Glucose Metabolism in High-Risk Prostate Cancer Patients throughout the Acute Disease Trajectory

by

Kathleen M. Di Sebastiano

A thesis
presented to the University of Waterloo
in fulfillment of the
thesis requirement for the degree of
Doctor of Philosophy
in
Kinesiology

Waterloo, Ontario, Canada, 2016

AUTHOR'S DECLARATION

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Abstract

Background: Hyperglycemia and insulin resistance are associated with obesity, physical inactivity, and poor diet, and may be related to hyperinsulinemia, disrupted lipid and adipokine signaling, and pro-inflammation. The independent and/or combination of these metabolic perturbations may increase prostate cancer risk, exacerbate during acute treatment, and worsen in survivorship. The interactions between prostate cancer, treatment, dysregulation of glucose metabolism, and the associated metabolic sequelae are complex and should be evaluated in an integrative manner to discern these relationships. The purpose of this thesis is to employ an integrative approach to examine glucose metabolism and related features at diagnosis and during the acute treatment trajectory (up to the first 6 months of treatment) of prostate cancer patients. **Objectives:** The objectives of this thesis were to: 1) characterize glucose and associated parameters (insulin, C-peptide, IGF-1, IGFBP-3) in relation to lipid measures (triglycerides, cholesterols), adipokines (adiponectin, leptin), body composition, physical activity, diet, and inflammation (CRP, IL-1β, IL-4. IL-6, IL-8, IL-10, TNF-α) prior to prostate cancer treatment, 2) examine potential changes in this comprehensive metabolic profile during the acute prostate cancer treatment trajectory (~6 months following radiation therapy), and 3) develop a cell culture model to examine the cellular mechanisms contributing to potential alterations in glucose metabolism observed during the treatment trajectory. **Methods:** In Study 1, fasting glucose, insulin, C-peptide, IGF-1, IGFBP-3, cholesterol, triglycerides, leptin, adiponectin, CRP were measured along with traditional moderators glucose metabolism (body composition, nutrition intake, habitual physical activity) to characterize interrelated metabolic features in men undergoing prostate biopsy. High-risk prostate cancer patients demonstrate the greatest C-peptide and leptin concentrations, as well as the greatest visceral adiposity in Study 1. In Study 2, more comprehensive evaluations, including an oral glucose tolerance test (OGTT), were prospectively performed on a separate group of high-risk prostate cancer patients prior to radiation therapy (baseline), as well as 7

weeks and 33 weeks following baseline. Patients were compared to a group of young healthy males and a group of age- and BMI-matched males to assess age- and cancer-related differences in glucose metabolism. To understand the underlying mechanisms of poor metabolic outcomes in high-risk prostate cancer patients, a novel cell culture model was developed in Study 3 where human skeletal muscle myoblasts (HSkMM) were incubated with human serum from prostate cancer patients across the acute treatment trajectory to examine glucose uptake. **Results:** At the time of the prostate biopsy (or time of diagnosis for the prostate cancer patients) in Study 1, prostate cancer patients diagnosed with aggressive cancer (Gleason $\geq$4+3) demonstrated greater insulin secretion assessed by C-peptide concentrations compared with patients that had less aggressive cancers (Gleason $\geq$4+3: 2.8$\pm$1.1 ng/mL vs Gleason 3+3 1.4$\pm$0.6 mg/mL vs Gleason 3+4: 1.3$\pm$0.8 mg/mL; p=0.002). Insulin concentrations and HOMA-IR tended to be greater in patients with the most aggressive cancer compared to the others. Greater insulin secretion, measured by C-peptide concentrations, was related to greater visceral adiposity, higher leptin and leptin:adiponectin ratio, suggesting these metabolic perturbations may relate to obesity. In Study 2, high-risk prostate cancer patients demonstrated impaired glucose tolerance by 2 hours of an OGTT prior to treatment (Normal: <7.8mM, Patients: 8.62$\pm$2.87mM), which improved at 7 weeks post-baseline (6.78$\pm$1.48 mM) and was maintained 33 weeks following baseline (5.69$\pm$2.14mM, p=0.007). Insulin, C-peptide, IGF-1, IGFBP-3, adipokines, triglycerides, cholesterols, inflammatory markers, physical activity, and nutrition intake did not change during the study trajectory. In Study 3, we evaluated the effects of serum from prostate cancer patients and matched males on differentiated HSkMM, which showed significantly reduced glucose uptake compared with young males; however there were no differences between prostate cancer patients and matched males, suggesting an age-related decrease in glucose uptake. **Conclusions:** At diagnosis, prostate cancer patients demonstrated a cluster of glucose-related metabolic perturbations that may contribute to prostate cancer aggressiveness. Significant improvements in glucose

metabolism were observed during acute treatment, despite the lack of change in traditional moderators of glucose metabolism, suggesting cancer-related effects on host glucose metabolism. New models are needed to elucidate the mechanisms driving these metabolic changes and to, ultimately, reduce the risk of cancer recurrence, diabetes, and cardiovascular disease in prostate cancer survivors.

Acknowledgements

I would first like to thank my supervisor, Dr. Marina Mourtzakis, for her support and guidance over the course of my PhD. I could not have asked for a better mentor, colleague, and friend during my degree. I truly appreciate everything you have done for me.

Secondly, I would like to thank my advisory committee, Dr. Joe Quadrilatero, Ken Stark, and Jehonathan Pinthus, for their guidance and advice in conducting my research. I would also like to thank Drs. Paul Spagnuolo and Stephanie Chevalier for serving as members of my examination committee.

There are numerous people who out whom this research could not be conducted. Specifically I would like to thank Marg Burnett, Jing Ouyang, and Dr. Eric Bombardier for their technical expertise as well as Dr. Joel Dubin for his statistical advice. Dr. Helga Duivenvoorden, Laurel Patterson and the staff at the Juravinski Cancer Centre deserve special recognition for their help with Study 1 of this thesis as well as Drs. Dilip Panjwani, Pierre Fortin, Ramana Rachakonda, and the Clinical Trials Staff at the Grand River Regional Cancer Centre for their assistance in recruiting the participants for Study 2. I would like to acknowledge the members of the Muscle Biology and Cell Death Laboratory at the University of Waterloo for their technical instruction, specifically Elliott McMillian and Andrew Mitchell, as well as all of the other members of the Physiology stream for their support over the years. I would also like to thank the past and present members of the Nutrition and Metabolism Laboratory for all of their support over the years. I would especially like to thank everyone who participated my research studies. Without you this research would not have be possible!

I would like to acknowledge the funding sources of this research including the Canadian Institutes of Health Research, Natural Sciences and Engineering Research Council of Canada, Canadian Foundation for Innovation, and the Motorcycle Ride for Dad Foundation, as well as the Ontario Graduate Scholarship Program and Prostate Cancer Canada for my personal funding.

Finally, I would like to thank all of family and friends for their love and support over course of this degree. In particular my parents, Mary Anne and Andy Di Sebastiano, without whom none of this would be possible. Also, I would like to thank my brother-in-law Mike and especially my sister Andrea, who inspired me to become a scientist in the first place. Thank you!

Table of Contents

viii

List of Figures

List of Tables

List of Abbreviations

1-RM – 1 Repetition Maximum

6MWT – 6 Minute Walk Test

ADT – Androgen Deprivation Therapy

AHA – American Heart Association

Akt/PKB –Protein Kinase B

AMPK – 5' Adenosine Monophosphate-activated Protein Kinase

ANOVA – Analysis of Variance

AR – Androgen Receptor

AU – Arbitrary Units

AUA – American Urology Association

AUC – Area Under the Curve

BAD – Bcl-2-associated Death Promotor

Bcl – B-cell Lymphoma

BMI – Body Mass Index

BPH – Benign Prostate Hyperplasia

CB – Cytochalasin B

CI – Confidence Interval

CRP – C-reactive Protein

CT – Computed Tomography

CUA – Canadian Urological Association

CVD – Cardiovascular Disease

DBP – Diastolic Blood Pressure

DHT – Dihydrotestosterone

DM – Differentiation Media

DMEM - Dulbecco's Modified Eagle Medium

DPBS - Dulbecco's Phosphate-Buffered Saline

DRE – Digital Rectal Exam

EAU – European Association of Urology

EGIR – European Group for the Study of Insulin Resistance

ERK – Extracellular Signal-regulated Kinase

ES – Effect Size

ESHA – Elizabeth Stewart Hands and Associates

FBS – Fetal Bovine Serum

FFM – Fat Free Mass

FM – Fat Mass

FOXO – Forkhead Box O

FSH – Follicle Stimulating Hormone

GLUT – Glucose Transporter Type

HbA1c – Glycated Hemoglobin

HBS – HEPES Buffered Saline

HC – Hip Circumference

HDL – High-density Lipoprotein

HEPES - 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

HOMA-IR – Homeostatic Model of Insulin Resistance

HR – Hazard Ratio

HS – Horse Serum

HSkMM – Human Skeletal Muscle Myoblasts

IDF – International Diabetes Federation

IGF-1 – Insulin-like Growth Factor - 1

IGFBP-3 - Insulin-like Growth Factor Binding Protein -3

IKKβ – IκB Kinase β

IL - Interleukin

IRS-1 – Insulin Receptor Substrate -1

JNK – c-Jun N-terminal Kinase

LDL – Low-density Lipoprotein

LH – Luteinizing Hormone

LHRH - Luteinizing Hormone Releasing Hormone

MAPK – Mitogen-activated Protein Kinase

MM – Matched Males

MR – Magnetic Resonance

MRI – Magnetic Resonance Imaging

mTOR – Mechanistic Target of Rapamycin

NC – No Cancer

NCEP-ATP III - National Cholesterol Education Program Adult Treatment Panel III

NEFA - Non-esterified Free Fatty Acids

NF-κB – Nuclear Factor - κB

OGTT – Oral Glucose Tolerance Test

OR – Odds Ratio

PI3K – Phosphoinositide 3-kinase

PPAR-α – Peroxisome Proliferator-activated Receptor-α

PSA – Prostate Specific Antigen

QUICKI – Quantitative Insulin Sensitivity Check Index

RIA – Radioimmunoassay

RR – Relative Risk

RT – Radiation Therapy

SBP – Systolic Blood Pressure

SD – Standard Deviation

SEARCH – Shared Equal Access Regional Cancer Hospital

SF-BIA – Single Frequency – Bioelectrical Impedance Analysis

SHBG – Sex Hormone-binding Globulin

SKGM – Skeletal Muscle Growth Media

SMI – Skeletal Muscle Index

SMM – Skeletal Muscle Mass

STAT – Signal Transducer and Activator of Transcription

TG - Triglycerides

TNF-α – Tumour Necrosis Factor-α

TNM – Tumour, Node, Metastasis Staging

US – United States of America

USDA – United State Department of Agriculture

VLDL – Very Low-denisty Lipoprotein

VO_{2peak} – Maximal Oxygen Consumption

WC – Waist Circumference

WHO – World Health Organization

YM – Young Males

Chapter 1
Statement of the Problem

1.1 Introduction

Prostate cancer is the most common form of cancer in Canadian men, with 1 in 7 men being diagnosed in their lifetime (1). Despite the prevalence of prostate cancer, the 5-year survival rate is exceptionally high at 96% (2), especially when it is compared to the survival rates for other types of cancer, such as pancreatic and lung cancer where the 5-year survival rates are 8% (2) and 14% in males, respectively (2). However, prostate cancer patients are typically at increased risk for metabolic diseases, such as metabolic syndrome (3-5), cardiovascular disease (5-8), and diabetes (5-8), in survivorship likely attributed to treatment. Increased risk of these metabolic diseases as well as increased risk of prostate cancer development is typically associated with obesity (9-11) and hyperinsulinemia (12, 13), commonly resulting from high fat diets (14, 15) and physical inactivity (16, 17). Little is known about the interrelationship between these metabolic and lifestyle perturbations and prostate cancer development, as well as the changes in these relationships during treatment. From these data, two distinct research questions emerge:

A) What metabolic factors associate with the development of prostate cancer?

B) How do metabolic factors change during prostate cancer treatment and contribute to quality of life in survivorship?

1.1.1 What metabolic factors associate with the development of prostate cancer?

A number of non-modifiable risk factors have been associated with the diagnosis of prostate cancer; these include age, family history and ethnicity (18-20). There are also a number of modifiable risk factors that have been associated with developing prostate cancer including, diet, physical inactivity, obesity and smoking. Most of the literature that relates these modifiable lifestyle factors, such as obesity, to prostate cancer is inconclusive. Some studies demonstrate positive

associations between prostate cancer and lifestyle factors, while others demonstrate no association (21-23). However, there is more conclusive evidence to indicate that these lifestyle factors, may contribute to the increased risk of aggressive prostate cancer and prostate cancer mortality (24). Comparing metabolic and lifestyle features in prostate cancer patients with non-malignant individuals may advance our understanding of potential factors that associate with prostate cancer diagnosis.

1.1.2 How do Lifestyle and Metabolic Factors Influence Prostate Cancer Treatment and contribute to Quality of Life in Survivorship?

Increased prevalence of prostate cancer combined with a 96% 5-year survival rate results in a continually increasing number of prostate cancer survivors (2). There are several definitions for the term *cancer survivor*; for the purpose of this thesis, *cancer survivor* will be defined as an individual who has completed primary treatment, focusing on post-treatment. A large body of literature suggests that prostate cancer patients are at increased risk of cardiovascular disease and diabetes in survivorship (25-29). This relationship is primarily described in patients who undergo androgen deprivation therapy (ADT) as part of their cancer treatment. ADT is a form of chemical castration that reduces testosterone and other androgens to extremely low concentrations. This reduction in testosterone, the primary anabolic stimulus in the male body, may result in skeletal muscle loss and adipose tissue gains (30). These features of body composition are associated with various unhealthy metabolic outcomes (i.e. impaired glucose metabolism; 31) and with the development of the co-morbidities in survivorship (25-29). There is also a growing body of literature examining how ADT influences glucose metabolism and diabetes (28, 32); however, few studies have examined glucose metabolism integrating interactions with body composition, physical activity and diet. The metabolic profile of prostate cancer patients at the time of cancer diagnosis may relate to risk of more aggressive prostate cancer and prostate cancer mortality (24).

ADT is just one of the treatment options available for prostate cancer patients and the studies investigating the metabolic effects of ADT usually focus on the long-term (years) consequences of treatment (25-29, 32). Little is known about the metabolic implications of radiation therapy. Higher BMI is associated with increased risk of biochemical recurrence following radiation therapy (33); however, the mechanisms and the impact of radiation therapy on the metabolism of prostate cancer patients are unknown. Further investigation of the metabolic health of men undergoing acute treatment with modalities other than ADT is warranted to elucidate the interaction between prostate cancer treatment, metabolic perturbations and the development of secondary diseases in prostate cancer survivors.

Chapter 2
Fundamentals of Prostate Cancer

2.1 Prostate Cancer in Canada

Prostate cancer is the most common form of cancer in Canadaian men with an estimated 24 000 new cases diagnosed in 2015, accounting for 23.9% of all male cancer diagnoses (2). Since the early 1990's, the number of cases of prostate cancer has increased due to improvements in prostate cancer screening procedures, culminating in two peaks in prostate cancer diagnosis in 1993 and 2001 following intensified screening using prostate specific antigen (PSA) testing. However, since 2001, the age-standardized incidence rate of prostate cancer has been declining (2). Despite these declining incidence rates, the number of prostate cancer diagnoses is still significantly above those of other cancers (an estimated 176 355 Canadian males have been diagnosed with prostate cancer since 1999); however, there has been a steady decline in the prostate cancer mortality, with 96% 5-year survival rates (2). These reductions in mortality are likely due to improvements in treatment (20), leading to a growing number of prostate cancer survivors in Canada.

2.2 Prostate Cancer Staging

With the prevalence of prostate cancer and the exceptionally high 5-year survival rates, severity of cancer diagnosis is a large determinant of not only survival outcomes, but also treatment approaches and outcomes. Currently, there are 3 tools to diagnose and assess cancer severity: cancer stage, PSA level, and Gleason score, together named stage grouping. Digital rectal exams (DRE), physical palpation of the rectum to identify abnormalities in the prostate, are also used to assess prostate cancer. However, the subjective nature of the DRE prevents its inclusion in stage grouping. As with many cancers, TNM (Tumour, Node, Metastasis) staging, which is the cancer staging system accepted by the International Union Against Cancer and the American Joint Committee on Cancer 7th

edition (34), is used to assess the progression of cancer by classifying size, lymphatic involvement, and metastases (34). TNM staging is based on the extent of the tumour (T) (i.e. size and number of tumours), whether or not it has spread to the lymph nodes (N), and the presence of distant metastasis (M) (i.e. whether the tumour has spread to other locations) (34). A number is assigned to each letter to represent the extent of the disease (Table 2.1). These factors are assessed through a variety of tests including physical exam (DRE), imaging (x-ray, CT, MRI, etc.), biomarker assessment, pathology reports, and surgical assessment (34). There are 4 general stages of cancer progression. In Stage 0 the carcinoma in situ, while Stages I, II, and III represent more extensive disease and Stage IV represents metastatic disease. Additional criteria are used in staging assess tumour grade or cell type.

Table 2.1: TNM Staging Classification System			
	Primary Tumour (T)	Regional Lymph Nodes (N)	Distant Metastasis (M)
X	Cannot be evaluated	Cannot be evaluated	Cannot be evaluated
0	No primary tumour	No involvement	No metastasis
In situ	Abnormal cells present, but not yet cancerous	-	-
1	Size and extent of primary tumour is assessed	Lymph nodes involved, number and extent assessed	Metastasis present
2			-
3			-
4		-	-

The second tool used to diagnose prostate cancer is PSA concentration. PSA is a serine protease that is produced by both normal and malignant prostate glands. In a non-malignant male, it is secreted in seminal fluid and little is released into the blood; however, in malignant tissue significant amounts of PSA leaks into the blood stream, raising its circulating levels (35). Thus, PSA is a potent biomarker for prostate cancer. Healthy circulating PSA levels are < 4 ug/L, while PSA levels between 4-10 ug/L are termed the "grey zone" where further investigation is required, and PSA levels >10 ug/L are considered significantly elevated (35). However, the efficacy of PSA testing is

often questioned and PSA screening is not currently recommended in Canada (36); however, its use is still widespread (37, 38).

The final tool used to diagnose and assess prostate cancer severity is Gleason score. This is a pathology marker that characterizes the pattern of cells within the tumour. There are different cell patterns that a pathologist evaluates and subsequently assigns 2 scores (each score ranges from 1-5). The first score is the most common cell pattern observed in the prostate tissue and the second score is the second most common cell pattern observed. These scores are then added together to determine Gleason score (a total score ranging from 2-10) (39). Please see Appendix II for a detailed description of the individual cell characteristics of Gleason scores. Generally, the higher the Gleason score, the more severe the cancer; however, a Gleason score of $\geq$7 is often defined in the literature as the cut point between moderate and severe prostate cancer diagnosis.

These three diagnostic tools, TNM staging, PSA and Gleason score, are used together to stratify prostate cancer risk. The most commonly cited method was developed by D'Amico and colleagues (40). This system stratifies patients into 3 risk categories: low-, intermediate- and high-risk cancer. This system predicts the likelihood that the patient will experience PSA or biochemical failure (defined as elevation of PSA following prostate cancer treatment) within 5 years and it is based on cancer stage, PSA levels at diagnosis and Gleason score. Low-risk patients are staged T1c-T3a, have PSA < 10 ug/L, and Gleason < 6. They are predicted to have a < 25% chance of PSA failure in 5 years. Intermediate-risk patients have between 25-50% chance of PSA failure in 5 years and are staged T2b, with PSA between 10-20 ug/L, and Gleason scores of 7. Finally, high-risk prostate cancer is defined as > 50% chance of PSA failure in 5 years with a staging $\geq$ T2c, PSA $\geq$ 20 ug/L and/or Gleason score $\geq$ 8 (40). Understanding of the classification systems of prostate cancer is important as low-, intermediate- and high-risk cancer have different risk factors, treatment plans, and outcomes.

2.3 Prostate Cancer Treatment

Stage of cancer at diagnosis is a large determinant of the type of treatment a prostate cancer patient will receive, as each stage provides its own unique treatment challenges. There are 5 major treatment options for prostate cancer patients: active surveillance, surgery, radiation therapy, hormone therapy, and chemotherapy. The use of these therapies is highly dependent on the individual cancer diagnosis and progression. Many of these therapies are employed in conjunction with one another.

2.3.1 Active Surveillance

Active surveillance is a process of close monitoring in low-risk prostate cancers. Essentially, patients with cancers at a very low-risk for progression, are not treated initially, but are instead repeatedly evaluated to determine if the cancer is progressing and therefore warrants treatment (41). There are no specific guidelines for the use of active surveillance from the Canadian Urological Association (CUA), American Urology Association (AUA), or European Association for Urology (EAU). Since no specific guidelines for active surveillance exist, descriptions of active surveillance vary; in general, active surveillance constitutes repeated check-ups generally every 3-6 months that include DRE, PSA tests, and/or repeat biopsies (42).

The following eligibility criteria have been proposed by the EUA to identify patients eligible for active surveillance: clinically confirmed prostate cancer (T1-T2), Gleason ≤6, ≤3 biopsy cores involved with cancer, ≤50% of each core defined with the presence of cancer, and PSA <10 ng/mL (43). Therapy is recommended when there is significant disease progression, which is defined by rapid PSA doubling time (cut off between ≤2 and ≤4 years), clinical progression with DRE, or grade progression (Gleason ≥7) or tumour volume progression with repeated biopsy (43, 44).

2.3.2 Surgery

Radical prostatectomy is the most common form of surgery for prostate cancer treatment (42). It involves the complete removal of the prostate gland, the seminal vesicles, and ampulla of the vas deferens (41). There are multiple variations of radical prostatectomy including the incision location (retropubic vs perineal), as well as laparoscopic and robotic-assisted techniques (41). There are also both nerve-sparing and non-nerve sparing options that are chosen based on tumour characteristics and sexual function in patients (43). For patients with truly localized disease (found only within the prostate gland), radical prostatectomy offers a potentially curative treatment; however, for higher risk, non-localized disease, this may not be possible (41). For more advanced prostate cancer, radical prostatectomy is performed in conjunction with a pelvic lymphadenectomy, removal of the surrounding lymph nodes (43). For men with high-risk cancer (Stage >cT2b, PSA >20ng/dl, Gleason $\geq$7), multiple-modal treatment is used and surgical methods may be used in conjunction with additional treatments (43).

2.3.3 Radiation Therapy

Prostate cancer patients can undergo either external beam radiation therapy or brachytherapy, though the latter is typically used only in less advanced cases. Brachytherapy involves inserting small radioactive "seeds" directly inside of the prostate to treat the cancerous tissue (41). The isotope, isotope intensity, and number of seeds are determined on a case-by-case basis; however, common treatment regimens include 120 Gy of palladium or 140 Gy of iodine-125 (41). This technique has been deemed safe and effective for low-risk prostate cancer patients. The EAU employs the following eligibility criteria for this treatment: stage cT1c-T2z, N0, M0; Gleason $\leq$6, initial PSA $\leq$10ng/mL, $\leq$50% biopsy cores involved in cancer; prostate volume <50mL, and a good international prostate system score.

External beam radiation therapy involves providing radiation to the prostate and surrounding tissues via an external device (41). Dosage is dependent on cancer severity, with the EAU suggesting at least 74 Gy for low-risk prostate cancer, 76-81 Gy for intermediate prostate cancer patients as these dosages have been shown to improve disease-free survival times (45). However, for high-risk prostate cancer patients, the EAU suggests that while escalating doses of external beam radiation beyond those recommended for intermediate-risk patients improves disease-free survival, external beam radiation is unable to mitigate the risk of systemic relapse (45). Consequently, radiation therapy for high-risk patients is usually used in conjunction with hormonal therapy.

2.3.4 Hormone Therapy

Hormone therapy, termed androgen deprivation therapy (ADT), is used in patients for whom curative therapy is not possible or appropriate (41). The aim of ADT is to reduce testosterone to castrate levels, as testosterone has been identified as one of the earliest promoters of prostate cancer growth (46). Traditionally, this was achieved through orchiectomy, or surgical removal of the testicles, which reduces testosterone level by 90-95%; however, this treatment type is currently rare, with most patients undergoing "chemical castration" through the use of various drugs (47). ADT can be used as primary treatment in early-stage prostate cancer or can be administered neo-adjuvantly, prior to local treatment, to reduce tumour size and improve treatment outcomes, or adjuvantly, following local treatment, as a precaution to treat cancerous cells elsewhere in the body (47).

Numerous categories of drugs can be used in ADT, including luteinizing hormone-releasing hormone (LHRH) agonist, LHRH antagonist, and anti-androgens. LHRH, also known as gonadotropin-releasing hormone, is responsible for the release of both follicle stimulating hormone (FSH) and luteinizing hormone (LH) and is considered the first step in the hypothalamic-pituitary-gonadal axis (48). LHRH agonist work by essentially depleting the pituitary and down-regulating the pituitary LHRH receptor, reducing the pituitary's ability to respond to LHRH (17). This treatment is

associated with an initial testosterone flare when first administered as the pituitary initially releases LH prior to its expected down-regulation, which typically occurs approximately 30 days following administration (48). LHRH antagonist work more quickly and do not cause the testosterone flare observed with LHRH agonist (48). The drugs stop LHRH from binding to its receptors in the pituitary, reducing LH secretion and ultimately reducing testosterone levels (47). Anti-androgens are often used in conjunction with LHRH agonists as they block the effects of androgens without suppressing their production (48). The combination of these drugs is known as total androgen blockade.

There are numerous side-effects associated with ADT including hot flashes, decreased libido and erectile dysfunction, breast enlargement and tenderness, irritability, depression and emotional disturbances, headache, dry skin, itching, and rashes, gastrointestinal distress, testicle shrinkage, osteoporosis, anemia, skeletal muscle loss, increased adiposity, and the risk of developing metabolic syndrome (a cluster of risk factors that increases the risk of cardiovascular disease and diabetes) (47). Skeletal muscle loss and increased adiposity are especially a concern because these body composition changes are associated with cardiovascular disease and diabetes in non-malignant populations (49, 50). Prostate cancer patients who receive ADT are at increased risk for cardiovascular disease and diabetes in survivorship (28).

2.3.5 Chemotherapy

Chemotherapy is not used in most cases of prostate cancer and is typically used to treat only metastatic and castration resistant prostate cancer. Docetaxel is the primary chemotherapy agent recommended by the CUA for the treatment of castration resistant prostate cancer (51). The advised dosage is 75mg/m^2 IV, every 3 weeks (51). Docetaxel significantly increased survival compared to other treatment regimens including mitoxantrone (52). For disease control, palliation and quality of

life, the CUA indicated that there is Level 2, Grade B evidence for the use of weekly docetaxel plus prednisone, or mitoxantrone plus prednisone (51).

2.4 Prostate Cancer Risk Factors

2.4.1 Non-modifiable Risk Factors

There are 3 primary non-modifiable risk factors that have been associated with prostate cancer: age, ethnicity, and family history. Age is the strongest known risk factor for prostate cancer. In Canada, 97.8% of all prostate cancer diagnoses occur in men over the age of 50, while less than 1% of cases occur in men under the age of 40 (18). By age 90, 80% of men have been shown to have cancerous cells in their prostate (53).

Apart from age, family history has been shown to be one of the strongest risk factors for prostate cancer. The literature suggests that 5 to 10% of all prostate cancer is associated with genetic predisposition, and this value increases between 30 to 40% in men diagnosed prior to the age of 55 years old (19,54). Risk further increases if a first-degree relative is diagnosed prior to the age of 60 (55).

Ethnicity or race has also been shown to significantly influence prostate cancer risk. There is a significant increase in prostate cancer incidence among African American men when compared to any other race (20). The age-adjusted incidence rates per 100 000 men are 248.5 for African Americans compared to 156.7 for Caucasians, 93.8 for Asians and 138 for Hispanic populations (20). African Americans are also more likely to be diagnosed with advanced disease (56) and are 2 times as likely to die of prostate cancer compared with Caucasian men (20, 57).

2.4.2 Modifiable Risk Factors

While the evidence supporting the associations between age, family history, ethnicity and prostate cancer development is strong, the literature linking modifiable risk factors such as obesity, diet, and physical activity to prostate cancer risk is less clear.

Obesity has been linked to increased risk of a number of different types of cancer including breast and colorectal; however, the link between obesity and prostate cancer is less clear. Numerous meta-analyses have examined the relationship between obesity, usually assessed via body mass index (BMI) and prostate cancer incidence (9, 10, 21-23, 58-60). Each meta-analysis reported a small but significant positive increase in relative risk for prostate cancer. The general conclusions from these analyses are that obesity is associated with increased risk of prostate cancer, and specifically, aggressive prostate cancer. The literature examining the relationship between low-risk or localized prostate cancer and obesity remains inconclusive. Discacciati et al (9) reported a protective effect of obesity against localized prostate cancer (RR: 0.94; 95% CI, 0.91-0.97), but an increased risk of aggressive prostate cancer with obesity (RR: 1.09; 95% CI, 1.02-1.16). Most studies used BMI as their marker of body composition, which is a crude measure of body composition and obesity; therefore, it may not be sensitive enough to determine associations between prostate cancer and obesity in individual studies. However, BMI is an effective measure of obesity at a population level, thus, the relationship between BMI and prostate cancer incidence becomes clearer in the meta-analyses when a larger sample size is considered.

While most meta-analyses reported a positive association between BMI and prostate cancer risk and specifically, aggressive prostate cancer, there is great heterogeneity in the literature. This is most likely due to study design and country of origin as obesity rates and prostate cancer screening procedures vary greatly from country to country. Geographical regions have been shown to influence the relationship between prostate cancer and obesity (61). North American studies reported no effect

of obesity on prostate cancer risk (relative risk (RR): 1.04; 95% CI 0.96-1.03), while European and Australian cohorts demonstrated a modest positive association between obesity and prostate cancer risk (RR: 1.04; 95% CI 1.01-1.07) (61). These discrepancies are thought to be due to differences in PSA screening procedures, as PSA screening is more common in North America and compared to Europe; thus, cancer diagnosis occurs at earlier stages in North America versus in Europe. In addition, PSA levels are typically lower in obese men (62), which results in fewer biopsies in this population, and consequently, fewer obese men are diagnosed at an earlier stage – this phenomenon is known as *detection bias hypothesis* and will be discussed in detail later in this literature review (Section 2.5.2).

Two other lifestyles factors, which have been known to contribute significantly to obesity, have been examined for their relationship to prostate cancer: diet and physical activity. The relationship between diet and prostate cancer has been examined in a variety of different ways including specific macronutrients like fat intake, food group analysis such as fruit and vegetables, meat, and dairy, specific foods such as soy and green tea and a number of specific vitamins and minerals including, lycopene and selenium (14, 15, 63-65). The majority of the literature in this area remains inconclusive with small-scale studies showing both positive and neutral association between these components of diet and prostate cancer risk (14, 15, 63, 64).

The relationship between diet and prostate cancer is further confounded when obesity and metabolic syndrome are considered. Both obesity and metabolic syndrome have independently been implicated in prostate cancer development; however, dietary intake significantly influences both of these conditions. Obesity and metabolic syndrome are associated with high carbohydrate (65, 66) and saturated fat intake (67). The traditional hypocaloric macronutrient distribution of 15% protein, <30% fat, and 50-55% carbohydrates to treat obesity and metabolic syndrome has come into question of late, with a higher protein distribution of 30% protein, 30% fat and 40% carbohydrates may be

13

more effective in ameliorating the negative implications of diet on body composition and metabolic syndrome (68). However, all of these factors complicate the relationship between diet and prostate cancer even further.

As with diet, the literature examining physical activity as a risk factor remains inconsistent. Overall, physical activity is reported to reduce the risk of prostate cancer by 10-20% with increasing physical activity levels (16).   One review evaluated 40 epidemiological studies examining physical activity as a protective mechanism against prostate cancer (17). Twenty-two of the studies reported a small, but significant protective effect of physical activity and prostate cancer risk, 14 demonstrated no association between physical activity levels and prostate cancer risk, while the remaining 4 demonstrated a negative effect of physical activity on prostate cancer risk (17). However, many of the discrepancies observed in these data may be related to differences in methodological approaches, type of exercise reported (including the use aerobic versus resistance training or combined protocols), intensity, duration, and frequency of these programs as well as heterogeneity in the populations studied. Another important consideration for all of these modifiable risk factors, obesity, diet, and physical activity, all relate to one another, confounding the data even further.

2.5 Aggressive versus Slow Growing Cancers

Much of the literature examining risk of prostate cancer and lifestyle factors remain inconclusive, especially with regards to the dichotomy between the aggressive or high-risk cancers and localized or low-risk cancers. This phenomenon is most often observed in the literature examining obesity and obesity-related risk factors in prostate cancer. Obesity has been shown to be a risk factor for high-risk prostate cancer and prostate cancer mortality, but has not consistently been shown to be a risk factor for general prostate cancer diagnosis (69). Metabolic syndrome has also demonstrated this relationship with prostate cancer (70). There are two primary hypotheses that may explain this phenomenon: the altered hormone hypothesis and the detection bias hypothesis.

14

2.5.1 Altered Hormone Hypothesis

The altered hormone hypothesis is based on the role of testosterone in both normal and malignant prostate tissue, and the differences in testosterone levels in obese versus lean men. In healthy prostate tissue, testosterone stimulates tissue growth; however, testosterone cannot distinguish between healthy and malignant tissue. Thus, when a tumour is present testosterone stimulates tumour growth along with healthy prostate cell growth. Obese men typically have lower testosterone levels compared to lean men (71). Consequently, there is a smaller tumour growth stimulus from testosterone in obese patients. However, in advanced prostate cancer, tumours often become testosterone independent – they grow without a testosterone stimulus – thus, obese patients with lower testosterone levels would be more likely to develop the more aggressive type of tumour (72).

2.5.2 Detection Bias Hypothesis

Detection bias hypothesis suggests that prostate cancer is more difficult to detect in obese patients. Obesity obscures the accuracy of the primary screening tools employed by physicians to detect prostate cancer. First, digital rectal exams are more difficult to perform in obese patients, limiting the physician's ability to accurately assess the prostate (72). Second, obese individuals have naturally lower PSA levels due to hemodilution (72). Thus, this biomarker is less likely to be elevated in obese patients. Finally, obese patients have larger prostate volumes, implying that there is increased likelihood that small, early stage tumours may be missed with a prostate biopsy (72). A typical prostate biopsy extracts 12 biopsy cores to be examined by a pathologist to provide a Gleason score. The larger the prostate, the increased chance that one of the 12 cores will miss a small tumour (72). These three factors work together to hinder a physician's ability to detect prostate cancer in obese patients. Therefore, prostate cancer is diagnosed at later stages in obese patients (72).

Both the altered hormone hypothesis and the detection bias hypothesis remain controversial and require further investigation. Some researchers suggest that a combination of factors from both

the altered hormone hypothesis and the detection bias hypothesis may explain the discrepancy in the relationship between obesity and risk of low-grade versus high-grade prostate cancer.

Chapter 3
Literature Review

3.1 Insulin and Glucose Metabolism in Prostate Cancer

As with much of the literature examining metabolic factors and their association with prostate cancer, the relationship between glucose metabolism and prostate cancer remains unclear. Few studies have examined glucose metabolism as a risk factor for prostate cancer, and those that have, demonstrate inconsistent results (73, 74). As well, the literature examining glucose metabolism in prostate cancer treatment and survivorship is only focused on ADT use.

3.1.1 Insulin as a Risk Factor for Prostate Cancer

Insulin has been implicated in a number of processes in tumour growth. Increased insulin secretion occurs with many cancers, which results in a decreased sensitivity in peripheral tissues and leading to insulin resistance in healthy tissues. Consequently, this increase in secretion and decrease in peripheral tissue sensitivity may result in systemic hyperinsulinemia, further exacerbating cancer growth (76-77). Some studies have demonstrated positive association between hyperinsulinemia and prostate cancer risk (12, 78-80), while other studies examined hyperinsulinemia as a risk factor for prostate cancer and found no association between elevated insulin and prostate cancer risk. (73, 74). Abnormal insulin levels are not the only potential risk factors for prostate cancer, fasting insulin levels near the upper end of the healthy ranges have also been positively associated with prostate cancer in a case-cohort study (80). In contrast, late stage diabetes – specifically, when insulin levels begin to decline due to β-cell burnout - is negatively associated with prostate cancer risk (81-83). Metformin, a common drug used to control blood sugar in type 2 diabetics, is associated with decreased risk of prostate cancer (84, 55). Despite this data, in a meta-analysis that examined insulin therapy and prostate cancer risk, no significant associations were found between insulin therapy and

prostate cancer risk (86). Taken together, this suggests that insulin may stimulate prostate cancer

growth but it is uncertain whether insulin therapy will reduce the risk of prostate cancer.

3.1.2 Mechanisms of Insulin Action in Prostate Cancer

There is a large body of literature to support insulin as a potent growth factor in prostate

cancer (Reviewed: 87-91). Insulin is also associated with growth of aggressive prostate tumour

growth and more advanced disease (92). Higher grade (aggressive) prostate tumours have also been

shown to have increased numbers of insulin receptors on their membrane (91).   The binding of

insulin to its receptor activates the intrinsic tyrosine receptor domain stimulating both the

PI3K/Akt/mTOR pathway as well as the MAP/ERK-kinase pathway (92). Activation of these

pathways ultimately results in cell proliferation, migration, and differentiation, as well as the

inhibition of apoptosis (92). Thus, elevated circulating insulin concentrations combined with

increased number of insulin receptors on aggressive prostate tumours supports the hypothesis of

insulin as a significant contributor to prostate cancer growth.

Beyond insulin itself, other factors that contribute to glucose metabolism have been

implicated in the development of prostate cancer. Insulin-like growth factor-1 (IGF-1) works through

the same signalling pathways as insulin and has been shown to increase prostate cancer risk (93, 94),

while its primary transport binding protein insulin-like growth factor binding protein-3 (IGFBP-3),

which when would to IGF_1 forms a stable complex,. is inversely associated with prostate cancer

risk (24). As well, elevated fasting C-peptide, a marker of insulin secretion due to it simultaneous

release for the pancreas with insulin and its short half-life, has been associated with prostate cancer

risk (95). Its measurement allows for the distinction between increase insulin secretion or decrease

clearance as the mechanism for hyperinsulinemia. However, most of the literature focuses on fasting

insulin, and C-peptide values, which may mask the body's ability to respond to a glucose challenge.

Only 2 studies have employed an oral glucose tolerance test (OGTT) in prostate cancer patients to

evaluate responses to a bolus of glucose (96, 97). Zamboni et al (96) demonstrated normal 2-hour

glucose concentrations following OGTT in prostate cancer patients with varying severity of disease.

Here, patients had higher fasting insulin and homeostatic model assessment insulin resistance

(HOMA-IR) compared with non-malignant controls. Despite the plethora of data that one could

obtain from an OGTT, Zamboni and colleagues only evaluated fasting and 2-hour glucose

measurements were considered in addition to fasting insulin. They did not measure 2-hour insulin and

C-peptide concentrations during the OGTT, which would better examine the body's ability to clear

glucose. Since insulin may have a role in aggressive prostate cancer growth, it would also be

important to distinguish glucose responses during an OGTT based on severity of disease.

Tekdogan et al (97) examined insulin, IGF-1 and IGFBP-3 during and OGTT in prostate

cancer patients compared to patients with benign prostate hyperplasia (BPH). There were no

differences in IGF-1 and IBGBP-3 during the OGTT between the prostate cancer patients and the

BPH patients; however, they noted elevated insulin levels in both groups, though the patients with

BPH had significantly higher insulin concentrations than the cancer patients (97). The use of

methodology beyond fasting metabolites and hormone concentrations is clearly important since

fasting measures may mask impairments that would be revealed through an OGTT or other

comprehensive approaches. Further investigation into the role of glucose metabolism during a

glucose challenge may be more reflective of the tissue exposure to insulin and its related hormones

and may facilitate the understanding of the complex relationship between prostate cancer and glucose

metabolism.

3.1.3 Glucose Metabolism and Prostate Cancer Treatment

ADT is a common treatment approach for high-risk prostate cancer and its use is associated

with diabetes as a secondary disease state (27). Impaired insulin sensitivity occurs within the first 3

months of ADT use (98, 99). In an OGTT following 12 weeks of ADT, glucose response was

identical following ADT relative to baseline; however, insulin levels were significantly elevated following the 12 weeks of treatment during the OGTT, suggesting decreased insulin sensitivity (99). As well, decreases in the insulin sensitivity index and increased glycated hemoglobin (a marker of long term perturbations in glucose metabolism) were also observed in prostate cancer patients undergoing 12 weeks of ADT (98). Fasting serum insulin levels (98, 100, 102) and fasting glucose levels have also been shown to increase following ADT (102). Despite this evidence, there are few large-scale studies examining longitudinal changes and incidence of ADT- related metabolic disorders (103).

The mechanisms underlying the relationship between ADT and the development of insulin resistance are poorly characterized. The main hypothesis suggests that the interaction between cytokines and testosterone influence the development of insulin resistance (103). Adipose tissue derived pro-inflammatory cytokines like interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α), elevated in chronic low-grade inflammation, have been associated with type II diabetes (104, 105). Low testosterone concentrations have been shown to contribute to insulin resistance in hypogonadal men with metabolic syndrome. In contrast, testosterone administration can lower plasma insulin concentrations and HOMA-IR (106), which may occur through inhibition of these inflammatory markers (106, 107). It is possible that cross-talk between sex steroids and pro-inflammatory cytokines during ADT may influence the development of insulin resistance in the patients (103). However, it is unknown whether increased pro-inflammatory cytokine levels cause insulin resistance or are a consequence of ADT use and altered sex hormone levels. Further, investigation into the mechanisms behind these associations is warranted to elucidate these complex interactions.

Beyond ADT, there is some evidence that treatments other than ADT may increase diabetes risk in prostate cancer survivors. Thong et al (108) examined incidence of diabetes following a prostate cancer diagnosis and demonstrated that 50.2% of patients had received radical

20

prostatectomy, 21.4% received radiation therapy, 16.3% were undergoing active surveillance and only 12.1% received hormone therapy as their primary treatment type (108). These data suggest that all prostate cancer patients, not just those receive ADT, are at risk of developing diabetes in survivorship. Remarkably, the vast majority of research has focused on the metabolic effects of hormone therapy. Clearly, there are other mechanisms driving the relationship between insulin and prostate cancer that warrant further study.

3.2 Body Composition and Prostate Cancer

Obesity has been implicated in the development of disrupted glucose metabolism, insulin resistance and eventually diabetes (109). However, it has also been implicated in the development of prostate cancer. It is one of the major modifiable risk factors for prostate cancer. In Section 2.4.2 (Modifiable Risk Factors), I discussed the influence of obesity on prostate cancer risk. Beyond risk, obesity may also significantly affect treatment and survivorship following a prostate cancer diagnosis.

3.2.1 Obesity and Prostate Cancer Treatment

Obesity may influence outcomes of the 3 primary categories of prostate cancer treatment: radical prostatectomy, radiation therapy, and androgen deprivation therapy (ADT). Obesity has been shown to significantly increase biochemical recurrence (a rise in blood PSA following surgery or radiation treatment) and prostate cancer specific mortality (23). A systematic review and dose-response meta-analysis by Hu et al (58) examined the relationship between BMI and biochemical recurrence following multiple treatment types. Across all treatment types a 5 kg/m^2 increase in BMI was associated with a 16% increase in biochemical recurrence (RR: 1.16; 95% CI: 1.08-1.24). Analysis of individual treatment types revealed a 17% increase in biochemical recurrence with radical prostatectomy (RR: 1.17; 95% CI: 1.07-1.28; Hu et al, 2014). Similarly, Cao and Ma (23) observed a

21% increase in biochemical recurrence (RR: 1.21; 95% CI: 1.11-1.31) and a 15% increase in

prostate-specific mortality (RR: 1.15; 95% CI; 1.06-1.25) per 5 kg/m^2 increase in BMI following

radical prostatectomy (23). The relationship between increased BMI and biochemical recurrence is

stronger in more recently treated men, and populations of PSA-detected cancer (110). However,

obesity increases the risk of biochemical recurrence following radical prostatectomy in long-term

survival as well (111). This relationship may also be influenced by the difficulty of operating on

obese patients (112); however, when poor surgical technique is adjusted for, obesity is still associated

with increased biochemical recurrence and prostate-specific mortality (23, 113). Taken together, these

data suggest poorer survival outcomes for obese patients compared to normal weight patients.

The literature examining the relationship between obesity and radiation therapy is limited – to

date, 2 studies have examined brachytherapy and 4 have investigated external beam radiation therapy

in relation to biochemical recurrence. In patients treated with external beam radiation therapy, obesity

was significantly associated with increased risk of biochemical recurrence in 3 of 4 studies (ES

Range: 1.16-1.61) (114-116). The fourth study reported no significant associations in risk of

biochemical recurrence in obese men (Effect Size (ES): 1.02, 95% CI: 0.68-1.54; 117). In the studies

examining brachytherapy, neither showed a significant effect of treatment on biochemical recurrence

(118, 119). When these data were combined in a meta-analysis, Hu et al (58) indicated no association

between obesity and brachytherapy (RR: 0.91; 95% CI: 0.64-1.28); however, external beam radiation

therapy was associated with a 19% increased risk of biochemical recurrence per 5 kg/m^2 increase in

BMI (RR: 1.19; 95% CI: 1.10-1.28) (58).

ADT chemically reduces testosterone to castrate levels to combat the proliferative effects of

testosterone on many prostate tumours. The impact of obesity on ADT is poorly characterized.

Patients receiving ADT lose significant amount of muscle and gain adipose tissue (29), which has

been associated with increased incidence of cardiovascular disease and diabetes in survivorship (27).

22

However, a study by Keto and colleagues (120) found that during ADT, obese patients had increased risk of castrate-resistance prostate cancer, metastases, and prostate cancer specific mortality compared to non-obese patients. The literature also demonstrates that testosterone levels are higher in obese men on ADT, suggesting incomplete testosterone suppression (121). Although ADT prescription is currently not dependent on body size, it might be important to consider body composition on patients receiving ADT as obese patients may be under-dosed (121).

3.2.2 Obesity and High-Risk Prostate Cancer

Overall, the literature reports a 15-20% increase in prostate cancer specific mortality per 5 kg/m^2 increase in BMI (23). Detection bias (as described in Section 2.5.2) alone cannot explain this association and there are 3 proposed primary underlying biological mechanisms driving this relationship (122). Increased insulin and IGF-1 concentrations, which are associated with obesity (123, 124), result in elevated circulating growth factors causing increased tumour proliferation, reduced tumour apoptosis and transitioning of prostate tumour cells to an androgen resistant state (60). Accelerated tumour growth in prostate cancer xenograph models has been reported as a result of diet-induced hyperinsulinemia (125, 126). Conversely, later stages of diabetes, where insulin levels begin to decline, has been associated with reduced prostate cancer risk (127). Similarly, higher serum C-peptide concentrations have been associated with increased prostate cancer specific mortality (128). Elevated circulating IGF-1 has also been linked to increased prostate cancer incidence (22, 129). Up-regulation of the IGF-1 receptor has been observed in studies where there is a transition of androgen dependent cell lines to androgen independent cells, as well as prostate cancer progression *in vivo* (130). Taken together, these findings support the hypothesis that increased insulin and IGF-1 levels, which are associated with obesity, result in elevated circulating growth factors that stimulate tumour proliferation and reducing tumour apoptosis (60).

Obese men have lower testosterone levels (as discussed in Section 2.5.1), which creates an environment conducive to the growth of more aggressive prostate tumour – testosterone-independent tumours (71). Lower testosterone levels, such as those observed in obese men, will result in the slower the growth of testosterone-dependent tumours - less aggressive prostate tumours (71). Thus, testosterone concentrations, as per body phenotype, may also influence tumour characteristics, independent of the effects of hormone treatments.

The final mechanism hypothesized to explain the link between obesity and aggressive prostate cancer is chronic subclinical inflammation via altered adipokine levels. Inflammation and altered adipokine signalling has also been linked to the development of insulin resistance (131). Leptin is elevated in obesity and has been shown to increase prostate cancer cell line proliferation and inhibit apoptosis in both androgen sensitive (LNCaP) and androgen resistant (PC-3 and DU145) cell lines (132-134). However, there is a stronger proliferative response to leptin in PC-3 and DU145, androgen resistant cell lines (132, 133), which occurs through inactivation of FOXO1 (135). *In vitro* studies have demonstrated robust relationship between leptin and prostate cancer aggressiveness; however the epidemiological evidence is inconsistent, where positive (136, 137) and null relationships are demonstrated (112, 138-140). Conversely to leptin, adiponectin is purported to have anti-tumour effects (140). Serum adiponectin concentrations are reduced in obese individuals (141, 142). Prostate cancer patients have demonstrated lower serum adiponectin (140, 143) and there is an inverse relationship observed between histological grade and disease stage, and plasma adiponectin levels (143). Numerous pathways have been hypothesized to explain the protective effect of adiponectin against carcinogenesis including AMPK, NF-κB, PPAR-α, and MAPK signalling, as well as the downstream moderator JNK and STAT3 (144,). Taken together, these data suggest an important role for adipokines and risk of aggressive prostate cancer; however, further investigation is

required to elucidate the complex interactions between adipokines, obesity and prostate cancer and how these factors contribute to insulin resistance.

3.2.3 Skeletal Muscle in Prostate Cancer

Obesity addresses only one component of body composition – adipose tissue. It does not consider skeletal muscle and its potential role in prostate cancer. Unlike obesity, which has a large body of literature investigating its relationship to prostate cancer risk and treatment outcomes, there is no literature that examines skeletal muscle mass and prostate cancer risk and treatment outcomes. The majority of the literature examining skeletal muscle in prostate cancer examines the relationship between ADT and skeletal muscle loss. Androgens are the primary anabolic stimulus in skeletal muscle in men, and consequently, ADT causes significant muscle loss (145) and adipose tissue gain (29, 146). In non-malignant populations, these body composition characteristics are associated with the development of insulin resistance and metabolic syndrome (147, 148). Hypogonadism is independently predictive of hyperinsulinemia and metabolic syndrome (149-151). It may also be related to increased saturated fat intake (152), inactivity (153) as well as unhealthy changes in body composition (154) during the treatment time-course in prostate cancer patients. The literature demonstrates a clear relationship between ADT use and skeletal muscle loss; however, there is a significant gap in our understanding of skeletal muscle in prostate cancer as to date no one has investigated skeletal muscle at prostate cancer diagnosis. This is especially important in men with high-risk prostate cancer who receive ADT and are at increased risk of cardiovascular disease and diabetes as a result of treatment.

3.3 Metabolic Syndrome and Prostate Cancer

More recently, investigators have begun to examine metabolic syndrome as a potential risk factor for prostate cancer. Many of the studies that suggest potential mechanisms to explain the

complex relationship between obesity and prostate cancer implicate components of metabolic syndrome, such as insulin resistance.

There are 3 definitions of metabolic syndrome commonly cited in the literature: the National Cholesterol Education Program – Adult Treatment Panel III (NCEP-ATP III; 155), the World Health Organization (WHO; 156) definition, and the International Diabetes Federation (IDF; 157). The NCEP-ATP III definition and the IDF definition are the two most highly cited in the literature; however, for the purpose of this thesis the IDF definition of metabolic syndrome will be used as it has been used previously in the prostate cancer literature (70). The European Group for the Study of Insulin Resistance (EGIR; 158) and the American Heart Association (AHA; 159) have also published definitions (Table 3.1). The IDF definition is now the consensus worldwide definition of metabolic syndrome.

Table 3.1: Metabolic Syndrome Definitions

	IDF, 2006[1] (157)	NCEP, 2001[8] (155)	WHO, 1999[9] (156)	EGIR, 1999[10] (158)	AHA, 2004 (159)
Central Obesity WC					
Men (cm)	>94[2] or >90[3]	≥102		≥94	>102
Women (cm)	>80	≥88		≥80	>88
Waist to Hip Ratio					
Men (AU)	-		>0.90		
Women (AU)	-		>0.85		
BMI (kg/m^2)	≥30[4]		>30		
Triglycerides (mg/dL)	>150[5]	≥150	>150	>177[5]	≥150
HDL Cholesterol (mg/dL)				<39[5]	
Men	<40	<40	<35		<40
Women	<50[6]	<50	<39		<50
Raised Blood Pressure (mmHg)					
Systolic	>130	>130	>140	≥140	≥130
Diastolic	>85[6]	>85[5]	≥90	≥90[6]	≥85[6]
Raised Fasting Glucose (mM)	>5.6[7]	>6.1	-	6.1	≥5.6[11]
Microalbumin (ug/min)	-	-	≥20	-	-

[1] Metabolic syndrome is Central Obesity with ≥2 other risk factors

[2] Europids, Sub-Saharan African, Eastern Mediterranean and Middle Eastern Populations

[3] South Asian, Chinese, Japanese

[4] If BMI is >30 kg/m^2 central obesity can be assumed

[5] Or treatment for this abnormality

[6] Or previous diagnosis or treatment for this abnormality

[7] Or diagnosis of type 2 diabetes

[8] Metabolic syndrome is identified with at least 3 risk factors

[9] Metabolic syndrome requires the presence of one of the following: diabetes, impaired glucose intolerance, impaired fasting glucose or insulin resistance and 2 other risk factors

[10] Metabolic syndrome requires insulin resistance (top 25% fasting insulin values among nondiabetics) and ≥2 other risk factors

[11] Or use of medication for hyperglycemia

3.3.1 Metabolic Syndrome and Prostate Cancer Risk

To understand the role of metabolic syndrome in the prostate cancer trajectory, I will start by discussing the evidence that associates metabolic syndrome with risk of prostate cancer development. Much of the literature examining metabolic syndrome as a risk factor for prostate cancer is inconclusive with positive and null associations demonstrated. In a meta-analysis, Esposito and colleagues (160) demonstrated no significant association between metabolic syndrome and prostate cancer risk (160). Some studies reported a positive association between prostate cancer and metabolic syndrome (RR: 1.56-3.36; 161-164), while others demonstrated no association (RR: 0.65-1.29; 95, 163-173). Severity of disease may contribute to this relationship as De Nunzio and colleagues (70) reported a significant association between patients with aggressive prostate cancer (Gleason score $\geq$ 7) and metabolic syndrome (Odds Ratio (OR): 3.82, 95% CI 1.33-10.9; 70). Metabolic syndrome is a multi-factorial condition and each component may influence prostate cancer risk. When the individual components of metabolic syndrome (waist circumference, hypertension, hyperglycemia, dyslipidemia) were considered, only hypertension and waist circumference were demonstrated to be significantly associated with increased risk of prostate cancer (Hypertension: RR 1.15; 95% CI: 1.01-1.30; Waist Circumference >102cm: RR 1.56; 95% CI: 1.15-2.15; 160).

Increased risk of prostate cancer has also been reported for African Americans with metabolic syndrome (RR: 1.71; 95% CI: 0.97-3.01; 172), while no association (Hazard Ratio (HR): 0.81, 95% CI: 0.2-3.3;168) and reduced risk (RR: 0.77; 95% CI: 0.51-1.05; 163) is reported for other ethnic groups with metabolic syndrome. One hypothesis to explain the observed variations in these studies is geographic region. Specifically, ethnic profile may influence the outcomes of these results as the US studies have a large number of African Americans (who are at increased risk of prostate cancer relative to other ethnic profiles) and Hispanic participants; whereas the Scandinavian and Italian cohorts are almost entirely Caucasian (70, 161-163, 167, 169). As well, obesity rates differ greatly in

28

these countries. The Scandinavian cohorts are slimmer with only 12-15% of the population being

obese and have lower metabolic syndrome rates at only19-22% of the population (161-163, 167,169).

In the Italian cohort, 44% of the population was classified as obese (70), while 24-40% in the US

populations are identified as obese (163, 168, 170, 172).

Because of the multifactorial nature of metabolic syndrome, there is much speculation as the

mechanisms driving increased prostate cancer risk in patients with metabolic syndrome. De Nunzio

and colleagues (174) suggest that increased inflammation, changes in adipokine concentrations,

insulin resistance, and alterations in hormone status, all of which are observed in metabolic syndrome,

contribute to more aggressive prostate tumour biology. Metabolic syndrome is associated with

chronic low-grade inflammation, which may lead to uncontrolled proliferation in rapidly dividing

cells such as cancer cells (175). As previously discussed in Section 3.2.2, leptin has been shown to

stimulate the growth prostate cancer cells *in vitro* while increased serum leptin is associated with

larger, high-grade tumours (112, 140, 176). Adiponectin has been demonstrated to have

antiangiogenetic and antitumour properties (112, 140, 176), while low serum adiponectin is

associated with more advanced prostate cancer (112, 140, 176).

3.3.2   Metabolic Syndrome in Prostate Cancer Treatment and Survivorship

When examining the relationship of metabolic syndrome beyond risk, the majority of the

literature investigates the relationship between ADT and metabolic syndrome. A meta-analysis

investigating the relationship between metabolic syndrome and ADT demonstrated a 75% increased

risk of developing metabolic syndrome with ADT use (RR: 1.75; 95% CI: 1.27-2.41; 3). In an

observational, multicentre, prospective study, Morote et al (177) demonstrated that after only 6

months of ADT use almost all components of metabolic syndrome (waist circumference, BMI, total

cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides, glucose

and HbA1c) significantly increased and these changes persisted with 12 months of ADT use (177).

Androgen suppression use has been shown to decrease insulin sensitivity (98), increase fasting insulin levels (100, 101), and deleteriously change serum lipoprotein levels (100, 101). An epidemiological study by Haffner et al (149) also demonstrated that low serum testosterone levels are associated with increased total cholesterol, LDL cholesterol, and triglycerides (149). It is unclear whether these consequences arise as a result of ADT use or tumour presence (or lingering effects of the tumour if post-treatment). However, hypogonadism, independent of a cancer diagnosis, has been shown to be an independent predictor of metabolic syndrome in males (151), suggesting that ADT itself has profound effects on metabolism.

There are a few key differences between prostate cancer patients diagnosed with metabolic syndrome who are receiving ADT compared with individuals who have metabolic syndrome but never been diagnosed with prostate cancer. The first difference is that ADT is associated with increased HDL cholesterol - an increase of approximately 8-20% in the first 3-12 months of ADT (100, 178,179). Secondly, adipose tissue gains as a result ADT occur in subcutaneous fat not visceral fat (99, 179). Central obesity, which measures visceral adiposity, is the key component of metabolic syndrome. Visceral adiposity has negative implications on fasting glucose, insulin, cholesterol and triglyceride concentrations compared with subcutaneous adiposity (180). Thirdly, ADT is associated with increased adiponectin and normal C-reactive protein (CRP) concentrations (179, 181), while metabolic syndrome is associated with low adiponectin (182) and increased CRP (183). This suggests that the metabolic perturbations observed in prostate cancer patients, especially those receiving ADT, should be considered differently from non-malignant populations as they may have a different pathology that leads to metabolic syndrome (184).

3.3.3 Dyslipidemia and Prostate Cancer

Dyslipidemia, including increased triglyceride and decreased HDL cholesterol concentrations, is a key component of metabolic syndrome; however, cholesterol and lipid

30

metabolism may be of particular importance in the development and proliferation of prostate cancer. The literature examining lipid profiles in prostate cancer is limited and divided into two primary categories: risk and ADT use. Evidence for dyslipidemia as a risk factor for prostate cancer is mixed. Decreased HDL cholesterol has been observed in prostate cancer patients compared with age and BMI matched non-malignant controls (26, 185), which aligns with data from non-malignant males with have metabolic syndrome. Prostate cancer patients also demonstrate higher LDL cholesterol compared with age-matched controls (185), which results in increased total cholesterol levels (26).

In an examination of lipid profiles in aggressive (Gleason $\geq$ 8) versus non-aggressive (Gleason <8) prostate cancer, Prabhat et al (79) showed that aggressive cancer is associated with lower HDL cholesterol, higher triglyceride and very low-density lipoprotein (VLDL) cholesterol concentrations, but no significant differences where observed in total cholesterol and LDL cholesterol (79). Conversely, two cohort studies have demonstrated no significant changes in any lipid parameters in newly diagnosed prostate cancer patients (186, 187). The results of these studies are inconsistent, and therefore inconclusive. These discrepancies may be explained by the differences in study design (cohort versus case-control) and the small sample sizes of the studies as well as by the heterogeneity of the body composition profiles of the patients within the studies.

A recent epidemiological investigation in the SEARCH database evaluated the association between dyslipidemia and risk of prostate cancer recurrence in a group of patients who underwent surgery as their primary treatment type. Allott et al (188) demonstrated that for every 10 mg/dl increase in serum triglycerides there was a 3% increase in risk of prostate cancer recurrence (HR: 1.03; 95% CI: 1.01-1.05) (189). While the authors noted no significant associations between total, LDL, and HDL cholesterol and cancer recurrence across all men, in men with dyslipidemia, every 10 mg/dl increase in total cholesterol was associated with a 9% increased risk of cancer recurrence (HR: 1.09; 95% CI 1.01-1.17) and every 10 mg/dl increase in HDL cholesterol was associated with a 39%

reduction in the risk of recurrence (HR: 0.61; 95% CI: 0.41-0.91). It has also been observed that statin use, a class of cholesterol-lowering drug, has been associated with decreased risk of prostate cancer (189).

The literature examining lipid profiles during ADT demonstrate more consistent findings. Multiple studies have demonstrated increased total cholesterol, triglycerides and HDL cholesterol with ADT use (100, 178, 179). Total cholesterol has been shown to increase between 9 and 11%, HDL increases between 8 and 11%, while triglycerides increase by approximately 27% 100, 179). HDL cholesterol and total cholesterol levels rise relatively quickly, within the first 3 months of ADT administration (100). However, in more recent studies, changes observed in lipid profile resembled more traditional dyslipidemia with increases in total cholesterol, LDL cholesterol and triglycerides and decreases in HDL cholesterol following 1 year of ADT use (102) and compared with a control group (190). While the observed changes in dyslipidemic patterns are poorly understood, there is indirect evidence that link these patters to length of ADT use.

3.4   Conclusions and Perspectives

Much of the literature examining obesity, metabolic syndrome, glucose metabolism and prostate cancer has revealed some interesting associations. However, most studies in these research domains focus on two distinct time points in the prostate cancer disease trajectory: 1) prior to cancer diagnosis or risk factors and, 2) the long-term implications of ADT - the only treatment investigated. Little is known about prostate cancer patients as they progress through the disease trajectory. Moreover, many mechanisms have been proposed, with little investigation, to explain the potential integrative relationship between obesity, metabolic syndrome, insulin resistance and prostate cancer, including alteration in sex hormones, adipokines, and pro-inflammatory cytokines. Thus, the use of

different methodological approaches is warranted to help elucidate the nature of the complex

mechanisms of interaction between body composition, glucose metabolism and prostate cancer.

Chapter 4
Thesis Rationale and Study Design

4.1 Justification

Glucose-related metabolic perturbations, such as hyperinsulinemia, are emerging as important risk factors for aggressive prostate cancer (73, 74, 78). However, these metabolic disturbances do not occur in isolation, but rather work in conjunction with numerous other metabolic perturbations, including dyslipidemia, changes in the adipokine profile and inflammation to create a metabolic environment conducive to prostate cancer (174). These same perturbations are associated with the development of secondary disease states, including cardiovascular disease and diabetes (27), as well as cancer recurrence in survivorship (191). Despite these associations, little is known about glucose metabolism in prostate cancer patients during the acute treatment trajectory. Most of the available literature fails to characterize glucose and related parameters (i.e. insulin C-peptide, IGF-1), as well as lipid parameters, adipokines, cytokines and lifestyle factors for prostate cancer patients in an integrative manner; instead, most studies focus on few components in large populations, which have led to inconsistencies in the literature. The aims of this thesis were to: 1) understand the metabolic features including glucose and related parameters (insulin, C-peptide, IGF-1, IGFBP-3), lipid related markers (triglycerides, cholesterols, adiponectin, leptin) and lifestyle factors (body composition, physical activity, nutritional intake) that may be related to prostate cancer diagnosis and severity of disease, 2) to evaluate how these factors change during the acute prostate cancer treatment trajectory, and 3) to investigate potential mechanisms contributing to potential alterations in this metabolic profile observed during the treatment trajectory.

4.2 Overall Thesis Purpose and Objectives

4.2.1 Overall Thesis Purpose

The purpose of this thesis is to examine metabolic features (as mentioned in Section 4.1) of prostate cancer patients at diagnosis and changes in theses parameters throughout the acute treatment trajectory. It will also investigate the mechanisms underlying the potential changes in these metabolic features, specifically in glucose metabolism. Also, it will examine lifestyle factors that may contribute to potential changes in glucose metabolism that may occur during treatment, such as body composition, diet and exercise.

4.2.2 Overall Thesis Objectives

1. To identify potential perturbations in glucose metabolism in newly diagnosed prostate cancer patients and to understand the influence of other metabolic parameters, such as lipids, adipokines, cytokines, body composition, nutrition intake and physical activity, on glucose metabolism of these prostate cancer patients.

2. To understand how glucose and other related metabolic parameters differ between prostate cancer patients and men of similar age and body size who do not have prostate cancer.

3. To examine potential changes that may develop in glucose and related parameters in prostate cancer patients during the acute prostate cancer treatment trajectory (immediately and ~ 6 months following radiation therapy).

4. To examine the potential mechanisms that may contribute to the potential alterations in metabolism and specifically glucose metabolism during the treatment trajectory.

4.3 Study 1: C-peptide, abdominal obesity, and adipokines are associated with higher Gleason scores in prostate cancer

4.3.1 Rationale

Metabolic syndrome, defined in Section 3.3 as a cluster of metabolic perturbations including obesity, hypertension, hyperglycemia, and dyslipidemia, has recently emerged as an important risk factor for prostate cancer (191). Its presence increases the risk of cardiovascular disease and diabetes, and has been associated with numerous other metabolic perturbations including inflammation (192), insulin resistance (193) and changes in the sex hormone profile (194). These metabolic sequelae have been associated with prostate cancer and specifically aggressive prostate cancer development (174); it creates an environment conducive to tumour growth and angiogenesis. Metabolic syndrome, by definition, is as multifactorial condition and it is associated with numerous other metabolic perturbations (192-194), together this suggests a complex metabolic profile that may facilitate prostate cancer growth (195); however, the literature lacks studies that integrate these factors. This study aims to develop an integrative approach to elucidate the complex interactions between metabolic syndrome and glucose-related metabolic perturbations and prostate cancer growth. We also aim to examine lifestyle factors (physical inactivity, nutrition intake, obesity) that may contribute to the development of these metabolic perturbations observed in prostate cancer.

The purpose of this study was to evaluate the presence of metabolic syndrome as well as specific features of glucose metabolism, lipid profile, body composition, nutrition intake and physical activity levels in individuals who were referred for a prostate biopsy (to assess the presence of prostate cancer). Moreover, we sought to identify specific components that associated with aggressiveness of prostate cancer, based on Gleason score obtained from pathology reports.

4.3.2 Objectives and Hypotheses

4.3.2.1 Objectives

In men who have been prospectively referred for a prostate biopsy:

1. To identify proportion of individuals with metabolic syndrome and evaluate the relationship between its presence and prospective prostate biopsy Gleason scores as a surrogate for cancer aggressiveness.

2. To evaluate the components of metabolic syndrome, additional metabolic measures (i.e. cytokines, adipokines, C-peptide, insulin, etc.) and lifestyle factors (i.e. activity levels, nutrition intake) that may explain the presence of metabolic syndrome and to how it may related to prospective prostate biopsy Gleason scores

3. To associate metabolic features (body composition, glucose- and lipid-related markers, inflammation) and lifestyle factors (habitual physical activity levels, functional capacity, nutritional intake) with the prospective prostate biopsy Gleason scores.

4.3.2.2 Hypotheses

1. Participants diagnosed with prostate cancer will be more likely to have metabolic syndrome as well as elevated HbA1C, fasting, glucose, insulin, and C-peptide compared with those who are not diagnosed with prostate cancer. Patients diagnosed with prostate cancer will likely be overweight or obese and possess lower than normal lean mass compared with participants not diagnosed with prostate cancer.

2. Participants with higher Gleason scores are more likely to present with features of metabolic syndrome, including obesity, glucose impairments, and dyslipidemia, compared with participants diagnosed with earlier stage prostate cancer and those not diagnosed with cancer.

4.4 Study 2: Prostate cancer patients experience impaired glucose tolerance following diagnosis, which is improved with radiation therapy independent of changes in traditional moderators of glucose metabolism

4.4.1 Rationale

Metabolic perturbations (metabolic syndrome, hyperglycemia, hyperinsulinemia, dyslipidemia, inflammation) are associated with prostate cancer, and specifically aggressive prostate cancer (12, 196-199). However, despite these associations, significant gaps exist in the literature in identifying the potential mechanisms that relate metabolic perturbations and prostate cancer diagnosis. It has been suggested that increased insulin, IGF-1, inflammatory cytokines, estradiol and leptin as well as decreased adiponectin, work together to create a more aggressive prostate cancer tumour biology in those with metabolic syndrome; however, these hypotheses remain untested (174). Results from Study 1 of this thesis suggest a cluster of metabolic perturbations, including elevated C-peptide, increased visceral adiposity, elevated leptin, and lower adiponectin , are associated with prostate cancer diagnosis. Increased C-peptide concentrations are indicative of increased insulin release from the pancreas (200). Since insulin is a potent cancer promoter (75-77), further investigations into the glucose metabolism of prostate cancer patients are warranted to further elucidate these findings.

As well, the relationship between the metabolic perturbation described by De Nunzio and colleagues (174) and prostate cancer development is most pronounced in high-risk prostate cancer patients (191). Findings from Study 1 of this thesis lend further support to this hypothesis whereby patients with the highest Gleason scores demonstrated the worst metabolic profile (elevated C-peptide, increased visceral adiposity, elevated leptin, lower adiponectin) compared to patients with lower Gleason scores. Thus, high-risk prostate cancer patients were chosen for Study 2 for more in-

38

depth evaluations of their glucose metabolism and related metabolic health because metabolic disturbances in these patients will be the most pronounced.

The metabolic health of high-risk prostate cancer patients is clinically important. Firstly, high-risk prostate cancer patients undergo numerous treatment options including radiation therapy (RT), prostatectomy, and androgen deprivation therapy (ADT) (40-42). However, little is known about the influence of these treatment types on host glucose and lipid metabolism. This is especially concerning since the ~26% of prostate cancer patients who develop diabetes following prostate cancer treatment undergo radiation therapy as their initial treatment, while ~45% undergo prostatectomy (108). Secondly, high-risk patients are most likely to receive ADT, which reduces testosterone to castrate levels and consequently decreases lean tissue and increases fat mass (29). Significant metabolic perturbations have been observed in these patients following long-term ADT use and these patients are at increased risk of diabetes and cardiovascular disease in survivorship (27); however, little is known about the acute treatment trajectory.

The purpose of the current study is to investigate and describe the potential changes in glucose metabolism and how they relate to body composition, other metabolic factors (i.e. inflammation), nutrition intake, and exercise characteristics in high-risk prostate cancer patients prior to treatment and during the acute phase of treatment (radiation therapy with or without ADT). Patients were examined prior to the start of treatment (baseline), immediately following the completion of either type of radiation therapy (~7 weeks), and after 6 months following completion of radiation therapy (~33 weeks).

In addition, glucose metabolism and related parameters (including insulin, C-peptide, lipid metabolism) become more impaired with aging. These 2 factors are associated with impaired glucose tolerance in non-malignant individuals (147, 148). Given that prostate cancer occurs in older men with increased body mass index (BMI) (17, 201), it is necessary to compare age- and BMI-matched

males to identify potential impairments in glucose metabolism that may be attributed to age and BMI. A comparison to a young healthy group of males may also identify the extent of any potential impairments in patients and matched males.

4.4.2 Objectives and Hypotheses

4.4.2.1 Objectives

1. To describe the metabolic profile, including glucose metabolism and related markers, body composition, lipid metabolism, inflammation, nutrition intake, physical activity, in high-risk prostate cancer patient prior to treatment initiation and to compare this profile to men of the same age and body size (matched males) and a young healthy comparison group (young males) to discern both age- and prostate cancer-related metabolic perturbations.

2. To describe potential changes in metabolism including glucose metabolism and related markers (body composition, lipid metabolism, inflammation, nutrition intake, physical activity) during the acute treatment trajectory (at 7 and 33 weeks from treatment initiation).

4.4.2.2 Hypotheses

1. Compared with age- and BMI-matched males, prostate cancer patients will have greater peak glucose, greater 2-hour glucose concentrations as well as greater area under the glucose curve following an oral glucose tolerance test (OGTT). Prostate cancer patients will also present with dyslipidemia, greater body fat mass, less muscle mass, and pro-inflammation compared with matched and young males.

2. During the course of treatment (~7 weeks) and 6 months following the end of treatment (~33 weeks), prostate cancer patients will present with further impairments in glucose metabolism and its related markers compared to pre-treatment. Prostate cancer patients will also demonstrate fat gains, lean tissue losses and increases in pro-inflammatory markers.

4.5 Study 3: Serum from high-risk prostate cancer patients does not induce cancer specific changes in glucose uptake in differentiated human skeletal muscle myotubes: developing a novel model to examine mechanisms of metabolic change in cancer patients

4.5.1 Rationale

One of the defining features of metabolic syndrome, an emerging independent risk factor for prostate cancer, is impaired glucose metabolism (157). The results of Study 2 demonstrated that, prior to treatment initiation, high-risk prostate cancer patients have normal fasting glucose concentrations (4.9±1.2mM). However, during an OGTT, patients exhibited impaired glucose tolerance with 2-hour glucose concentrations (the time point clinically used to determine impairments in glucose tolerance) >7 mM (8.7±2.9mM). Following 7 weeks of radiation therapy glucose tolerance was improved, as 2-hour glucose concentrations were <7 mM (6.8±1.5mM) and this improvement was maintained 37 weeks from baseline (2-hour glucose: 5.7±2.1mM). There were also no significant changes in physical activity levels or nutrition intake, which suggests improvements in glucose tolerance were independent of lifestyle changes . No significant changes were noted in insulin and C-peptide throughout the treatment trajectory, which suggested increased peripheral insulin sensitivity . This implies that there may be a systemic influence at the level of the muscle and possibly other peripheral tissues (liver, adipose). The presence of prostate cancer may influence glucose tolerance in these patients, independent of changes in glucose related hormones, such as insulin and C-peptide, and lifestyle determinants of glucose metabolism.

It has been suggested that metabolic perturbations associated with metabolic syndrome, such as increased inflammatory cytokines, increased insulin and IGF-1, altered the adipokine profile (increased leptin and decreased adiponectin) and increased estradiol, work together to create a more aggressive prostate cancer tumour biology in high risk-prostate cancer patients (174). These

perturbations may exacerbate impairments in glucose metabolism observed at diagnosis, such as in Study 2 ; however, the underlying mechanisms explaining these interactions are unclear. Cell culture and animal model studies are an appropriate starting point for these investigations, given the limited knowledge in this area and the difficulty of conducting invasive metabolic testing in a cancer patient population.

To investigate this response further, a novel *in vitro* cell culture model was developed, as cell culture allows complete control of the environment surrounding the cell. Skeletal muscle accounts for 80% of whole body post-prandial glucose disposal (202), and human skeletal muscle myotubes in culture respond to insulin *in vitro* allowing comparison between glucose uptake in both basal and insulin stimulated conditions (203,204); therefore, they were selected as a the model in which to observed changes in glucose metabolism. The use of skeletal muscle will allow us to investigate if there is a systemic signal driving the changes in glucose metabolism observed *in vivo*. The myotubes were exposed to serum collected from prostate cancer patients, prior to treatment to induce any cancer-related changes in glucose metabolism. Fasting serum was used to account to variability in hormone levels in the post-prandial state. Myotubes were also exposed to fasting serum from age- and BMI-matched males and young healthy males, to help explain for cancer- and age-related changes in glucose uptake. Serum from post-treatment (7 weeks and 33 weeks post-treatment initiation) was also used in the culture model to examine how glucose uptake may change during the acute treatment trajectory. This model allowed the investigation of systemically driven cancer-related changes in glucose uptake in skeletal muscle, the tissue that accounts for most glucose disposal of any tissue *in vivo*. These potentially unknown factors released from tumours to induce whole body changes in host glucose metabolism have previously been identified in pancreatic cancer patients (206). Basso and colleagues identified a 2030MW putative pancreatic cancer-associated factor that

was present in the serum of patients and was also capable of impairing glucose metabolism in hepatocytes in an *in vitro* model (206).

The purpose of this study was to investigate the capability of this novel method to measure impairments in glucose uptake in human skeletal muscle cells when exposed to serum from prostate cancer patients and compare this with serum from an age- and BMI-matched and a young healthy group of males. Moreover, the effects of patient serum collected throughout the treatment trajectory (baseline - prior to treatment, following ~7 weeks of RT, and ~37 weeks from baseline) on glucose uptake in cultured skeletal muscle myotubes was also investigated.

4.5.2 Objectives and Hypotheses

4.5.2.1 Objectives

1. To examine the effects of serum, collected from prostate cancer patients, on glucose uptake in cultured human skeletal muscle myoblasts compared with serum from age- and BMI-matched non-malignant males and a young healthy comparison group.

2. To examine the differences in glucose uptake in cultured human skeletal muscle cells incubated with serum collected from prostate cancer patients at baseline, following ~7 weeks of radiation therapy, and ~37 weeks following baseline evaluations.

4.5.2.2 Hypotheses

1. Glucose uptake will be decreased in human skeletal muscle cells cultured with prostate cancer serum as compared with an age- and BMI-matched comparison group and a young healthy comparison group.

2. Glucose uptake will be lower in prostate cancer patients prior to the initiation of cancer treatment. Following 7 weeks of radiation therapy, glucose uptake will increase and this improvement will be maintained at ~33 weeks following baseline.

Chapter 5

Study 1: C-peptide, Abdominal Obesity, and Adipokines, known Cardiovascular and Diabetes Risk Factors, are Associated with Higher Gleason Scores in Prostate Cancer

5.1 Authors

Katie M. Di Sebastiano[1], Jehonathan H. Pinthus[2], Wilhelmina C.M. Duivenvoorden[2], Laurel Patterson[2], Joel A. Dubin[3, 4], Marina Mourtzakis[1]

5.2 Affiliations

[1] Department of Kinesiology, University of Waterloo, Waterloo, ON, Canada

[2] Department of Surgery, Division of Urology, McMaster University, Hamilton, ON, Canada

[3] School of Public Health and Health Systems, University of Waterloo, Waterloo, ON, Canada

[4] Department of Statistics and Actuarial Science, University of Waterloo, Waterloo, ON, Canada

5.3 Abstract

Purpose: Prostate cancer development has been associated with numerous lifestyle factors (obesity, physical activity, nutrition intake) and metabolic perturbations. We aimed to characterize an integrative metabolic profile of men undergoing diagnostic prostate biopsies.

Materials and Methods: We prospectively evaluated 51 consecutive men for body composition, metabolic syndrome, glucose- and lipid-related measures and lifestyle factors prior to prostate biopsy. These factors were related to biopsy outcomes for: 1) presence or absence of cancer, and 2) where cancer was present, Gleason score.

Results: Serum C-peptide concentrations were significantly greater in participants with Gleason scores ≥4+3 (2.8±1.1 ng/mL) compared to those with Gleason 3+3 (1.4±0.6 ng/mL) or Gleason 3+4 (1.3±0.8 ng/mL). There were no significant differences in fasting glucose concentrations; however, insulin and insulin resistance (HOMA-IR) tended to be greater participants with Gleason ≥4+3, suggesting greater insulin secretion, compared to participants with Gleason 3+3 and 3+4. Central adiposity, measured by waist circumference, was significantly greater in participants with Gleason ≥4+3 compared to those with lower Gleason scores (Gleason ≥4+3: 110.1±7.4 cm vs Gleason 3+4: 102.0±9.5 cm). Men with Gleason ≥4+3 had significantly greater leptin concentrations than those with lower Gleason scores (Gleason ≥4+3: 15.6±3.3 ng/mL vs Gleason 3+4: 811±8.1 ng/mL, $p<0.05$) and leptin: adiponectin ratio (Gleason ≥4+3: 2.4±2.1 AU vs Gleason 3+4: 2.9±3.2 AU, $p<0.05$).

Conclusions: This is the first study to integratively show that serum C-peptide, visceral adiposity, serum leptin and leptin: adiponectin ratio are associated with high Gleason scores, suggesting a cluster of obesity-related metabolic perturbations that may contribute to aggressiveness in prostate cancer.

5.4 Introduction

Lifestyle factors including obesity (201), physical inactivity (206), and high-fat diets (207) are associated with prostate cancer. These factors are also associated with metabolic syndrome, an emerging risk factor for prostate cancer (160, 174, 208). Metabolic syndrome, a cluster of interrelated risk factors, is purported to increase risk of diabetes and cardiovascular disease (157). Despite the numerous definitions that exist for metabolic syndrome, the International Diabetes Federation (IDF) definition will be used here: central obesity (waist circumference (WC) >94 cm in males), with at least 2 other risk factors, including hypertension (SBP ≥130mmHg, DBP ≥85mmHg), raised fasting glucose (≥5.6 mM), raised triglycerides (TG, ≥1.7mM), and/or reduced high-density lipoprotein cholesterol (HDL, <1.0mM; 157). Of these components, central obesity and impaired glucose metabolism have been specifically associated with prostate cancer development (160, 174).

Abnormal metabolic features like obesity and insulin resistance may not affect prostate cancer in isolation, but instead work together to create a metabolic environment favourable for prostate cancer growth (174). Physical inactivity and high-fat diets contribute to obesity (210), and may contribute to metabolic perturbations associated with prostate cancer development (174). A comprehensive metabolic phenotype of prostate cancer patients will elucidate interactions between these features and identify potential predictors of prostate cancer development and aggressiveness.

To our knowledge, the integrative examination of these metabolic and clinical characteristics employed here is novel. The primary objective of this prospective observational study was to characterize the proportion of participants with metabolic syndrome, concurrently evaluating other metabolic parameters that might explain the presence or absence of criteria of the metabolic syndrome (WC, glucose, blood pressure, TG and HDL) including body composition (hip-circumference (HC), fat mass (FM), fat free mass (FFM), % body fat), glucose-related measures (insulin, C-peptide, insulin-like growth factor-1 (IGF-1), insulin-like growth factor binding protein-3

(IGFBP-3), lactate), additional features of lipid metabolism (total cholesterol, low-density lipoprotein cholesterol (LDL)), C-reactive protein (CRP), adiponectin, and leptin. To interpret these metabolic findings, lifestyle factors (habitual physical activity, functional capacity, nutrition intake) were also assessed. Our secondary objective was to associate these metabolic features with the surrogate measures of cancer aggressiveness using Gleason scores resulting from the prospective biopsy.

5.5 Methods

5.5.1 Participants

We consecutively and prospectively screened 139 men, with 51 men completing the study (Figure 5.1). Men were referred to a single uro-oncologist (JHP) with a clinical suspicion of prostate cancer presenting with elevated prostate specific antigen (PSA) levels and/or abnormal digital rectal exam (n=36). A second group (n=15) of low-risk prostate cancer patients under active surveillance were recruited prior to their surveillance biopsy. Exclusion criteria included previous diagnosis of cancer (other than basal cell carcinoma) not in remission for at least 3 years, current anti-neoplastic treatment, use of corticosteroids and chronic anti-pain medication for any reason. Participants' clinical characteristics are summarized in Tables 5.1 and 5.2. This study was reviewed and cleared by the University Of Waterloo Office Of Research Ethics and by Hamilton Health Sciences/Faculty of Health Sciences Research Ethics Board.
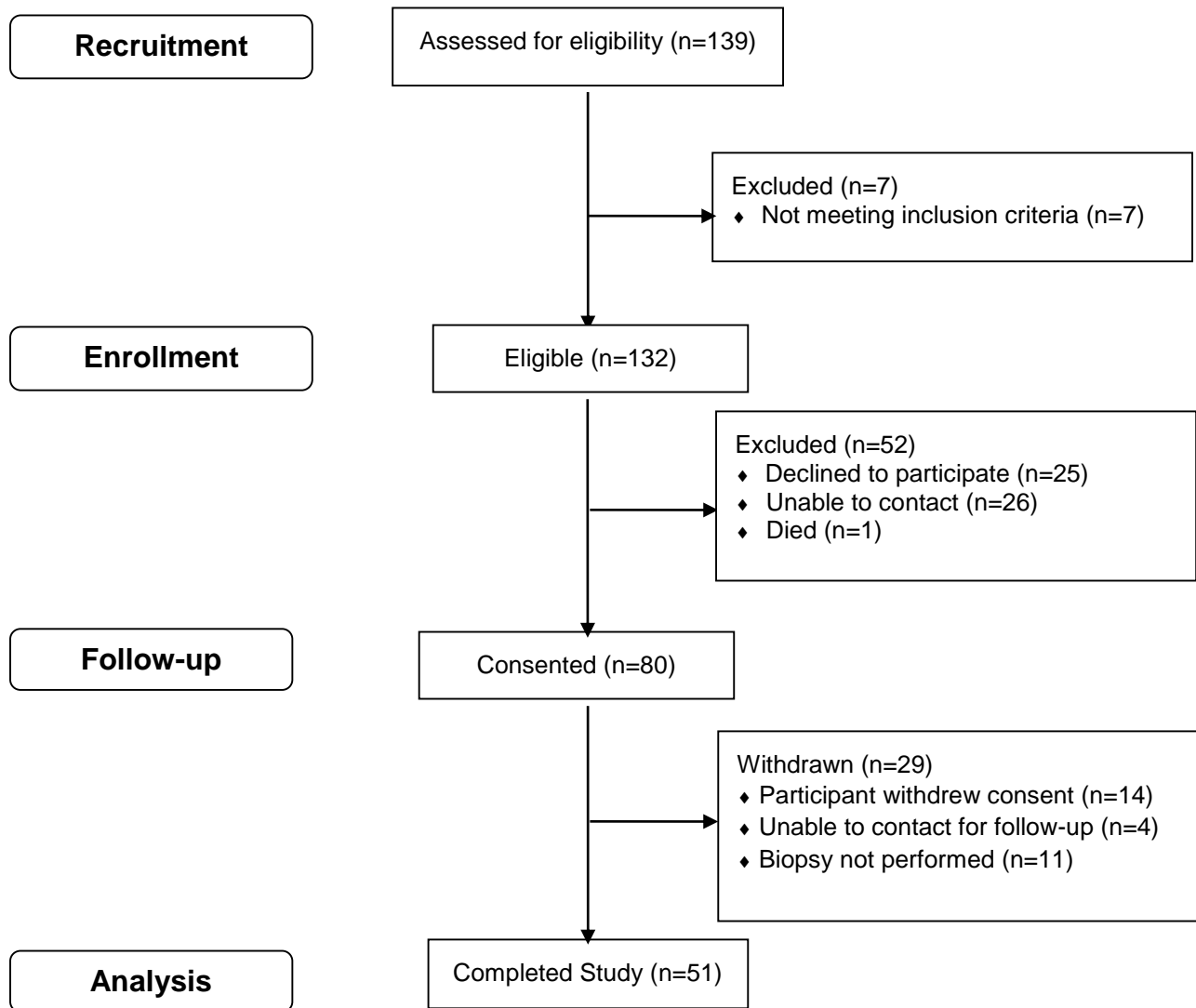
Figure 5.1: Participant recruitment flow diagram. This consort diagram describes the recruitment, enrolment, follow-up and analysis for the current study.

5.5.2 General Study Design

Participants were recruited prior to their prostate biopsy; the biopsy ultimately confirmed a positive or negative cancer diagnosis. To limit clinic visits and reduce participant burden, all study procedures were conducted prior to the biopsy on a single day. However, when this was not possible (n=2), study assessments were scheduled within 2 weeks of the biopsy date. Following data collection, biopsy pathology reports were used to stratify participants into 4 Gleason score categories: No cancer, Gleason 3+3, Gleason 3+4, and Gleason ≥4+3.

5.5.3 Biopsy Protocol

A single uro-oncologist (JHP) performed all transrectal ultrasound-guided biopsies. For patients with prostate cancer positive biopsies, a minimum of 16 cores were obtained (n=15) including 3 cores from the base, 3 cores from the mid, and 2 cores from both the right and left apex, including the far lateral aspects of these zones (211). Twenty-one patients had 26 core saturation biopsies, including the same 16 core template plus 2 cores from the transitional zone and 3 cores from both the left and right anterior (211).

5.5.4 Clinical Data

Medical history was assessed using chart review and participant self-report methods. Family history of cancer (in general and prostate cancer), active surveillance prior to current biopsy, PSA levels, treatment received following biopsy, and presence of bone metastases was collected via chart review. Previous diagnosis of cancer, other medical conditions (i.e. hypertension, high cholesterol, diabetes), current medications, and smoking status were collected using a medical screening questionnaire. Blood pressure was measured with a sphygmomanometer.

5.5.5 Blood Sampling

Blood was withdrawn after an overnight fast (8-12 hours with no food or drink except for water). Glycated hemoglobin (HbA1c) was analyzed using fresh whole blood. The remaining sample was allowed to clot, spun and serum was collected, aliquoted and stored until analyzed for glucose-related measures (glucose, insulin, C-peptide, IGF-1, IGFBP-3, lactate), lipid profiles (TG, total cholesterol, LDL, HDL), CRP, and adipokines (adiponectin, leptin).

5.5.6 Biochemical Analysis

HbA1c was analyzed using the commercially available A1CNow+ (Bayer, Sunnydale, CA). Glucose and lactate were assessed using spectrophotometric methods, as previously described (212). Insulin and C-peptide were analyzed using commercially available radioimmunoassay kits (Siemans Healthcare Diagnostics; Deerfield, IL). Lipid profiles including TG, total cholesterol, HDL, and LDL were analyzed spectrofluorophotometrically using commercially available regents (Pointe Scientific; Canton, MI). Leptin, adiponectin, IGF-1, IGFBP-3, and CRP were assessed using sandwich enzyme-linked immunosorbant assays (R&D Systems Inc, Minneapolis, MN)**.**

5.5.7 Insulin Resistance Calculations

Insulin resistance was assessed using fasting glucose and insulin values and the homeostatic model assessment for insulin resistance (HOMA-IR) equation (213).

5.5.8 Metabolic Syndrome Assessments

The IDF definition was used to define metabolic syndrome as follows: central obesity (WC >94 cm in males), with at least 2 other risk factors, including hypertension (SBP $\geq$ 135mmHg, DBP $\geq$85), raised fasting glucose ($\geq$5.6 mM), raised TG ($\geq$1.7 mM), and/or reduced HDL (<1.0 mM; 8).

5.5.9 Body Composition

BMI (kg/m^2) was calculated from weight and height recorded from medical charts. WC, taken (in cm) at the top of the iliac crests, and hip circumference (HC, in cm), at the level of greatest gluteal prominence (214), were used to assess waist-to-hip ratio, surrogate measures of visceral adiposity.

Single frequency-bioelectrical impedance analysis (SF-BIA; BIA-101S, RJL Systems, Clinton TWP, MI) was used to calculate FFM, FM, % body fat, SMM and SMI. Fasted participants lay supine while electrodes were placed below the knuckles on the prone side of the right hand and right wrist, and behind the toes of the right foot and right ankle. Reactance and resistance values were generated and used to estimate FFM with the equation described by Kyle et al (215). FFM was then used to estimate FM and % body fat. SMM was calculated using the equation described by Janssen et al (216). This value was then divided by height squared (m^2) to determine SMI (kg/m^2).

5.5.10 Functional Assessments and Questionnaires

Functional assessments included 6-minute walk test (6MWT), hand-grip strength test and the Godin Leisure Time Activity Questionnaire. The Godin Leisure Time Activity Questionnaire provided an evaluation of habitual activity (217). For the 6MWT, participants walked as quickly as possible on a 50m course for 6 minutes and distance travelled was recorded (218). Hand-grip strength was assessed using a Takie A5001 analogue hand-grip dynamometer. Participants held their elbow against their body at 90º with a neutral wrist position. They squeezed the hand dynamometer as hard as possible with their dominant hand and maximum force was recorded. Three trials separated by 1-minute breaks were recorded with the highest result deemed maximal strength (219).

5.5.11 Nutrition Intake

Participants completed a 3-day food diary over 2 weekdays and 1 weekend day during the week of their assessments (220). Participants were instructed to record all food and beverages

consumed each day and the location the food was consumed. Participants were also asked to record

any supplement take and whether the recorded eating pattern matched their usual eating patterns.

Caloric intake and macronutrient breakdown (% fat, % carbohydrate and % protein) was determined

from these records using ESHA Food Processor software and the Canadian Nutrient Files where

available and the USDA National Nutrient Database for Standard Reference when Canadian

information was not available.

5.5.12 Statistical Analysis

Values are presented as mean±SD. Statistical calculations were performed on Sigma Plot ®

version 11.2 (Systat Software Inc.; San Jose, CA). As the data met the assumptions of parametric

statistics, a one-way Analysis of Variance (ANOVA) was used for comparisons between the 4 groups

(no cancer, Gleason 3+3, Gleason 3+4, Gleason ≥4+3) for all measures using Tukey's post-hoc

analysis for pairwise comparisons. Linear regression was used to model the relationship between

cancer severity and metabolic, lifestyle and body composition measures using the best subset

regression approach to select the model. To limit repeated comparisons and parameters investigated,

only measures that were statistically significant or approaching significance ($p < 0.100$) among the

group comparisons were considered for linear regression. When a regression variable included

multiple components (i.e. HOMA-IR is calculated from glucose and insulin), individual components

as well as the multiple component variable were considered in the regression analysis (i.e. glucose,

insulin, and HOMA-IR were considered). Significance was identified at $p < 0.050$.

5.6 Results

Overall, participants were 66±7 years old (Range: 53-82 years old), with a BMI of 28.2±4.4

kg/m^2 (Table 5.1). Of the 51 patients, 38 patients (75%) were diagnosed with prostate cancer, 17 with

Gleason 3+3, 14 with Gleason 3+4, 5 with Gleason 4+3 and 2 patients with a Gleason Score >7. For

the purpose of analysis in this study, patients with Gleason 4+3 and Gleason >7 have been grouped together (Gleason ≥4+3). Treatment distribution is outlined in Table 5.2. Interestingly, patients without cancer were significantly younger than patients with Gleason ≥4+3 (62±7 vs 72±2 years, p=0.008; Table 5.2). Further analysis demonstrates that patients with Gleason ≥4+3 (61-76 years) have a narrower age range compared to all other groups. As expected, PSA levels were elevated (defined as >4.0 ug/L; 20) across the entire cohort (6.2±2.9 ug/L, Table 5.2); patients with Gleason ≥4+3 had significantly higher PSA levels compared to the no cancer group (p=0.019, Table 5.2).

Table 5.1: Participant Characteristics (n=51)	
Age (years)	66 ± 7
Systolic Blood Pressure (mmHg)	135 ± 14
Diastolic Blood Pressure (mmHg)	85 ± 8
Height (cm)	175.7 ± 6.4
Weight (kg)	89.3 ± 14.9
BMI (kg/m^2)	28.9 ± 4.4
Underweight: BMI <18.5 kg/m^2 (n)	0 (0%)
Normal: BMI 18.5 – 24.9 kg/m^2 (n)	8 (17.0%)
Overweight: BMI 25.0 – 29.9 kg/m^2 (n)	21 (44.7%)
Obese: BMI 30.0 – 34.9 kg/m^2 (n)	13 (27.7%)
Obese: BMI >35.0 kg/m^2 (n)	5 (10.6%)

Metabolic syndrome was identified in 32 of 51 (63%) participants based on IDF criteria (8), 21 (66%) of which were diagnosed with cancer (Table 5.3). Remarkably, all 51 patients had at least one metabolic syndrome risk factor (Table 5.3). Also, 24 participants self-identified as hypertensive and were prescribed medication, 24 participants self-identified with lipid abnormalities (hypercholesterolemia) and were prescribed medication, and 9 participants self-identified as diabetic or prediabetic (Table 5.3). Interestingly, a large proportion of participants still had metabolic syndrome, despite receiving medical treatment to manage elements of this condition.

Table 5.2: Clinical Characteristics

	All participants (n=51)	No Cancer (n=13)	Gleason 3+3 (n=17)	Gleason 3+4 (n=14)	Gleason ≥4+3 (n=7)
Patient Outcomes					
Age (years)	66 ± 7	62 ± 6	65 ± 7	67 ± 7	71 ± 5*
Range	53-82	53-82	53-76	53-76	61-76
PSA (ng/mL)	6.1 ± 3.3	4.3 ± 2.0	6.2 ± 2.9	6.7 ± 2.2	8.2 ± 4.2*
Active Surveillance Prior to Current Biopsy (n)	15	-	9	4	2
Treatment					
Active Surveillance (n)	17	-	15	2	0
Radiation Therapy (n)	5	-	1	0	4
Androgen Deprivation Therapy (n)	2	-	0	1	1
Radical Prostatectomy (n)	13	-	1	10	2
Bone Metastases (n)	2	-	0	1	1
Number of Positive Biopsy Cores (n)	5 ± 4	-	3 ± 2	8 ± 3	7 ± 3
Percent Tissue Involved (%)	7.0 ± 10.7	-	3.4 ± 8.0	7.3 ± 7.0	20 ± 20
Medical History					
Family History of Cancer (n)	24	8	10	3	3
Family History of Prostate Cancer (n)	9	3	4	1	1
Previous Cancer (n)	12	3	2	6	1
Basel Cell Carcinoma (n)	7	2	1	4	0
Colorectal (n)	1	0	0	1	0
Bladder (n)	1	0	1	0	0
Kidney (n)	1	0	0	1	0
Testicular (n)	1	0	0	0	1
Male Breast Cancer (n)	1	1	0	0	0
Hypertension (n)	24	9	3	8	4
High Cholesterol (n)	24	5	9	5	5
Diabetes (n)	9	2	2	3	2
Smoking					
Current (n)	7	4	1	2	0
Ex (n)	25	4	12	3	6
Never (n)	19	6	4	8	1

* indicates significantly different from all other groups

Table 5.3: Assessment of the components of metabolic syndrome					
	All participants (n=51)	No Cancer (n=13)	Gleason 3+3 (n=17)	Gleason 3+4 (n=14)	Gleason ≥4+3 (n=7)
Metabolic syndrome (n)	32 (63%)	11 (84%)	12 (70%)	3 (17%)	6 (86%)
Central Obesity + 1 Risk Factor (n)	39 (76%)	13 (100%)	14 (82%)	5 (36%)	7(100%)
At least 1 Risk Factor (n)	51 (100%)	13 (100%)	17 (100%)	14 (100%)	7 (100%)
Central Obesity (n)	39 (76%)	12 (92%)	14 (82%)	5 (36%)	7 (100%)
Hypertension (n)	46 (90%)	13 (100%)	15 (88%)	11 (79%)	6 (86%)
Abnormal Fasting Glucose (n)	18 (35%)	7 (54%)	4 (23%)	4 (29%)	3 (43%)
Abnormal TG (n)	8 (15%)	5 (38%)	0 (0%)	2 (14%)	1 (14%)
Abnormal HDL (n)	22 (43%)	5 (38%)	8 (47%)	3 (17%)	5 (71%)
Using Lipid Altering Medications (n)	24 (47%)	5 (38%)	9 (52%)	5 (36%)	5 (71%)

5.6.1 Glucose and Insulin Metabolism

Fasting C-peptide concentrations (indicative of insulin secretion) were significantly greater in Gleason ≥4+3 patients versus Gleason 3+3 and Gleason 3+4 (Gleason ≥4+3: 2.8±1.1 ng/mL; Gleason 3+3 1.4±0.6 mg/mL; Gleason 3+4: 1.3±0.8 mg/mL, p=0.002; Figure 5.2C), despite no differences in fasting glucose (p=0.101, Figure 5.2A), lactate (p=0.885, Table 5.4) and HbA1c values (p=0.834, Table 4.4). Fasting insulin was 17.5±10.0 uIU/mL in Gleason ≥4+3 patients compared to 7.5±4.6 uIU/mL in Gleason 3+4, 8.6±3.9 uIU/mL in Gleason 3+3 and, 11.7±10.2 uIU/mL in the No cancer group (p=0.087; Figure 5.2B). Insulin resistance, measured by HOMA-IR, was highest in patients with Gleason ≥4+3 though this did not reach statistical significance (p=0.07, Figure 5.2D). Despite that IGF-1 shares a signalling cascade with insulin, no differences were observed in IGF-1 (p=0.546), IGFBP-3 (p=0.432) or IGF-1:IGFBP-3 ratio between any of the groups (p=0.123, Table 5.4). Collectively, these data suggest emerging insulin resistance in Gleason ≥4+3 patients.

Figure 5.2: Glucose metabolism related measures: All indicate the combined averages of All participants, NC represents the No Cancer groups; 3+3 represents the Gleason 3+3 group, 3+4 represents the Gleason 3+4 group, and ≥4+3 represented the Gleason ≥4+3 group.

56

5.4: Glucose- and fat-related measures, and inflammatory markers

	All participants	No Cancer (n=13)	Gleason 3+3 (n=17)	Gleason 3+4 (n=14)	Gleason ≥4+3 (n=7)	Reference Value
Glucose-Related Measures						
HbA1c (%)	5.9 ± 0.9	5.9 ± 0.5	5.7 ± 0.4	6.1 ± 1.5	6.0 ± 1.0	<6.0
Lactate (mM)	3.8 ± 1.4	3.8 ± 1.3	3.8 ± 1.5	3.9 ± 1.7	3.5 ± 1.5	<2
IGF-1 (ng/mL)	92.3 ± 24.4	95.1 ± 26.3	90.0 ± 27.6	87.0 ± 19.0	102.9 ± 23.4	105
IGFBP-3 (ng/mL)	2315.6 ± 583.2	2327.6 ± 798.6	2197.7 ± 523.3	2522.0 ± 454.1	2170.7 ± 467.6	~2375
IGF-1:IGFBP-3 Ratio (AU)	0.18 ± 0.05	0.20 ± 0.06	0.18 ± 0.05	0.15 ± 0.04	0.17 ± 0.03	
Lipid-Related Measures						
Total Cholesterol (mM)	4.8 ± 1.1	4.9 ± 1.0	5.0 ± 0.9	4.7 ± 1.3	4.2 ± 1.3	<5.2
HDL (mm)	1.1 ± 0.5	1.0 ± 0.3	1.2 ± 0.6	1.3 ± 0.4	0.9 ± 0.4	>1.0
LDL (mM)	3.1 ± 1.1	3.1 ± 0.9	3.4 ± 0.9	3.0 ± 1.3	2.5 ± 1.0	<3.3
TG (mM)	1.2 ± 0.7	1.6 ± 1.0	0.9 ± 0.4	1.2 ± 0.6	1.5 ± 0.4	<1.7
Inflammation						
CRP (mg/L)	2.1 ± 1.7	1.5 ± 0.7	2.6 ± 2.6	1.8 ± 0.9	2.8 ± 1.6	<3

5.6.2 Body Composition

Given that obesity is associated with abnormal insulin signaling (222), body composition may elucidate the relationship between increased insulin secretion and aggressive cancers. Approximately, 80% of participants were overweight (BMI 25.0–29.9 kg/m^2, n=21) or obese (BMI >30.0 kg/m^2, n=20), while no participants were underweight (BMI <18.5 kg/m^2) (Table 5.1). Although it was not statistically significant, patients with Gleason ≥4+3 had the largest BMI (p=0.090, Table 5.5). On average, WC was 102.8±11.7 cm, indicative of abdominal obesity (IDF cut-point: >102 cm; Table 5.5). Patients with Gleason ≥4+3 had significantly larger WC when compared to patients with Gleason 3+4 (112.4±6.7 cm vs 97.5±13.7 cm, p=0.028, respectively, Figure 5.3A). Similarly, Gleason ≥4+3 patients had significantly greater HC than Gleason 3+4 patients (110.1 ± 7.4 cm vs 102.0 ± 9.5 cm, p=0.034, respectively, Figure 5.3B). As well, there was a main effect for waist-to-hip ratio, though no interactions were identified (p=0.048, Figure 5.3C). Though not statistically significant, patients with Gleason ≥4+3 had the highest estimates of FM, FM Index, % body fat, and SMM compared to the other groups (FM: p=0.090; FM Index: p=0.087; % body fat: p=0.058, Table 5.5). There were no significant differences were observed in FFM, however, estimated SMM was highest in participants no cancer, though this did not reach significance (p=0.080). Body fat, specifically visceral adiposity, may contribute to aggressive prostate cancer development based on these data.

Table 5.5: Anthropometric and BIA derived body composition measures					
	All participants	No Cancer (n=13)	Gleason 3+3 (n=17)	Gleason 3+4 (n=14)	Gleason ≥4+3 (n=7)
Height (cm)	175.7 ± 6.4	177.5 ± 6.2	176.8 ± 5.5	175.4 ± 6.9	169.6 ± 5.4
Weight (kg)	89.3 ± 14.9	93.7 ± 13.5	89.4 ± 14.8	83.9 ± 17.4	92.9 ± 9.7
BMI (kg/m^2)	28.9 ± 4.4	29.7 ± 3.3	28.6 ± 4.3	27.3 ± 5.0	32.2 ± 2.8
Fat Free Mass (kg)	66.4 ± 10.1	70.7 ± 7.9	65.8 ± 9.5	65.8 ± 12.4	61.3 ± 8.9
Fat Free Mass Index (kg/m^2)	21.4 ±2.8	22.4 ± 2.1	20.9 ± 2.6	21.1 ± 3.7	21.3 ± 2.6
Fat Mass (kg)	24.0 ± 9.6	23.0 ± 7.2	24.3 ± 7.2	20.8 ± 13.3	31.6 ± 7.8
Fat Mass Index (kg/m^2)	7.8 ± 3.1	7.2 ± 2.0	7.8 ± 2.4	6.7 ± 4.0	11.0 ± 2.8
% Body Fat	26.0 ± 8.1	24.1 ± 4.9	26.6 ± 4.7	23.3 ± 11.6	33.9 ± 8.0
Skeletal Muscle Mass (kg)	33.5 ± 6.4	36.0 ± 4.2	33.8 ± 7.1	32.0 ± 7.3	31.0 ± 4.0
SMM Index (kg/m^2)	10.8 ± 1.8	11.4 ±1.0	10.7 ± 2.0	10.4 ± 2.2	10.8 ± 1.2

[†] indicates significantly different from Gleason 3+4; [‡] indicates main effect
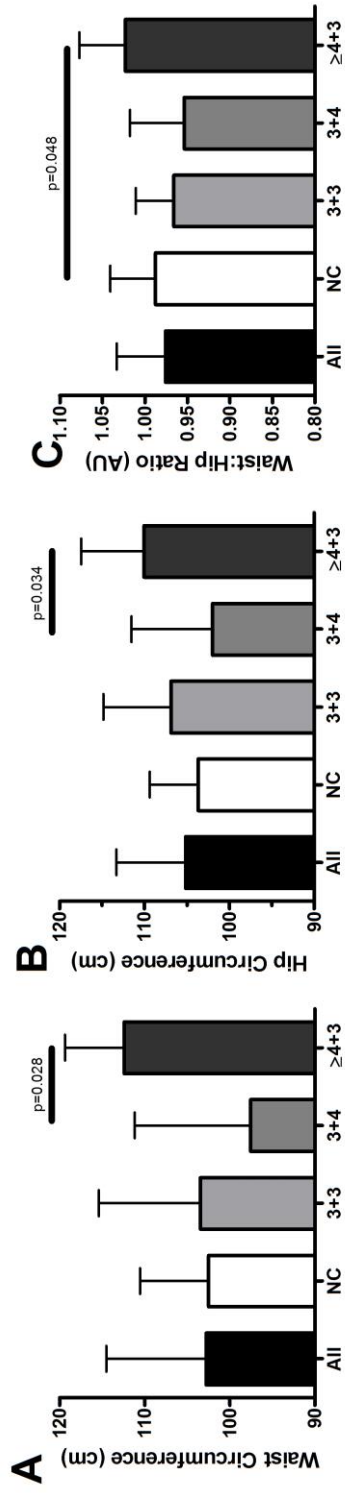
Figure 5.3: Circumference Assessments: All indicate the combined averages of All participants, NC represents the No Cancer groups; 3+3 represents the Gleason 3+3 group, 3+4 represents the Gleason 3+4 group, and ≥4+3 represented the Gleason ≥4+3 group.

60

5.6.3 Adipokines, C-reactive Protein, and Lipid Metabolism

Adipokines are the signalling molecules linking obesity to insulin resistance (223). Gleason ≥4+3 patients had higher leptin levels compared with Gleason 3+4, but not other groups (Gleason ≥4+3: 15.6±3.3 ng/mL, Gleason 3+4; 8.1±8.1 ng/mL, Gleason 3+3: 8.5±5.4ng/mL, No cancer: 7.9±4.0 ng/mL, p=0.013; Figure 5.4A). Adiponectin concentrations were lower in Gleason ≥4+3 patients compared with the other groups, but this did not reach statistical significance (No cancer: 5.87±3.69 pg/mL; Gleason 3+3: 9.65±6.91 pg/mL; Gleason 3+4: 8.05±5.93 pg/mL; Gleason ≥4+3: 5.05±5.10, pg/mL, p=0.069, Figure 5.4B). Thus, leptin: adiponectin ratio was highest in Gleason ≥4+3 patients (Gleason 3+3: 2.4±2.1 AU, Gleason 3+4; 2.9±3.2, Gleason ≥4+3: 9.7±6.1 AU, p=0.013, Figure 5.4C). There were no significant differences observed in CRP between any of the groups (p=0.265, Table 5.4).

There were no significant differences in the lipid profile (total cholesterol, HDL, LDL, TG) between any of the groups (Table 5.5). However, only patients with Gleason ≥4+3 had HDL levels (0.9 ± 0.4 mM) below the IDF cut point (1.0mM; 157).
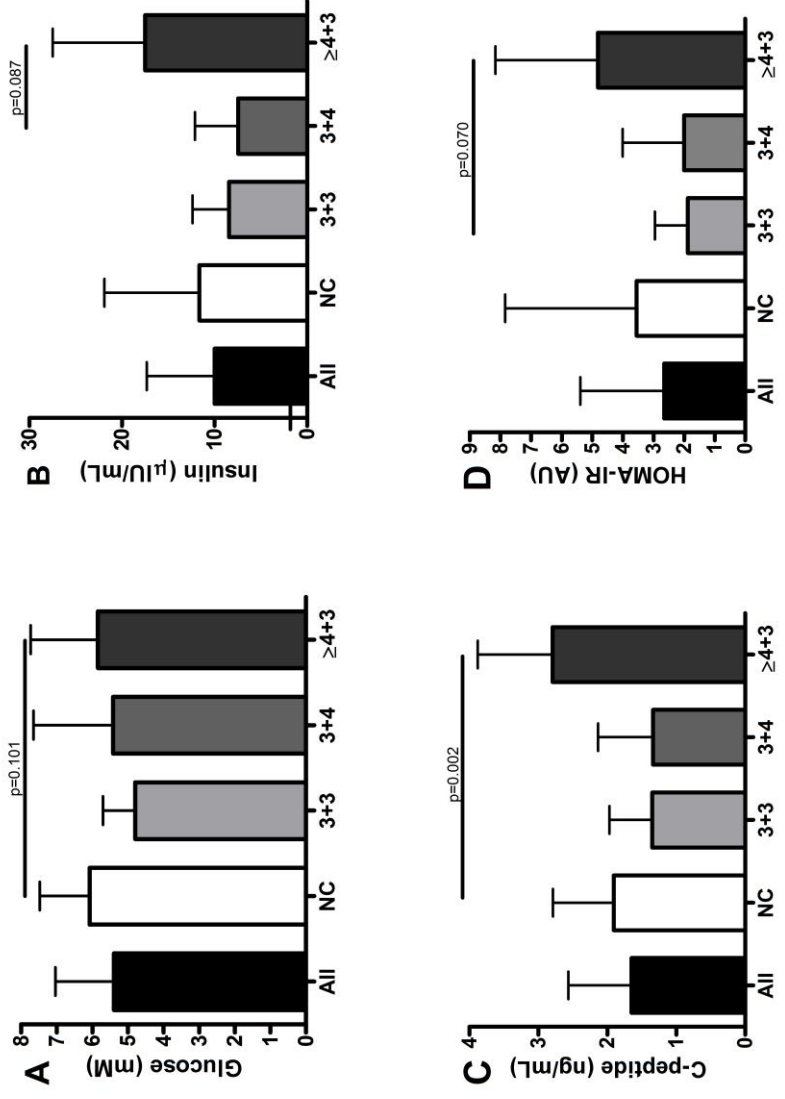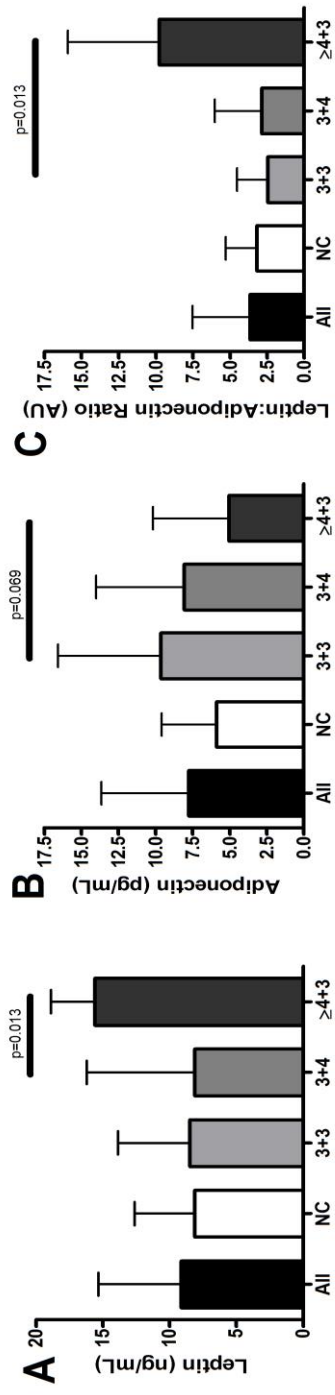
Figure 5.4: Adipokine Assessments All indicate the combined averages of All participants, NC represents the No Cancer groups; 3+3 represents the Gleason 3+3 group, 3+4 represents the Gleason 3+4 group, and ≥4+3 represented the Gleason ≥4+3 group.

5.6.4 Functional Assessment, Habitual Physical Activity Levels, and Dietary Intake

Traditional lifestyle factors that moderate of glucose metabolism, functional capacity, habitual physical activity, nutritional intake, and macronutrient distribution, were not significantly different between any of the groups (Table 5.6). Patients walked 546.5±88.7m in 6 minutes, which was 104±16% of their predicted walking distance (218). Average handgrip strength was 44.5±8.6 kg (Reference 40±8.3; 219) and habitual physical activity measured by Godin Score was 28±23 AU (Reference: 38±34; 217). On average participants consumed 2363±631 kcal per day, consisting of 43±8% carbohydrate, 19±4% protein and 35±5% fat (Table 5.6).

Table 5.6: Functional Measures and Nutritional Intake						
	All participants	No Cancer (n=13)	Gleason 3+3 (n=17)	Gleason 3+4 (n=14)	Gleason ≥4+3 (n=7)	Reference Value
Functional Measures						
6-minute Walk Test (m)	546.5 ± 88.7	583.4 ± 46.9	528.8 ± 93.4	574.3 ± 102.8	493.6 ± 65.2	104 ± 16% of predicted
Handgrip Strength (kg)	44.5 ± 8.6	45.9 ± 8.4	43.9 ± 10.2	45.8 ± 7.9	39.9 ± 5.5	~40
Godin Score (AU)	28 ± 23	23 ± 22	24 ± 21	38 ± 28	27 ± 21	~38
Nutritional Intake						
Total Calories (kcal)	2363 ± 631	2540 ± 1001	2292 ± 409	2368 ± 407	2095 ± 346	~1900
Carbohydrates (%)	43 ± 8	41 ± 8	45 ± 7	45 ± 9	40 ± 11.5	45-60
Protein (%)	19 ± 4	20 ± 5	17 ± 4	18 ± 5	22 ± 2	15-20
Fat (%)	35 ± 5	36 ± 4	35 ± 5	34 ± 4	36 ± 7	20-35

5.6.5 Linear Regression Analysis

Multiple linear regression revealed age, PSA, leptin: adiponectin ratio, and HC were significantly related to Gleason scores. Leptin: adiponectin ratio and HC were correlated; thus,

including both in the model was unnecessary. The following model was found to modestly but significantly explain the variation in Gleason score:

$$Gleason\ Score = (0.0456 \times Age\ (years) + \left(0.103 \times PSA\ \left(\frac{ng}{mL}\right)\right)$$

$$+ \left(0.000138 \times Leptin{:}Adiponectin\ (AU)\right) - 2.630$$

This model provides an r^2 value of 0.398. Age, PSA, and leptin to adiponectin ratio were statistically significant in the model (p=0.013, p=0.021 and p=0.027, respectively).

5.7 Discussion

This is the first study, to our knowledge, to comprehensively integrate and evaluate the metabolic characteristics of men, prospectively and consecutively recruited following referral to an uro-oncologist for prostate biopsy. After obtaining biopsy outcomes, these characteristics were associated with corresponding Gleason scores.

C-peptide concentrations were highest in participants with Gleason ≥4+3 compared with other Gleason scores, in line with tendencies exhibited in fasting insulin concentrations and HOMA-IR. Central adiposity (measured by WC) is associated with insulin resistance (222) and was significantly larger in Gleason ≥4+3 patients. Gleason ≥4+3 patients also had significantly greater leptin and leptin: adiponectin ratios compared with all other groups. These adipokine perturbations may be associated with insulin resistance (223) and visceral adiposity (223, 224). Collectively, these data suggest adiposity-related metabolic sequelae that contribute to aggressive prostate cancer.

5.7.1 Impaired Markers of Glucose Metabolism are Associated with Higher Gleason Scores

Despite similar fasting glucose concentrations across Gleason score categories, C-peptide concentrations were greatest in men with Gleason ≥4+3 suggesting more insulin secretion in this group. Previous work has demonstrated both positive (138) and neutral (225) associations between

64

prostate cancer aggressiveness and C-peptide concentrations.   Higher baseline C-peptide

concentrations have also been shown to increase the likelihood of prostate cancer-specific death

(128).

Elevated C-peptide concentrations aligned with fasting insulin concentrations and the

HOMA-IR that approached significance in participants with Gleason ≥4+3. Hyperinsulinemia is

hypothesized to link obesity to prostate cancer development, whereby insulin creates a metabolic

milieu favourable for cancer growth. Insulin receptors are found in abundance on human prostate

tumours27, allowing activation of Akt and MAPK pathways, ultimately resulting in proliferation and

apoptosis inhibition (88). IGF-1 is also proposed to stimulate prostate cancer cell growth through the

same mechanisms (226). Increased IGFBP-3 binding, the major binding protein of IGF-1, prohibits

IGF-1 binding to the prostate cancer cell, ultimately reducing proliferation (226). We found no

differences across Gleason scores for IGF-1, IGFBP-3, and IGF-1:IGFBP-3 ratio; however, it may

not be possible to detect differences across Gleason scores in these measures (227).

5.7.2 Visceral Adiposity, a feature of metabolic syndrome, is related to insulin resistance

The majority of the study participants (63%) had metabolic syndrome, and all participants

had a least one risk factor of metabolic syndrome. Metabolic syndrome by definition is a

multifactorial diagnosis; however, central obesity measured by WC is required criteria for all patients

diagnosed with this syndrome (157). Patients with Gleason ≥4+3 had larger WC and waist-to-hip

ratio than those with lower Gleason scores. WC >94 cm, the metabolic syndrome cut-point, may be

an independent risk factor for prostate cancer (160), with both positive (160) and neutral (228)

associations demonstrated. WC is a measure of visceral adiposity, a key factor numerous disease

states including metabolic syndrome, diabetes and cardiovascular disease (223). Increased visceral

adiposity is associated with increased basal insulin and C-peptide levels (228), supporting the

elevations in C-peptide and insulin observed here. Similarly, HC and waist-to-hip ratio were significantly greater in patients with the Gleason ≥4+3. Few studies consider waist-to-hip ratio as a marker of visceral adiposity in prostate cancer, but like WC positive (229) and neutral (228) associations have been demonstrated.

This variability may be related to study design and participants because geographic and cultural differences lead to different metabolic, dietary and activity profiles. However, considerations of visceral adiposity may need to include other metabolic features including glucose- (insulin, C-peptide) and lipid-related parameters, such as adipokines, as visceral adiposity is associated with increased leptin and decreased adiponectin levels (223).

5.7.3 Adipokines are Associated with Visceral Adiposity and Hyperinsulinemia

Leptin and leptin: adiponectin ratios were significantly higher in patients with Gleason ≥4+3. Both leptin and adiponectin are independently associated with prostate cancer development (132, 230). Leptin can stimulate cancer cell growth and angiogenesis (132), while adiponectin may have anti-proliferative functions (230). Leptin: adiponectin ratio is emerging as an important predictor of prostate cancer risk, with Burton et al reporting elevated leptin, decreased adiponectin, and higher leptin: adiponectin ratios in aggressive prostate cancer (198).

Adiponectin and leptin are hypothesized to link adiposity to the development of insulin resistance. Increased adiposity is associated with higher leptin and decreased adiponectin levels and hyperinsulinemia may result from changes in circulating adipokines, potentially stimulating prostate cancer proliferation beyond the independent effects of leptin and adiponectin (223). We observed increased visceral adiposity, leptin and C-peptide levels in patients with Gleason ≥4+3. This suggests a cluster of metabolic disturbances working via numerous pathways, creating a metabolic environment conducive to aggressive prostate cancer development.

66

5.7.4 Age is an Important Risk Factor for Prostate Cancer

Age is the strongest known risk factor for prostate cancer. In Canada, 97.8% of all prostate cancer diagnoses occur in men over the age of 50 (17) and by age 90, 80% of men have been shown to have cancerous cells in their prostate (52). Increased age is also associated with more aggressive cancers (231), and therefore, it is unsurprising that in the current study patients with the most aggressive cancers are significantly older than those with no cancer. However, age is also associated with increased insulin resistance (232) and obesity (233). Consequently, as patients with the most aggressive cancers (Gleason score ≥4+3) were significantly older than patients with less aggressive cancers, some of the differences in C-peptide and central obesity may be explained by the increased age of this group.

5.7.5 The Metabolic Sequelae of Aggressive Cancer may Impact Prostate Cancer Survivorship

We describe a cluster of metabolic perturbations related to aggressive prostate cancer; however, this cluster may also pose significant risk for prostate cancer survivors. Obesity (234), hypertension (234), impaired fasting glucose (235), and dyslipidemia (235), features of metabolic syndrome, have been associated with increased risk of prostate cancer recurrence. However, recurrence is not the only concern for aggressive prostate cancer patients, as they are more likely to receive ADT. ADT causes significant metabolic perturbation (236) and is associated with diabetes and cardiovascular disease in survivorship (27). The negative effects of ADT may be exacerbated in patients with aggressive prostate cancer as they exhibit metabolic perturbations at diagnosis.

5.7.6 Study Considerations

The prospective study design allowed investigators to be blinded to biopsy outcomes and facilitated the inclusion of the no cancer group. Though, no cancer was confirmed by a negative

extended prostate biopsy (≥16 cores), this group may differ from men who have never had a prostate biopsy. This single-institution cohort is relatively small, consequently, a limited number of variables were considered in the linear regression analysis. However, the small sample size allowed for characterization of a comprehensive metabolic profile, resulting in the novel integration of the metabolic and clinical characteristics of these men. These data will serve as the foundation for future, larger-scale studies to further examine these interactions.

5.8 Conclusions

Overall, this investigation revealed a cluster of adiposity related abnormalities in participants with high Gleason scores, when compared to participants with lower Gleason scores. Specifically, patients with Gleason ≥4+3 had increased C-peptide concentrations, increased visceral adiposity, lower than normal HDL, increased leptin, and leptin: adiponectin ratio. These finding suggest aggressive prostate cancer is associated with a set of adiposity driven metabolic perturbations. Further investigations into these metabolic sequelae and their association with high-risk disease is warranted to elucidate the mechanisms driving this development and to identify interventions to combat this profile.

Chapter 6

Study 2: Prostate Cancer Patients Experience Impaired Glucose Metabolism Following Diagnosis but Improves Following Radiation Treatment

6.1 Authors

Katie M. Di Sebastiano[1], Kirsten E. Bell[2], Andrew S. Mitchell[1], Joe Quadrilatero[1], Pierre Fortin[3], Ramana Rachakonda[3], Joel A. Dubin[4, 5], Marina Mourtzakis[1]

6.2 Affiliations

[1] Department of Kinesiology, University of Waterloo, Waterloo, ON, Canada

[2] Department of Kinesiology, McMaster University, Hamilton, ON, Canada

[3] Grand River Regional Cancer Centre, Grand River Hospital, Kitchener, ON, Canada

[4] School of Public Health and Health Systems, University of Waterloo, Waterloo, ON, Canada

[5] Department of Statistics and Actuarial Science, University of Waterloo, Waterloo, ON, Canada

6.3 Abstract

Background: Obesity and age, key risk factors for aggressive prostate cancer, are associated with insulin resistance. Glucose-related parameters in aggressive prostate cancer patients were compared with 2 reference groups: men of similar age and body mass index (BMI) without cancer and healthy young men. Acute changes in these parameters following radiation treatment were also evaluated.

 Methods: Nine high-risk prostate cancer patients underwent metabolic assessments prior to treatment (baseline), 7 and 33 weeks post-baseline (post-treatment initiation). Baseline measures were compared with the 2 reference groups. Evaluations included: 1) fasting and oral glucose tolerance test (OGTT) blood samples for glucose, C-peptide, and insulin, 2) fasting blood samples for triglycerides, cholesterols, leptin, adiponectin, IL-6, and TNF-α, 3) body composition, 4) nutrition intake, and 5) physical activity.

Results: At baseline, patients had normal fasting glucose concentrations (<5.6mM; 4.9±1.2mM) but impaired 2-hours OGTT glucose concentrations (>7.8mM; 8.7±2.9mM). Both reference groups had normal fasting (matched males: 4.2±0.5mM; young males: 3.7±0.4mM) and 2-hour OGTT glucose concentrations (matched males: 5.6±1.8mM; young males: 3.1±0.1mM) that were significantly lower than patient values. During the OGTT, patients had higher insulin (120 minutes) and C-peptide (45, 60, 90, 120 minutes) concentrations compared to the matched males. At 7 weeks, 2-hour OGTT glucose concentrations in patients improved to healthy ranges without changes in insulin, C-peptide, IGF-1, IGFBP-3 or other metabolic parameters.

Conclusions: At baseline, high-risk prostate cancer patients demonstrated impaired glucose tolerance compared with men of similar age and body size. Following treatment, glucose tolerance improved in absence of changes in expected modifiers of glucose metabolism. These improvements may be related to treatment.

6.4 Introduction

Age is the strongest known non-modifiable risk factor for prostate cancer whereby 97.8% of all Canadian prostate cancer diagnoses occur in men over 50 years old, while <1% of cases occur in men under the age of 40 (17). Obesity (201) and metabolic syndrome (160, 174) have emerged as key modifiable risk factors for prostate cancer development. Impaired insulin and adipokine signalling develop with aging (237) and obesity (238, 239). Prostate cancer patients typically exhibit hyperinsulinemia (12, 196), elevated C-peptide concentrations (128), dyslipidemia (196, 188), adipokine perturbations (198), and/or pro-inflammation (199) that contribute to tumour development and influence the presence of comorbidities. To further complicate these interrelationships, prostate cancer diagnosis is independently associated with increased number of cardiovascular events (240). Given the prevalence of insulin resistance and prostate cancer in older men, it is important to distinguish whether these metabolic perturbations are related to the cancer or underlying co-morbidities that often accompany older men with prostate cancer. Understanding the underlying mechanisms of these interrelated metabolic deviations, independent of age and body size, is also important in preventing/managing comorbidities and cancer recurrence in survivorship.

Different treatment regimens may possess unique metabolic disturbances that may contribute to development of CVD and diabetes or exacerbate existing metabolic conditions. Androgen deprivation therapy (ADT) has been associated with increased risk of diabetes and cardiovascular disease (CVD) in prostate cancer survivors (27, 241), and it is thought that these risks are related to muscle loss and adipose tissue gains during ADT. In prostate cancer patients who developed diabetes after diagnosis, 50.2% had received a prostatectomy, 21.4% received radiation therapy (RT) 12.1% received hormonal therapy, and 16.3% underwent active surveillance as their primary treatment type (108). Interestingly, RT is often used in conjunction with prostatectomy and ADT, yet the specific

71

metabolic consequences (i.e. glucose, insulin, C-peptide, adipokine metabolism) of these treatments, including RT, are largely unknown.

The purpose of this study was to examine the metabolic consequences of prostate cancer diagnosis and potential changes during the acute treatment trajectory (primarily RT with secondary ADT). Since age and body size influence the development of CVD and diabetes in non-malignant and prostate cancer populations, our first objective was to characterize and describe differences in glucose metabolism as well as lipids, body composition, cytokines, dietary intake, and physical activity between newly diagnosed prostate cancer patients, men of the same age and body size (matched males), and a young healthy comparison group (young males). Moreover, RT is intended to have localized effects on the tumour, which may directly or indirectly lead to metabolic perturbations on host-metabolism. Hence, our second objective was to describe potential changes in these measures during the acute treatment trajectory (~33 weeks from treatment initiation).

6.5 Methods

6.5.1 General Study Design

High-risk prostate cancer patients were recruited for this study. Treatment plan was either conventional radiation treatment with upfront ADT (n=4) or hypofractionated radiation therapy with salvage ADT (n=5). To distinguish metabolic effects observed in prostate cancer patients related to aging and body size, 2 separate comparison groups were used: a group of males age- and BMI-matched to the prostate cancer participants (matched males) and a young healthy group of males (young males).

For prostate cancer patients, clinical and metabolic assessments were conducted over 2 days that were >48 hours apart but no more than 1 week apart. Assessments on prostate cancer patients were performed at: Baseline (prior to treatment initiation), ~7 weeks (end of RT), and ~33 weeks

from baseline (6 months following the end of RT). Assessing patients following diagnosis and prior to treatment will distinguish between metabolic changes that lead to the development of prostate cancer and provide a true baseline for the examination of metabolism during the acute trajectory. Assessments included the following measures: 1) fasting blood samples followed by an OGTT, 2) body composition analysis (weight, BMI, Single Frequency-Bioelectrical Impedance Analysis (SF-BIA)), 3) systolic and diastolic blood pressure and resting heart rate, 4) physical activity measures (VO$_{2peak}$, repeated 1-RM, physical activity questionnaire), and 5) dietary intake (by 3-day food diary). This study was reviewed by the University of Waterloo Office of Research Ethics (all groups) and by Tri-Hospital Research Ethics Board (prostate cancer patients only).

6.5.2 Participants

6.5.2.1 Prostate Cancer Patients

Nine high-risk (Gleason score >8, PSA >20ng/mL, or Stage >T3A) prostate cancer patients were recruited from the Grand River Regional Cancer Centre (Kitchener, ON) following their cancer diagnosis, but prior treatment initiation. Patients included in the study were of any age (≥18 years old), BMI, and fitness level. However, patients were excluded from participation if their fasting glucose was >7.0 mM or if they had any diagnosis of metabolic disease (cardiovascular disease, diabetes).

6.5.2.2 Non-Malignant Reference Groups

Men without cancer were recruited from the community and we sought to individually match them to corresponding prostate cancer patients in terms of age (±5 years) and BMI (±3 kg/m^2). Matched males were also required to be weight stable over the last 6 months, have a fasting blood glucose <7.0mM, and no history of metabolic disease (diabetes, cardiovascular disease).

To evaluate the extent of potential impairment in glucose and lipid metabolism, prostate cancer patients and the individuals in the matched males group were compared with the young male group who were between the ages of 20-30 years old, weight stable with normal BMI (18.5-24.9 kg/m^2), active with VO$_{2peak}$ >45 mL/kg/min, without metabolic disease and had fasting blood glucose <5.6mM.

6.5.3 Blood Sampling

Blood was sampled following an overnight fast (8-12 hours with no food or drink except for water). A sterile catheter was inserted into an antecubital vein of the participant's preferred arm and 25 mL of blood was drawn (Timepoint: -30). Following 30 minutes (Timepoint 0) a second fasting sample of 5 mL was drawn, to account for variability in measures such as fasting blood glucose. An OGTT was subsequently performed to investigate the body's response to a glucose challenge. Participants consumed a 75g glucose drink (Trutol Glucose Tolerance Beverage, ThermoFisher Scientific; East Providence, RI) within 10 minutes. Post-prandial blood samples (5 mL) were drawn 15, 30, 45, 60, 90, and 120 minutes following consumption of the drink the timer was set and 5 mL of blood was drawn at (total time of OGTT = 150 minutes). Blood samples were obtained from 8 of 9 prostate cancer patients due to sampling difficulties; thus n=8 for all blood measures.

Fasting blood samples were analyzed for glucose-related parameters (glucose, insulin, C-peptide), lipid profile (total cholesterol, LDL cholesterol, HDL cholesterol, TG), adipokines (leptin, adiponectin), inflammatory markers (IL-1β, IL-4, IL-6, IL-8, IL-10, TNF-α, CRP) as well as testosterone, IGF-1, and IGFBP-3. Blood samples collected at all other time-points during the OGTT were assessed for glucose, insulin, and C-peptide.

6.5.4 Biochemical Analysis

Blood samples were allowed to clot for 30 minutes, centrifuged and serum collected for metabolite analysis. Glucose was analyzed using spectrofluorometric methods (212). Insulin and C-peptide were analyzed using commercially available kits (Insulin: Human Insulin Specific RIA kit, EDM Millipore, St. Charles, MO; C-peptide Double Antibody RIA Kit, Siemans Healthcare Diagnostics; Deerfield, IL). Triglycerides, total cholesterol, HDL and LDL cholesterol were analyzed using a spectrofluorophotometer and commercially available reagents (Pointe Scientific; Canton, MI). Cytokines (IL-1β, IL-4, IL-6, IL-8, IL-10, TNF-α) were analyzed using BD Cytometric Bead Array and BD FACSCalibur flow cytometer (BD Biosciences; Mississauga, ON). Leptin, adiponectin, IGF-1, IGFBP-3, and C-reactive protein were assessed using sandwich enzyme-linked immunosorbant assay (R&D Systems Inc, Minneapolis, MN). Testosterone was measured using a Parameter assay (R&D Systems Inc, Minneapolis, MN).

6.5.5 Body Composition

Anthropometric measures included height, weight, and calculated BMI. Weight was determined using a balance beam scale (Detecto Scales Inc, Brooklyn, NY) and recorded to the nearest 0.1 kilogram. Height was determined using a stadiometer and recorded to the nearest 0.1 cm. These values were used to calculate BMI.

Fat free mass (FFM), fat mass (FM), percent body fat, skeletal muscle mass (SMM) and skeletal muscle index (SMI) were determined via SF-BIA. Reactance and resistance values were generated and used to estimate FFM using the equation describe by Kyle et al (215).

FM was estimated from calculated FFM and % body fat was calculated as FM divided by total weight multiplied by 100. Skeletal muscle mass (SMM) was calculated using the equation described by Janssen et al (216).

SMI (kg/m^2) was determined by dividing SMM by height squared (m^2). Estimated SMI was compared to sarcopenic cut points from the literature defined as SMI ≤ 8.5 kg/m^2 in men (242).

6.5.6 Physical Activity Assessment

Prior to the exercise tests, resting blood pressure was assessed using a sphygmomanometer and stethoscope and was recorded to the nearest 2 mmHg. Participants were excluded from participation in the exercise assessment if systolic blood pressure ≥ 150 mmHg or diastolic pressure of ≥ 90 mmHg on the day of the assessment.

VO_{2peak} tests were conducted on a treadmill (Bodyguard Fitness, St. Georges, QC) using the Vmax breath-by-breath system (Vmax; SensorMedics, Yorba Linda, CA). Three (3) minutes of resting data were collected while sitting in a chair and in standing position. Patients and matched males warmed-up by walking at 2 mph (no incline) for 3 minutes. Treadmill speed and grade was increased based on a modified Balke protocol (243) for patients and matched. The University of Waterloo Treadmill Protocol was used for the young males. Following resting measures, young males warmed-up by walking at 3.5 mph (no incline) for 2 minutes. The speed of the treadmill was then increased to a pre-determined speed between 7-8 mph (no incline) and this speed was maintained for the entire test. The treadmill grade was increased by 2% every 2 minutes until the participant reached exhaustion. Blood pressure, using a manual sphygmomanometer, and Borg's rating of perceived exertion were recorded at every stage of the test. Predicted tests (patients and matched males) were terminated when participants reached $\geq 85\%$ of their age-predicted maximal heart rate , or when the participant requested to stop (244).

Strength was assessed using predictive 1RM tests for upright bench press and leg extension using the American College of Sport Medicine (245) procedure. 1RM was predicted using the O'Connor et al equation (246).

Habitual physical activity levels were evaluated using the Godin Leisure Time Physical Activity Questionnaire. This questionnaire provides a physical activity score, which can be compared to a healthy reference group and has been used in a population of prostate cancer patient previously(247).

6.5.7 Nutrition Intake

During the week of the exercise and metabolic assessments, participants completed a 3-day food diary over the 2 weekdays and 1 weekend day (220). Participants were instructed to record all foods, beverages and supplements in detail. Daily caloric intake and macronutrient breakdown (% fat, % carbohydrate and % protein) were determined using ESHA Food Processor software and the Canadian Nutrient Files where available. The USDA National Nutrient Database for Standard Reference was used when Canadian information was not available.

6.5.8 Calculations

Area under the curve (AUC) was calculated using the incremental area method for glucose, insulin, and C-peptide (248). HOMA-IR (213), QUICKI (249), and Mastuda Index (250) were calculated as previously described. Molar ratio for IFG-1: IGFBP-3 and for leptin: adiponectin were calculated as previously described using the following molecular masses: IGF-1: 7.5kDa (251); IGFBP-3: 30.5kDa (251), leptin: 16kDa (252), adiponectin: 30kDa (252).

6.5.9 Statistical Analysis

Values are presented as mean±SD. All statistical calculations were completed using Sigma Plot ® version 11.2 (Systat Software Inc.; San Jose, CA). Statistical significance was determined at p<0.05. One-way repeated-measures ANOVAs were used to determine differences in measurements between each of the time points (baseline, 7, and 33 week post-baseline) with Tukey's post-hoc analysis for pairwise comparisons. Two-way repeated-measures ANOVAs with Tukey's post-hoc

analysis was also be used to compare average fasting glucose, insulin, C-peptide, and lactate to values obtained 15, 30, 45, 60, 90, and 120 min of the OGTT for all groups. Comparison between multiple groups (prostate cancer patients, matched males, young males) were determined using One-way ANOVAs with Tukey's post-hoc analysis.

6.6 Results

Patients were, on average, 71±6 (60-77) years old and had a BMI of 28.2±6.1 (21.0-37.7) kg/m^2. On average, patients were classified as overweight as per BMI, with 5 of 9 patients being overweight or obese (BMI ≥25.0 kg/m^2) and average BIA-derived % body fat characterized these patients as obese (>30%; Table 6.1). For matched males, 353 individuals who were >40 years old and did not have prostate cancer were assessed for eligibility for the matched reference group. Of these, 71 were eligible and 32 were interested in participating, and were screened. We chose 9 participants who best met our criteria to participate in the study (Figure 6.1). For 3 patients who had a BMI >33.0 kg/m^2, we were unable to find eligible matches. These patients were matched with the screened individual with the closest age and BMI criteria to the patients and who met all other inclusion criteria; as such, data were then analyzed as groups. There were no significant differences between patients and matched males in age (72±5 years old, p=0.582) or BMI (27.0±4.0 kg/m^2, p=0.634; Table 5.1) or other body composition assessments (Table 6.1). For the young male reference group, participants were 26±3 years old, had BMI 23.5±1.6kg/m^2 and had lower fat mass (12.1±4.1 kg) and percentage body fat (16.5±4.7%) than the prostate cancer patients. Patients, matched males and younger males had normal muscularity based on BIA calculations (Table 6.1).
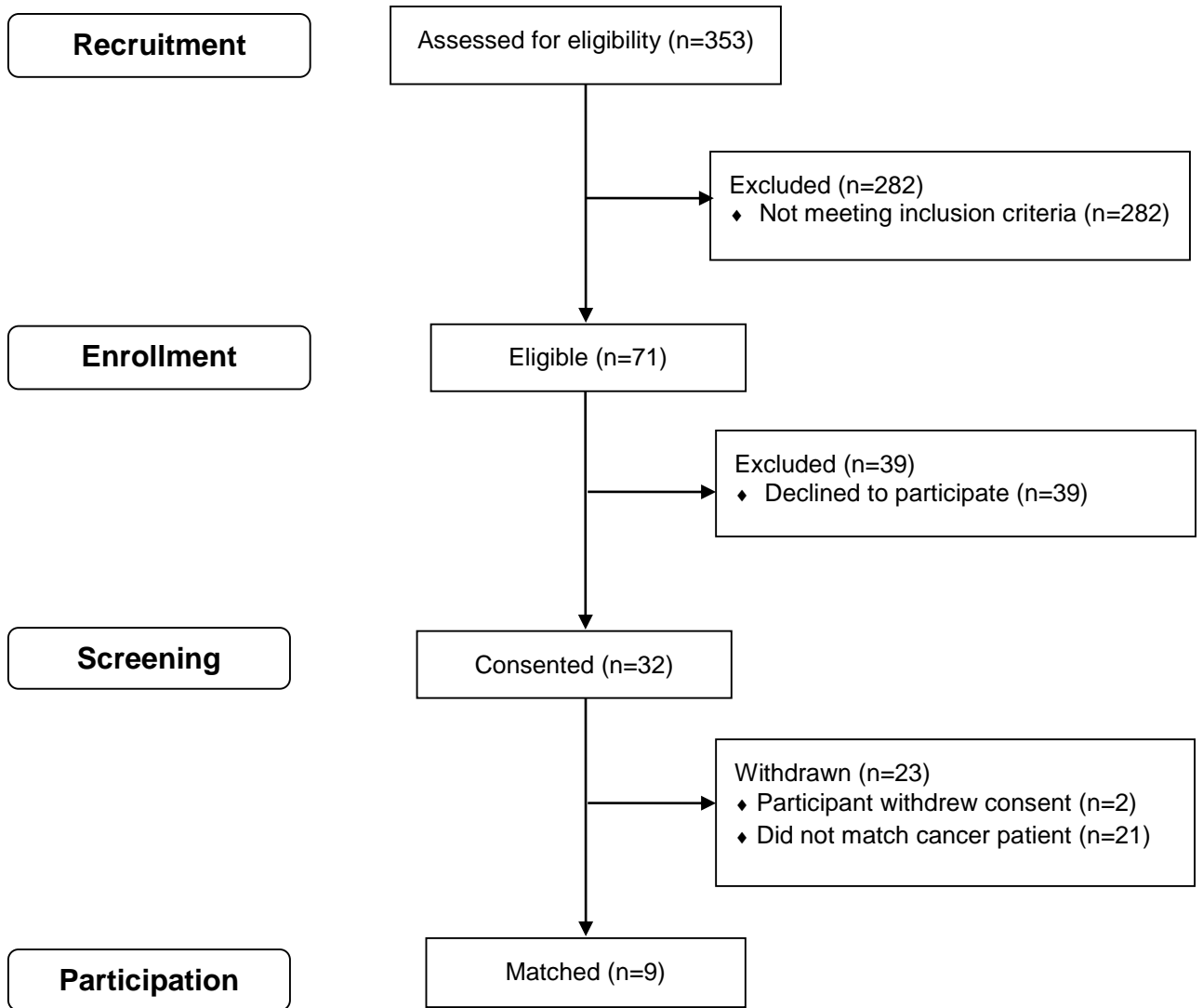
Figure 6.1: Participant recruitment flow diagram. This consort diagram describes the recruitment, enrolment, follow-up and analysis for the current study.

Table 6.1A: Physical and Clinical Characteristics					
Characteristic	Prostate Cancer Patients			Non-Malignant Comparison Groups	
	Baseline (n=9)	7 Weeks (n=9)	33 Weeks (n=9)	Matched Males (n=9)	Young Males (n=10)
Age (years)	71±6			72±5	26±3*†
Systolic Blood Pressure (mmHg)	164 ±20	147±12[a]	148±16[a]	135±19*	119±9*
Diastolic Blood Pressure (mmHg)	85±11	77±14[a]	80±12	83±13	76±6
Resting Heart Rate (bpm)	72 ±10	73±9	72±10	73±13	70±12
Testosterone (ng/mL)	29.4±3.3	17.8±10.8[a]	18.2±8.1[a]	17.6±12.1*	23.7±4.8
Table 6.1B: Body Composition Characteristics					
Characteristic	Prostate Cancer Patients		Non-Malignant Comparison Groups		
	Baseline (n=9)	7 Weeks (n=9)	33 Weeks (n=9)	Matched Males (n=9)	Young Males (n=10)
Height (cm)	173.8±0.08			174.0±5.2	175.8±4.7
Weight (kg)	86.1±23.6	85.6±22.3	85.8±20.6	81.8±12.8	72.7±6.5
Body Mass Index (kg/m²)	28.2±6.1	28.1±5.7	28.1±5.2	27.0±4.0	23.5±1.6
BIA-Derived Estimates of Body Composition					
Fat Free Mass (kg)	58.8±14.4	63.7±12.2	60.4±10.1	61.3±6.0	60.5±5.9
Fat Mass (kg)	27.3±13.4	21.8±15.4	25.4±11.9	20.6±8.6	12.1±4.1*
% Body Fat	30.6±10.9	23.4±15.2	28.4±7.8	24.4±7.0	16.5±4.7†
Skeletal Muscle Mass (kg)	29.1±7.3	29.6±7.5	29.9±5.3	31.9±3.8	34.4±3.8
Skeletal Muscle Mass Index (km/m²)	9.5 ±1.7	9.7±1.9	9.8±0.97	10.5±0.90	11.1±1.2

Letters represent significance between Repeated Measures (Baseline, 7 Weeks, 33 Weeks). [a] indicates significant difference from Baseline; [b] indicates significant difference between 7 Weeks and 33 Weeks. Letters represent significance between Baseline assessments in Patients and comparison groups. * indicates significant difference between Patients and comparison group; † indicates significant difference between Matched Males and Young Males; § indicates a main effect

6.6.1 Clinical Characteristics of Prostate Cancer Patients versus Comparison Groups

At baseline, all patients met the stage IIB requirements, 6 prostate cancer patients were classified as tumour stage T1C, 2 patients were classified as T2C and 1 was classified as T3C. Patients had average baseline PSA levels of 17.4±17.8 ng/dL while pathology revealed 3 patients had Gleason scores of 7 and 6 patients had Gleason scores of 8. Patients were on average classified as hypertensive (SBP>140mmHg) despite that 4 were prescribed hypertensive medication. In contrast, matched and young males had mean SBP that was in the normal range. Four matched males were taking hypertensive medication. Patients exhibited significant improvements in SBP at 7 and 33 weeks post-baseline but SBP remained >140mmHg (Table 6.1). Baseline testosterone concentrations were significantly higher in the prostate cancer patients compared with the matched males (p=0.013, Table 6.1).

6.6.2 Prostate cancer patients exhibited impaired glucose tolerance and related parameters at baseline compared with matched males

At baseline, patients had normal fasting glucose values of <5.6mM (4.9±1.2mM, Figure 6.2A) but exhibited impaired glucose concentrations at 2 hours following an OGTT (Impaired OGTT defined as >7.8mM by WHO/IFD (157); 8.62±2.87mM, Figure 6.2A). While fasting glucose concentrations were similar to matched males (4.2±0.5 mM, p=0.683), 2-hour glucose concentrations during the OGTT were significantly lower (5.5±2.2 mM, p=0.015, Table 6.2). Despite this, prostate cancer patients and matched males demonstrated similar peak glucose concentrations (11.9±1.9mM and 9.8±3.2mM, respectively) and glucose AUC (Figure 6.2B). Young males demonstrated lower fasting (3.7±0.4 mM), peak (7.2±1.6mM), 2-hour (3.1±0.99 mM, p<0.001; Table 6.2) glucose concentrations and glucose AUC (Figure 6.2B) than prostate cancer patients. Peak glucose also occurred at an earlier time point (27.0±14.2 minutes) than in prostate cancer patients (63.8±23.7 minutes; Table 6.2).

Figure 6.2 Glucose related measures: Letters represent significance between Repeated Measures (Baseline, 7 Weeks, 33 Weeks). [a] indicates significant difference from Baseline; [b] indicates significant difference between 7 Weeks and 33 Weeks. Letters represent significance between Baseline assessments in Patients and comparison groups. * indicates significant difference between Patients and comparison group; † indicates significant difference between Matched Males and Young Males; § indicates a main effect

Fasting insulin and C-peptide concentrations in prostate cancer patients did not differ with the comparison groups (Figures 6.2B and 6.2C). However, during the OGTT, prostate cancer patients exhibited greater serum insulin concentrations at 120 minutes compared with the matched males and at 45, 60, 90 and 120 minutes compared with the young males (Figure 6.2C). C-peptide concentrations in prostate cancer patients were also significantly greater than both the matched and young males at 45, 60, 90, and 120 minutes into the OGTT (Figure 6.2D). HOMA-IR and Matsuda Index revealed lower insulin sensitivity in the prostate cancer patients compared to the matched males (Table 6.2). As expected, young males were significantly more insulin sensitive than the prostate cancer patients when using the HOMA-IR and QUICKI (Table 6.2). IGF-1 was similar across the 3 groups but prostate cancer patients and matched males had significantly lower concentrations of IGFBP-3 compared with young males (Table 6.2).

Table 6.2: Glucose and Related Hormones					
Characteristic	Prostate Cancer Patients			Non-Malignant Comparison Groups	
	Baseline (n=8)	7 Weeks (n=8)	33 Weeks (n=8)	Matched Males (n=9)	Young Males (n=10)
Glucose Response during OGTT					
Fasting Glucose (mM)	4.9±1.2	4.6±0.99	4.5±0.3	4.2±0.5	3.7±0.4*
2-hour Glucose (mM)	8.7±2.9	6.8±1.5	5.7±2.1[a]	5.6±1.8*	3.1±0.99*[†]
Peak Glucose Concentration (mM)	11.9±1.9	9.4±1.9[a]	9.8±1.5[a]	9.8±3.2	7.2±1.6*
Time to Peak Glucose (min)	63.8±23.7	65.6±26.5	56.3±15.5	43.3±21.8	27.0±14.2*
Glucose-related Measures and Calculations					
Fasting Insulin (µIU/mL)	14.6±5.4	17.8±7.8	14.7±6.1	10.1±4.3	6.2±2.5
Fasting C-peptide (ng/mL)	3.3±0.96	2.9±1.0	3.3±0.78	1.8±0.43	0.65±0.31[†]
HOMA-IR	3.3±1.7	3.9±2.1	3.1±1.4	2.0±0.64*	1.1±0.50*
QUICKI	0.32±0.02	0.32±0.02	0.33±0.02	0.35±0.02	0.39±0.03*[†]
Matsuda Index	2.7±1.0	3.3±1.9	3.2±1.1	1.2±0.47*	2.8±0.8[†]
IGF-1 (ng/mL)	107.6±52.8	99.2±48.8	89.6±38.7[a]	97.8±28.4	143.1±39.2
IGFBP-3 (ng/mL)	2534.1±748.1	2416.1±530.0	2417.1±727.0	2367.8±265.8	3326.3±507.2 *[†]
IGF-1: IGFBP-3 Ratio (AU)	0.15±0.05	0.15±0.06	0.14±0.04	0.15±0.04	0.16±0.05

Letters represent significance between Repeated Measures. [a] indicates significant difference from Baseline; [b] indicates significant difference between 7 Weeks and 33 Weeks. Letters represent significance between Baseline assessments in Patients and comparison groups. * indicates significant difference between Patients and comparison group; † indicates significant difference between Matched Males and Young Males; § indicates a main effect

6.6.3 Lipids, Cytokines, and Adipokines in Prostate Cancer Patients Compared with Matched and Young Males

Considering that 2 patients had been prescribed lipid-altering medications, on average, prostate cancer patients had total cholesterol, LDL and HDL cholesterol, as well as triglyceride concentrations in normal ranges at baseline. Triglycerides were greater in prostate cancer patients, otherwise, all other parameters were similar to concentrations in matched males (considering that 4 matched participants were taking lipid-altering medication). Total cholesterol and triglyceride levels were greater and LDL cholesterol were greater in prostate cancer patients compared with young males though this did not reach statistical significance (Table 6.3).

Table 6.3: Lipid-Related Metabolic Markers					
Characteristic	Prostate Cancer Patients			Non-Malignant Comparison Groups	
	Baseline (n=8)	7 Weeks (n=8)	33 Weeks (n=8)	Matched Males (n=9)	Young Males (n=10)
Lipid Profile					
Total Cholesterol (mM)	4.7±0.9	4.9±1.0	4.9±1.5	4.6±1.0	3.3±0.7*†
LDL (mM)	2.6±1.7	3.0±1.0	2.9±1.4	2.8±0.9	1.8±0.7†‡
HDL (mM)	1.2±0.5	1.3±0.4	1.2±0.5	1.1±0.3	1.3±0.8
Triglycerides (mM)	1.7±1.3	1.6±0.9	1.6±0.9	0.9±0.6§	0.8±0.3*
Adipokines					
Leptin (ng/mL)	7.9±6.0	9.1±7.3	9.4±5.8	7.5±4.5	2.2±2.2*†
Adiponectin (ng/mL)	6743.8±2759.6	6172.0±2796.3	6998.2±3175.9	8823.6±4212.4	6320.7±2385.4
Leptin: Adiponectin Ratio (AU)	0.79±0.70	0.95±1.2	0.54±0.45	0.87±0.66	2.8±2.8§

Letters represent significance between Repeated Measures. [a] indicates significant difference from Baseline; [b] indicates significant difference between 7 Weeks and 33 Weeks. Letters represent significance between Baseline assessments in Patients and comparison groups. * indicates significant difference between Patients and comparison group; † indicates significant difference between Matched Males and Young Males; § indicates a main effect

Leptin and adiponectin, which are typically linked to obesity and insulin resistance, were not different between prostate cancer patients and matched males. As expected, based on adiposity and OGTT results, leptin concentrations were significantly greater in the prostate cancer patients as compared with the young males. We also observed a main effect for the leptin: adiponectin ratio (Table 6.3).

Increased pro-inflammatory markers have been linked to insulin resistance (253) and obesity (254). CRP, TNF-α, IL-6 and IL-8 were greater in prostate cancer patients and matched males compared with young males and IL-1β was similar across the groups (Table 6.4). Anti-inflammatory cytokines IL-4 and IL-10 were significantly greater in prostate cancer patients compared with young males, and only IL-10 was significantly greater in prostate cancer patients than matched males (Table 6.4).

6.6.4 Nutrition Intake and Physical Activity in Prostate Cancer Patients Compared with Matched and Young Males

Prostate cancer patients and matched males consumed significantly less protein compared with young males (Table 6.5), which align with the lower body fat measurements observed in the young males. There were no differences in habitual activity levels, VO_{2peak}, leg extension and vertical bench press between prostate cancer patients and matched males (Table 6.5). Prostate cancer patients and matched males had significantly lower VO_{2peak}, leg extension and vertical bench press results than young males (Table 6.5).

Table 6.4: Inflammatory Markers

Characteristic	Prostate Cancer Patients			Non-Malignant Comparison Groups	
	Baseline (n=8)	7 Weeks (n=8)	33 Weeks (n=8)	Matched Males (n=9)	Young Males (n=10)
C-Reactive Protein (ng/mL)	2.3±1.3	2.8±1.1	3.2±1.6^c	2.0±1.5	0.61±0.44*[†]
TNF (pg/mL)	12.4±1.8	11.9±2.6	12.6±3.0	7.7±1.6	4.9±0.84*[†]
IL-1β (pg/mL)	7.4±1.1 (n=7)	8.7±1.36 (n=7)	7.6±0.86 (n=6)	6.8±1.2 (n=6)	7.7±1.0
IL-4 (pg/mL)	11.2±1.5	10.9±1.3	10.7±1.7	6.2±1.2 (n=5)	1.7±0.40*
IL-6 (pg/mL)	11.9±1.2	13.1±3.4	12.7±0.78	7.6±2.5	1.3±0.23*[†] (n=8)
IL-8 (pg/mL)	26.6±3.6	27.2±2.8	26.3±5.2	15.1±5.7	8.2±0.81*[†]
IL-10 (pg/mL)	11.0±0.84	11.2±1.8	11.1±1.2	7.5±1.1*	3.8±0.92*[†]

Table 6.5: Lifestyle Factors

Characteristic	Prostate Cancer Patients			Non-Malignant Comparison Groups	
	Baseline (n=9)	7 Weeks (n=9)	33 Weeks (n=9)	Matched Males (n=9)	Young Males (n=10)
Physical Activity					
Godin Score (AU)	25.9±20.9	19.4±15.9	19.4±12.1	41.7±22.0	63.9±18.2*
VO$_2$peak (mL/kg/min)	27.5±9.2 (n=5)	24.8±4.6 (n=7)	28.5±7.8 (n=5)	31.5±3.4 (n=7)	61.2±5.4*[†]
Leg Extension (kg)	31.4±10.1	30.5±8.4	28.6±9.7 (n=8)	35.5±5.0 (n=7)	52.5±12.7*[†]
Vertical Bench Press (kg)	42.7±17.4	39.4±17.9	39.5±15.5 (n=8)	47.9±5.2	109.0±28.9*[†]
Nutrition Intake					
Total Calories (kcal)	2245.6±348.4	2132.0±420.8	2211.6±356.2	2571.8±543.4	2461.1±628.1
Carbohydrates (%)	45.4±7.9	48.1±5.9	44.2±8.0	43.5±6.7	47.5±10.1
Fat (%)	35.2±5.1	32.0±5.3	34.8±5.7	33.4±4.0	29.2±7.8
Protein (%)	15.8±2.6	16.6±3.8	16.4±3.9	16.8±2.4	21.8±6.0*[†]

Letters represent significance between Repeated Measures. [a] indicates significant difference from Baseline; [b] indicates significant difference between 7 Weeks and 33 Weeks. Letters represent significance between Baseline assessments in Patients and comparison groups. * indicates significant difference between Patients and comparison group; † indicates significant difference between Matched Males and Young Males; § indicates a main effect

6.6.5 Prostate cancer patients demonstrated longitudinal improvements in glucose and related parameters

Prostate cancer patients continued to have healthy fasting glucose concentrations at 7 and 33 weeks following baseline (Baseline: 4.9±1.2mM, 7 weeks: 4.6±1.0mM, 33 weeks: 4.5±0.3mM; p=0.792; Table 6.2); however, 2-hour glucose values during the OGTT demonstrated improvements during the acute treatment trajectory, returning to normal ranges of < 7.8mM by 7 and 33 weeks (Baseline: 8.62±2.87mM; 7 weeks: 6.78±1.48mM and 33 weeks: 5.69±2.14mM, respectively; p<0.007; Table 6.2). Peak glucose concentrations during the OGTT were also significantly lower at 7 and 33 weeks compared with baseline (Baseline: 11.9±1.9mM, 7 Weeks: 9.4±1.9mM, 33 weeks: 9.8±1.9; p<0.001; Table 6.2). Prostate cancer patients also demonstrated significant decreases in AUC at both 7 and 33 weeks (396.7±111.9 mMxmin and 354.6±174.3 mMxmin, respectively) from baseline (585.5±193.6 mMXmin; p=0.002; Figure 6.2B).

Fasting and OGTT insulin and C-peptide concentrations did not change across the study trajectory in prostate cancer patients (Figures 6.2C and 6.2D). HOMA-IR, QUICKI, and Matsuda Index did not change across the prostate cancer trajectory (Table 6.2). IGF-1 decreased by 33 weeks in prostate cancer patients, but IGFBP-3 did not change over 33 weeks in the cancer patients.

These changes in glucose metabolism were not related to body composition (Table 6.1), lipid parameters (Table 6.3), or adipokines (Table 6.3) as these measures did not change during the 33-week study trajectory.

Testosterone concentrations significantly decreased by 33 weeks in prostate cancer patients (Table 6.3), which also does not explain the improvements in glucose metabolism. The reduction in average testosterone, however, were likely driven by 3 patients who received ADT during the study and showed significantly decreased testosterone at 7 and 33 weeks (Baseline: 30.9 ± 3.6 ng/mL; 7 weeks: 5.4 ± 2.8 ng/mL, p<0.001; 33 weeks: 10.8 ± 7.2 ng/mL, p<0.001) while the other 5 patients

demonstrated decreased testosterone that approached significance (n=5; Baseline: 28.6 ± 3.2 ng/mL; 7 weeks: 25.3 ± 1.9 ng/mL, p=0.387; 33 weeks: 22.6 ± 4.8 ng/mL, p=0.065). We compared patients receiving ADT (n=3) with those not receive ADT throughout the trajectory and observed no significant differences in any glucose-related parameters between the 2 treatment regimens (data not shown).

Interestingly, CRP tended to increase over 33 weeks in prostate cancer patients (Table 6.4), which would typically result in impaired glucose metabolism. Nutritional intake, physical activity and fitness measures did not change from baseline to 33 weeks in prostate cancer patients, suggesting that these modifiable risk factors did not contribute to the improved glucose metabolism that was observed post-treatment.

6.7 Discussion

This was the first study, to our knowledge, to investigate glucose metabolism in prostate cancer patients in a manner that accounts for age and body size. This is also a novel, integrative evaluation of changes in glucose metabolism and related parameters during the acute treatment trajectory. In the current study, we observed higher peak glucose concentrations and impaired glucose tolerance tests in prostate cancer patients at baseline (prior to treatment) compared with matched males. This is supported by greater insulin and C-peptide concentrations at 2 hours during an OGTT as well as worse HOMA-IR and Matsuda Index in patients compared with matched males. Given that matched males were similar age and body size, these data suggest impaired glucose tolerance at prostate cancer diagnosis is likely related to the presence of the cancer rather than age and body size. Prostate cancer patients also exhibited greater testosterone, triglycerides, and IL-10 compared with matched males, which may lend further support to the idea that impaired glucose metabolism is related to high-risk prostate cancer.

89

While glucose response during the OGTT's returned to normal ranges by 7 weeks, there were no changes in related hormone responses (i.e. insulin, C-peptide curves) or insulin sensitivity (HOMA-IR, Matsuda Index. The observed improvements in glucose response to the OGTT could not be explained by inflammatory mediators or lifestyle parameters (i.e. body composition, physical activity, nutrition intake) as these did not change over the 33-week period. Based on our findings, it is possible that the improvements in glucose metabolism observed over 33 weeks were related to treatment, potentially supporting the relationship between host glucose metabolism, treatment and aggressive tumour presence/mitigation.

6.7.1 Prostate cancer patients demonstrate impaired glucose metabolism compared to matched males, despite similar age and body size

While impaired OGTT was exhibited in prostate cancer patients at baseline compared with matched males, there were no differences in body composition measures, nutrition intake, and physical activity level. Matched males had normal glucose response during the OGTT but it was significantly worse compared with young males. These findings are consistent with impairments in glucose tolerance exhibited with aging (255). Since prostate cancer patients had greater impairments in glucose tolerance during the baseline OGTT compared with matched males, it is likely that these impairments are related to the tumour presence.

There is little literature that uses OGTT in prostate cancer patients to better understand the body's ability to clear a bolus of glucose. Zamboni et al (96) demonstrated normal 2-hour glucose concentrations following OGTT in prostate cancer patients with varying severity of disease; however, patients had higher fasting insulin and HOMA-IR compared with non-malignant controls (96). This study only used fasting and 2-hour glucose measurements and they did not measure 2-hour insulin concentrations. The use of methodology beyond fasting glucose concentrations is clearly important since fasting measures may mask impairments that would be revealed through an OGTT or other

comprehensive approaches. We also found significantly higher insulin and C-peptide concentrations at 45, 60, and 120 minutes of the OGTT in the prostate cancer patients compared with the matched males, suggesting that prostate cancer patients may experience higher post-prandial circulating insulin. Increased circulating insulin levels have been shown to increase tumour size (126), consequently, reducing fasting and post-prandial concentrations of insulin maybe essential for mitigating the potential for tumour proliferation.

Prostate cancer patients at baseline had significantly greater IL-10, testosterone, and triglyceride concentrations compared with matched males. The anti-inflammatory mediator, IL-10, was significantly greater in prostate cancer patients compared with matched males; however, no significant differences were observed in any other inflammatory markers between the 2 groups. IL-10 is hypothesized to have tumour-promoting potential by allowing tumour cells to evade the host's immune surveillance (256); however, animal and *in vitro* models suggest IL-10 may decrease tumour growth and angiogenesis (256). Consequently, observational studies examining IL-10 in prostate cancer remain inconclusive. IL-10 has also been shown to have insulin sensitizing properties and may attenuate diet-induced insulin resistance (257). Presence of glucose intolerance in patients in this study suggests that IL-10 did not contribute to insulin resistance. These data imply that elevated concentrations of IL-10 in high-risk patients were related to the presence of the tumour, potentially contributing to tumour growth and aggressiveness. Future work is needed to characterize the interactions between IL-10, insulin sensitivity and tumour growth in prostate cancer patients to delineate the role of IL-10 in prostate cancer. Despite seeing no significant differences in the remaining 5 cytokines that were examined, it is important to consider that various other cytokines may influence the role of IL-10, and importantly, the relationship between prostate cancer and glucose tolerance. Cytokines also demonstrate pleiotropic actions as well as autocrine and paracrine

effects. Here we used a whole body measure of a limited number of cytokines, thus further investigations into these complex interactions are warranted.

Testosterone is thought to facilitate prostate cancer growth via the androgen receptor (AR), stimulating numerous genes that increase PSA, cell growth and survival (258), while low testosterone associates with insulin resistance (259). Increased adiposity and age are associated with lower testosterone but these factors are also related to insulin resistance. Here, adiposity and age were similar between prostate cancer patients and matched males, and testosterone was greater in the patient group. Since none of these relationships explain impaired glucose tolerance in the patient group, these lack of findings imply that glucose impairments in our study may relate to the presence or absence of the tumour rather than age.

The relationship between hyperinsulinemia and dyslipidemia is cyclical, with dyslipidemia shown to contribute to hyperinsulinemia (260) and hyperinsulinemia shown to contribute to both hypertriglyceridemia and increased LDL cholesterol (261). Prostate cancer patients had normal ranges of total cholesterol, HDL and LDL concentrations at baseline, but triglyceride levels were greater than matched males. Greater circulating triglycerides may contribute to the observed impairments in glucose tolerance in the prostate cancer patients through activation of the IKK pathway [262]. Elevated triglycerides are also associated with increased risk of prostate cancer (263, 264) and prostate cancer recurrence (188); consequently, managing the lipid profile of prostate cancer survivors is warranted to counter the risk of impaired glucose metabolism and recurrence in survivorship.

Despite the association between elevated leptin, decreased adiponectin and increased risk of prostate cancer and prostate cancer recurrence via the IRS-1–PI3K–Akt signalling cascade (132), we did not observe differences in these adipokines between prostate cancer patients and the matched males. This phenotype of adipokines has typically been used to explain the development of insulin

resistance attributed to obesity. Since adipokines and body composition were similar between matched males and prostate cancer patients, these features may not relate to tumour presence and are unlikely to explain impaired glucose tolerance in the prostate cancer patients relative to the matched males.

6.7.2 Impairments in glucose metabolism improve during the acute treatment trajectory in prostate cancer patients

During the study trajectory, prostate cancer patients demonstrated decreased peak glucose concentrations (7 and 33 weeks) as well as decreased glucose concentrations at 2 hours (33 weeks) during the OGTT compared with baseline. These improvements occurred in absence of changes in lifestyle factors, such as nutritional intake (e.g. fat) and physical activity, as well as body composition. No significant changes in insulin and C-peptide concentrations occurred at any point during the OGTT (fasting and post-prandial) at 7 or 33 weeks compared with baseline, together with decreased 2-hour OGTT glucose concentrations suggest greater insulin sensitivity in peripheral tissues. Similarly, IGF-1 decreased at 33 weeks post-baseline aligning with the idea that low IGF-1 concentrations may enhance insulin sensitivity (as observed in this study). However, it is important to note that if IGF-1 concentrations are too low, glucose clearance may become impaired (265, 266). Interestingly, CRP approached significance by 33 weeks in the prostate cancer trajectory. Elevated CRP concentrations are typically associated with insulin resistance and have been correlated with several markers of insulin resistance including fasting glucose, insulin and HOMA-IR in non-diabetic patients (267). Despite no changes in glucose tolerance or hormonal response, this may suggest a shift toward insulin resistance at this time point and warrants further investigation.

Changes in glucose tolerance may be attributed to the removal of the tumour following radiation therapy. *In vitro* studies have demonstrated a more glycolytic profile in aggressive prostate cancer cell lines (PC3, DU145) compared with less aggressive cell lines (LNCaP; 268), suggesting a

greater reliance on anaerobic glucose metabolism as the primary fuel source in these cell lines to satisfy the metabolic demand of these cells. Therefore, there is potential that, *in vivo,* the presence of the tumour may have the ability to disrupt host metabolism. Consequently, removal of the tumour may result in a return to normal glucose metabolism in the host.

6.8 Conclusions

This was the first study to integratively investigate the metabolic changes of prostate cancer patients independent of age and body size during the acute treatment trajectory. While caution is needed to interpret the findings of this study due to its small sample size, we observed impaired glucose tolerance in the prostate cancer patients as compared to the matched males that significantly improved during the acute treatment trajectory independent of changes in the traditional moderators of glucose metabolism, including hormone response, adipokines, cytokines, body composition, physical activity, and nutritional intake. We also noted significant decreases in testosterone and IGF-1, known prostate cancer promoters, during the treatment trajectory, while lipid profile, adipokine, and inflammatory markers remained unchanged. These improvements in glucose tolerance may be due to mitigation of the tumour itself. Future, larger scale studies will advance these findings to better understand the influence of the tumour on host glucose metabolism.

Chapter 7

Serum from high-risk prostate cancer patients does not induce cancer specific changes in glucose uptake in differentiated human skeletal muscle myotubes: developing a novel model to examine mechanisms of metabolic change in cancer patients

7.1 Authors

Katie M. Di Sebastiano[1], Elliott M. McMillian[1], Joe Quadrilatero[1], Evangelia L. Tsiani[2], Marina Mourtzakis[1]

7.2 Affiliations

[1] Department of Kinesiology, University of Waterloo, Waterloo, ON

[2] Department of Health Sciences, Brock University, St. Catherine's, ON

7.3 Abstract

Background: We have demonstrated that prostate cancer patients have impaired glucose tolerance at diagnosis compared with men of the same age and body size, but glucose tolerance improves over the acute treatment trajectory. Methodology to investigate the mechanisms to explain the findings observed in glucose metabolism is limited in cancer patients. Thus, here we developed a novel *in vitro* method to evaluate glucose uptake in human muscle cells that were exposed to human serum, and used it to assess this technique's ability to explain our previous findings.

Methods: Human skeletal muscle myoblasts were differentiated and incubated with human serum from 3 groups: prostate cancer patients prior to treatment, males of the same age and body size (matched males), and young males. Muscle cells exposed to serum from patients prior to treatment (baseline) were also compared with cells exposed to serum collected 7 and 33 weeks following baseline, reflecting the acute treatment trajectory. A 2-[^{3}H]-deoxy-D-glucose uptake assay with insulin stimulation was used to assess differences in glucose uptake between the groups.

Results: Glucose uptake in myotubes incubated with the serum of prostate cancer patients at baseline was significantly lower compared to cells incubated with serum from young males (114.0±6.3% vs 134.5±9.3% respectively, p<0.001), but not different from cells incubated with serum from matched males (114.8±4.5%, p=0.972). There were no significant differences in the glucose uptake among cells incubated with serum from prostate cancer patients across the treatment trajectory (Baseline: 114.0±6.3%; 7 Weeks; 117.5±10.5%; 33 Weeks: 119.2±9.2%, p=0.366).

Conclusions: In this novel model, serum from aged individuals (prostate cancer patients and matched males) significantly decreases the ability of the skeletal muscle cells to respond to insulin and take up glucose. Cancer-related alterations in glucose uptake were not observed with this method.

7.4 Introduction

Insulin resistance may contribute to aggressive prostate cancer as insulin and IGF-I have been shown to stimulate *in vitro* growth of both androgen sensitive and independent cells (125, 126). Both hyperinsulinemia and high IGF-I levels have been associated with increased prostate cancer risk (125-127). Interestingly, prostate cancer risk decreases with increased time after a diagnosis of type 2 diabetes, when insulin levels begin to decline following decreased beta cell function (127)

Hyperinsulinemia and related metabolic parameters are typically associated with age and obesity (269). Our work (Study 2, Chapter 6) suggests that high-risk prostate cancer patients have greater impairments in glucose tolerance at the time of cancer diagnosis compared with men of the same age and body size. Following acute treatment (primarily radiation therapy), patients demonstrated significantly improved glucose tolerance, independent of changes in insulin, C-peptide, inflammation, body composition, diet, and physical activity levels. Skeletal muscle may contribute to these changes in glucose metabolism because it accounts for 80% of total glucose disposal (202). Isolated skeletal muscle cells from pancreatic cancer patients is less responsive to physiological concentrations of insulin compared with non-malignant controls, which was attributed to changes in glucose transport across the sarcolemma, and PI3K activity (270). However, there are no studies that have examined changes in muscle glucose uptake across the prostate cancer trajectory.

Here, we developed a novel method to explore glucose uptake and insulin signalling using cultured human skeletal muscle cells in order to better understand glucose metabolism in high-risk prostate cancer patients. The objectives of this study were to: 1) compare glucose uptake in human skeletal muscle cells exposed to serum from prostate cancer patients, serum from age- and BMI-matched non-malignant males as well as serum from a young healthy comparison group, and 2) compare glucose uptake in human skeletal muscle cells cultured with serum collected from prostate cancer patients at baseline (near diagnosis), and following treatment at 7 weeks and 33 weeks from

baseline. In concordance with our previous work (Study 2, Chapter 6), we hypothesized that glucose

uptake would be decreased in human skeletal muscle cells exposed to serum from prostate cancer

patients compared with both serum from age- and BMI-matched males and serum from a young

healthy group. We also hypothesized that glucose uptake would be elevated in muscle cells exposed

to serum from prostate cancer patients prior to the initiation of cancer treatment.

7.5 Methods

7.5.1 General Study Design

After an overnight fast, serum from prostate cancer patients was collected throughout the

treatment trajectory, prior to initiation of treatment (baseline), following 7, and 33 weeks from

baseline. Fasting serum was also collected from two separate comparison groups that included an age-

and BMI-matched group (matched males) as well as a young healthy comparison group (young

males). These blood samples were collected as part of a larger study described in detail in Study 2

(Chapter 6); the data presented here has not been previously reported. Human skeletal muscle

myoblasts (HSkMM, Lonza, Rochester, NY) were differentiated into myotubes. Glucose uptake was

measured following a 24 hour acute conditioning period with serum collected from the cancer

patients and comparison groups. The 2-[^{3}H]-Deoxy-D-glucose uptake assay was used a marker of *in

vitro* glucose uptake.

7.5.2 Participants

Eight high-risk prostate cancer patients (Stage IIB: Gleason score >8, PSA >20ng/mL, or

Stage >T3A), prior to the initiation of treatment, were recruited for this study. Patients were eligible

for study participation if their planned treatment included radiation therapy with or without the use of

androgen deprivation therapy. Serum was collected from patients prior to initiation of treatment

(baseline), 7, and 33 weeks from baseline.   For patients, no limits were placed on age, body size,

determined by body mass index (BMI), and fitness level; however, patients were excluded from participation if their fasting glucose was >7.0 mM, or had any diagnosis of metabolic disease. For the comparison groups, the matched males individually corresponded to each prostate cancer patients in terms of age (±5 years) and BMI (±3 kg/m^2). Matched males were also required to be weight stable, have a fasting blood glucose <7.0mM, and no history of metabolic disease. For 3 patients who had a BMI >33.0 kg/m^2, we were unable to find matches without a history of metabolic disease. These patients were matched with the closest age and BMI criteria but met all other criteria. Young males were required to be between the ages of 20-30 years, weight stable with normal BMI (18.5-24.9 kg/m^2), active with VO$_{2peak}$ >45 mL/kg/min, fasting blood glucose <6.0mM, and no history of metabolic disease. This study was reviewed and received ethical clearance by the University of Waterloo Office of Research Ethics (all groups) and by Tri-Hospital Research Ethics Board (prostate cancer patients only).

7.5.3 Cell Culture Procedures

Human skeletal muscle myoblasts (HSkMM, Lonza, Rochester, NY) were obtained. The donor of these HSkMM line was from the quadriceps muscle of a 19-year old female who had a BMI of 19 kg/m^2. According to the manufacturer, these cells have been optimized for cellular differentiation and for use in insulin uptake experiments. The cells were seeded in 100mm polystyrene dishes at 3500 cell/cm^2. Cells were grown in 8-10mL of Skeletal Muscle Growth Media-2 (SKGM-2) with 10% fetal bovine serum (FBS), 1% L-glutamine, 0.1% human epidermal growth factor, 0.1% dexamethasone, and 0.1% Gentamicin/Amphotericin-B (SKGM-2 Bullet Kit, Lonza, Rochester, NY). This media has been specifically developed by the manufacturer to ensure optimal growth of the HSkMM cell line. Cell were incubated at 37° C and 5% CO$_2$. SKGM-2 media was replaced every 48 hours and cells were washed with each media change with Dulbecco's Phosphate-

Buffered Saline (DPBS; Lonza). Preliminary work revealed growth rates and glucose uptake declined at $\geq$12 cell passages; thus, all experiments were conducted between passages 7-10.

Sub-culturing was performed by removing media from the plate and washing the plates 2x with DPBS, trypisinzed at 37°C and 5% CO_2 for 5 minutes. SKGM-2 Media was added and cells were collected, and centrifuged at 500g for 5 minutes. Media was aspirated and the pellets was re-suspended in 2mL of SKGM-2 Media. Cells were counted a Z2 Coulter Counter (Beckman Coulter) to determine cell concentrations for reseeding.

For experimentation, HSkMM were seeded into a 12-well polystyrene culture plate a density of 50 000 cells per well and were maintained in 1mL of SKGM-2 media per well until ~90% confluence was achieved (3-4 days). SKGM-2 media was changed every 48 hours until differentiation. To stimulate differentiation, the SKGM-2 media was removed, cells were washed 2x with DPBS, and 1 ml of differentiation media was added to each well. Differentiation media (DM) consisted of low-glucose Dulbecco's Modified Eagle Medium (DMEM), containing 2% horse serum (HS) with 1% penicillin streptomycin (P/S). Cells were maintained at 37°C and 5% CO_2, with media changed every 48 hours for 4 days. Myotube formation was confirmed using microscopy.

7.5.4 Incubations with Human Serum

Fusion and myotube formation are typically observed 3 days following the initiation of differentiation in HSkMM (271). Pilot work also revealed that glucose uptake was highest on day 5 post-differentiation (Figure 7.1A). Thus, on day 4 of differentiation, cells were washed 2x with DPBS and differentiation media was replaced with experimental media consisting of DMEM with 1% P/S and 10% human serum from one participant of the following groups, prostate cancer patients at baseline, 7 weeks, and 33 weeks post baseline, matched males, young males, as this concentration of human serum was found to have the most robust effects in pilot work (Figure 7.1B). Cells were incubated for 24 hours at 37°C and 5% CO_2. Glucose uptake procedures were conducted on day 5

post-differentiation in all cells. Each set of experiments (1 plate) consisted of 4 technical replicates maintained in DM (Control: DM only), 4 technical replicates maintained in human serum unstimulated with insulin (Unstimulated: DMEM, 10% human serum, 1% P/S) and 4 technical replicates in human serum stimulated with insulin (Stimulated: DMEM, 10% human serum, 1% P/S, insulin). Two biological replicates were used for each participant sample, as each set of human serum experiments was run in duplicate.
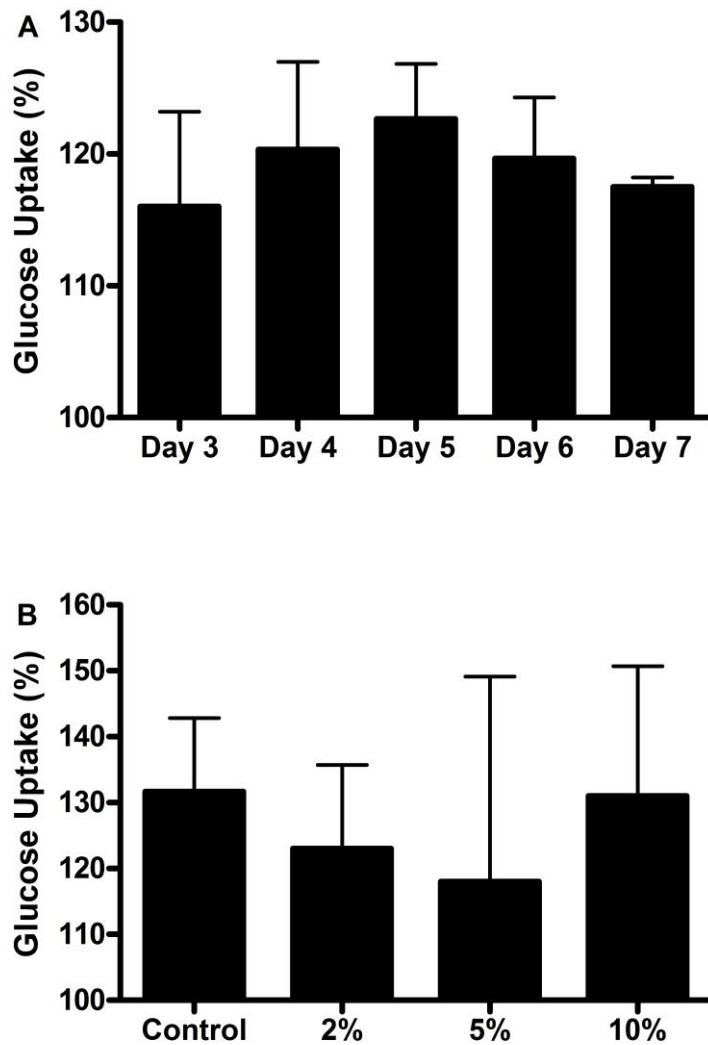
Figure 7.1: Results of pilot work. A. Y-axis represent insulin-stimulated glucose uptake relative to basal glucose uptake levels in cells incubated with DM only for the number of days indicated on the x-axis. Insulin stimulation and glucose uptake procedures are as described in sections 7.5.5 and 7.5.6. B. Y-axis represent insulin-stimulated glucose uptake relative to basal glucose uptake levels in cells incubated with DM only (Control) or human serum in the percentages indicated on the x-axis. Insulin stimulation and glucose uptake procedures are as described in sections 7.5.5 and 7.5.6.

7.5.5 Insulin Stimulation Procedures

To wash-out the effects of insulin and other glucose uptake stimulating compounds present in the serum, differentiated HSkMM were washed 2x with DPBS and media was changed to serum-free DMEM with 1% P/S and incubated for 3 hours at $37^{\circ}C$ and 5% CO_2. Longer washout periods were not found to have any further benefit in pilot work. To examine differences in the cells ability to respond to insulin some cells were stimulated with serum-free-DMEM 1% P/S supplemented with 3.4 mM insulin for 30 minutes since this has been shown to be the optimal treatment time for glucose uptake (203, 204) and this concentration demonstrated the most potent response in pilot work.

7.5.6 2-[^{3}H]-Deoxy-D-glucose Uptake Assay

Following insulin stimulation, media was removed and cells were washed 2x with DPBS. Cells were incubated for 10 minutes at room temperature with HEPES buffered solution (HBS) which contained 140mM NaCl, 5mM KCl, 20mM HEPES, 2.5 mM $MgSO_4$, 1mM $CaCl_2$, pH 7.4 and 50 μM 2-[^{3}H]-Deoxy-D-glucose and 50 μM 2-Deoxy-D-glucose. Cells were then washed with cold 0.9% NaCl (w/v) and lysed with 1mL of 0.05M NaOH. Lysates were collected and added to 5mL of scintillation cocktail and was assessed for radioactivity using liquid scintillation counting. An addition of 10 μM Cytochalasin B (CB; Sigma- Aldrich) to one well of each condition was used to control for non-specific glucose uptake. CB inhibits glucose transporters on the cell periphery accounting for glucose uptake that does not occur through the GLUT transporters (272).

7.5.7 Statistics

Values are presented as mean ± SD. All statistical calculations were completed using Sigma Plot ® version 11.2 (Systat Software Inc.; San Jose, CA). Statistical significance was determined at p < 0.05. One-way repeated-measures ANOVAs were used to determine differences in measurements between each of the time points (baseline, 7, and 33 week post-baseline). Comparisons between

multiple groups (prostate cancer patients, matched males, young males) were determined using One-way ANOVAs with Tukey's post-hoc analysis.

To understand whether traditional markers (glucose, insulin, C-peptide) of glucose metabolism were related to glucose uptake, simple univariate linear regression was performed using the following parameters: glucose uptake, fasting glucose, insulin, and C-peptide levels, 2-hour glucose, insulin, and C-peptide levels during an oral glucose tolerance test (OGTT), and glucose, insulin, and C-peptide levels area under the curve (AUC). This series of data was described in Study 2 (Chapter 6) and are used here to develop regression analysis to better understand the relationship between our *in vitro* findings with measures from our *in vivo* studies.

7.6 Results

7.6.1 Participants

Physical characteristics of participants have been described in detail in Study 2 (Chapter 6); however, basic physical characteristics (Table 7.1) of participants and factors that may contribute to alterations in glucose metabolism can be found in Table 7.2.

Table 7.1: Physical Characteristics					
Characteristic	Prostate Cancer Patients			Non-Malignant Comparison Groups	
	Baseline (n=8)	7 Weeks (n=8)	33 Weeks (n=8)	Matched Males (n=9)	Young Males (n=10)
Age (years)	71±6	-	-	72±5	26±3*
Height (cm)	173.5±8.8	-	-	174.0±5.2	175.8±4.7
Weight (kg)	86.1±25.2	85.5±23.8	84.6±21.7	81.8±12.8	72.7±6.5
Body Mass Index (kg/m^2)	28.3±6.6	28.1±6.1	27.8±5.5	27.0±4.0	23.5±1.6*
% Body Fat	30.8±11.6	23.0±16.2	28.0±8.5	24.4±7.0	16.5±4.7*

Letters represent significance between Repeated Measures. * indicates significant difference between Patients and comparison group; † indicates significant difference between Matched Males and Young Males; § indicates a main effect

Table 7.2: Serum Glucose Metabolism Parameters

Characteristic	Prostate Cancer Patients			Non-Malignant Comparison Groups	
	Baseline (n=8)	7 Weeks (n=8)	33 Weeks (n=8)	Matched Males (n=9)	Young Males (n=10)
Fasting Glucose (mM)	4.9±1.2	4.6±0.99	4.5±0.3	4.2±0.5	3.7±0.4*
2-hour Glucose (mM)	8.7±2.9	6.8±1.5	5.7±2.1^a	5.6±1.8*	3.1±0.99^{a*}†
Glucose AUC (mM*min)	585.5±193.7	396.7±113.7	354.6±174.3	377.2±261.9	104.4±130.0
Fasting Insulin (μIU/mL)	14.6±5.4	17.8±7.8	14.7±6.1	10.1±4.3	6.2±2.5
2-hour Insulin (μIU/mL)	105.2±98.2	87.5±57.5	66.2±63.8	51.1±24.3	13.2±9.3
Insulin AUC (μIU/mL*min)	9502.8±8013.6	7427.3±4505±2	7965.3±4505.2	9765.8±4850.2	3691.0±1723.9
Fasting C-peptide (pmol/L)	3.3±0.96	2.9±1.0	3.3±0.78	1.8±0.43	0.65±0.31†
2 hour C-peptide (pmol/L)	11.3±2.9	11.0±3.0	9.8±3.0	7.5±2.6	2.9±1.3
C-peptide AUC (pM*min)	881.0±269.4	742.7±223.8	711.1±163.0	637.5±223.5	279.6±157.5
HOMA-IR (AU)	3.3±1.7	3.9±2.1	3.1±1.4	2.0±0.64*	1.1±0.50*

Letters represent significance between Repeated Measures. ^{a} indicates significant difference from Baseline; ^{b} indicates significant difference between 7 Weeks and 33 Weeks;. Letters represent significance between Baseline assessments in Patients and comparison groups. * indicates significant difference between Patients and comparison group; † indicates significant difference between Matched Males and Young Males; § indicates a main effect.

7.6.2 Glucose Uptake

Pilot experiments revealed that the maximum glucose uptake of differentiated HSkMM (maintained in differentiation media for 5 day) following acute insulin stimulation was 128.0±13.4%. Insulin-stimulated glucose uptake in cells incubated with serum from young males was not significantly different from the control-stimulated cells (young males: 134.5±9.3% vs Controls: 128.0±13.4%, p=0.202). Thus, glucose uptake of cells incubated with young male serum was found

to be optimal and used as the reference point in subsequent analysis.

Insulin stimulated glucose uptake in cells incubated with the serum of prostate cancer patients at baseline was found to be significantly lower than insulin stimulated glucose uptake of cells incubated with young male serum (114.0±6.3% vs 134.5±9.3% of basal glucose uptake respectively, p<0.001, Figure 7.2A). Glucose uptake was also significantly lower in cells incubated with the serum of matched males compared to young males (114.8±4.5% vs 134.5±9.3% of basal glucose uptake, respectively, p<0.001, Figure 7.2A), with no significant differences in glucose uptake in cells incubated with prostate cancer patients at baseline versus matched males. There were also no significant differences in the glucose uptake among cells incubated with serum from prostate cancer patients across the treatment trajectory (Baseline: 114.0±6.3%; 7 Weeks; 117.5±10.5%; 33 Weeks: 119.2 ±9.2% of basal glucose uptake, p=0.366, Figure 7.2A).

To investigate the effects of human serum of glucose uptake independent of group, unstimulated control cells (maintained in differentiation media) were included in every experiment. Comparison of basal glucose uptake between cells exposed to differentiation media and media containing serum indicates that glucose uptake was increased from ~15-23% by the presence of human serum alone (Figure 7.2B); however, there were no significant differences between any of the groups (Patient baseline: 117.7±13.25%; matched males: 115.3±15.4%; young males 118.7±13.6%, p=0.869; Figure 7.2B) or across the trajectory (Baseline: 117.7±13.2%; 7 Weeks; 117.0±7.0%; 33 Weeks: 123.9±7.9%, p=0.419, Figure 7.2B). We also examined insulin-stimulated glucose uptake in cells incubated with human serum compared to unstimulated control cells (Figure 7.2C). Insulin-stimulated glucose uptake tended to be higher in cells incubated with serum from young males compared to patients at baseline and matched males when unstimulated control cells were used as a reference (Patient Baseline: 135.2±24.0%; matched males: 135.6±22.3%; young males 154.1±15.2%, p=0.093; Figure 7.2C). However, no significant differences in insulin-stimulated glucose uptake

were observed across the treatment trajectory when unstimulated control cells were used as the unstimulated reference in the analysis (Baseline: $135.2\pm24.0\%$; 7 Weeks; $136.7\pm14.0\%$; 33 Weeks: $147.3\pm14.7\%$, p=0.362, Figure 7.2C).   When all of these data are considered, age- but not cancer-related changes in *in vitro* glucose uptake are revealed.
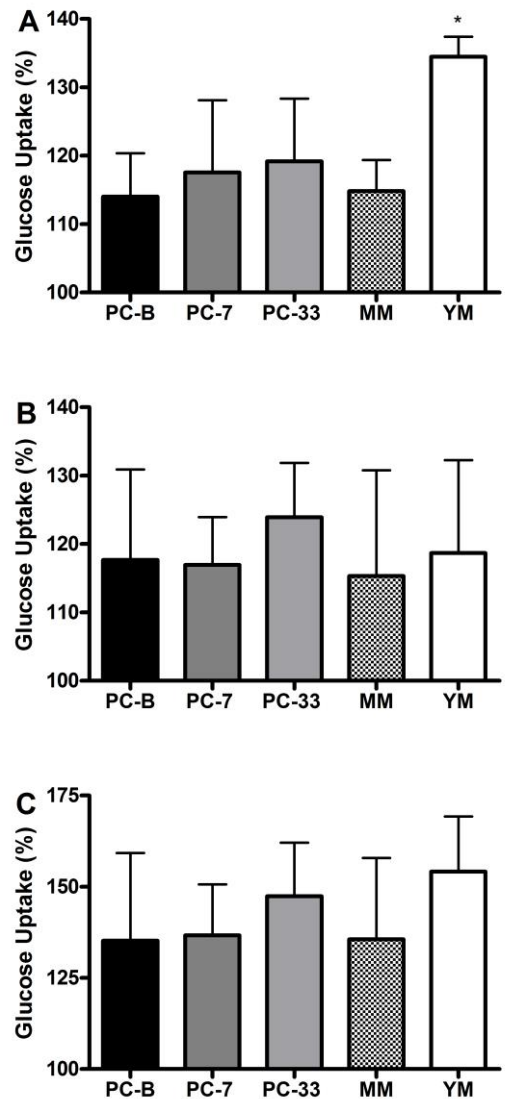
Figure 7.2: Glucose uptake in HSkMM incubated with human serum spiked media and differentiation media. * indicates significantly different from all groups. PC-B represents prostate cancer patients at baseline, PC-7 represents prostate cancer patients 7 weeks post-baseline, PC-33 represents prostate cancer patients 33 week post-baseline, MM represents matched males and YM represents young males.. A. Y-axis represent insulin-stimulated glucose uptake relative to basal glucose uptake levels in cells incubated with each human serum stimulated group. B. Y-axis represent insulin-stimulated glucose uptake of cells incubated with indicated human serum to insulin stimulated glucose uptake of cells incubated with DM only. C. Y-axis represent basal glucose uptake of cells incubated with indicated human serum to basal glucose uptake of cells incubated with DM only.

7.6.3 Does insulin stimulated glucose uptake in human serum condition cells reflect traditional markers of glucose uptake?

Linear regression modeling was used to examine the relationships between glucose uptake measures and traditional markers of glucose metabolism, which were obtained in Study 2 (Chapter 6). Regression analysis revealed moderate negative but significant relationships between fasting glucose (p=0.010), 2-hour glucose (p=0.009), glucose AUC (p=0.014), fasting C-peptide (p=0.015), 2-hour C-peptide (p=0.010), C-peptide AUC (p=0.012) and HOMA-IR (p=0.015) and *in vitro* glucose uptake (Table 6.3). Fasting insulin concentrations e (p=0.064, Table 6.3), 2-hour insulin (p=0.331) and insulin AUC (p=0.169) were not related to glucose uptake. Taken together, these data suggest that this *in vitro* model of glucose metabolism reflects changes observed in *in vivo* models.

Table 7.3: Simple Univariate Linear Regression Analysis of Human Serum Insulin Stimulated Glucose Uptake (Dependent Variable) and Glucose Metabolism Parameters

Parameter (n=43)	Co-efficient	R	R-squared	p
Fasting Glucose	-0.0451	0.387	0.150	0.010
2-hour Glucose	-0.0168	0.394	0.155	0.009
Glucose AUC	-0.000177	0.373	0.139	0.014
Fasting Insulin	-0.00455	0.285	0.081	0.064
2-hour Insulin	-0.000265	0.152	0.023	0.331
Insulin AUC	-0.00000446	0.214	0.0457	0.169
Fasting C-peptide	-0.0328	0.369	0.136	0.015
2 hour C-peptide	-0.0105	0.387	0.150	0.010
C-peptide AUC	-0.000146	0.381	0.145	0.012
HOMA-IR	-0.0245	0.367	0.135	0.015

7.7 Discussion

This study aimed to develop a new method to explore glucose and insulin signalling using cultured human skeletal muscle cells and, subsequently, evaluated glucose uptake in these muscle cells after exposure to serum from prostate cancer patients, matched males, and young males. By examining the mechanisms that alter glucose metabolism in prostate cancer patients, targeted

interventions can be used to ameliorate the negative effects of impaired glucose tolerance. Three major components of this model were novel. First glucose uptake had never been assessed in HSkMM. Secondly, human serum had never been used as a treatment on any type of muscle cell lines, and finally, the effects of prostate cancer on HSkMM has never been investigated *in vitro.*

The presence of human serum in the conditioning media had the ability to increase glucose uptake by approximately 20% and insulin-stimulated glucose uptake in differentiated HSkMM by ~30% regardless of which serum was used. These findings suggest that the administration of human serum alone has robust effect on the ability of the cells ability to take-up glucose. Glucose uptake in cells incubated with the serum of prostate cancer patients at baseline was found to be significantly lower than the glucose uptake of cells incubated with young male serum. However, glucose uptake was similar between cells incubated with serum from prostate cancer patients at baseline and cells exposed to serum from matched males, and similar across the patients' treatment trajectory (baseline vs 7 or 33 weeks). Glucose uptake correlated moderately with both fasting and 2-hour glucose and C-peptide concentrations, but did not correlate with serum insulin concentration (fasting and 2-hour). These findings suggest an age-related, but not cancer-related decline in glucose uptake; thus further investigation is warranted.

7.7.1 Glucose uptake was lower in HSkMM exposed to serum from prostate cancer patients compared with young males

In Study 2 (Chapter 6), we found that prostate cancer patients had greater impairments in glucose tolerance during an OGTT compared with matched males; however, they exhibited improved glucose tolerance over the treatment trajectory. Given that nutrition intake, physical activity, and body composition were similar to matched males and these factors did not change over time, we hypothesized that glucose uptake would be lesser at baseline compared with matched males but would improve at 7 and 33 weeks. The only significant difference observed in this study was that

glucose uptake was greater in young males compared with prostate cancer patients and matched males, suggesting that there may not have been any changes in muscle glucose uptake related to tumour presence/absence. Age-related declines in glucose uptake in skeletal muscle occurred as expected. Previous *in vivo* work using glycemic clamp techniques have revealed that in aged individuals, differences in glucose tolerance are attributed to changes in peripheral tissue sensitivity to insulin (273, 274).

There are several perspectives that may explain our results: 1) this method may not be sensitive enough to detect changes in glucose uptake beyond a certain threshold, 2) factors that elicit significant changes in glucose uptake in prostate cancer patients are not present in fasting serum, 3) 24 hours of human serum pre-conditioning was not sufficient to induce differences in glucose uptake between the group or 24 hours of human serum pre-conditioning was too long and acute changes in glucose uptake were blunted due to feedback control mechanisms in the cell, and 4) observed changes in glucose tolerance in prostate cancer patients may not occur at the muscle. Future working characterizing the metabolic pathways is warranted to examine if, despite not observing changes in glucose uptake, alteration occurs in muscle cell insulin signalling pathways.

Most studies use L6 myoblasts to investigate glucose uptake due to their increased ability to take-up glucose *in vitro* and the decreased cell to cell variability; however, primary human skeletal muscle cells have been shown to be suitable for examining glucose uptake in culture (9, 10). Previous literature has suggested that serum from prostate cancer patients has the ability to induce significant changes in prostate cancer cells *in vitro* (275-277). While our data demonstrates greater glucose uptake for muscle cells exposed to serum from young males compared with either aged participants (prostate cancer or matched males), there is large variability in glucose uptake across all participants. Consequently, no significant differences were observed between the cancer patients and the matched males or across the treatment trajectory. In Study 2 (Chapter 6), we noted large heterogeneity in most

metabolic parameters (glucose, insulin, C-peptide, IGF-1, etc.) from the participants, which is suspected to have contributed the variability in glucose uptake in *in vitro*.

This may be addressed in one of two ways: combining serum from all cancer patients to create a single serum stock from each group or through using pre-conditioned media. By combining the serum from multiple participants together, you eliminate variability in the concentration of various metabolites in the stock serum; however, you lose the ability to examine differences between individuals, if any occur. This method would not guarantee any observed differences between the groups, but would rule out metabolite differences as a reason for the variability in glucose uptake. To use pre-conditioned media prior to treating the skeletal muscle cells with media, prostate cancer cells are incubated with the experimental serum to create a potent prostate cancer stimulus eliminating the variability associated with human serum. This methodology may create a more potent prostate cancer related stimulus; however, it would not account for whole body metabolic perturbations that may influence glucose metabolism *in vivo*. Employing both of these models may elucidate what contributes to the variability observed in glucose uptake in the current model.

As part of the Study 2 (Chapter 6), an OGTT was performed and post-prandial blood samples were collected from all participants. However, we chose to use fasting serum in all experiments to eliminate variation that may occur in the post-prandial state of the OGTT; in other words, fasting glucose concentrations fell within a fairly narrow range compared with using serum during the OGTT. However, in Study 2 (Chapter 6), we only observed elevated glucose, insulin, and C-peptide during the OGTT in prostate cancer patients compared with matched males and not in fasting conditions. Using serum from non-fasting time-points during the OGTT may help identify an impairment in glucose metabolism during the glucose challenge that would also be reflected in muscle glucose uptake. Another consideration is that tumour-related factors that induce changes in glucose metabolism *in vivo* may act, to a greater extent on other tissues and their signalling cascades

112

such as liver and not muscle. Another important consideration is that an OGTT is a whole body measure of glucose tolerance, and is unable to distinguish between insulin resistance in liver, adipose tissue, muscle, or other tissues. Other invasive measures of insulin resistance such as hyperinsulinemic-euglycemic clamps or measures of hepatic glucose production *in vivo* may elucidate complex tissue interactions and facilitate the development of future models to investigate them.

HSkMM exposure to serum *in vitro* is significantly different that muscle exposure to whole blood *in vivo*. Firstly, *in vivo*, muscle cells are exposed to plasma not serum and the clotting factors present in plasma may alter glucose uptake *in vitro*. As well, storage of the serum may alter the concentrations of some components of the serum such as cytokine, which have a short half-life (278), as well as many lipids, which have been shown to degrade with storage (279). As well HSkMM are intrinsically different from muscle cells *in vivo*. There is no fascia surrounding the HSkMM, which alters the way in which the cells and media can interact. There is also no fat infiltration, as is often observed in skeletal muscle *in vivo,* which may alter myocyte insulin signalling compared to HSkMM *in vitro*. Consequently, the insulin stimulation of glucose *in vitro* may be significantly different than that observed *in vivo*.

All pilot experiments in this paper used human serum for a 24-hour incubation period, and suggesting that this timeframe is sufficient to induce changes in HSkMM. During the experiments, 24 hours was sufficient to induce age-related changes in glucose uptake, though no cancer related changes in glucose uptake were observed. However, *in vivo*, changes in skeletal muscle that alter whole body glucose metabolism may occur over a prolonged period of time. Prostate cancer development also occurs over a prolonged period of time, which may convolute the ability to optimize this method for this patient cohort. Therefore, longer incubation periods with human serum may create a more potent effect. However, the length of the incubation period is limited as glucose uptake in HSkMM decline as the myotubes spend longer time in culture as revealed in pilot work

113

(Figure 6.1A). However, it is also possible that the 24-hour incubation was too long and acute changes in glucose uptake were lost due to negative feedback mechanisms. Serum from prostate cancer patients may have induced acute changes in glucose uptake in the HSkMM, within the first minutes to hours of incubation. However, by the time the glucose uptake assay was completed 24 hours following human serum administration, feedback mechanism within the cells may have been initiated to return glucose uptake levels to those of the matched males. Further investigation of earlier time points (i.e. 10 minutes, 1 hours) is needed to elucidate these hypotheses.

A concentration of 10% human serum was selected to incubate the HSkMM as there was little difference between the glucose uptake in other concentrations provided and 10% human serum media has been used previously in the literature (280). However, like the 24-hour incubation period, this may not be an appropriate stimulus to detect differences in glucose uptake between the groups.

7.7.2 Glucose uptake measured in HSkMM was moderately correlated with whole body measures of glucose metabolism

Study 2 (Chapter 6) examined various parameters related to glucose metabolism following an overnight fast and during an OGTT. Fasting glucose, 2-hour glucose, glucose AUC, fasting C-peptide, 2-hour C-peptide, C-peptide AUC, and HOMA-IR were moderately but significantly correlated with glucose uptake. Fasting insulin, 2-hour insulin and insulin AUC, were not related to glucose uptake. These significant relationships between *in vitro* glucose uptake and whole body markers of glucose metabolism, indicate that this model of *in vitro* glucose uptake reflects the metabolite concentration in the serum samples used in the experiment.

HOMA-IR, 2-hour glucose, and C-peptide as well as glucose and C-peptide AUC were significantly related to glucose uptake. With the exception of HOMA-IR, these are dynamic measures and reflect the body's ability to respond to a glucose challenge. We hypothesized that there would be no relationship between these dynamic measures and glucose uptake as this model would

114

not be able to account for the transient nature of these measures. These markers reflect post-prandial serum during the OGTT, which was not used in the cell culture model; however, it was in these post-prandial measures of glucose metabolism that differences in glucose tolerance were observed in Study 2 (Chapter 6). These data suggest that the 24 hours of incubation with human serum induced changes in the HSkMM to moderately reflect whole body glucose metabolism *in vivo*. This agrees with the hypothesis that observed changes in glucose tolerance in prostate cancer patients in Study 2 (Chapter 6) may not occur at the muscle.

There were no relationships observed between 2-hour insulin and insulin AUC and glucose uptake; however, the design of the 2-[^{3}H]-Deoxy-D-glucose uptake assay requires that cells are provided with exogenous insulin, at significantly greater concentrations than those found in serum. Thus, it is not surprising that any potential relationships were washed out.

While we observed no significant differences in glucose uptake between prostate cancer patients and matched males, significant differences were observed in glucose uptake between young males and aged males (bothmatched males and prostate cancer patients). Numerous differences were observed between the serum of young males and aged males including lower fasting glucose, c-peptide and IGFBP-3 concentrations in Study 2 (Chapter 6). Lipid profile components total cholesterol, LDL cholesterol and triglycerides were also lower in young male as were many inflammatory marker including IL-4, IL-6, IL-8, IL-10, and CRP. These results suggest that insulin secretion, lipid profile, and inflammation may not be mediating the differences in glucose tolerance observed in Study 2 (Chapter 6). Future investigations into these pathways are warranted to investigate this hypothesis.

7.8 Conclusions

We developed a new model in which to investigate potential changes in insulin stimulated glucose uptake in cells conditioned with human serum. Human serum was found to increase glucose

uptake by approximately 20%, and insulin having the ability to stimulate glucose uptake in differentiated HSkMM by approximately 30% regardless of serum used. Serum from aged individuals significantly decreases the cells ability to respond to insulin; however, this technique did not detect any cancer-related changes in glucose uptake. However, linear regression revealed that this model of glucose uptake related to many traditional moderators of glucose metabolism.

Chapter 8
Integrated Discussion

Numerous lifestyle factors including obesity (201), physical inactivity (206), high-fat diets (281), and metabolic syndrome (160, 174) have been associated with the development of prostate cancer. De Nunzio et al. (174) proposed an integrative model of prostate cancer development in which these lifestyle factors create a deleterious metabolic profile resulting in insulin resistance, visceral adiposity, pro-inflammation, and hormonal changes that create an environment conducive to more aggressive tumour growth (174). However, much of the current literature fails to characterize these features in an integrative manner, choosing epidemiological approaches that focus on few parameters using large samples. This thesis aimed to comprehensively characterize the metabolic features of prostate cancer patients near the time of diagnosis and to examine changes in this profile during acute treatment, immediately and 6-months following radiation therapy. Specifically, features of both metabolic syndrome and glucose metabolism were the primary areas of investigation. Body composition, features of lipid metabolism, adipokines, inflammatory markers, physical activity, and nutrition intake were also characterized to better understand the presences of metabolic syndrome and impairments in glucose metabolism in the prostate cancer patients.   Figure 8.1 illustrates the integrative model of prostate cancer investigated in this thesis.
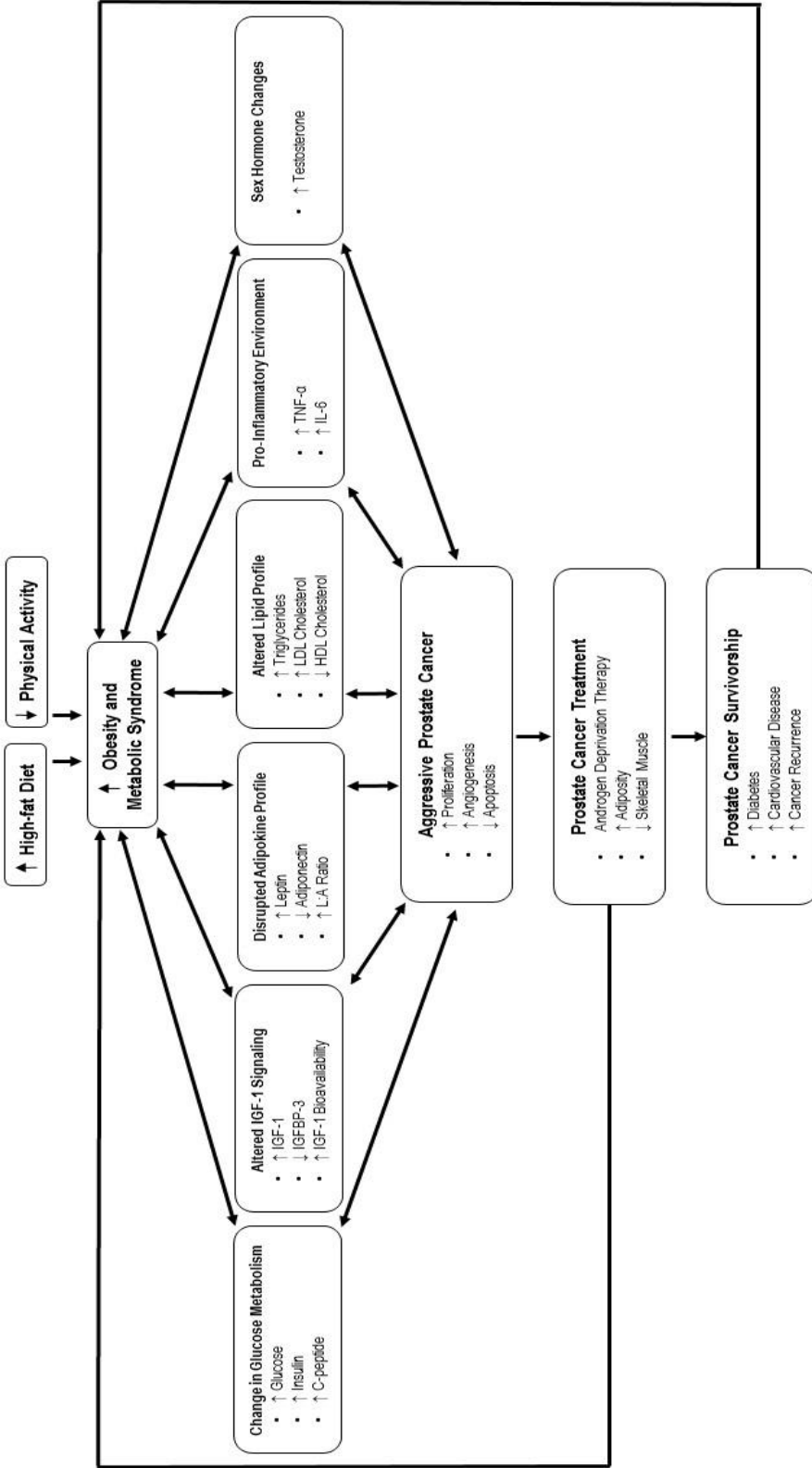
Figure 8.1 illustrates the integrative model of prostate cancer investigated in this thesis.

8.1 Prostate cancer patients present with a cluster of glucose-related metabolic perturbations that may contribute to aggressive prostate cancer growth

Three major metabolic perturbations were identified in patients with the most aggressive cancers (indicated by the highest Gleason scores) at the time of diagnosis in Study 1 (Chapter 5). First, elevated C-peptide concentrations were observed, suggesting increased insulin secretion; this finding was supported by insulin concentrations and HOMA-IR calculations that approached significance in patients with the most aggressive cancers. Together, these findings suggest that patients with higher Gleason scores may have developed impairments in glucose metabolism and insulin resistance, which may relate to with the aggressiveness of their cancer. C-peptide is an important indicator of insulin secretion as elevations in C-peptide concentrations indicate that high insulin concentrations are the result of insulin secretion and not poor insulin clearance (281) or peripheral insensitivity.   Second, greater visceral adiposity, measured by waist circumference, was identified in patients with the higher Gleason scores (4+3 or greater) compared with patients with Gleason 3+4. Large waist circumferences are typically observed with insulin resistance in non-malignant populations (282), and large waist circumference is one of the defining characteristics of metabolic syndrome (157). Thirdly, altered adipokine levels (increased leptin, and increased leptin: adiponectin ratio) were observed in men with the highest Gleason scores, another phenotype commonly observed in insulin resistance (284). Patients with Gleason score $\geq$4+3 were significantly older than patients with no cancer.

These factors were then further considered in men with high-risk prostate cancer relative to men without cancer but who were of similar age and body size in Study 2 (Chapter 6). Despite the lack of differences in body composition or adipokines between the high-risk cancer patients and the matched males, high-risk prostate cancer was associated with a greater magnitude of impaired

119

glucose tolerance during an OGTT compared with the matched males. Thus, perturbations in glucose

metabolism in high-risk prostate cancer patients at diagnosis are distinct from those of similar age and

size who do not have cancer, and these perturbations may also contribute to the development of

aggressive prostate cancer. This also suggests that the increased age of patients with Gleason score

≥4+3 in Study 1 cannot fully account for the metabolic perturbation observed.

8.1.1 Impaired glucose metabolism is associated with aggressive prostate cancer

Fasting glucose concentrations were not different between patients with different Gleason

scores (Study 1, Chapter 5), nor were they different in treatment-naïve high-risk prostate cancer

patients compared with matched males (Study 2, Chapter 6). However, fasting glucose measures may

not be sensitive enough to depict impairments in glucose metabolism in these patients (285). The

examination of fasting C-peptide, insulin, and HOMA-IR as well as OGTT measures including

glucose, insulin, and C-peptide, demonstrate impairments in glucose metabolism in prostate cancer

patients with high Gleason scores, compared to patients with lower Gleason scores (Study 1, Chapter

5) and matched males (Study 2, Chapter 6). Despite that fasting glucose is often used to identify

impaired glucose metabolism it may not be the best biomarker, as it reflects the net balance of

glucose metabolism and long-term ability of the body to metabolize glucose (285).

Elevated fasting C-peptide concentrations have been associated with high-grade prostate

cancer as well as increased likelihood of prostate cancer-specific death (128, 286); however, other

studies demonstrate no association between C-peptide concentrations and prostate cancer risk (138,

225). Insulin has been implicated in prostate cancer development through two major pathways to

promote tumour growth: 1) MAPK signalling resulting in an anti-apoptotic effect via STAT3

signalling, and 2) PI3K/Akt pathway, promoting proliferation, cell cycle progression and anti-

apoptotic effects via activation of BAD, Bcl, and FOXO complexes (287, 288). PI3K/Akt also has

120

the ability to stimulate mTOR signalling, consequently promoting protein synthesis and promoting cell proliferation in the tumour (287, 288).

Insulin and IGF-1 act through the same metabolic pathways to promote prostate cancer; however, fasting IGF-1, IGFBP-3, and the molar ratio IGF-1:IGFBP-3 were not significantly different between any of the groups in Study 1 (Chapter 5) or between the cancer patients and the matched males in Study 2 (Chapter 6). Since IGF-1 has similar functions with insulin, IGF-1 has been associated with increased risk of prostate cancer development (93, 289). IGF-1 may be linked to early stage prostate cancer initiation, however, multiple studies have characterized a decrease in IGF-1 action and a subsequent down-regulation of the IGF-1 receptors in advanced and metastatic disease (290, 291). This down-regulation facilitates survival of the cancer cells once they enter circulation (290, 291), which may explain the lack of difference observed in the aggressive prostate cancer patients compared with matched males and throughout the trajectory in this thesis.

Obesity may contribute to the relationship between impairments in glucose metabolism and aggressive prostate cancer as is associated with hyperinsulinemia, insulin resistance, and type 2 diabetes. Obesity is also association with increases in bioactive IGF-1 concentrations (292, 293). Bioavailability of IGF-1 is regulated through the IGFBPs, with IGFBP-3 identified as having the largest capacity to reduce IGF-1 bioavailability (294). Higher IGFBP-3 resulting in lower active IGF-1 has been associated with decreased risk of prostate cancer (93). IGFBP-3 may not be an ideal biomarker to relate obesity with prostate cancer development. While increased bioactive IGF-1 is associated with obesity, serum IGFBP-3 concentrations have not been associated with obesity (295, 296). This increase in free IGF-1 was related to decreased IGFBP-1 and IGFBP-2 in obese versus normal weight subjects, not IGFBP-3 (296).

Despite matching age and BMI for males in Study 2 (Chapter 6), high-risk prostate cancer patients' demonstrated impaired glucose tolerance to a greater extent than matched males without

121

cancer and thus, these collective perturbations in glucose metabolism of high-risk prostate cancer patients may be distinctly related to presence of the tumour. This hypothesis is further supported by the significant improvements observed in glucose concentrations during the OGTT performed immediately following treatment (7 weeks) and was maintained at the 33-week follow-up. There were no significant changes in lifestyle factors, such as body composition, physical activity, and nutritional intake over the course of Study 2 (Chapter 6), despite that these factors usually contribute to changes in glucose metabolism in non-malignant populations (123, 297, 298). However, it is possible that if the tumour contributes to impairments in host glucose metabolism, then radiation therapy to mitigate the tumour would also account for the observed improvements in glucose tolerance following radiation therapy. We noted no significant differences in insulin and C-peptide response following radiation therapy, which suggest increased insulin sensitivity in peripheral tissues (such as skeletal muscle) for glucose clearance.

To better understand possible effects of treatment on peripheral glucose uptake, muscle biopsies would be needed. Muscle biopsies would allow thorough examination (expression, regulation, activation) of numerous proteins involved in peripheral glucose uptake, including components of the insulin and IGF-1 signalling cascades to determine where potential impairments in glucose uptake are occurring. However, it was not feasible to obtain muscle biopsies for this thesis. Thus, to investigate potential mechanisms for the observed improvements in glucose metabolism following treatment, a novel cell culture model was developed which incubated human skeletal muscle cells and serum collected for prostate cancer patients to examine how systemic factors found in the serum may influence glucose metabolism in the muscle. While no previous literature has examined interactions between prostate cancer and skeletal muscle *in vitro*, the idea was conceived from studies that have used serum from prostate cancer patients prior to and following lifestyle interventions to induce changes in prostate cancer cell proliferation (275-277, 299-303). These

122

studies demonstrated that human serum has the potential to induce significant change in *in vitro*

models using prostate cancer cells (275-277, 299-303). We hypothesized that it may be possible to

induce changes in HSkMM using serum from prostate cancer patients to understand tumour– skeletal

muscle interactions.

Tumour resection has been shown to significantly improve glucose metabolism (304),

suggest that the tumour itself may contribute to changes in glucose tolerance. Peripheral insulin

resistance has also been shown to contribute to alterations in glucose metabolism in pancreatic cancer

patients due to changes in glucose transport in to skeletal muscle as well as alterations in PI3K

activity (270). These peripheral effects may occur though a tumour derived factor. Basso et al (205)

identified a putative pancreatic cancer factor, present in the serum of patient capable of inducing

impairments in glucose metabolism. Thus, this model was designed to examine if systemically driven

cancer-related changes in glucose uptake could be observed in cultured human skeletal muscle cells.

With this model it was found that skeletal muscle cells incubated with serum from high-risk

prostate cancer patients at baseline and the matched males demonstrated significantly lower glucose

uptake compared to the cells incubated with serum from young males. This suggested an age and

body size-related, but not cancer-related, decline in glucose uptake. There were no significant

differences in glucose uptake in cells incubated with serum for prostate cancer patients at baseline,

following radiation therapy (7 weeks) and at 33 weeks post-baseline.

8.1.1.1 Limitation to the novel model

The novel cell culture model was unable to detect significant differences in glucose uptake

between HSkMM incubated with serum from cancer patient and serum form matched males.

Hypothesized reasons for the inability of the model to detect these differences include the use of

fasting serum, insensitivity of the model, length of the incubation time, and the use of skeletal muscle

as the experimental tissue cells. First, fasting serum was used in all experiments to eliminate the

influence of variable post-prandial hormone levels on HSkMM *in vitro*. Study 2 (Chapter 6) reveal no significant differences in fasting glucose, insulin, C-peptide, and other glucose-related parameters between the high-risk prostate cancer patients and the matched males and impaired glucose tolerance was revealed in the prostate cancer patients through the glucose challenge of the OGTT. Consequently, fasting serum may not contain the factors necessary to induce significant changes in HSkMM and alter glucose uptake *in vitro.*

Second, previous work has shown that human skeletal muscle myoblasts are suitable for examining glucose uptake in culture (203, 204) and pilot work suggested it may be possible to detect differences between prostate cancer patients and the matched males; however, once glucose uptake was averaged over numerous participants, there were no significant differences between the cancer patients and the matched males. Thus, it is possible that the method was not sensitive to detect differences in glucose due to variability in the human serum. Perhaps a more potent stimulus through the use of pre-conditioned media, concentrating the systemic factors released from the prostate cancer cells is required to induce changes in skeletal muscle *in vitro*. Not only would the use of pre-conditioned media create a more potent prostate cancer stimulus but it may eliminate the variability observed when using human serum.

Third, the lack of differences observed between cancer patients and the matched males may be related to incubation time. Either, 24 hours of incubation may not be long enough to induce cancer-specific changes in glucose uptake or it was too long of an incubation time and acute changes in glucose uptake were lost due to negative feedback mechanisms. However, it is important to note that 24 hour incubation period was sufficient to induce age and body size related changes in glucose uptake. Conversely, 24 hours of incubation may have too long and acute changes in glucose uptake were lost due to negative feedback mechanisms. Serum from prostate cancer patients may have induced acute changes in glucose uptake in the HSkMM, within the first minutes to hours of

124

incubation. However, by the time the glucose uptake assay was completed, 24 hours following

human serum administration, feedback mechanism within the cells may have been initiated to return

glucose uptake levels to those of the matched males.

Finally, changes in glucose tolerance in prostate cancer patients may not occur in skeletal

muscle. Skeletal muscle accounts for the largest proportion (~80%) of insulin stimulated glucose

disposal in the human body (202), and was therefore chosen as the tissue of investigation. However,

the *in vitro* studies examining insulin resistance and pancreatic cancer were conducted in hepatocytes

(205), suggesting that the liver may be a potential area of investigation. It might only be possible to

address the improvements in glucose clearance following treatment *in vivo*, using more invasive

techniques such as hyperinsulinemic-euglycemic clamp or by measuring hepatic glucose production

(via artiovenous-difference techniques, isotope dilution techniques, or labeled nuclear MR

spectroscopy). These methods may provide a better indication of where insulin resistance is occurring

and where improvements were occurring post-treatment. Given the invasiveness and burden of these

methods may be too high for a proper research study in cancer patients, an animal model may be a

better choice to examine interactions between glucose metabolism and prostate cancer.

8.1.2 Poor glucose tolerance may contribute to the development of secondary disease states in cancer survivors

Patients with aggressive prostate cancer usually receive androgen deprivation therapy (ADT)

as part of their treatment (305). While acute treatment and specifically, radiation therapy, was the

focus of this thesis, 4 of 9 patients received ADT during the study and more may have received ADT

following study conclusion (Chapter 6). ADT reduces androgens to castrate levels, essentially

eliminating the primary anabolic stimulus for maintaining or gaining muscle (29). Consequently,

patients lose significant amounts of skeletal muscle and gain adipose tissue (29, 145, 146), which is

associated with significant metabolic perturbations leading to cardiovascular disease and diabetes (9).

Obesity has also been associated with increased risk of advanced disease (10, 11, 21, 22, 57, 58); consequently, obese patients already possess a potent risk factor for diabetes, which are further exacerbated due to treatment. Collier et al, have suggested that prostate cancer patients should be screened for symptoms relating to cardiovascular disease, diabetes and metabolic syndrome prior to ADT use to help mitigate the negative metabolic consequences of this treatments (25).

Beyond ADT, there is emerging evidence suggesting that prostate cancer patients who receive other forms of treatment may be at risk of diabetes as well. Thong et al examined incidence of diabetes following prostate cancer diagnosis and demonstrated that 50.2% of patients had received radical prostatectomy, 21.4% received radiation therapy, 12.1% received hormone therapy and 16.3% were undergoing active surveillance as their primary treatment type (108). These data suggest that all prostate cancer patients, not just those who receive ADT, are at risk of developing diabetes in survivorship.

The mechanism that drives the accelerated development of diabetes in men receiving ADT is largely unknown (306). However, there is evidence to suggest that the development of diabetes may be related to observed changes in body composition (101). ADT is primarily used to treat more aggressive forms of prostate cancer, which as noted in this thesis are associated with greater metabolic perturbations, like hyperinsulinemia, increased leptin, decreased adiponectin, inflammation and visceral adiposity (Chapter 5 and Chapter 6). No significant changes in body composition were observed over the acute treatment trajectory in Study 2 (Chapter 6); however, insulin and C-peptide response to the OGTT suggests increased insulin secretion despite normal fasting glucose and insulin levels. Hyperinsulinemia, reflective of peripheral insulin resistance, has been shown linked to the development of insulin resistance in non-malignant populations (261, 307). Peripheral tissues are unable to response to insulin and take-up circulating glucose resulting in hyperglycemia (261, 307). Hyperglycemia stimulates further insulin secretion, exacerbating this cycle and eventually resulting in

diabetes (261, 307). Prolonged elevation in insulin secretion such as those observed during the OGTT in patients following 7 and 33 weeks following treatment many contribute to the development of insulin resistance and ultimately diabetes in prostate cancer survivors.

8.1.3 Obesity, specifically visceral adiposity, and its associated metabolic sequelae may contribute to impairments in glucose metabolism observed in prostate cancer patients

Obesity causes many metabolic perturbations including changes in the IGF-1 and insulin signalling axis as well alterations in adipokine production, sex hormones and inflammation in non-malignant populations (308), which may contribute to aggressive cancer development. However, obesity in most cases has been associated with increased risk of prostate cancer (10, 11, 21, 22, 57, 58). The literature that links obesity to prostate cancer risk in humans remains inconclusive because numerous obesity-related metabolic perturbations have also been linked to prostate cancer development, including insulin, IGF-1, leptin and sex hormones (308).

Based on average BMI, participants in both Studies 1 (Chapter 5) and 2 (Chapter 6) were classified as overweight. While BIA, which provides fat and fat free mass distributions classified these patients at obese (% body fat >24% in male; 64). In Study 1 (Chapter 5), patients with Gleason score ≥3+4 had 33.9±8.0% body fat, which was the highest among any of the groups, but was similar to the high-risk prostate cancer patients in Study 2 (30.6±10.9%; Chapter 6).   Animal models demonstrate that excessive energy intake may stimulate prostate tumour growth directly and through increased angiogenesis by altering the expression of many angiogenic growth factors, specifically one of the most potent factors, vascular endothelial growth factor (310).

The adipokine profile of prostate cancer patients is thought to link obesity and prostate cancer development. Elevated leptin and decreased adiponectin levels have been associated with increased risk of prostate cancer (132). Leptin primarily functions as a satiety signal, inhibiting lipogenesis and

127

stimulating lipolysis (311), and leptin concentrations are significantly elevated in obese versus normal weight humans (312). Conversely, adiponectin, stimulates fatty acid uptake and increases fatty acid oxidation (311), and it has been shown to decrease in obese subjects (313). Leptin acts as a growth factor in pathological conditions stimulating cancer cell growth and angiogenesis (132), while adiponectin has been shown to modulate proliferation resulting in decreased growth (230). In Study 1 (Chapter 5), we observed significant increases in leptin and leptin to adiponectin ratio in patients with the most aggressive cancers. However, in Study 2 (Chapter 6), prostate cancer patients were matched to individuals of similar BMI and thus had no differences in body composition, which may explain the similar adipokine profiles between these two groups.

In Study 2 (Chapter 6), the leptin levels of the prostate cancer patients were significantly higher than the young males; however, there were no differences across the trajectory or with the matched males. This adipokine profile, (elevated leptin, decreased adiponectin) may contribute to hyperinsulinemia and the development of diabetes in prostate cancer survivors. Adiponectin has potent insulin sensitizing effects, activating AMPK and PPAR-α (314), while leptin resistance, observed with rising leptin levels, and has the opposite effect. Under normal conditions, leptin inhibits appetite, increases fatty acid oxidation, decreases glucose and ultimately reduces body weight and fat; however, in leptin resistance obesity, decreased fatty acid oxidation and increased glucose concentrations are observed, which may result in hyperinsulinemia and insulin resistance (314). Increased leptin and decreased adiponectin are associated with most aggressive forms of prostate cancer (as demonstrated in Study 1, Chapter 5); this may be of particular concern as these patients are most likely to receive ADT. ADT is also associated with increased adipose tissue (29, 145, 146), which may further exacerbate the negative effects of elevated leptin and decreased adiponectin in circulation.

Sex hormones, in particular testosterone, are hugely implicated in prostate cancer development.   Free testosterone enters the prostate cell and it is converted into dihydrotestosterone (DHT) by the 5α-reductase enzyme, which binds to the androgen receptor (AR). This causes dissociation from heat-shock proteins and phosphorylation of the AR (258). The AR dimerizes, and then enters the nucleus and binds to the androgen responsive elements in the promoter regions of numerous genes that increase PSA levels, cell growth and survival (258). However, testosterone levels decline with obesity, which would appear to be beneficial for prostate cancer patients. It is possible that free testosterone levels may not be a good biomarker of prostate cancer development (315). Kaaks and Stattin suggest that sex hormone-binding globulin (SHBG), a carrier protein that binds to testosterone and DHT and reduces the bioavailability of these hormones, may be a better biomarker (315). SHBG is inversely correlated with BMI, thus, obese individual has more bioavailable testosterone to facilitate prostate cancer growth (315). SHBG levels have been inversely associated with prostate cancer risk (316). In addition to the potential role of SHBG, the majority of interprostatic DHT is formed locally, and has little correlations with circulating testosterone levels; therefore, the declining testosterone levels observed in obesity are of less consequence than the interprostatic DHT and 5α-reductase levels, key regulators of androgen action on prostate cancer (315). Evidence for this relationship comes from examining the relationship between finasteride, a 5α-reductase inhibitor, and prostate cancer risk. Data from the Prostate Cancer Prevention Trial demonstrated a 22% reduction in risk of high-grade cancers in patients received finasteride and even higher reductions in patients with low-grade tumours (317). Despite these hypotheses, the role for androgens has not been completely eliminated (318). Here, higher testosterone levels were identified in the prostate cancer patients compared to the matched males, despite similar body composition; however, SHBG was not assess and therefore it was not possible to determine the bioactivity of

testosterone in our patients. The interprostatic DHT and 5α-reductase levels were also not characterized and therefore were unable to investigate this relationship further.

Inflammation had also been identified as a potential link between obesity and aggressive prostate cancer growth, specifically through increases in IL-6 and TNF-α. Obesity is associated with low-grade systemic inflammation, which facilitates cancer growth through several transcription factors including NF-κB and STAT3, to promote cell proliferation, survival, angiogenesis, tumour-cell migration and metastasis (319). There were no significant differences in CRP between any of the Gleason score groups in Study 1 (Chapter 5); however, CRP, IL-4, IL-6, IL-8, and IL-10, were significantly greater in the prostate cancer patients than the young males, which is indicative of a pro-inflammatory environment. Compared to the prostate cancer patients, young males had significantly lower fat mass and % body fat, which suggests that the observed increases in inflammatory marker may be related to increased adiposity. There were no significant differences in inflammatory profile between the cancer patients and the matched males, who demonstrated similar body composition. Systemic inflammation is also implicated in the development of obesity and insulin resistance in non-malignant populations. Obesity activates the IKKβ/NF-κβ and JNK pathways in various tissues. JNK activation phosphorylates of serine residues in the IRS-1 complex. This phosphorylation prevents normal activation of the IRS-1 complex via the insulin receptor and its tyrosine kinases cascade (253). IKKβ activation cause the translocation of NF-κβ, which stimulates the transcription of numerous genes associated with the development of insulin resistance including IL-6, IL-8, IL-10, and TNF-α (253). This creates a cycle of further JNK and NF-κβ activation leading to insulin resistance.

Visceral adiposity may be specifically related to aggressive prostate cancer development (320, 321) and has be previously associated with higher Gleason score (321, 322). Study 1 (Chapter 5) also noted increased abdominal adiposity, measured by waist circumference, in patients with

Gleason ≥4+3. Numerous hormonal changes occur as a result of increased visceral adiposity including decreased adiponectin, increased leptin, and hyperinsulinemia (323). Changes in adipokine profiles (increase leptin, decreased adiponectin) increase cancer cell proliferation and angiogenesis (132, 230), as described above. We observed increase leptin and decreased adiponectin levels in patients with the most aggressive cancers in Study 1 (Chapter 5), who also had the largest waist circumferences. Visceral fat increases hyperinsulinemia by increasing the liver's exposure to non-esterified fatty acids (NEFA; 324). Increased NEFA exposure can decrease the liver's ability to respond to insulin and consequently results in increased gluconeogenesis and systemic hyperinsulinemia (324). Systemic hyperinsulinemia may increase peripheral insulin resistance, consequently increasing circulating glucose levels and insulin release from the pancreas (261, 307). In Study 1 (Chapter 5), increased C-peptide, suggesting increased insulin secretion, was demonstrated in patients with the most aggressive cancers, who also demonstrated the largest waist circumferences. In Study 2 (Chapter 6), there were no direct measures of visceral adiposity, however, patients were categorized as overweight based on BMI and obese based on % body fat; these patients demonstrated impaired glucose tolerance following an OGTT which suggests impairments in the peripheral tissues' ability to respond to insulin. Taken together, visceral adiposity may be contributing to not only aggressive prostate cancer developments but a cluster of obesity-related metabolic perturbations that facilitate prostate cancer growth.

8.2 Future Directions

8.2.1 There are significant metabolic sequelae associated with aggressive prostate cancer: Does the relationship between glucose metabolism and aggressive prostate cancer change depending on geographical location?

Much of the literature that attempts to relate metabolism and lifestyle factors to prostate cancer remain inconclusive, often with contrasting results from various studies depending of the

variable of interest, such as glucose (83-85), insulin (73, 74, 325) or obesity (10, 11, 21, 22, 57, 58). One hypothesis to explain the heterogeneity of results considers differences in the geographical location of the studies. Firstly, there are widely different prostate cancer screening protocols in different countries (43, 44, 326). For example, PSA screening is widespread in North America, which consequently, results in a large number of prostate cancer diagnoses. Cancers are usually identified early and are therefore highly treatable. Thus, the 5-year survival rates for non-metastatic diseases in both Canada and the US are almost 100% (2, 327). PSA screening is not as widespread in other counties, such as those in Europe, where prevalence of new cases of prostate cancer is highly different (43, 326). The diversity in screening may contribute to a large range if metabolic disruptions observed between cohorts from North America and Europe. The second major difference between different geographical locations is diet and physical activity levels vary significantly between countries, which potentially results in significantly different metabolic profiles of prostate cancer patients. The studies in this thesis use small, single-site Canadian populations, who typically undergo more frequent PSA screening, and may consume higher saturated fats, fewer vegetables and may be less active than Mediterranean and Scandinavian men (328, 329). As such, future multi-centre work should examine the metabolic diversity across difference geographic regions.

8.2.2 Glucose tolerance improves immediately following radiation therapy: What changes occur during other forms of acute treatment?

In this thesis, high-risk prostate cancer patients demonstrate significant improvements in glucose tolerance immediately following radiation therapy. Patients with aggressive cancer present with a poor metabolic profile (obesity, increased insulin secretions, increased leptin decreased adiponectin; Chapter 6) and these patients are also most likely to receive ADT (305), which will likely exacerbate this poor metabolic profile. Understanding what is happening in the early stages of

the treatment trajectory, before significant metabolic changes occur will allow for the development of optimal lifestyle interventions to combat these metabolic perturbations.

As well, investigation into the acute treatment trajectory must go beyond hormone therapy. The literature focuses on ADT; however, there are numerous other forms of prostate cancer treatment including radiation therapy, prostatectomy, and chemotherapy. There is little literature on the metabolic health of patients who receive other forms of treatment. Here, a combination of radiation therapy with or without ADT was examined and contrary to our hypothesis observed significant improvements in glucose tolerance immediately following 7 weeks of radiation therapy. This demonstrates that, early in the treatment trajectory, radiation treatment with or without ADT has a unique metabolic response. Thong et al also demonstrated that patients who receive multiple types of treatment are diagnosed with diabetes in survivorship, not just those who receive hormone therapy (108). Each treatment type possesses its own metabolic implications for survivors and understanding the impact of each treatment will facilitate the individualized interventions for different treatment types and improve quality of life for prostate cancer survivors.

8.2.3 Immediately following treatment may be the best time for lifestyle interventions to combat the negative metabolic implications of prostate cancer

Both Study 1 and Study 2 (Chapter 5 and 6) demonstrate that prior to treatment, prostate cancer patients demonstrate significant metabolic disruption, including increased insulin secretion, impaired glucose tolerance, obesity, increased leptin, and decreased adiponectin concentrations (Chapter 5, 6). However, glucose tolerance is improved with radiation treatment, despite no changes in many of the other metabolic markers examined such as body composition, physical activity and nutrition intake. There is a significant body of literature looking to mitigate the detrimental changes in body composition that contribute to these metabolic disruptions and secondary disease state (cardiovascular disease, diabetes) via diet and exercise in patients who receive ADT (Reviewed: 330-

332). Significant improvements in glucose tolerance were noted immediately following radiation treatment, suggesting this may be an ideal time to intervene to not only combat changes in body composition but to also exploit the improved metabolic profile of prostate cancer patients at this time during the acute treatment trajectory. Investigating early versus later lifestyle interventions will help identify if it is possible to exploit improvements in glucose metabolism immediately following radiation therapy. If it is possible to mitigate or even prevent changes in metabolism and body composition in prostate cancer, it may be possible to mitigate the long-term consequences of prostate cancer treatment such as the development of cardiovascular disease and diabetes as has been demonstrated in non-malignant populations (333, 334).

8.2.4 Glucose metabolism is significantly altered in prostate cancer patients: What are the mechanisms?

This thesis has demonstrated that there are specific metabolic sequelae that occur in prostate cancer; however, the mechanisms that cause these disruptions in metabolism are unknown in this population. In this thesis, an OGTT was used to examine the body's ability to clear glucose from the blood, but the use of clamp techniques may provide further insight. A hyperglycemic clamp acutely raises plasma glucose concentrations above fasting levels, via a continuous glucose infusion, which can be used as an index of insulin secretion (273). A hyperinsulinemic-euglycemic clamp, reflective of insulin sensitivity raises insulin concentration to 100µU/mL, while glucose concentrations are held at fasting levels via continuous infusions until homeostasis is achieved (273). Further details about hepatic and skeletal muscle insulin sensitivity could be revealed via artiovenous-difference techniques (335-333), isotope dilution techniques (339), or labeled nuclear MR spectroscopy (340); however, these techniques are invasive and a difficult to perform in cancer population due to the high patient burden.   The use of human skeletal muscle biopsies would also allow the characterization of the metabolic pathways involved in prostate cancer-induced impairments in glucose tolerance, but again

134

there is a high-burden associated with these techniques. The use of tracer methodology for both glucose and lipid kinetics may also provide valuable insight into alteration in these processes. The development of novel models to investigate the relationship between insulin resistance and prostate cancer are warranted to help elucidate the complex interactions between insulin signalling and aggressive prostate cancer development.

Study 3 attempted to develop a model in which to investigate these interactions; however, it did not detect cancer related change in glucose metabolism. Thus, the development of other models, both cell and animal, to investigate these interactions in necessary. This thesis has demonstrated that there is a complex series of interactions between glucose metabolism, obesity, and prostate cancer development and progression. Understanding the mechanism of these interactions will help at numerous time points across the treatment trajectory, including reducing occurrence, preventing cancer recurrence, and decreasing the risk of secondary disease states for prostate cancer survivors. There is a significant disconnect in the literature between what factors are associated with prostate cancer through observational studies and the physiological mechanism that drive these associations. Understanding these physiological mechanisms will allow the development of the most appropriate interventions for prostate cancer patients to create the best outcomes.

8.3 Conclusions

With this thesis I aimed to create a comprehensive metabolic profile of prostate cancer patients at the time of diagnosis to examine which metabolic factors may contribute to the most aggressive forms of prostate cancer. I also sought to examine how this metabolic profile changed during the acute treatment trajectory in patient with the more aggressive cancer. Patients with higher Gleason scores were found to have higher C-peptide levels, suggesting increased insulin release. These patients also had greater abdominal adiposity, measured by waist circumference and a disrupted adipokines profile compared with patients with lower Gleason scores. All of these

135

metabolic perturbations are not only associated with the most aggressive forms of prostate cancer as well as the development of insulin resistance. The metabolic profile of high-risk prostate cancer patients across the acute treatment trajectory, prior to treatment, demonstrated impaired glucose tolerance which improved following radiation treatment and this improvement was maintained 33 weeks post-baseline, independent of changes in traditional moderators of glucose metabolism. This is beneficial for these patients as they are the patients most likely to receive ADT as part of their cancer treatment. ADT is associated with numerous body composition and metabolic changes that increase the risk of secondary disease states including diabetes in survivorship. Thus, prostate cancer patients have a poor metabolic profile at the time of diagnosis, which can increase the risk of prostate cancer recurrence and secondary disease states in survivorship including diabetes. This profile improves following treatment; however, these men are susceptible to the metabolic consequences of ADT, which will exacerbate these metabolic sequelae and lead to the development of secondary disease states such as diabetes. Combating the changes in the metabolic profile of prostate cancer patients throughout the acute treatment trajectory may mitigate the development of secondary disease states in prostate cancer survivors.

References

1. Canadian Cancer Society Steering Committee on Statistics. *Canadian Cancer Statistics 2012*. Toronto, ON: Canadian Cancer Society, 2012.
2. Canadian Cancer Society's Advisory Committee on Cancer Statistics. *Canadian Cancer Statistics 2015*. Toronto, ON: Canadian Cancer Society, 2015.
3. Bosco C, Crawley D, Adolfsson J, Rudman S, Van Hemelrijck M. Quantifying the evidence of the risk of metabolic syndrome and its components following androgen deprivation therapy for prostate cancer: a meta-analysis. *PLoS One.* 2015; 10:e0117344.
4. Morote J, Gómez-Caamaño A, Alvarez-Ossorio JL, et al. The metabolic syndrome and its components in patients with prostate cancer on androgen deprivation therapy. *J Urol.* 2015; 193:1963-1969.
5. Kintzel PE, Chase SL, Schultz LM, et al. Increased risk of metabolic syndrome, diabetes mellitus, and cardiovascular disease in men receiving androgen deprivation therapy for prostate cancer. *Pharmacotherapy.* 2008; 25:1511-1522.
6. Nguyen PL, Alibhai SMH, Basaria S, et al. Adverse effects of androgen deprivation therapy and strategies to mitigate them. *Eur Urol.* 2015; 67:825-836.
7. Tivesten A, Pinthus JH, Clarke N, et al. Cardiovascular risk with androgen deprivation therapy for prostate cancer: potential mechanisms. *Urol Oncol.* 2015; 33:464-475.
8. Smith MR. Androgen deprivation therapy and risk of cardiovascular disease in prostate cancer survivors. *Curr Urol Rep.* 2008; 9:197-202.
9. Discacciati A, Orsini N, Wolk A. Body mass index and incidence of localized and advanced prostate cancer – a dose-response meta-analysis of prospective studies. *Ann Oncol.* 2012; 23:1665-1671.
10. Keum N, Greenwood, DC, Lee DH, et al. Adult weight gain and adiposity related cancers: a dose-response meta-analysis of prospective observational studies. *J Natl Cancer Inst.* 2015; 107: djv088.
11. Zhang X, Zhou G, Sun B, et al. Impact of obesity upon prostate cancer-associated mortality: a meta-analysis of 17 cohort studies. *Oncol Letters.* 2015*;* 9:1307-1312.
12. Pandeya DR, Mittal A, Sathian B, et al. Role of hyperinsulinemia in increased risk of prostate cancer: a case control study of the Kathmandu Valley. *Asian Pac J Cancer Prev.* 2014; 15:1031-1033.
13. Di Sebastiano KM, Mourtzakis M. The role of dietary fat throughout the prostate cancer treatment trajectory. *Nutrients.* 2014; 6:6095-6109.
14. Ma RW, Chapman K. A systematic review of the effects of diet in prostate cancer prevention and treatment. *J Hum Nutr Diet.* 2009; 22:187-199.
15. Kruk J, Czerniak U. Physical activity and its relation to cancer risk: updating the evidence. *Asian Pac J Cancer Prev.* 2014; 14:3993-4003.
16. Young-McCaughan S. Potential for prostate cancer prevention through physical activity. *World J Urol.* 2012; 30:167-179.
17. McCormak M, Saad F. *Understanding Prostate Cancer*. Montreal, QC: Rogers Media Publishing; 2004.
18. Bratt O. Hereditary prostate cancer: clinical aspects *J Urol.* 2002; 168, 906–913.
19. Fradet Y, Klotz L, Trachtenberg J, et al. The burden of prostate cancer in Canada. *Can Urol Assoc J.* 2009; 3(3 Suppl 2), S92–S100.
20. Bergström A, Pisani P, Tenet V, et al. Overweight as an avoidable cause of cancer in Europe. *Int J Cancer.* 2001; 91(3):421–430.

21. Macinnis RJ, English DR. Body size and composition and prostate cancer risk: systematic review and meta-regression analysis. *Cancer Causes Control*, 2006; 17:989–1003.
22. Renehan AG, Tyson M, Egger M, et al. Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies, *Lancet*, 2008; 371:569–578.
23. Cao Y, Ma J. Body mass index, prostate cancer-specific mortality, and biochemical recurrence: a systematic review and meta-analysis. *Cancer Prev Res*, 2011; 4:486–501.
24. Cattabiani C, Basaria S, Ceda GP, et al. Relationship between testosterone deficiency and cardiovascular risk and mortality in adult men. *J Endocrinol Invest.* 2012; 35:104–120.
25. Collier A, Ghosh S, McGlynn B, et al. Prostate cancer, androgen deprivation therapy, obesity, the metabolic syndrome, type 2 diabetes, and cardiovascular disease: a review *Am J Clin Oncol.* 2012; 35:504–509.
26. Grosman H, Fabre B, Mesch V, et al. Lipoproteins, sex hormones and inflammatory markers in association with prostate cancer. *Aging Male.* 2010; 13:87–92.
27. Keating NL, O'Malley AJ, Freedland SJ, et al. Diabetes and cardiovascular disease during androgen deprivation therapy: observational study of veterans with prostate cancer. *J Natl Cancer Inst.* 2010; 102:39–46.
28. Ribeiro AF, Camara C, Segre CA, et al. Cardiovascular risks of androgen deprivation therapy. *Arq Bras Cardiol*, 2010; 95:412–415.
29. Galvão DA, Spry NA, Taaffe DR, et al. Changes in muscle, fat and bone mass after 36 weeks of maximal androgen blockade for prostate cancer. *BJU Int*. 2008; 102:44–47.
30. Kim TN, Choi KM. The implication of sarcopenia and sarcopenic obesity on cardiometabolic disease. *J Cell Biochem.* 2015; 116:1171—1178.
31. Keating NL, Liu PH, O'Malley AJ, et al. Androgen-deprivation Therapy and Diabetes Control Among Diabetic Men with Prostate Cancer. *Eur Urol.* 2014; 65:816-824.
32. Wang LA, Murphy CT, Ruth K, et al. The impact of obesity on outcomes after definitive dose-escalated intensity-modulated radiotherapy for localized prostate cancer. *Cancer.* 2015; 121:3010-3017.
33. Edge S, Byrd DR, Compton CC et al. (Eds) *AJCC Cancer Staging Manual.* 7th edition. New York, NY: Springer-Verlag New York, 2010.
34. Stenman UH, Leinonen J, Zhang WM, et al. Prostate-specific antigen. *Semin Cancer Biol.* 1999; 9:83-93.
35. Canadian Task Force on Preventative Health Care, Bell, N, Connor Gorber S, et al. Recommendations on screening for prostate cancer with the prostate-specific antigen test. *CMAJ.* 2014; 186:1225-1234.
36. Levy I. Prostate cancer: the epidemiological perspective. *Can J Oncol.* 1994; 4 Suple 1: 4-7.
37. Li J, Djenaba JA, Soman A, et al. Recent trends in prostate cancer incidence by age, cancer state, and grade in the United States, 2001-2007. *Prostate Cancer.* 2012;2012:691380.
38. Gleason DF. Histological grading of prostate cancer: a perspective. *Hum Pathol.* 1992;23:273-279.
39. D'Amico AV, Whittington R, Malkowicz SB, et al. Biochemical outcome after radical prostatectomy, external beam radiation therapy, or interstitial radiation therapy of clinical localized prostate cancer. *JAMA.* 1998; 16:969-974.
40. Thompson I, Thrasher JB, Aus G et al. Guideline for the Management of Clinically Localized Prostate Cancer: 2007 Update. *J Urol.* 2007; 177:2106-2131.
41. Canadian Cancer Society. Treatment of Prostate Cancer. Accessed 16 January 2016. From http://www.cancer.ca/en/cancer-information/cancer-type/prostate/treatment/?region=sk.

42. Heidenreich A, Aus G, Bolla M, et al. EUA guideline on prostate cancer. *Eur Urol.* 2008; 53:68-80.
43. Izawa JI, Klotz L, Siemens DR, et al. Prostate cancer screening: Canadian Guidelines 2011. *Can Urol J.* 2011; 5:235-240.
44. Heindenreich A, Bellmunt J, Bolla M, et al. EUA guidelines on prostate cancer. Part 1: Screening, diagnosis, and treatment of clinically localized disease. *Eur Urol.* 2011; 59:61-71.
45. Huggins C, Hodges CV. Studies on prostatic cancer 1: The effect of castration, or estrogen and of androgen injection on serum phosphatases in metastatic carcinoma of the prostate. *Cancer Res.* 1994. 1:293-297.
46. Prostate Cancer Canada. Treatment options. Accessed 16 January 2016. From http://www.prostatecancer.ca/Prostate-Cancer/Treatment/Treatment-Options-(1).
47. Crawford ED, Hou AH. The role of LHRH antagonists in the treatment of prostate cancer. *Oncology (Williston Park).* 2009; 23:626-630.
48. Després JP, Moorjani S, Lupien PJ, et al. Regional distribution of body fat, plasma lipoproteins, and cardiovascular disease. *Arteriosclerosis.* 1990; 10:497-511.
49. Heshka S, Ruggiero A, Bray GA, et al. Altered body composition I ntype 2 diabetes mellitus. *Int J Obesity.* 2008; 32:780-787.
50. Saad F, chi KM, Finelli A, et al. The 2015 CUA- CUOG guidelines for the management of castration-resistant prostate cancer (CRPC). *Can Urol Assoc J.* 2015; 9: 90-96.
51. Michels J, Montemurro T, Murray N, et al. First- and second-line chemotherapy with docetaxel or mitoxantrone in patients with hormone-refractory prostate cancer: does sequence matter? *Cancer.* 2006; 106:1041-1046.
52. Haas GP, Delongchamps NB, Jones RF, et al. Needle biopsies on autopsy prostates: sensitivity of cancer detection based on true prevalence. *J Natl Cancer Inst.* 2007; 99:1484-1489.
53. Carter BS, Beaty TH, Steinberg GD, et al. Mendelian inheritance of familial prostate cancer. *Proc Natl Acad Sci USA,* 1992; 89:3367–3371.
54. Johns LE, Houlston RS. A systematic review and meta-analysis of familial prostate cancer risk. *BJU Int,* 2003; 91:789–794.
55. Hoffman RM, Gilliland FD, Eley JW, et al. Racial and ethnic differences in advanced-stage prostate cancer: the Prostate Cancer Outcomes Study. *J Natl Cancer Inst.* 2001; 93:388–395.
56. Rosen EM, Fan S, Goldberg ID. BRCA1 and prostate cancer. *Cancer Invest*, 2001; 19: 396–412

57. Guh DP, Zhang W, Bansback N, et al, The incidence of co-morbidities related to obesity and overweight: a systematic review and meta-analysis. *BMC Public Health.* 2009; 9:88.
58. Hu MB, Liu SH, Jiang HW, et al. Obesity affects the biopsy-mediated detection of prostate cancer, particularly higher-grade prostate cancer: a dose-response meta-analysis. *PLoS One,* 2014; 9:e106677
59. Zhang X, Zhou G, Sun B, et al. Impact of obesity upon prostate cancer-associated mortality: a meta-analysis of 17 cohort studies. *Oncol Letters.* 2015*;* 9:1307-1312.
60. Allott EH, Masko EM, Freedland SJ. Obesity and prostate cancer: weighing the evidence *Eur Urol,* 2013; 63: 800–809.
61. Bañez LL, Hamilton RJ, Partin AW, et al. Obesity-related plasma hemodilution and PSA concentration among men with prostate cancer *JAMA*, 2007; 298:2275–2280.
62. Demark-Wahnefried W. Dietary interventions in prostate cancer. *Curr Urol Rep*, 2008; 9:217–225.

63. Hurst R, Hooper L, Norat T, et al. Selenium and prostate cancer: systematic review and meta-analysis. *Am J Clin Nutr,* 2012; 96:111-122.
64. Kenfield SA, Chang ST, Chan JM. Diet and Lifestyle Interventions in Active Surveillance Patients with Favorable-Risk Prostate Cancer. *Curr Treat Options Oncol,* 2007; 8:173–196.
65. Sacks FM, Bray GA, Carey VJ, et al. Comparison of weight-loss diets with different compositions of fat, protein, and Carbohydrates. *N Engl J Med,* 2009; 360:859-873.
66. Shai I, Schwarzfuchs D, Henkin Y, et al. Weight loss with low-carbohydrate, Mediterranean, or low-fat diet. *N Engl J Med.* 2008; 359:229-241.
67. Field AE, Willett WC, Lissner L, et al. Dietary fat and weight gain among women in the in the Nurses' Health Study. *Obesity.* 2007; 15:967-976.
68. Abete I, Astrup A, Martínez JA, et al. Obesity and the metabolic syndrome: role of different dietary macronutrients distribution patterns and specific nutritional components on weight loss and maintenance. *Nutr Rev.* 2010; 68:214-231.
69. Gong Z, Neuhouser ML, Goodman PJ, et al. Obesity, diabetes, and risk of prostate cancer: results from the Prostate Cancer Prevention Trial. *Cancer Epidemiol Biomarkers Prev*, 2006; 15:1977–1983.
70. De Nunzio C, Freedland SJ, Miano R, et al, Metabolic syndrome is associated with high grade Gleason score when prostate cancer is diagnosed on biopsy. *Prostate.* 2011; 71:1492-1498.
71. De Maddalena C, Vodo S, Petroni A, et al. Impact of testosterone on body fat composition. *J Cell Physiol,* 2012; 227:3744–3748.
72. Freedland SJ, Platz EA. Obesity and prostate cancer: making sense out of apparently conflicting data. *Epidemiol Rev,* 2007; 29:88–97.
73. Chen C, Lewis SK, Voigt L, et al. Prostate carcinoma incidence in relation to prediagnostic circulating levels of insulin-like growth factor I, insulin-like growth factor binding protein 3, and insulin. *Cancer,* 2004; 103:76–84.
74. Stattin P, Rinaldi S, Biessy C, et al. High levels of circulating insulin-like growth factor-I increase prostate cancer risk: a prospective study in a population-based nonscreened cohort. *J Clin Oncol,* 2004; 22:3104–3112.
75. Argilés JM, López-Soriano F J. Insulin and cancer (Review) *Int J Oncol,* 2001; 18: 683–687.
76. Boyd, B. Insulin and Cancer. *Integr Cancer Ther.* 2003; 2:315-329.
77. Gallagher EJ, Leroith D. Minireview: IFG, Insulin, and Cancer. *Endocrinol.* 2011; 152:2546-2551.
78. Hsing AW, Gao YT, Chua S, et al. Insulin resistance and prostate cancer risk. *J Natl Cancer Inst.* 2003; 95: 67–71.
79. Prabhat P, Tewari R, Natu SM, et al. Is central obesity, hyperinsulinemia and dyslipidemia associated with high-grade prostate cancer? A descriptive cross-sectional study. *Indian J Urol,* 2010; 26:502–506.
80. Albanes D, Weinstein SJ, Wright ME, et al. Serum insulin, glucose, indices of insulin resistance, and risk of prostate cancer. *J Natl Cancer Inst.* 2009; 101: 1272–1279.
81. Bonovas S, Filioussi K, Tsantes A. Diabetes mellitus and risk of prostate cancer: a meta-analysis *Diabetologia*, 2004; 47:1071–1078.
82. Kasper JS, Giovannucci E. A meta-analysis of diabetes mellitus and the risk of prostate cancer. *Cancer Epidemiol, Biomarkers Prev,* 2006;15: 2056–2062.
83. Darbinian JA, Ferrara AM, Van Den Eeden SK, et al. Glycemic status and risk of prostate cancer. *Cancer Epidemiol, Biomarkers Prev,* 2008; 17:628-635.

84. Preston MA, Riis AH, Ehrenstein V, et al. Metformin use and prostate cancer risk. *Eur Urol.* 2014; 66:1012-1020.
85. Wright JL, Stanford JL. Metformin use and prostate cancer risk in Caucasian men: results from a population-based case-control study. *Cancer Causes Control.* 2009; 20:1617-1622.
86. Chen YB, Chen Q, Wang Z, et al. Insulin therapy and risk of prostate cancer: a systematic review and meta-analysis of observational studies. *PLos One.*2013: 8:e815954.
87. Lawlor MA, Alessi DR. PKB/Akt: a key mediator of cell proliferation, survival and insulin responses. *J Cell Sci.* 2001; 114: 2903–2910.
88. Nandeesha N. Insulin: a novel agent in the pathogenesis of prostate cancer. *Int Urol Nephrol.* 2009; 41:267-272.
89. Monti S, Proietti-Pannunzi L, Sciarra A, et al. The IGF axis in prostate cancer. *Curr Pharm Des.* 2007; 13:719-727.
90. Pollak M. Insulin and insulin-like growth factor signalling in neoplasia. *Nat Rev Cancer.* 2008; 8:915-928.
91. Cox ME, Gleave ME, Zakikhani M, et al. Insulin receptor expression by human prostate cancers The Prostate, 2009;69(1):33–40.
92. Arcidiacono B, Iiritano S, Nocera A, et al. Insulin resistance and cancer risk: an overview of the pathogenetic mechanisms. *Ex Diabetes Res.* 2012; 2012:789174.
93. Chan JM, Stampfer MJ, Giovannucci E, et al. Plasma insulin-like growth factor-1 and prostate cancer risk: a prospective study. *Science.* 1998; 279:563-566.
94. Renehan AG, Zwahlen M, Minder C, et al. Insulin-like growth factor (IGF)-1, IGF binding protein-3, and cancer risk: a systematic review and meta-analysis. *Lancet.* 2004; 363:1346-1353.
95. Stocks T, Lukanova A, Rinaldi S, et al. Insulin resistance is inversely related to prostate cancer: A prospective study in Northern Sweden. *Int J Cancer.* 2007;120: 2678–2686.
96. Zamboni PF, Simone M, Passaro A, et al. Metabolic profile in patients with benign prostate hyperplasia or prostate cancer and normal glucose tolerance. *Horm Metab Res.* 2003; 35:296-300.
97. Tekdoğan UY, Bağcioğlu M, Özcan S, et al. The effect of oral glucose tolerance test on insulin and some related indicators in elderly male patients with prostate cancer and benign prostat hyperplasia. *Turkish J Geriatrics.* 2015;18:10-14.
98. Smith MR. Insulin sensitivity during combined androgen blockade for prostate cancer. *J Clin Endocrinol Metabol,* 2006; 91: 1305–1308.
99. Smith MR, Lee H, Mcgovern F, et al. Metabolic changes during gonadotropin-releasing hormone agonist therapy for prostate cancer. *Cancer,* 2008; 112: 2188–2194.
100. Dockery F, Bulpitt CJ, Agarwal S, et al. Testosterone suppression in men with prostate cancer leads to an increase in arterial stiffness and hyperinsulinaemia. *Clin Sci (London, England: 1979),* 2003:104: 195–201.
101. Smith JC. The effects of induced hypogonadism on arterial stiffness, body composition, and metabolic parameters in males with prostate cancer. *J Clin Endocrinol Metabol*, 2001;86: 4261–4267.
102. Sağlam HS, Köse O, Kumsar Ş, et al. Fasting blood glucose and lipid profile alterations following twelve-month androgen deprivation therapy in men with prostate cancer. *Scientific World J,* 2012; 2012: 696329.

103. Hara N. Prostate carcinogenesis with diabetes and androgen-deprivation-therapy-related diabetes: an update. *Exp Diabetes Res,* 2012; 2012: 801610.

104. Dennis RJ, Maldonado D, Rojas MX, et al. Inadequate glucose control in type 2 diabetes is associated with impaired lung function and systemic inflammation: a cross-sectional study. *BMC Pulmonary Med,* 2010;10: 38.

105. Omori K, Ohira T, Uchida Y, et al. Priming of neutrophil oxidative burst in diabetes requires preassembly of the NADPH oxidase. *J Leukocyte Biol,* 2008;84: 292–301.

106. Kalinchenko SY, Tishova YA, Mskhalaya GJ, et al. Effects of testosterone supplementation on markers of the metabolic syndrome and inflammation in hypogonadal men with the metabolic syndrome: the double-blinded placebo-controlled Moscow study. *Clin Endocrinol,* 2010; 73: 602–612.

107. Norata GD, Tibolla G, Seccomandi PM, et al. Dihydrotestosterone decreases tumor necrosis factor-alpha and lipopolysaccharide-induced inflammatory response in human endothelial cells. *J Clin Endocrinol Metabol* 2006; 91: 546–554

108. Thong MSY, van de Poll-Franse L, Hoffman RM, et al. Diabetes mellitus and health-related quality of life in prostate cancer: 5-year results from the Prostate Cancer Outcomes Study. *BJU Int.* 2010; 107:1223-1231.

109. Qatanani M, Lazar MA. Mechanisms of obesity-associated insulin resistance: many choices on the menu. *Genes Dev.* 2007; 21:1443-1455.

110. Freedland SJ, Sun L, Kane CJ, et al. Obesity and oncological outcome after radical prostatectomy: impact of prostate-specific antigen-based prostate cancer screening: results from the Shared Equal Access Regional Cancer Hospital and Duke Prostate Center databases. *BJU Int,* 2008; 102; 969–974.

111. Chalfin HJ, Lee SB, Jeong BC, et al. Obesity and long-term survival after radical prostatectomy. *J Urol.* 2014; 192: 1100-1104.

112. Freedland SJ, Sokoll LJ, Mangold, LA, et al. Serum leptin and pathological findings at the time of radical prostatectomy. *J Urol,* 2005;173: 773–776.

113. Okotie OT, Aronson WJ, Wieder JA, et al. Predictors of metastatic disease in men with biochemical failure following radical prostatectomy. *J Urol,* 2004; 171:2260–2264.

114. Stroup SP, Cullen J, Auge BK, et al. Effect of obesity on prostate-specific antigen recurrence after radiation therapy for localized prostate cancer as measured by the 2006 Radiation Therapy Oncology Group-American Society for Therapeutic Radiation and Oncology (RTOG-ASTRO) Phoenix consensus definition. *Cancer.* 2007;110(5):1003–1009.

115. Strom SS, Kamat AM, Gruschkus SK, et al. Influence of obesity on biochemical and clinical failure after external-beam radiotherapy for localized prostate cancer. *Cancer.* 2006; 107:631–639.

116. Efstathiou JA, Chen M-H, Renshaw AA, et al. Influence of body mass index on prostate-specific antigen failure after androgen suppression and radiation therapy for localized prostate cancer. *Cancer.* 2007;109:1493–8.

117. Geinitz H, Thamm R, Mueller T, et al. Impact of body mass index on outcomes after conformal radiotherapy in patients with prostate cancer. *Int J Radiat Oncol Biol Phys.* 2011;81:16–22.

118. Efstathiou JA, Skowronski RY, Coen JJ, et al. Body mass index and prostate-specific antigen failure following brachytherapy for localized prostate cancer. *Int J Radiat Oncol Biol Phys.* 2008;71:1302–1308.

119. Van Roermund JGH, Hinnen KA, Battermann JJ, et al. Body mass index is not a prognostic marker for prostate-specific antigen failure and survival in Dutch men treated with brachytherapy. *BJU Int.* 2010;105:42–8.

120. Keto CJ, Aronson WJ, Terris MK, et al. Obesity is associated with castration-resistant disease and metastasis in men treated with androgen deprivation therapy after radical prostatectomy: results from the SEARCH database *BJU Int,* 2012;110: 492–498.

121. Smith MR. Obesity and sex steroids during gonadotropin-releasing hormone agonist treatment for prostate cancer. *Clin Cancer Res,* 2007;13: 241–245.

122. Roberts DL, Dive C, Renehan AG. Biological mechanisms linking obesity and cancer risk: new perspectives. *Ann Rev Med,* 2010; 61: 301–316.

123. Kahn SE, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature.* 2006;444:840-846.

124. Cohen DH, LeRoith D. Obesity, type 2 diabetes, and cancer: the insulin and IGF connection. *Endocrine-Related Cancer.* 2012; 19:F27-F45.

125. Freedland SJ, Mavropoulos J, Wang A, et al. Carbohydrate restriction, prostate cancer growth, and the insulin-like growth factor axis. *The Prostate,* 2007;68: 11–19.

126. Venkateswaran V, Haddad AQ, Fleshner NE, et al. Association of diet-induced hyperinsulinemia with accelerated growth of prostate cancer (LNCaP) xenografts. *Cancer Spectrum Knowledge Environment,* 2007;99: 1793–1800.

127. Kasper JS, Liu Y, Giovannucci E. Diabetes mellitus and risk of prostate cancer in the health professionals follow-up study. *Int J Cancer*, 2009; 124: 1398–1403.

128. Ma J, Li H, Giovannucci E, et al. Prediagnostic body-mass index, plasma C-peptide concentration, and prostate cancer-specific mortality in men with prostate cancer: a long-term survival analysis. *Lancet Oncol,* 2008; 9: 1039–1047.

129. Rowlands MA, Gunnell D, Harris R, et al. Circulating insulin-like growth factor peptides and prostate cancer risk: a systematic review and meta-analysis, *Int J Cancer.* 2009;124: 2416–2429.

130. Nickerson T, Chang F, Lorimer D, et al. In vivo progression of LAPC-9 and LNCaP prostate cancer models to androgen independence is associated with increased expression of insulin-like growth factor I (IGF-I) and IGF-I receptor (IGF-IR). *Cancer Res,* 2001; 6: 6276–6280.

131. Bastard JP, Maachi M, Lagathu C, et al. Recent advances in the relationship between obesity, inflammation, and insulin resistance. *Eur Cytokine Netw.* 2006;17: 4-12.

132. Alshaker H, Sacco K, Alfraidi A, et al. Leptin signalling, obesity and prostate cancer: molecular and clinical perspective on the old dilemma. *Oncotarget,* 2015;6: 35556-35563.

133. Hoda MR, Popken G. Mitogenic and anti-apoptotic actions of adipocyte-derived hormone leptin in prostate cancer cells. *BJU Int.* 2008; 102: 383–388.

134. Somasundar P, Frankenberry KA, Skinner H, et al. Prostate cancer cell proliferation is influenced by leptin. *J Surgical Res,* 2004;118: 71–82.

135. Noda T, Kikugawa T, Tanji N, et al. Long-term exposure to leptin enhances the growth of prostate cancer cells. *Int J Onocol.* 2015; 46:1535-1542.

136. Stattin P, Söderberg S, Hallmans G, et al. Leptin is associated with increased prostate cancer risk: a nested case-referent study. *J Clin Endocrinol Metab.* 2001; 86: 1341- 1345.

137. Gade-Andavolu R, Cone LA, Shu S, et al. Molecular interactions of leptin and prostate cancer. *Cancer J.* 2006; 12:201-206.

138. Lai GY, Giovannucci EL, Pollak MN, et al. Association of C-peptide and leptin with prostate cancer incidence in the Health Professionals Follow-up Study. *Cancer Causes Contol.* 2014; 25:625-632.

139. Baillargeon J, Platz EA, Rose DP, et al. Obesity, adipokines, and prostate cancer in a prospective population-based study. *Cancer Epidemiol Biomarkers Prev,* 2006; 15:, 1331–1335.

140. Li H, Stampfer MJ, Mucci L, et al. A 25-year prospective study of plasma adiponectin and leptin concentrations and prostate cancer risk and survival. *Clin Chem,* 2010; 56:, 34–43.

141. De Rosa A, Monaco ML, Capasso M, et al. Adiponectin oligomers as potential indicators of adipose tissue improvement in obese subjects. *Eur J Endocrinol.* 2013; 169:34-43.

142. Nigro E, Scudiero O, Monaco ML, et al. New insight into adiponectin role in obesity and obesity-related diseases. *Biomed Res Int.* 2014; 2014:658913.

143. Goktas S, Yilmaz MI, Caglar K, et al. Prostate cancer and adiponectin. *Urol.* 2005; 65:1168-1172.

144. Kelesidis I, Kelesidis T, Mantzoros CS. Adiponectin and cancer: a systematic review. *Br J Cancer.* 2006; 94:1221-1225.

145. Bylow K, Mohile SG, Stadler WM, et al. Does androgen-deprivation therapy accelerate the development of frailty in older men with prostate cancer?: a conceptual review. *Cancer,* 2007;110: 2604–2613.

146. Chen Z, Maricic M, Nguyen P, et al. Low bone density and high percentage of body fat among men who were treated with androgen deprivation therapy for prostate carcinoma. *Cancer,* 2002; 95: 2136–2144.

147. Després J-P, Cartier A, Côté M, et al. The concept of cardiometabolic risk: Bridging the fields of diabetology and cardiology. *Ann Med,* 2008; 40: 514–523.

148. Robinson LE, Graham TE. Metabolic syndrome, a cardiovascular disease risk factor: role of adipocytokines and impact of diet and physical activity. *Can J Appl Physiol,* 2004; 29; 808–829.

149. Haffner SM, Valdez RA, Mykkänen L, et al. Decreased testosterone and dehydroepiandrosterone sulfate concentrations are associated with increased insulin and glucose concentrations in nondiabetic men. *Metab Clin Experimental,* 1994; 43: 599–603.

150. Laaksonen DE, Niskanen L, Punnonen K, et al. Testosterone and sex hormone-binding globulin predict the metabolic syndrome and diabetes in middle-aged men. *Diabetes Care,* 2004;27: 1036–1041.

151. Muller M, Grobbee DE, Tonkelaar I, et al. Endogenous sex hormones and metabolic syndrome in aging men, *J Clin Endocrinol Metab.* 2005; 90: 2618–2623.

152. Meyer F, Bairati I, Shadmani R, et al. Dietary fat and prostate cancer survival. *Cancer Causes Control,* 1999; 10: 245–251.

153. Ornish D, Weidner G, Fair WR, et al. Intensive lifestyle changes may affect the progression of prostate cancer. *J Urol.* 2005;174: 1065–1069.

154. van Londen GJ, Levy ME, Perera S, et al. Body composition changes during androgen deprivation therapy for prostate cancer: a 2-year prospective study. *Crit Rev Oncol Hematol.* 2008; 68: 172–177.

155. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (May 2001). Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA.* 285: 2486–97.

156. Alberti KG, Zimmet PZ. Definition, Diagnosis, and Classification of Diabetes Mellitus and its Complications Part 1: Diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabetes Med.* 1998; 15:539-553.

157. Alberti G, Zimmet P, Shaw J, et al. The IDF consensus worldwide definition of the metabolic syndrome. *(PDF). IDF communications.* 2006.

158. Balkua B, Charles MA. Comment on the provisional report form the WHO consultation. European Group for the Study of Insulin Resistance (EGIR). *Diabetes Med.* 1999; 16:442-443.

159. Grundy SM, Brewer B, Cleeman JI, et al. Definition of metabolic syndrome: report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. *Circulation.* 2004; 109:433-438.

160. Esposito K, Chiodini P, Cauano A, et al. Effects of metabolic syndrome and it components on prostate cancer risk: meta-analysis. *J Endocrinol Invest.* 2013; 36:132-139.

161. Laukkanen JA, Laaksonen DE, Niskanen L, et al. Metabolic syndrome and the risk of prostate cancer in Finnish men: A population-based study. *Cancer Epidemiol Biomarkers Prev* 2004, 13: 1646-50.

162. Lund Haheim L, Wisloff TF, Holme I, et al. Metabolic syndrome predicts prostate cancer in a cohort of middle-aged Norwegian men followed for 27 years. *Am J Epidemiol.* 2006, 164: 769-74.

163. Tuohimaa P, Tenkanen L, Syvala H, et al. Interaction of factors related to the metabolic syndrome and vitamin D on risk of prostate cancer. *Cancer Epidemiol Biomarkers Prev.* 2007, 16: 302-7.

164. Pelucchi C, Serraino D, Negri E, et al. The metabolic syndrome and risk of prostate cancer in Italy. *Ann Epidemiol* 2011, 21: 835-41.

165. Tande AJ, Platz EA, Folsom AR. The metabolic syndrome is associated with reduced risk of prostate cancer. *Am J Epidemiol* 2006, 164: 1094-102.

166. Inoue M, Noda M, Kurahashi N, et al; Japan Public Health Center based Prospective Study Group. Impact of metabolic factors on subsequent cancer risk: results from a large-scale population-based cohort study in Japan. *Eur J Cancer Prev* 2009, 18: 240-7.

167. Martin RM, Vatten L, Gunnell D, et al. Components of the metabolic syndrome and risk of prostate cancer: the HUNT 2 cohort, Norway. *Cancer Causes Control* 2009, 20: 1181-92.

168. Wallner LP, Morgenstern H, McGree ME, et al. The effects of metabolic conditions on prostate cancer incidence over 15 years of follow-up: results from the Olmsted County Study. *BJU Int* 2010, 107: 929-35.

169. Grundmark B, Garmo H, Loda M et al. The metabolic syndrome and the risk of prostate cancer under competing risks of death from other causes. *Cancer Epidemiol Biomarkers Prev.* 2010, 19: 2088-96.

170. Beebe-Dimmer JL, Dunn RL, Sarma AV, et al Features of the metabolic syndrome and prostate cancer in African-American men. *Cancer.* 2007, 109: 875-81.

171. Russo A, Autelitano M, Bisanti L. Metabolic syndrome and cancer risk. *Eur J Cancer* 2008, 44: 293-7.
172. Beebe-Dimmer JL, Nock NL, Neslund-Dudas C, et al. Racial differences in risk of prostate cancer associated with metabolic syndrome. *Urology.* 2009, 74: 185-90.
173. Osaki Y, Taniguchi S-I, Tahara A, et al. Metabolic syndrome and incidence of liver and breast cancers in Japan. *Cancer Epidemiol.* 2012, 36: 141-7.
174. De Nunzio C, Aronson W, Freedland SJ, et al. The correlation between metabolic syndrome and prostatic diseases. *Eur Urol,* 2012;61: 560–570.
175. De Marzo AM, Platz EA, Sutcliffe S, et al. Inflammation in prostate carcinogenesis. *Nature Rev Cancer,* 2007;7: 256–269.
176. Buschemeyer WC III, Freedland SJ. Obesity and Prostate Cancer: Epidemiology and Clinical Implications. *Eur Urol.* 2007; 52: 331–343.
177. Morote J, Gómez-Caamaño A, Alvarez-Ossorio JL, et al. The metabolic syndrome and it components in patients with prostate cancer on androgen deprivation therapy. *J Urol.* 2015; 193:1963-1969.
178. Eri LM, Urdal P, Bechensteen AG. Effects of the luteinizing hormone-releasing hormone agonist leuprolide on lipoproteins, fibrinogen and plasminogen activator inhibitor in patients with benign prostatic hyperplasia. *J Urol,* 1995;154: 100–104.
179. Smith MR. Changes in Body Composition during Androgen Deprivation Therapy for Prostate Cancer. *J Clin Endocrinol Metab,* 2002; 87: 599–603.
180. Fujioka S, Matsuzawa Y, Tokunaga K, et al. Contribution of intra-abdominal fat accumulation to the impairment of glucose and lipid metabolism in human obesity. *Metabolism.* 1987; 36:54-59.
181. Smith MR, Lee H, Fallon MA, et al. Adipocytokines, obesity, and insulin resistance during combined androgen blockade for prostate cancer. *Urol,* 2008; 71: 318–322.
182. Trujillo ME, Scherer PE. Adiponectin--journey from an adipocyte secretory protein to biomarker of the metabolic syndrome. *J Internal Med,* 2005; 257: 167–175.
183. Haffner SM. The metabolic syndrome: inflammation, diabetes mellitus, and cardiovascular disease. *Am J Cardiol*, 2006;97: 3A–11A.
184. Saylor PJ, Smith MR. Metabolic Complications of Androgen Deprivation Therapy for Prostate Cancer. *J Urol.*, 2009; 181: 1998–2008.
185. Nandeesha H, Koner BC, Dorairajan LN. Altered insulin sensitivity, insulin secretion and lipid profile in non-diabetic prostate carcinoma. *Acta Physiologica Hungarica,* 2008;95: 97–105.
186. Jain D, Ray A, Bahadur AK, et al. Status of epidermal growth factor receptors family in hormone-dependent carcinomas of the breast and prostate with reference to serum lipids and lipoproteins. *Indian J Clin Biochem,* 2001; 16: 42–51.
187. Mydlo JH, Tieng NL, Volpe MA, et al. A pilot study analyzing PSA, serum testosterone, lipid profile, body mass index and race in a small sample of patients with and without carcinoma of the prostate. *Prostate cancer Prostatic Diseases,* 2001;4: 101–105.
188. Allott EH, Howard, LE, Cooperberg, MR, et al. Serum lipid profile and risk of prostate cancer recurrence: results from the SEARCH database, *Cancer Epidemiol Biomarkers Prev.* 0214; 23:2349-2356.
189. Zhong S, Zhang X, Chen L, et al. Statin use and mortality in cancer patients: systematic review and meta-analysis of observational studies. *Cancer Treatment Rev.* 2015; 41:554-567.
190. Braga-Basaria M, Muller DC, Carducci MA, et al. Lipoprotein profile in men with prostate cancer undergoing androgen deprivation therapy. *Int J Impotence Res*, 2006; 18: 494–498.

191. Xiang Y-Z, Xiong H, Cui Z-I, et al. The association between metabolic syndrome and risk of prostate cancer, high-grade prostate cancer, advanced prostate cancer, prostate cancer-specific mortality and biochemical recurrence. *J Exp Clin Cancer Res.* 2013: 32:9.

192. Monteiro R, Azevedo I. Chronic inflammation in obesity and the metabolic syndrome. Mediators of Inflammation. 2010; 2010:289645.

193. Gundy SM. Hypertryglyceridemia, insulin resistance, and the metabolic syndrome. Am J Cardiol. 1999; 83: 25-29.

194. Taish AM, Guay A, Feeley R, et al. The dark side of testosterone deficiency: I. Metabolic syndrome and erectile dysfunction. *J Androl.* 2009; 30:10-22.

195. Hsing AW, Sakoda LC, Chua SC Jr. Obesity, metabolic syndrome, and prostate cancer. *Am J Clin Nutr.* 2007; 86:843S-857S.

196. Rhee H, Vela I, Chung E. Metabolic syndrome and prostate cancer: a review of complex interplay amongst various endocrine factors in the pathophysiology and progression of prostate cancer. *Horm Canc.* Epub 2015 Nov 6.

197. Ma J, Li H, Giovannucci E, Mucci L, Qiu W, Nguyen PL, Gaziano JM, Pollak M, Stampfer MJ. Prediagnostic body-mass indices, plasma C-peptide concentration, and prostate cancer-specific mortality in men with prostate cancer: a long-term survival analysis. *Lancer Oncol.* 2008; 9:1039-1047.

198. Burton A, Martin RM, Holly J, Lane JA, Donovan JL, Hamdy FC. Neal DE, Tilling K. Associations of adiponectin and leptin with stage and grade of PSA-detected prostate cancer: the ProtecT study. *Cancer Causes Control.* 2013; 24:323-334.

199. Graff JN, Beer TM, Lui B, Sonpavde G, Taioli E. Pooled analysis of c-reactive protein levels and mortality in prostate cancer patients. *Clin Genitoutin Cancer.* 2015; 13:e217-e221.

200. Polonsky KS, Rubenstein AH. C-peptide as a measure of the secretion and hepatic extraction of insulin. Pitfalls and limitations. *Diabetes.* 1984; 33:486-494.

201. Golabek T, Bukowczan J, Chlosta P, Powroznik J, Dobruch J, Borowka A. Obesity and prostate cancer incidence and mortality: a systematic review of prospective cohort studies. *Urol Int.* 2014; 92:7-14.

202. Thiebaud D, Jacot E, Defronzo RA, et al. The effect of graded doses of insulin on total glucose uptake, glucose oxidation, and glucose storage in man *Diabetes*, 1982;*31*: 957–963.

203. Sarabia V, Lam L, Burdett E, et al. Glucose transport in human skeletal muscle cells in culture: stimulation by insulin and metformin. *J Clin Invest.* 1992;90: 1386-1395.

204. Sarabia V, Ramlal T, Klip A. Glucose uptake is human and animal muscle cells in culture. *Biochem Cell Bio.* 1989; 68:536-542.

205. Basso D, Valerio A, Seraglia R, et al. Putative pancreatic cancer-associated diabetogenic factor: 2030MW peptide. *Pancreas.* 2002; 24:8-14.

206. De Nunzio C, Presicce F, Lombardo R, et al. Physical activity as a risk factor for prostate cancer diagnosis: a prospective biopsy cohort analysis. *BJU Int.* Epub: 2015 Apr 24.

207. Schwab U, Lauritzen L, Tholstrup T, et al. Effects of the amount and type of dietary fat on cardiometabolic risk factors and risk of developing type 2 diabetes, cardiovascular diseases, and cancer: a systematic review. *Food Nutr Res.* 2014; 58:25145.

208. Ford ES, Khol WH III, Mokdad AH, et al. Sedentary behavior, physical activity, and the metabolic syndrome among U.S. adults. *Obesity.* 2005; 12:608-614.

209. Riccardi G, Giacco R, Rivellese AA. Dietary fat, insulin sensitivity and the metabolic syndrome. *Clin Nutr.* 2004; 23:447-456.

210. Jebb SA, Moore MS. Contribution of a sedentary lifestyle and inactivity to the etiology of overweight and obesity: current evidence and research issues. *Med Sci Sport Exerc.* 1999;31(11 Suppl):S534-S541.

211. Cole E, Margel D, Greenspan M, et al. Is there a role for anterior zone sampling as part of saturation trans-rectal ultrasound guided prostate biopsy?. *BMC Urol.* 2014; 14:34.

212. Lowery OH, Passonneau JV. *The flexible system of enzymatic analysis.* 1972. New York: Academic Press.

213. Matthews DR, Hosker JP, Rudenski AS, et al. Homesostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia.* 1985; 28:412-419.

214. World Health Organization. Waist Circumference and Waist-Hip Ratio: Report of a WHO Expert Consultation. *WHO Press.* 2011. Available from: http://apps.who.int/iris/bitstream/10665/44583/1/9789241501491_eng.pdf?ua=1,

215. Kyle UG, Genton L, Slosman DO, et al. Fat-free and fat mass percentiles in 5225 healthy subjects aged 15-98. *Nutrition.* 2001; 17:534-541.

216. Janssen I, Heymsfield SB, Baumgartner RN, et al. Estimation of skeletal muscle mass by bioelectrical impedance analysis. *J Appl Physiol (1985).* 2000; 89:465-471.

217. Godin G, Shepard RJ. A simple method to assess exercise behavior in the community. *Can J Appl Sport Sci.* 1985; 10:141-146.

218. Enright PL. The 6-min walk test: a quick measure of functional status in healthy adults. *Chest.* 2003; 123:387-398.

219. Massy-Wastropp NM, Gill TK, Taylor AW, et al. Hand grip strength: age and gender stratified normative data in a population-based study. *BMC Research Notes.* 2011; 4:127.

220. Thompson FE, Byers T. Dietary assessment resource manual. *J Nutr.* 1994; 124:2245S-2317S.

221. Thompson IM, Pauler DK, Goodman PJ, et al. Prevalence of prostate cancer among men with prostate-specific antigen levels < or =4.0 mg/mL. *N Engl J Med.* 2004; 350: 2239-2246.

222. Macor C, Ruggeri A, Mazzonetto P, et al. Visceral adipose tissue impairs insulin secretion and insulin sensitivity but not energy expenditure in obesity. *Metabolism.* 1997; 46:123-129.

223. López-Jaramillo P, Gómez-Arbeláez D, López- López J, et al. The role of leptin/adiponectin ratio in metabolic syndrome and diabetes. *Horm Mol Biol Clin Investig.* 2014; 18:37-45.

224. Calabro P, Yeh ET. Intra-abdominal adiposity, inflammation, and cardiovascular risk: new insight into global cardiometabolic risk. *Curr Hypertens Rep.* 2008; 10:32-38.

225. Stevens VL, Jacobs EJ, Sun J, et al. No association of plasma levels of adiponectin and C-peptide with risk of aggressive prostate cancer in the cancer prevention study II nutrition cohort. *Cancer Epidemiol Biomarkers Prev.* 2014; 23:890-892.

226. Adhami VM, Afag F, Mukhtar H. Insulin-like growth factor-I axis and a pathway for chemoprevention. *Clin Cancer Res.* 2006; 12:5611-5614.

227. Correa LL, Neto LV, Lima GA, et al. Insulin-like growth factor (IGF)-1, IGF binding protein-3, and prostate cancer: correlation Gleason score. *Int Braz J Urol.* 2015; 41:110-115.

228. Harding JL, Shaw JE, Anstey KJ, et al. Comparison of anthropometric measures as predictors of cancer incidence: A pooled collaborative analysis of 11 Australian cohorts. *Int J Cancer.* 2015; 137:1699-1708.

229. Tewari R, Rajender S, Natu SM, et al. Significance of obesity markers and adipocytokines in high grade and high state prostate cancer in North Indian men- a cross-sectional study. *Cytokine.* 2013; 63:130-134.

230. Gao Q, Zheng J. Adiponectin-induced antitumor activity on prostatic cancer through inhibiting proliferation. *Cell Biochem Biophys.* 2014; 70:461-465.

231. Hussein S, Satturwar S, Van der Kwast T/ Young-age prostate cancer. *J Clin Pathol.* 2015; 68:511-515.

232. Barbieri M, Rizzo MR, Manzella D, et al. Age-related insulin resistance: is it an obligatory finding? The lesion from healthy centenarians. *Diabetes Metab Res Rev.* 2001; 17:19-26.

233. Villareal DT, Apovian CM, Kushner RF, et al. Obesity in older adults: technical review and position statement of the American Society for Nutrition and NAASO, The Obesity Society. *Am J Clin Nutr.* 2005; 82:923-934.

234. Asmar R, Beebe-Dimmer JL, Korgavkar K, et al. Hypertension, obesity and prostate cancer biochemical recurrence after radical prostatectomy. *Prostate Cancer Prostatic Dis.* 2013; 16: 62-66.

235. Macleod LC, Chery EY, Zeliadt SB, et al. Metabolic syndrome, dyslipiedamia and prostate cancer recurrence after primary surgery or radiation in a veterans cohort. *Prostate Cancer Prostatic Dis.* 2015; 18:190-195.

236. Choi SM, Kam SC. Metabolic effects of androgen deprivation therapy. *Korean J Urol.* 2015; 56:12-18.

237. Roberts SB, Rosenberg, I. Nutrition and aging: changes in the regulation of energy metabolism with aging. *Physiol Rev.* 2006; 86:651-667.

238. Redinger RN. The pathophysiology of obesity and its clinical manifestations. *Gastroenterol Hepatol (NY).* 2007; 3:856-863.

239. Lean ME. Pathophysiology of obesity. *Proc Nutr Soc.* 2000; 59:331-336.

240. Van Hemelrijck M, Garmo H, Holmberg L, Stattin P, Adolfsson J. Multiple events of fracture and cardiovascular and thromboembolic disease following prostate cancer diagnosis: results from the population-based PCBaSe Sweden. *Eur Urol.* 2012; 61:690-700.

241. Tsai HT, Keating NL, Van Den Eeden SK, Haque R, Cassidy-Bushrow AE, Ulcickas Yood M, Smith MR, Potosky AL. Risk of diabetes among patients receiving primary androgen deprivation therapy for clinically localized prostate cancer. *J Urol.* 2015; 193:1956-1962

242. Janssen I, Baumgartner RN, Ross R, Rosenberg IH, Roubenoff R. Skeletal muscle cutpoints associated with elevated physical disability risk in older men and women. *Am J Epidemiol.* 2004; 159:413-421.

243. Froelicher VF, Brammell H, Davis G, Noguera I, Stewart A, Lancaster MC. A comparison of three maximal treadmill exercise protocols. *J Appl Phyiol.* 1974; 36:720-725.

244. Darby LA, Yaekle BC. Physiological responses during two types of exercise performed on land and in the water. *J Sports Med Phys Fitness.* 2000; 40:303-311.

245. Mahler DA, Froelicher VF, Houston Miller N. ASCM's Guidelines for Exercise Testing and Prescription 5th Ed. Media, PA: William & Wilkins; 1995

246. O'Connor B, Simmons J, O'Shea P. Weight training today. St. Paul, MN: West, 1989.

247. Antonelli JA, Jones LW, Bañez LL, Thomas JA, Anderson K, Taylor LA, Gerber L, Anderson T, Hoyo C, Grant D, Freedland SJ. Exercise and prostate cancer risk in a cohort of veterans undergoing prostate needle biopsy. *J Urol.* 2009; 182:2226-2231.

248. Allison DB, Paultre F, Maggio C, Mezzitis N, Pi-Sunyer FX. The use of area under curves in diabetes research. *Diabetes Care.* 1995; 18:245-250.

249. Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, Quon MJ. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *J Clin Endocrinol Metab.* 2000; 85:2402-2410.

250. Matsuda M and DeFronzo RA, Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care.* 1999; 22:1462-1470.

251. Granada ML, Murillo J, Lucas A, Salinas I, Llopis MA, Castells I, Foz M, Sanmartí A. Diagnostic efficiency of serum IGF-I, IGF-binding protein-3 (IGFBP-3), IGF-I/IGFBP-3 molar ratio and urinary GH measurements in the diagnosis of adult GH deficiency: importance of an appropriate reference population. *Eur J Endocrinol.* 2000; 142:243-53.

252. Meier U, Gressner AM. Endocrine regulation of energy metabolism: review of pathobiochemical and clinical chemical aspects of leptin, Ghrelin, adiponectin, and resistin. *Clin Chem.* 2004; 50:1511-1525.

253. Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. *J Clin Invest.* 2006; 116:1793-1801.

254. Trayhurn P, Wood IS. Signalling role of adipose tissue: adipokines and inflammation in obesity. *Biochem Soc Trans.* 2005; 33:1078-1081.

255. Davidson MB. The effect of aging on carbohydrate metabolism: a review of the English literature and a practival approach to diagnosis of diabetes mellitus in the elderly. *Metabolism.* 1979; 28:688-705.

256. Faupel-Badger JM, Kidd LCR, Albanes D, Virtamo J, Woodson K, Tangrea JA. Association of IL-10 polymorphism with prostate cancer risk and grade of disease. *Cancer Causes Control.* 2008; 19:119-124.

257. Hong EG, Ko HJ, Cho YR, Kim HJ, Ma Z, Yu TY, Friedline RH, Kurt-Jones E, Finberg R, Fischer MA,Granger EL, Norbury CC, Hauschka SD, Philbrick WM, Lee CG, Elias JA, Kim JK. Interleukin-10 prevents diet-induced insulin resistance by attenuating macrophage and cytokine response in skeletal muscle. *Diabetes.* 2009; 58:252-2535.

258. Feldman BJ, Feldman F, The development of androgen-independent prostate cancer. *Nature Rev Cancer.* 2001; 1:34-45.

259. Kapoor D, Malkin CJ, Channert KS, Jones TH. Androgens insulin resistance and vascular disease in men. *Clin Endocrinol.* 2005; 63:239-250,

260. Patel TP, Rawal K, Bagchi AK, Akolkar G, Bernardes N, da Silva Dias D, Gupta S, Singal PK. Insulin resistance: and additional risk factor in the pathogenesis of cardiovascular disease in type 2 diabetes. *Heart Fail Rev.* Epub: 5 Nov 2015.

261. DeFronzo RA, Ferrannini E, Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. *Diabetes Care.* 1991; 14:173-194.

262. Lam DW, LeRoith D. Metabolic Syndrome. [Updated 2015 May 19]. In: De Groot LJ, Beck-Peccoz P, Chrousos G, et al., editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000-. Available from: http://www.ncbi.nlm.nih.gov/books/NBK278936/.

263. Wuermli L, Joerger M, Henz S, Schmid HP, Riesen WF, Thamas G, Krek W, Cerny T, Gillesen S. Hypertrigliceridemia as a possible risk factor for prostate cancer. *Prostate Cancer Prostatic Dis.* 2005; 8:316-320.

264. Mondul AM, Weinstein SJ, Virtamo J, Albanes D. Serum total and HDL cholesterol and risk of prostate cancer. *Cancer Causes Control.* 2011; 22:1545-1552.

265. Ren J, Anversa P. the insulin-like growth factor I system: physiological and pathophysiological implication in cardiovascular diseases associated with metabolic syndrome. *Biochem Pharmacol.* 2015; 93:409-417.

266. Friedrich N, Thuesen B, Jørgensen T, Juul A, Spielhagen C, Wallaschofksi H, Linneberg A. The association between IGF-I and insulin resistance: a general population study in Danish adults. *Diabetes Care.* 2012; 35:768-73.

267. Singh B, Saxena A. Surrogate markers of insulin resistance: a review. *World J Diabetes.* 2010; 1:36-37.

268. Higgins LH, Withers HG, Garbens A, et al. Hypoxia and the metabolic phenotype of prostate cancer cells. *Biochimica et Biophysica Acta.* 2009; 1787: 1433-1443.

269. Ryan AS. Insulin resistance with aging: Effects of diet and exercise. *Spots Med,* 2000; 30: 327-345.

270. Isaksson B, Strommer L, Friess H, et al. Impaired insulin action on phosphatidylinositol 3-kinase activity and glucose transport in skeletal muscle of pancreatic cancer patients. *Pancreas* 2003; 26:173–17.

271. Owens J, Moreira K, Bain G. Characterization of primary skeletal muscle cells from multiple commercial sources. *In Vitro Cell Dev Biol – Animal,* 2013; 49:695-705.

272. Jung CY, Pampal AL. Cytochalasin B binding sites and glucose transport carrier in human erythrocyte ghost. *J Biol Chem.* 1977; 252:5456-5463.

273. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Phyiol.* 1979; 237:E214-E223.

274. Meneilly GS, Ryan AS, Minaker KL, et al. The effect of age and glycemic level on the response of beta-cell to glucose-dependent insulinotropic polypeptide and peripheral tissue sensitivity to endogenously release insulin. *Clin Endocrinol Metab.* 1998; 83:2925-2932.

275. Aronson WJ, Barnard RJ, Freedland SJ, et al. Growth inhibitory effect of low fat diet on prostate cancer cells: results of a prospective, randomized dietary intervention trial in men with prostate cancer. *J Urol.* 2010; 183:345-350

276. Barnard RJ, Kobayashi N, Aronson WJ. Effect of diet and exercise intervention on the growth of prostate epithelial cells. *Prostate Cancer Prostatic Dis.* 2008;11:362-366

277. Barnard RJ, Leung PS, Aronson WJ, et al. A mechanism to explain how regular exercise might reduce the risk for clinical prostate cancer. *Eur J Cancer Prev.* 2007; 16:415-421.

278. Oliver JC, Bland LA, Oettinger CW et al. Cytokine kinetics in an in vitro whole blood model following an endo toxin challenge. *Lymphokine Cytokine Res.* 1993; 12:115-120.

279. Metherel AH, Stark KD. The stability of blood fatty acids during storage and potential mechanisms of degradation: a review. *Prostaglandins Leukot Essent Fatty Acids.* 2016; 104:33-43.

280. Isacc C, De Mattos CN, Co Rego FMP, et al. Replacement of fetal calf serum by human serum as a supplementation for human fibroblast culture. *Rev Bras Cir Plást.* 2011; 26: 379-84.

281. Schwab U, Lauritzen L, Tholstrup T, et al. Effects of the amount and type of dietary fat on cardiometabolic risk factors and risk of developing type 2 diabetes, cardiovascular diseases, and cancer: a systematic review. Food Nutr Res. 2014; 58:25145.

282. Zhang M, Hu T, Zhang S, et al. Associations of difference adipose tissue depots with insulin resistance: a systematic review and meta-analysis of observational studies. Sci Rep. 2015; 5:18495.

283. Polonsky KS, Rubenstein AH. C-peptide as a measure of the secretion and hepatic extraction of insulin. Pitfalls and limitations. *Diabetes.* 1984; 33:486-494.

284. Rabe K, Lehrke M, Parhofer KF, et al. Adipokines and insulin resistance. Mol Med. 2008; 14:741-751.

285. Giugliano D, Ceriello A, Esposito K. Glucose metabolism and hyperglycemia. Am J Clin Nutr. 2008; 87:217S-222S.

286. Neuhouser ML, Till C, Kristal A, et al. Finasteride modifies the relation between serum C-peptide and prostate cancer risk: Results from the Prostate Cancer Prevention Trial. Cancer Prev Res. 2010; 3: 279-289.

287. Djiogue S, Kamdje AHN, Vecchio L, et al. Insulin resistance and cancer: the role of insulin and IGFs. Endocr Relat Cancer. 2013; 20: R1-R17.

288. Lima GA, Corrêa LL, Gabrich R, et al. IGF-I, insulin and prostate cancer. Arg Bras Endocrinol Metabol. 2009; 53: 969-975.

289. Wolk A, Mantzoros CS, Andersson S-O, et al. Insulin-like growth factor 1 and prostate cancer risk: a population-based, case-control study. J Natl Cancer Inst. 1998; 90: 911-915.

290. Papatsoris AG, Karamouzis MV, Papvassiliou AG, Novel insights into the implication of the IGF-1 network in prostate cancer. TRENDS Mol Med. 2005; 11:52-55.

291. Roberts CT Jr. IGF-1 and prostate cancer. Novartis Found Symp. 2004; 262:193-199.

292. Kaaks R, Lukanova A, Sommersberg B. Plasma androgens, IGF-1, body size, and prostate cancer risk: a synthetic review. Prostate Cancer Prostatic Dis. 2000; 3:157-172.

293. Maccario M, Tassone F, Grottoli S, et al. Neuroendocrine and metabolic determinants of the adaptation of GH/IGF-1 axis to obesity. Ann Endocrinol (Paris). 2002; 6:140-144.

294. Martin JL, Baxter RC. Insulin-like growth factor-binding protein from human plasma. Purification and characterization. J Biol Chem. 1986; 261:8754-8760.

295. Schoen RE, Schragin J, Weissfeld JL, et al. Lack of association between adipose tissue distribution an dIGF-1 and IGFBP-3 in men and women. Cancer Epidemiol Biomarkers Prev. 2002; 11: 581-586.

296. Nam SY, Lee EJ, Kim KR, et al. Effect of obesity on total and free insulin-like growth factor (IGF)-1, and their relationship to IGF-binding protein (BP)-1. IGFBP-2, IGFBP-3, insulin and growth hormone. Int J Obes Relat Metab Disord. 1997; 21:355-359.

297. Meyers-Davies EJ, D'Agostino R, Kerter AJ, et al. Intensity and amount of physical activity in relation to insulin sensitivity: the insulin resistance atherosclerosis study. JAMA. 1998; 279: 669-674.

298. Williams KJ, Wu X. Imbalanced insulin action in chronic over nutrition: clinical harm, molecular mechanisms. And a way forward. Atherosclerosis. 2016; 247: 225-282.

299. Leung PS, Aronson WJ, Ngo TH, et al. Exercise alters the IGF axis in vivo and increases p53 protein in prostate tumor cells in vitro. J Appl Physiol (1985). 2004: 96: 450-454.

300. Ngo TH, Barnard RJ, Leung PS, et al. Insulin-like growth factor I (IGF-I) and IGF binding protein-1 modulate prostate cancer cell growth and apoptosis: possible mediators for the effects of diet and exercise on cancer cell survival. *Endocrinology.* 2003; 144:2319-2324.

301. Ngo TH, Barnard RJ, Tymchuk CN, et al. Effects of diet and exercise on serum insulin, IGF-1, and IGFBP-1 levels and growth of LNCaP cells in vitro (United States). *Cancer Causes Control.* 2002; 13:929-935.

302. Tymchuk CN, Barnard RJ, Heber D, et al. Evidence of an inhibitory effect of diet and exercise on prostate cancer cell growth. *J Urol.* 2001; 166: 1185-1189.

303. Tymchuk CN, Barnard, Ngo TH, et al. Role of testosterone, estradiol, and insulin in diet- and exercise-induced reductions in serum-stimulated prostate cancer cell growth in vitro. *Nutr Cancer.* 2002; 42:112-116.

304. Saruç M, Karaarsian M, Rasa K, et al. Pancreatic cancer and glucose metabolism. Turk J Gastroenterol. 2009; 20:257-260.

305. Canadian Urological Association. Hormone therapy for prostate cancer. Dorval, QC: Canadian Urological Association, 2014.

306. Yu, IC, Lin HY, Sparks JD, et al. Androgen receptor roles in insulin resistance and obesity in males: the linkage of androgen-deprivation therapy to metabolic syndrome. Diabetes. 2014; 63:3180-3188.

307. Samuel VT, Shulman GI. Mechanisms for insulin resistance: Common threads and missing links. Cell. 148:852-871.

308. Freedland SJ, Aronson WJ. Examining the relationship between obesity and prostate cancer. Rev Urol. 2004; 6: 73-81.

309. Jeukendrup A, Gleeson M. Normal ranges of body weight and body fat. In Sport Nutrition. 2nd Edition. Champaign, IL: Human Kinetics; 2010.

310. Mukherjee P, Sotnikov AV, Mangain HJ, et al. Energy intake and prostate tumor growth, angiogenesis, and vascular endothelial growth factor expression. J Natl Cancer Inst. 1999; 91:512-523.

311. Balistreri CR, Caruso C, Candore G. The role of adipose tissue and adipokines in obesity related inflammatory diseases. Mediators Inflamm. 2010; 2010:802078.

312. Considine RV, Sinha MK, Heiman ML, et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med. 1996; 334:292-295.

313. Kern PA, Gregorio GB, Lu T, et al. Adiponectin expression from human adipose tissue: relation to obesity, insulin resistance, and tumor necrosis factor-α. Diabetes. 2003; 52:1779-1785.

314. Yadav A, Kataria MA, Saini, et al. role of leptin and adiponectin in insulin resistance. Clinica Chimica Acta. 2013; 417:80-84.

315. Kaaks R, Stattin P. Obesity, endogenous hormone metabolism, and prostate cancer risk: a conundrum of "highs" and "lows". Cancer Prev Res. 2010; 3:259-262.

316. Roddam AW, Allen NE, Appleby P, et al. Endogenous sex hormones and prostate cancer: a collaborative analysis of 18 prospective studies. *J Natl Cancer Inst.* 2008; 100; 170-183.

317. Kaplan SA, Roehrborn CG, Meehan AG, et al. PCPT: Evidence that finasteride reduces risk of most frequently detected intermediate- and high0grande (Gleason score 6 and 7) cancer. *Urology.* 2009; 73:935-939.

318. Hsing AW, Chu LW, Stanczyk FZ. Androgen and prostate cancer: is the hypothesis dead? *Cancer Epidemiol Biomarkers Prev.* 2008; 17:2525-2530.

319. Mantovani A, Allavena P, Sica A, et al. Cancer-related inflammation. *Nature.* 2008; 454:436-444.

320. Boehm K, Sun M, Larcher A, et al. Waist circumference, waist-hip ratio, body mass index, and prostate cancer risk: results form he North-American case-control study Prostate Cancer & Environment Study. *Urol Oncol.* 2015; 33:494.e1-7/

321. De Nunzio C, Albisinni S, Freedland SJ, et al. Abdominal obesity as a risk factor for prostate camcer diagnosis and high grade disease: a prospective multicenter Italian cohort study. *Urol Oncol.* 2013; 31: 997-1002.

322. Zilli T, Chagnon M, Van Nguyen T, et al. Influence of abdominal adiposity, waist circumference, and body mass index on clinical and pathological findings in patients treated with radiotherapy for localized prostate cancer. *Cancer.* 2010; 116:5650-5658.

323. Kapoor D, Goodwin E, Channer KS, et al. Testosterone replacement therapy improves insulin resistance, glycaemic control, visceral adiposity and hypercholesterolaemia in hypogonadal men with type 2 diabetes. *Eur J Endocrinol.* 2006; 154: 899-906.

324. Ebbert JO and Jensen MD. Fat depots, free fatty acids, and dyslipidemia. *Nutrients.* 2013; 5:498-508.

325. Hsing AW, Chua Jr S, Gao YT, et al. Prostate cancer risk and serum levels of insulin and leptin: a population based study. *J Natl Cancer Inst.* 2001; 93:783-789.

326. Heidenreich A, Aus G, Bolla M, et al. EUA guideline on prostate cancer. *Eur Urol.* 2008; 53:68-80.

327. American Cancer Society. *Prostate Cancer Survival Rates.* Accessed 23 March 2016. Fromhttp://www.cancer.org/cancer/prostatecancer/detailedguide/prostate-cancer-survival-rates

328. Ogce F, Ceber E, Ekit R, et al. Comparison of Mediterranean, Western and Japanese diets. *Asian Pacific J Cancer Prev.* 2008; 9; 351-356.

329. Hallal PC, Andersen LB, Bull FC, et al. Global physical activity levels: surveillance progress, pitfalls, and prospects. *Lancet.* 380; 247-257.

330. Haseen F, Murray LJ, Cardwell DR, et al. The effects of androgen deprivation therapy on body composition in men with prostate cancer: systematic review and meta-analysis. *J Cancer Surviv.* 2010; 4: 128-139.

331. Hasenoehrl T, Keilani M, Sedghi Komanadj T, et al. The effects if resistance exercise on physical performance and health-related quality of life in prostate cancer patient: a systematic review. *Support Care Cancer.* 2015; 23:2479-2497.

332. Keogh JW, MacLeod RD. Body composition, physical fitness, functional performance, quality of life, and fatigue benefits of exercise for prostate cancer patients: a systematic review. *J Pain Symptom Manage.* 2012; 43: 96-110.

333. Diabetes Prevention Program Research Group. Reduction in the incidence of type @ diabetes with lifestyle interventions or metformin. *N Engl J Med.* 2002; 346: 393-403.

334. Thompson PD, Buchner D, Piña IL, et al. Exercise and Physical Activity in the Prevention and Treatment of Atherosclerotic Cardiovascular Disease: A Statement From the Council on

Clinical Cardiology (Subcommittee on Exercise, Rehabilitation, and Prevention) and the Council on Nutrition, Physical Activity, and Metabolism (Subcommittee on Physical Activity). *Circulation.* 2003; 107:3109-3116.

335. Somogyi M. Studies of arteriovenous differences in blood sugar: Effects of alimentary hyperglycemia on the rate of extrahepatic glucose assimilation. *J Biol Chem.* 1948; 174:189-200.

336. Somogyi M. Studies of arteriovenous differences in blood sugar: Effects of hypoglycemia on the rate of extrahepatic glucose assimilation. *J Biol Chem.* 1948; 174:597-603.

337. Somogyi M. Studies of arteriovenous differences in blood sugar: Effects of insulin administered intravenously in the post absorptive state. *J Biol Chem.* 1949; 179:217-233.

338. Somogyi M. Studies of arteriovenous differences in blood sugar: Effects of intravenous insulin and simultaneous feeding. *J Biol Chem.* 1949; 179:1289-1297.

339. Steele R, Wall JS, de Bodo RC, et al. Measurement of size and turnover rate of body glucose pool by the isotope dilution method. *Am J Physiol.* 1956; 187:15-24.

340. Shulman GI, Rothman DL, Smith D, et al. Mechanism of liver glycogen repletion in vivo by nuclear magnetic resonance spectroscopy. *J Clin Invest.* 1985; 76:1229-1236.

Appendix A
Glossary of Relevant Medical Terms

5-year Survival Rate: the percentage of patients alive 5 years following diagnosis. Used to estimate prognosis of a particular disease.

Androgen Deprivation Therapy (ADT): any treatment that reduced the levels of androgen in the body to prevent them from reaching prostate cancer cells and stimulating growth. There are two primary types of ADT, chemical and surgical. An orchiectomy, surgical removal of the testicles, luteinizing hormone-releasing hormone analogs, luteinizing hormone-releasing hormone antagonists, anti-androgen are common forms of ADT. All serve to reduce androgen to castrate levels.

Cancer Survivor: An individual who has completed primary treatment.

Digital Rectal Exam (DRE): physical medical examination involving the insertion of a digit (finger) into the rectum to palpate for abnormalities in the prostate and surrounding tissue.

Metastasis: the spread of cancer from one organ or tissue to distant, non-adjacent organs or tissue.

Prostate Specific Antigen (PSA): a serine protease produced by the prostate, used as a blood biomarker for prostate cancer. In non-malignant tissue it is secreted in seminal fluid, while in malignant tissue, it leaks into the blood stream raising circulating levels.
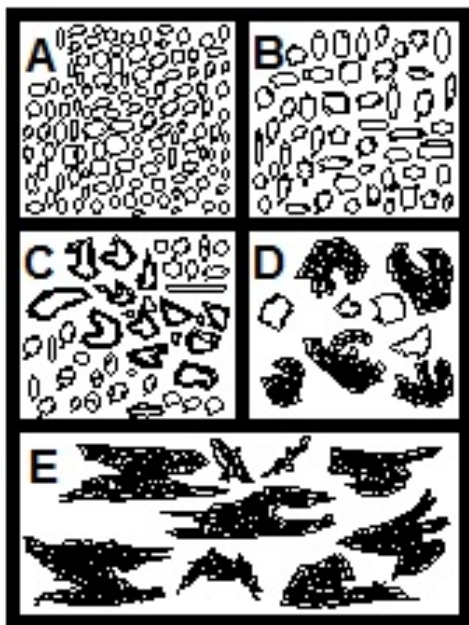
PSA Failure: elevation of circulating PSA levels following prostate cancer treatment, indicating cancer recurrence.

Appendix B
Gleason Scores

Use of Gleason Scores as a Pathology Marker of Prostate Cancer

The Gleason scores is most common pathology marker used to assess prostate cancer severity. The pathologist evaluates the patterns of the cells within the biopsy core and subsequently assigns 2 scores (each score ranges from1-5) to the tissue, based on the pattern of cell growth. The first score is the most common cell pattern and the second score is the second most common cell pattern. These scores are then added together to determine Gleason score (a total score ranging from 2-10). A Gleason score of 1 indicates small uniform, tightly packed gland cells (Figure 1A), while larger more spaced cells with loosely defined edges indicates a Gleason score of 2 (Figure 1B). With a Gleason score of 3 the cells begin to take on irregular shapes and spacing (Figure 1C); a Gleason score of 4 indicates that the glands have begun to fuse into cords or sheets (Figure 1D). Finally, a score of 5 indicates that the cells have taken on a ragged sheet like appearance (Figure 1E) (Gleason, 1992). Generally, the higher the Gleason score, the more severe the cancer; however, a Gleason score of ≥ 7 is often defined in the literature as the cut point between moderate and severe prostate cancer diagnosis.

Figure 1: Standardized drawing of prostatic adenocarcinoma histological patterns. **A.** Gleason 1: Small uniform, tightly packed gland cells. **B.** Gleason 2: Larger, more spaced cells. **C.** Gleason 3: Irregular shape and spacing. **D.** Gleason 4: Cells have fused into sheets. **E.** Gleason 5: Ragged sheet like appearance.

Appendix C
Detailed Methodology

Biochemical Analysis

Serum Glucose

Serum glucose was measured using a spectrophotometer (Shimadzu UV160U UV-Visible Recording Spectrophotometer; Columbia, MD) and peroxidase/glucose oxidase enzymatic reaction. Test tubes were filled in triplicate with 10 μL distilled water (blank), glucose standard or serum sample. We added 2.5 mL of a reagent solution containing peroxidase, glucose oxidase and o-dianisidine dihydrochloride to each tube. Tubes were then vortexed and incubated at 37° C for 30 minutes. During the incubation period, glucose in the blanks, standards and samples reacts with glucose oxidase, releasing hydrogen peroxide. Peroxidase then catalyzes a reaction between the liberated hydrogen peroxide and o-dianisidine dihydrochloride, forming oxidized o-dianisidine. Oxidized o-dianisidine produces a colour that can be read at 450 nm by the spectrophotometer. The intensity of the colour in the samples in comparison to the intensity of the colour in the standards provides an indication of glucose concentration.

Serum Insulin

Serum insulin was measured in duplicate using the Coat-A-Count Insulin Radioimmunoassay kit (Study 1,Siemans Healthcare Diagnostics; Deerfield, IL) or the Millipore Human Insulin Specific Radioimmunoassay kit (Study 2, EMD Millipore, Billerica, MA) as the Coat-A-Count assay was discontinued.

For the Coat-A-Count kit, 200 μL of blank, standard or sample was added to polypropylene tubes pre-coated with insulin antibody. To all tubes 1.0 mL of ^{125}I-labeled insulin was added. Tubes were vortexed and incubated at room temperature for 24 hours. During the incubation period, [125]I-

labeled insulin competes with insulin in the sample or standard for binding sites on the insulin antibody molecules, which are fixed to the polypropylene tube walls. After 24 hours, the supernatant was aspirated and tubes were counted for 1 minute using a gamma counter (Wallac Wizard 1470 Automatic Gamma Counter; PerkinElmer Life and Analytical Sciences; Woodbridge, ON). Samples or standards with high concentrations of insulin bind less ^{125}I-labeled insulin and are less radioactive.

For the Millipore kit, 100μL of assay buffer was added to each of the glass test tubes required, followed by 100μL of sample, standard, or blank, 100μL of hydrated ^{125}I-labeled insulin and 100μL of human insulin antibody. Tubes were vortexed, covered and left to incubate over-night (24 hours) at room temperature. The following day 1mL of cold precipitating reagent was added to all tube. The tubes were vortexed and incubated for 20 minutes at 4°C. Tubes were then centrifuged at 3000g for 20 minutes, after which the supernatant was aspirated and the precipitate was counted using the same gamma counter for 1 minutes. This assay works under the same principle as the Siemens assays; samples or standards with high concentrations of insulin bind less ^{125}I-labeled insulin and are less radioactive.

Serum C-peptide

Serum C-peptide was assessed in duplicate using a C-peptide Double Antibody Radioimmunoassay kit (Siemans Healthcare Diagnostics; Deerfield, IL). During the assay, 25 μL of standard or serum was combined with 100 μL ^{125}I-labeled C-peptide and 100 μL C-peptide antibody in polypropylene tubes. The tubes were vortexed and incubated for 4 hours at room temperature. During the incubation, C-peptide in the standard and sample competed with ^{125}I-labeled C-peptide for binding sites on the C-peptide antibody. After the 4-hour incubation, 1.0 mL of cold (4° C) precipitating solution was added to each tube. The tubes were vortexed and then centrifuged at 3000g for 15 minutes. After centrifugation, the supernatant was aspirated and the tubes were counted for 1

minute in a gamma counter. Radioactivity is inversely correlated with C-peptide concentration.

Concentration of C-peptide was determined by interpolating samples from a graph of known C-

peptide concentrations.

Glycated Hemoglobin (HbA1C)

Glycated hemoglobin was determined from fresh whole blood samples using the

commercially available A1CNow$^+$ kit (Bayer Healthcare LLC, Sunnyvale, CA), which uses

immunological reactions to determine glycated hemoglobin and a chemical reaction to determine total

hemoglobin. These values are then used to calculate the percentage of glycated hemoglobin. For the

assay, 5 μL of whole blood was added to the blood collector provided by the manufacturer. Once

collected, the blood collector is than combined with the manufacturer sampler body and shaken to

adequately dilute the sample. The diluted sample was then added to the test cartridge where blue

microparticles conjugate to the anti-HbA1c antibodies and migrate along reagent strips to the

detectors. The amount of blue microparticles present on the strip is proportional to the amount of

HbA1C in the sample. Total hemoglobin is measured using basic chemistry. The sample diluent

reacts with the hemoglobin present in the sample and converts hemoglobin to met-hemoglobin, which

is red-brown in colour. The intensity of the red-brown is measured on the reagent strips and is

proportional to the concentration of hemoglobin. Quantification of both forms of hemoglobin

occurred using reflectance photometry and two LED lights. The monitoring device than provided a

reading of the percent of glycated hemoglobin.

Perchloric Acid (PCA) Extraction for Serum Lactate

Serum contains many extraneous proteins that may interfere with the reading of certain

metabolites such as lactate. To remove these extra proteins, a solution of 0.6 M perchloric acid was

prepared by combining perchloric acid stock solution and water. Following which 500 μL of this

solution was combined with 100 μL of serum in an eppendorf tube. All tubes and solutions were kept

on ice throughout the procedure. Tubes were vortexed and centrifuged at 4° C at 15 000g for 2

minutes. After centrifugation, 250 μL of 1.25 M potassium bicarbonate was added to each tube.

Tubes were incubated on ice for 10 minutes, and centrifugation was repeated. The supernatant was

extracted, transferred to new eppendorf tubes and stored at -80 °C until use. A dilution factor of 8.5

is introduced with the PCA extraction; the dilution factor was taken into account when calculating

final concentrations of lactate.

Serum Lactate following PCA Extraction

Serum lactate was measured using a spectroflourophotometric assay similar that described by

(1). A reagent solution was first prepared containing 15 mL of hydrazine, 15 mL glycine, and 1500

μL of NAD^+, brought to a volume of 150 mL with distilled water and the pH was adjusted to 10.0.

Lactate dehydrogenase (5200 U/mL) was diluted by combining 250 uL of lactate dehydrogenase

(Sigma-Alderich, St. Louis, MO) with 1 mL of the reagent. Subsequently, 25 μL of dilute blank,

standard, and sample was added to glass test tubes in triplicate. Dilute reagent was added to each test

tube at a volume of 1 mL and test tubes were vortexed. Baseline readings were taken on the

spectroflourophotometer (RF-1501; Chimadzu, Columbia, MD) with the absorbance set between 365

nm and 455 nm. After baseline readings, 25 μL of dilute lactate dehydrogenase was added to each

test tube. Tubes were vortexed and incubated in the dark for 120 minutes. Following incubation, a

final reading of each tube was completed and the final absorbency of each tube was determined by

subtracting the baseline reading from the final reading. During the incubation, lactate present in the

tubes reacts with NAD^+ to form pyruvate and NADH as catalyzed by lactate dehydrogenase.

Pyruvate then reacts with the hydrazine present in the reagent to drive the first reaction to completion.

161

The fluorescence of NADH – created from the reduction of NAD$^+$ - is directly proportional to the concentration of each sample.

Serum Triglycerides

Fasting triglycerides concentrations were measured using a commercially available triglyceride-GPO regent set (Pointe Scientific, Canton, MI). Triglyceride reagent (active ingredients: ATP 1.0mM, Magnesium Sale >5.9mM, 3-hydroxy-2,3,4-tribomobenzoic acid (TBHB) 2.0mM, glycerol phosphate oxidase (GPO) >2000 U/L, lipase >200 000 U/L, glycerol kinase (GK) 1000 U/L peroxidase >500 U/L) was prepared according to the manufacturer's instructions, after which 1 mL was pipetted into required glass test tubes and incubated at 37°C for 5 minutes.   For the assay, 10 µL of blank, standard or sample was added to each tube and incubated again for 5 minutes at 37°C. During incubation, lipase catalyzes the degradation of triglycerides into glycerol and non-esterified free fatty acids (NEFAs). GK then catalyzes the reaction between glycerol and ATP to form glycerol-3-phosphate (G3P) and ADP. The newly formed G3P reacts with oxygen in the presence of GPO to form hydrogen peroxide and dihydroxyacetone phosphate. Peroxidase than catalyzes the reaction between hydrogen peroxide and TBHB to produce a red coloured quinoneimine dye. The absorbance of each tube was read at 540 nm on the spectrophotometer (Spectramax Plus 384; Molecular Devides, Sunnvale, CA). The increase in absorbance at 540nm is directly proportional to the concentration of triglyceride in the sample.

Total Cholesterol

Fasting serum cholesterol will be evaluated using a commercially available cholesterol reagent set (Pointe Scientific, Canton, MI). The cholesterol reagent (active ingredients: 4-aminoantipyrine 0.6mM, sodium cholate 8.0mM, cholesterol esterase >150 U/L, cholesterol oxidase

> 200 U/L, horseradish peroxidase 1000 U/L, ρ-hydroxybenzene (ρ-HBS) sulfonate 20mM) was prepared according to the manufacturer's instructions and 1mL was added to glass test tubes as required. Reagent was pre-warmed at 37°C for 5 minutes. Sample, standard or control was added to each tube in a volume of 10μL. Tubes were incubated at 37°C for 5 minutes. During the incubation, free cholesterol and fatty acids are liberated from cholesterol esters via cholesterol esterase. Cholesterol oxidase than catalyzes the reaction of free serum cholesterol to cholestol-3-one and hydrogen peroxide. Hydrogen peroxide couples with 4-aminoantipyrine and ρ-HBS in the presence of peroxidase to produce quinoneimine and water. The absorbance of each tube was read at 520 nm on a spectrophotometer (Spectramax Plus 384; Molecular Devides, Sunnvale, CA). The amount of quinonemine is directly proportional to the concentration of total cholesterol in the sample.

High-density Lipoprotein (HDL)

Fasting serum HDL cholesterol will be measured using the HDL Cholesterol Precipitating Reagent Set (Dextran Sulfate) and the cholesterol reagent set (Pointe Scientific, Canton, MI). This two-part assay involves first removing LDL and VLDL cholesterol fractions and leaving the HDL fraction in solution. The HDL concentration is then determine in the supernatant using the cholesterol reagent set as described above.

The HDL cholesterol precipitating solution was prepared using the manufacturer's instructions (active ingredients: dextran sulfate (50 000MW) 10g/L, Magnesium ions 500mM). Samples and controls were diluted 1:1 with saline, after which 500μL of diluted sample and controls were added to each tube. Then, 50μL of reagent was added and the tubes were vortexed and

incubated for 5 minutes at room temperature. Tubes were vortexed again and centrifuged at 2000g for 5 minutes. The supernatant was then used in the cholesterol assay described above.

Low-density Lipoprotein (LDL)

Fasting serum LDL was assessed indirectly using the procedures described by Friedewald et al (2). Total cholesterol, HDL and TG was assessed using the procedures described above. LDL concentrations was then estimated based on the following equation (2).

$$LDL \ (mmol/L) = total \ cholesterol \ (mmol/L) - HDL(mmol/L) - (TG(mmol/L) \times 2.2)$$

Serum TNF-α, IL-6, IL-4, IL-8, IL-10

Serum cytokines were analyzed using the BD Cytometric Bead Array Human Soluble Protein Master Buffer Kit and BD FACSCalibur flow cytometer (BD Biosciences; Mississauga, ON). The kit provides 6 types of capture beads coated with an antibody specific to TNF-α, IL-1β, IL-6, IL-4, IL-8 or IL-10. Each bead has a matching detection reagent that fluoresces at a specific activity. Prior to beginning the assay, all beads were combined into a single tube and vortexed and all detection reagents were combined into single tube. Serum samples were diluted in a 1:4 ratio with serum diluent provided by the manufacturer.

During the assay, 50 μL of each standard or diluted sample was added to appropriately labelled tubes and 50 μL of the Mixed Capture Beads was then added to each tube. Tubes were vortexed and incubated at room temperature for 1 hour. During the incubation, the beads form complexes with the cytokines matching their antibodies. After 1 hour, 50 μL of Mixed Detection Reagents was added to each tube, tubes were vortexed and then incubated for 2 hours at room temperature. During the 2-hour incubation, the detection reagent specific to each bead associates with the bead/cytokine complex, forming a sandwich structure. After 2 hours, 1 mL of wash buffer

164

was added to all tubes. Tubes were vortexed and centrifuged at 200g for 5 minutes, after which time the supernatant was aspirated. Each pellet was re-suspended with 300 μL wash buffer and acquired on the flow cytometer. Each of the 6-bead/cytokine/detection reagent complexes is reflected as a different population. Concentrations were determined by comparing the mean fluorescence of the population to the standard curve for each cytokine.

Serum C-reactive Protein (CRP)

Serum CRP will be measured using an enzyme-linked immunosorbant assay (ELISA) (R&D Systems Inc, Minneapolis, MN). Wells of the ELISA plate are pre-coated with monoclonal antibody specific for human CRP. All regents were prepared according to manufacturers' specifications and samples were diluted 100-fold. To prepare, 100μL of assay diluent was added to each well, followed by 50μL of standard, control or diluted sample. The plate was incubated at room temperature for 2 hours, following which the plates was aspirated and washed 4 times with the manufacturer's wash buffer. 200μL of CRP conjugate was added to each well and incubated again for 2 hours at room temperature. The wash process was repeated and 200μL of substrate solution was added to each well. A third 30 minute incubation followed protecting the plate from light, followed by the addition of 50 μL of stop solution. Optical density was determined using a microplate reader at 450nm. The amount of colour is directly proportional to the concentration of CRP in the sample.

Serum Adiponectin

Serum adiponectin was measured using an enzyme-linked immunosorbant assay (ELISA) (R&D Systems Inc, Minneapolis, MN). Wells of the ELISA plate are pre-coated with a monoclonal antibody specific to the adiponectin globular domain. All regents were prepared according to manufacturers' specifications and samples were diluted 100-fold. To prepare, 100μL of assay diluent

165

was added to each well, followed by 50 μL of standard, control or diluted sample. The plate was incubated at room temperature for 2 hours, following which the plate was aspirated and washed 4 times with the manufacturer's wash buffer. 200μL of adiponectin conjugate was added to each well and incubated again for 2 hours at room temperature. The wash process was repeated and 200μL of substrate solution was added to each well. A third 30 minute incubation followed, protecting the plate from light, followed by the addition of 50 μL of stop solution. Optical density was determined using a microplate reader at 450nm. The amount of colour is directly proportional to the concentration of adiponectin in the sample.

Serum Leptin

Serum leptin will be measured using the Human Leptin sandwich enzyme ELISA immunoassay (R&D Systems Inc, Minneapolis, MN). Wells of the ELISA plate are pre-coated with human specific monoclonal leptin antibody. Prior to the assay samples were diluted 50-150-fold and all regents were prepared as instructed by the manufacturer. For the assay, 100 μL of Assay Diluent was added to each well and 100 μL of either standard, control, and sample. The plate was incubated at room temperature for 2 hours. During the incubation period, leptin in the standards or samples becomes bound to the antibody-coated microwells. The plate was then washed and 200 μL of Leptin Conjugate was added and the plate was incubated for 1 hour at room temperature. During the second incubation period, the immunoconjugate binds to the leptin-antibody complex affixed to the wells. After the incubation, the plate was washed again and 200 μL of Substrate Solution was added to each well. The plate will again be incubated at room temperature for 30 minutes, protected from light, during which time a blue colour will develop as the TMB reacts with hydrogen peroxide. Finally, 50 μL of stop solution was added to each well causing the colour of the well to change from blue to

yellow. The plate was then read at a wavelength of 450 nm. The amount of colour is directly

proportional to the concentration of leptin in the sample.

Insulin-like Growth Factor -1 (IGF-1)

IGF-1 was measured using a commercially available enzyme-linked immunosorbent ELISA

kit (R&D Systems Inc, Minneapolis, MN). Prior to the start of the assay, samples were extracted and

diluted 100-fold to liberate IGF-1 from its binding proteins. For the extraction, 380μL of an acidic

dissociation solution was combined with 20μL of sample and incubated for 10 minutes at room

temperature. Following which, 50 uL of this solutions was added to 200μL of buffered proteins and

were assayed immediately. All regents were prepared according to manufacturers' specifications. To

begin, 150μL of assay diluent was added to each well, followed by 50 μL of standard, control or

liberated sample. The plate was incubated at 2°C for 2 hours, following which the plate was aspirated

and washed 4 times with the manufacturer's wash buffer. 200μL of cold IGF-1 conjugate was added

to each well and incubated again for 1 hours at 2°C. The wash process was repeated and 200μL of

substrate solution was added to each well. A third, 30 minute incubation followed, protecting the

plate from light, followed by the addition of 50 μL of stop solution. Optical density was determined

using a microplate reader at 450nm. The amount of colour is directly proportional to the

concentration of IGF-1 in the sample.

Insulin-like Growth Factor Binding Protein-3 (IGFBP-3)

IGFBP-3 was measured using a commercially available enzyme-linked immunosorbent

ELISA kit (R&D Systems Inc, Minneapolis, MN). Wells of the ELISA plate are pre-coated with

human specific monoclonal IGFBP-3 antibody. Prior to the assay samples were diluted 100-fold and

all regents were prepared as instructed by the manufacturer. To begin, 100μL of assay diluent was

added to each well, followed by 100 µL of standard, control or diluted sample. The plate was incubated at 2℃ for 2 hours, following which the plate was aspirated and washed 4 times with the manufacturer's wash buffer. 200µL of cold IGFBP-3 conjugate was added to each well and incubated again for 1 hour at 2℃. The wash process was repeated and 200µL of substrate solution was added to each well. A third, 30 minute incubation followed, protecting the plate from light, followed by the addition of 50 µL of stop solution. Optical density was determined using a microplate reader at 450nm. The amount of colour is directly proportional to the concentration of IGFBP-3 in the sample.

Serum Testosterone

Testosterone was measured using the commercially available Testosterone Parameter assay (R&D Systems Inc, Minneapolis, MN). The assay is based on the competitive binding technique and the intensity of the colour at the end of the assay is inversely proportional to the concentration of testosterone in the sample. The supplied microplate comes coated with a goat anti-mouse antibody to which monoclonal antibody specific for testosterone binds. The testosterone in the sample competes with a fixed amount of horseradish peroxidase labelled testosterone for sites on the monoclonal antibody. Samples were diluted 10-fold prior to the assay and all regents were prepared according to the manufacturer's instructions. To begin, 50µL of primary antibody solution was added to each well and incubated at room temperature for 1 hours on a horizontal orbital microplate shaker at 500 rpm. The plate was aspirated and washed 4 times with the manufacturer's wash buffer and 100µL of standard, control, diluted sample, or blank was added to each well. Following which, 50µL of testosterone conjugate was added to each well. The plate incubated at room temperature for 3 hours on a horizontal orbital microplate shaker at 500 rpm. Washing procedures were repeated and 200µL of substrate solution was added to each well. The plate was incubated for 30 minutes at room

temperature protected from light. Stop solution was added in a volume of 50μL to each well and

optical density was determined at 450 nm using a microplate reader.

References

1. McLellan AC, Phillips SA, Thornalley PJ. Flourimetric assay of D-lactate. *Anal Biochem.* 1992; 206: 12-16.

2. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein in plasma, without the use of preparative untracentrifuge. *Clin Chem.* 1972; 18:499-502.