0 and 10.0 μg/ml adiponectin) (Figure 16).

Figure 18 – PCNA expression from adiponectin treatment on LNCaP cells for 24 h

Western blot analysis of PCNA protein expression following adiponectin treatment (at 1.0 and 10.0 μg/ml) for 24 h. Bottom figure represents densitometry analysis of PCNA standardized against β-Actin as percent change from control (set as 100%). (Graph results represent average between experimental repeats).

Adiponectin did not significantly affect the expression of PCNA protein levels in LNCaP cells (p=0.820) (Figure 17).
Adiponectin did not significantly affect the expression of BC or P-BC protein levels in LNCaP cells (p=0.182 and p=0.107 respectively) (Figure 18 and 19).
Adiponectin treatment of LNCaP cells significantly decreased the protein expression of GSK-3β at 1.0 and 10.0 µg/ml (mean change = 94% and 87% respectively, p=0.032) (Figure 20). The relationship only remained significant for 10.0 µg/ml adiponectin treatment with post-hoc analysis (p=0.042). Adiponectin treatment of LNCaP cells significantly increased the protein expression of P-GSK-3β at 1.0 and 10.0 µg/ml (mean change = 125% and 127% respectively, p=0.032) (Figure 21).
The relationship failed to remain significant for 10.0 μg/ml adiponectin treatment with post-hoc analysis (p=0.057).

**Effect of Adiponectin and Resistin using LNCaP cells on the Expression of Protein Markers**

Results from the first set of Western blot analyses led to a second set of experiments where the expression of various proteins were analyzed following adiponectin and resistin treatments alone and in combination for 24 h in LNCaP cells (1.0 and 10.0 μg/ml adiponectin, 5.0 μg/ml resistin, and 10.0 μg/ml adiponectin with 5.0 μg/ml resistin in combination).
There appeared to be increases in the protein expression of both AR and PSA with the various treatment groups, however, due to large variability, no relationships were significant (p=0.355 and p=0.708 respectively) (Figure 22 and 23).
Western blot analysis of PCNA protein expression following adiponectin and resistin treatment (at 1.0 and 10.0 μg/ml adiponectin, 5.0 μg/ml resistin, and 10.0 μg/ml adiponectin and 5.0 μg/ml resistin together) for 24 h. Bottom figure represents densitometry analysis of PCNA standardized against β-Actin as percent change from control (set as 100%). (Graph results represent average between experimental repeats).

Neither PCNA nor PARP protein levels significantly changed with the various treatment groups in LNCaP cells (p=0.751 and p=0.617 respectively) (Figure 24 and 25).
Western blot analysis of p21 protein expression following adiponectin and resistin treatment (at 1.0 and 10.0 μg/ml adiponectin, 5.0 μg/ml resistin, and 10.0 μg/ml adiponectin and 5.0 μg/ml resistin together) for 24 h. Bottom figure represents densitometry analysis of p21 standardized against β-Actin as percent change from control (set as 100%). (Graph results represent average between experimental repeats).

P21 protein expression appeared to increase two-fold with 10.0 μg/ml adiponectin treatment (mean change = 216%) and nearly three-fold with combination treatment (mean change = 285%) (Figure 26). However, due to large variation, the relationship merely approached significance (p=0.072). P27 protein expression did not change with the various treatment groups (p=0.924) (Figure 27).
Western blot analysis of cyclin D1 protein expression following adiponectin and resistin treatment (at 1.0 and 10.0 μg/ml adiponectin, 5.0 μg/ml resistin, and 10.0 μg/ml adiponectin and 5.0 μg/ml resistin together) for 24 h. Bottom figure represents densitometry analysis of cyclin D1 standardized against β-Actin as percent change from control (set as 100%). (Graph results represent average between experimental repeats).

Cyclin D1 protein expression did not change with the various treatment groups (p=0.640) (Figure 28).
**Figure 30** – Akt expression from adiponectin and/or resistin treatment on LNCaP cells for 24 h

Western blot analysis of Akt protein expression following adiponectin and resistin treatment (at 1.0 and 10.0 μg/ml adiponectin, 5.0 μg/ml resistin, and 10.0 μg/ml adiponectin and 5.0 μg/ml resistin together) for 24 h. Bottom figure represents densitometry analysis of Akt standardized against β-Actin as percent change from control (set as 100%). (Graph results represent average between experimental repeats).

Due to large variability, neither Akt nor P-Akt protein expression changed with the various treatment groups (p=0.464 and p=0.598 respectively) (**Figure 29 and 30**).

**Figure 31** – P-Akt expression from adiponectin and/or resistin treatment on LNCaP cells for 24 h

Western blot analysis of P-Akt protein expression following adiponectin and resistin treatment (at 1.0 and 10.0 μg/ml adiponectin, 5.0 μg/ml resistin, and 10.0 μg/ml adiponectin and 5.0 μg/ml resistin together) for 24 h. Bottom figure represents densitometry analysis of P-Akt standardized against β-Actin as percent change from control (set as 100%). (Graph results represent average between experimental repeats).
Western blot analysis of BC protein expression following adiponectin and resistin treatment (at 1.0 and 10.0 μg/ml adiponectin, 5.0 μg/ml resistin, and 10.0 μg/ml adiponectin and 5.0 μg/ml resistin together) for 24 h. Bottom figure represents densitometry analysis of BC standardized against β-Actin as percent change from control (set as 100%). (Graph results represent average between experimental repeats). *P≤0.05.

BC protein expression significantly increased with the various treatment groups in LNCaP cells (p=0.011) (Figure 31). Adiponectin and resistin combination treatment increased BC protein expression by 139% (p=0.044 post-hoc). Due to large variability, P-BC protein expression levels did not change with the various treatment groups (p=0.590) (Figure 32).
There appeared to be an increase in GSK-3β protein expression with combination treatment (mean change = 147%) (However, due to large variability the relationship was not significant, p=0.363) (Figure 33). Since only one experimental repeat was done for P-GSK-3β, statistical analysis was unable to be conducted. Resistin at 5.0 μg/ml treatment, and adiponectin and resistin combination treatment both
increased P-GSK-3β protein expression nearly twofold (mean change = 196% and 190% respectively) (Figure 34).

There was no CC3 protein expression with any of the adiponectin and/or resistin treatment groups (data not shown).

4.2.5 Discussion

Obesity has been associated with high-grade PC, BCR, and PC-specific mortality. One prominent mechanism whereby obesity may affect PC progression is through dysfunctional adipokine signaling. In the present study, various adipokines were tested for their effects on PC cell proliferation in vitro. Visfatin, NGF, and resistin decreased LNCaP and PC3 cell proliferation, while adiponectin decreased LNCaP and PC3-AR2, and increased DU145 cell proliferation in vitro (Figure 6, 7, 8, and 9). Resistin appeared to counteract the anti-proliferative effect of adiponectin with LNCaP cells (Figure 10). Adiponectin and resistin separately and in combination had no significant effect on PC3 cell migration or invasion (Figure 11 and 12). It was found that adiponectin decreased GSK-3β, and increased P-GSK-3β protein expression in LNCaP cells (Figure 20 and 21). Lastly, adiponectin and resistin in combination increased BC protein expression in LNCaP cells (Figure 31).

The present data regarding NGF, visfatin, and resistin and PC cell proliferation is not in accordance with previous findings. NGF has in fact been reported to increase LNCaP cell proliferation via the high-affinity NGF receptor, p140-TrkA (Sortino, et al. 2000). NGF was shown to increase the growth rate of LNCaP, DU145, and PC3 cells (Angelsen, et al. 1998). Similarly, visfatin was shown to
increase PC3 cell proliferation via MAPKs ERK-1/2 and p38 (Patel, et al. 2010).

Inhibition of visfatin with FK866 suppressed LNCaP cell proliferation (Wang, et al. 2011). Lastly, resistin was also shown to stimulate PC3 and DU145 cell proliferation via PI3K/Akt (Kim, et al. 2011).

The lack of harmony between the present study’s findings and previous studies may be due to variation in several factors, including the source and concentration of the adipokine, cell density, use of FBS or serum-free media, time to cell attachment and time to treatment, length of treatment, passage number of cell line used, and type of proliferation assay incorporated. We are confident of our results since all experiments were repeated three times, in triplicate wells per 96-well plate, with two or more time points, and using several cell lines per adipokine.

The present study’s results regarding adiponectin were more in harmony with previous findings. Adiponectin has been shown to inhibit LNCaP-FGC, DU145, and PC3 cell proliferation at subphysiological doses (Bub, Miyazaki and Iwamoto 2006). Contrary to the present study, which found that adiponectin had no effect on PC cell migration, previous data has shown that adiponectin increased PC3 and DU145 cell migration (via α5β1 integrin expression) (Tang and Lu 2009).

Several reports have indicated that adiponectin may interact with other adipokines either potentiating or attenuating its effects on PC cells. For instance, adiponectin in combination with leptin significantly decreased PC3 cell proliferation (Mistry, Digby and Desai, et al. 2008). Moreover, adiponectin negated leptin-induced decreases in p53 expression in PC3 cells (Mistry, Digby and Desai, et al. 2008). Adiponectin may also interact with resistin. Adiponectin was shown to decrease
resistin-induced increases in vascular smooth muscle cell proliferation and MAPK phosphorylation (Hirai, et al. 2013). In the present study, we found that adiponectin alone decreased PC cell proliferation, and adiponectin in combination with resistin had no effect on PC cell proliferation in vitro (p<0.001).

Dysregulation of Wnt signaling can lead to the development of several types of cancer, including PC (Krypta and Waxman 2012). One hallmark of the Wnt signaling pathway is stabilization of the transcription factor BC (Krypta and Waxman 2012). No reports have investigated the action of adiponectin and its effects on Wnt signaling in PC biology. However, one report demonstrated that adiponectin blocked GSK-3β phosphorylation (part of the destruction complex that hyperphosphorylates BC, tagging it for degradation) and suppressed intracellular accumulation of BC (effectively diminishing Wnt signalling) in MDA-MB-231 breast cancer cells (Wang, Lam, et al. 2006). Unexpectedly, the present study found that adiponectin treatment of LNCaP cells significantly decreased the protein expression of GSK-3β (p=0.042), and increased the protein expression of P-GSK-3β (p=0.057). A combination of adiponectin and resistin resulted in increased expression of BC protein (p=0.044 post-hoc). Activation of the Wnt signaling pathway would be expected to have potentiated cell proliferation, but the present data indicated that adiponectin decreased LNCaP cell proliferation. Further mechanistic studies need to be conducted in order to accurately elucidate whether adiponectin and/or resistin are signaling through Wnt proteins in mediating their effects on PC cell proliferation in vitro.
The present study contains several strengths and limitations. The screened adipokines were analyzed in a variety of contexts, with various cell lines at different time points. A variety of cellular effects were assessed following adiponectin and/or resistin treatment, including proliferation, migration, invasion, and protein expression. Furthermore, a variety of proteins were assessed for changes in expression levels following adiponectin and/or resistin treatment. However, the study could have been strengthened if further experiments were carried out that included different measures for cell growth (including clonogenic assays), gene expression analysis (i.e. RT-PCR, coupled with protein expression), and overexpression of the adipokines using siRNA (in contrast to exogenous treatment). Interactions between adiponectin and the other adipokines screened, including NGF or visfatin would also have been informative. Velocity sedimentation analysis has shown that the active form of adiponectin in circulation affecting PC cell proliferation is the high molecular weight form (Bub, Miyazaki and Iwamoto 2006). The present experiments did not separate the different oligomeric forms of adiponectin. In vitro AdipoR1 and AdipoR2 knockout models would have been helpful to assess whether adiponectin signaled through its own receptors in its modulation of the Wnt signaling pathway. Lastly, a heterochthonous in vivo xenograft model could have been used to analyze the effects of adiponectin and/or resistin overexpression on tumor progression and outcomes.

In conclusion, adipokines NGF, visfatin, resistin, and adiponectin modulate PC cell proliferation in vitro. Resistin reverses the antiproliferative effects of adiponectin in LNCaP cells. Adiponectin and resistin separately and in combination
have no significant effect on PC cell migration or invasion. Adiponectin may affect PC cells partly through components of the Wnt signaling pathway, BC, GSK-3β and P-GSK-3β. Further investigations should be conducted to explore the effect of adiponectin and resistin on Wnt signalling in the context of PC biology.
CHAPTER FIVE

5. Conclusion and Future Directions

Obesity has been associated with a variety of health complications, including CVD, T2D, and cancer. Dysfunctional adiposity poses particular problems for those cancer sites that lie in close proximity to fat pads, including PC, where the prostate is surrounded by periprostatic adipose tissue. The accumulation of visceral adipose depots promotes communication between dysfunctional adipocytes and cancer cells via paracrine and endocrine signals, potentiating tumor development and progression. Obesity may affect PC biology through biological mechanisms involving depressed T levels, elevated estrogen levels, dysfunctional IGF/insulin signaling, and dysfunctional adipokine signaling.

The present research, included in chapter three and four, examined the association between endocrine-related signaling molecules, specifically adipokines, and in vitro PC cell proliferation, migration, invasion, and cell signaling. Overall, the results from these two studies support the notion that adipokine signalling explains in part how obesity can negatively or positively impact PC progression.

In chapter three, it was concluded that serum from obese PC patients, compared to non-obese PC patients, induces higher amounts of PC cell migration, and lower amounts of PC cell proliferation and invasion in vitro. Serum from obese PC patients has aberrant levels of HGF, TNF-α, and resistin indicating that obesity affects PC progression through serum-born signaling molecules. These results only
partially confirmed the original hypothesis, which stated that obese PC patient serum would do the opposite and increase PC cell proliferation and invasion in vitro.

In chapter four, it was concluded that human-derived adipokines NGF, resistin, visfatin, and adiponectin, modulate PC cell proliferation in androgen-sensitive and insensitive cell lines in vitro. Resistin counteracts the antiproliferative effect of adiponectin in an androgen-sensitive cell line in vitro. Additionally, adiponectin may affect PC cells partly through components of the Wnt signaling pathway, including BC, GSK-3β and P-GSK-3β. Similar to chapter three, these results only partially confirmed the original hypothesis, which stated that resistin would ameliorate the effects of adiponectin on PC cell migration and invasion in vitro. However, neither adiponectin nor resistin separately or in combination had any significant effect on PC cell migration or invasion in vitro.

There are many avenues that future research can take to explore adipokine signalling in the obesity-PC relationship. The clinical study in chapter three, which used patient serum to treat PC cells in vitro, needs to be conducted with a larger number of patients to increase the power to detect subtle changes in adipokine serum levels between obese and non-obese PC patients. A larger panel of adipokines and cytokines needs to be screened for, including leptin, VEGF, osteopontin, and adiponectin, in addition to markers for glucose (e.g. glycosylated haemoglobin). Together, glucose and insulin promote cell proliferation, and thus need to be controlled for in patient serum. Obesity is a metabolic disorder that involves dysregulation of energy metabolism, and several of the adipokines under consideration are associated with glucose regulation and fatty acid metabolism.
(Gharibeh, et al. 2010, Pajvani, et al. 2003). A mediating factor between obesity and
PC appears to be dysregulated de novo fatty acid synthesis (Nguyen, et al. 2010),
and thus future studies could explore the role of adipokines in mediating this effect.

The present data shows that HGF serum levels are significantly elevated in
obese and PC patient serum. HGF has been shown to promote PC cell migration and
invasion (Dai and Siemann 2010). Body adiposity, rather than hyperinsulinemia,
mediates plasma HGF levels in humans (de Courten, et al. 2013). Therefore, future
studies could examine the interaction between HGF and other adipokines that are
directly associated with body adiposity, such as adiponectin and leptin. HGF’s effects
on PC cell migration or invasion may be promoted or inhibited by other adipokines.

Only two cell lines, LNCaP and PC3, were used in assessing PC cell
proliferation, migration, and invasion in chapter three. LNCaP and PC3 cells are
derived from lymph node and bone metastatic lesions respectively, and therefore
only represent a particular subset of PC cells (Sobel and Sadar 2005). Other cell lines
could be used in future research, such as 22Rv1, DU-145, DuCaP, VCaP, LAPC-4, etc.
The impact of AR status in mediating obese PC patient’s serum on PC cell
characteristics could also be assessed comparing PC3 and PC3-AR2 cells.

The wound-healing assay used in chapter three and four failed to produce
specific and consistent results due to large amounts of variation along the wound
border. Future studies could utilize alternative methods for measuring cell
migration distance. Additionally, other assays that offer advantages over the scratch
assay could be used to measure cell migration, such as Electric Cell-substrate
Impedance Sensing, Boyden Chamber, and Microfluidics-based system (Liang, Park and Guan 2007).

Many of the adipokines are modulated by circadian rhythms, and rise or fall in post-prandial or hypoglycaemic states. Therefore, this research would benefit from a prospective study that controls for the time of day and fasting state during blood draw. Furthermore, BMI is a crude marker for body adiposity, and fails to appreciate the ratio of subcutaneous to visceral adipose tissue, which plays an important role in the pathophysiology of obesity-related diseases. Thus, future research needs to measure WHR, waist circumference, and/or periprostatic fat density/width.

In chapter four, the results from the in vitro study on adipokines present promising data regarding the role for adipokines (NGF, visfatin, resistin, and adiponectin) in either attenuating or potentiating PC progression. Though definitive results could not be obtained, our data indicates that adiponectin may affect p21 protein expression in LNCaP cells. The expression of p21 has been shown to predict biochemical failure in patients with locally advanced PC treated with RP (Lacombe, et al. 2001). P21 also has a cell cycle regulatory function (Dash and El-Deiry 2005). Future studies could explore the impact of adiponectin and resistin on cell cycle progression using flow cytometry and analysis of cell cycle markers, with particular attention on p21 signalling.

The oral biguanide, antidiabetic drug metformin has been shown to improve ADT-induced metabolic syndrome and exhibit antineoplastic activity in PC (Clements, et al. 2011). A recent study reported that adiponectin and metformin
alone or in combination antagonize IL-1β-regulated malignant potential in human and mouse colon cancer cell lines (Moon and Mantzoros 2013). Future studies should explore the interaction between adipokines such as adiponectin and/or resistin with metformin in influencing PC progression.

It has previously been shown that T and its metabolites suppress adipocyte production of adiponectin (Page, et al. 2005), though not all data support this conclusion (Horenburg, et al. 2008). Furthermore, the Wnt signalling pathway plays a critical role in androgen-mediated transcription and PC cell growth (Verras, et al. 2004). Future studies could explore the interactions between T and adipokines, such as adiponectin and resistin, in the context of Wnt signalling.

Moreover, further analyses need to explore the gene expression of the proteins involved in adiponectin and/or resistin signalling. There are different bioactive forms of adiponectin that should be separated in future analyses, including full-length and globular, low-, medium-, and high-molecular weight adiponectin, all of which have been shown to exhibit different biological activities. Furthermore, in vivo experiments are lacking in the published literature, exploring adiponectin and resistin signalling in relation to PC biology. Future studies should utilize xenograft models with altered expression of, or treated with particular adipokines to assess impact on PC progression and outcomes.
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