Elevated C-peptides, Abdominal Obesity, and Abnormal Adipokine Profile Are Associated with Higher Gleason Scores in Prostate Cancer

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Running Title: Metabolic profile of prostate cancer patients

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Abstract

Background: Prostate cancer development is associated with numerous lifestyle factors (i.e. physical activity, nutrition intake) and metabolic perturbations. These factors have been studied independently; here, we used an integrative approach to characterize these lifestyle and metabolic parameters in men undergoing diagnostic prostate biopsies.

Methods: We prospectively evaluated 51 consecutive men for body composition, metabolic factors including glucose- and lipid-related measures, as well as lifestyle factors prior to prostate biopsy. Evaluations were performed in a blinded manner and were subsequently 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, p=0.002), suggesting greater insulin secretion despite lack of differences in fasting glucose concentrations. Central adiposity, measured by waist circumference, was significantly greater in participants with Gleason ≥4+3 (110.1±7.4 cm) compared to those with Gleason 3+4 (102.0±9.5 cm, p=0.028). Men with Gleason ≥4+3 also had significantly greater leptin concentrations than those with lower Gleason scores (Gleason ≥4+3: 15.6±3.3 ng/mL vs. Gleason 3+4: 8.1±8.1 ng/mL, p<0.05) and leptin:adiponectin ratio (Gleason ≥4+3: 9.7±6.1 AU, Gleason 3+4: 2.9±3.2, Gleason 3+3: 2.4±2.1 AU, p=0.013).

Conclusions: We profiled a cluster of obesity-related metabolic perturbations (C-peptide, central adiposity, leptin and leptin:adiponectin ratios) which may associate with more aggressive prostate cancer histology.

Key Words: insulin, waist circumference, central obesity, leptin, adiponectin

Introduction

Lifestyle factors (obesity (1), physical inactivity (2), high-fat diets (3)) are associated with prostate cancer (PC) as well as metabolic syndrome, an emerging risk factor for PC (4-7). Metabolic syndrome, a cluster of interrelated risk factors for diabetes and cardiovascular disease (8), is defined by the International Diabetes Federation (IDF) as central obesity (waist circumference (WC) >94cm in males), with at least two other risk factors, including hypertension (SBP ≥130mmHg, DBP ≥85mmHg), hyperglycemia (≥5.6mM), hypertriglyceridemia (TG, ≥150mg/dL), and/or reduced high-density lipoprotein cholesterol (HDL, <40mg/dL) (8). Specifically, central obesity and impaired glucose metabolism have been associated with PC development (4,5).

Metabolic abnormalities, such as obesity and insulin resistance may not affect PC in isolation, but work together to create a metabolic environment favourable for tumour growth (5). Physical inactivity and high-fat diets contribute to obesity (9), and may contribute to metabolic perturbations associated with PC (5). A comprehensive metabolic phenotype of PC patients will elucidate interactions between these features and identify potential predictors of PC 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, and concurrently evaluate other metabolic parameters that may 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 (LDL) cholesterol), C-reactive protein (CRP), adiponectin, and leptin. To interpret these metabolic findings, lifestyle factors (habitual physical activity, functional capacity, nutritional intake) were also assessed. Our secondary objective was to associate these metabolic features with the biopsy Gleason score as a surrogate measure of PC aggressiveness.

Materials and Methods

Participants

We consecutively and prospectively screened 139 men, with 51 men completing the study (Figure 1). Two groups of participants were included in this study: 1) men referred to a single uro-oncologist (JHP) with a clinical suspicion of PC presenting with elevated prostate-specific antigen (PSA) levels and/or abnormal digital rectal exam (n=36), and 2) low-risk PC patients under active surveillance recruited prior to their surveillance biopsy (n=15). 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 or chronic anti-pain medication. Participants' clinical characteristics are summarized in Tables 1 and 2. This protocol was reviewed and received clearance from the University of Waterloo's Office of Research Ethics and Hamilton Health Sciences/Faculty of Health Sciences (McMaster) Research Ethics Board.

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 (described below) were conducted prior to the biopsy on a single day.

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However, when this was not possible (n=2), assessments were scheduled within 2 weeks of the biopsy date. Assessments were performed by a single investigator (KMDS), who was blinded to the biopsy results until data collection was complete. Once data collection was completed, 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.

Biopsy Protocol

A single uro-oncologist (JHP) performed all transrectal ultrasound-guided 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 (10). 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 (10).

Clinical Data

Medical history was assessed using chart review and participant self-report. Family history of cancer (in general and PC), 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, hypercholesterolemia, diabetes), current medications, and smoking status were collected using a screening questionnaire. Blood pressure was measured with a sphygmomanometer.

Blood Sampling and Analyses

Blood was withdrawn after an overnight fast (8-12 hours with no food or drink except for water). Glycated hemoglobin (HbA1c) was analyzed with A1CNow+ (Bayer, Sunnydale, CA, USA) using fresh whole blood. The remaining sample was allowed to clot, spun and serum was collected, aliquoted and stored until analysis for glucose, insulin, C-peptide, IGF-1, IGFBP-3,

lactate, lipid profiles (TG, total cholesterol, LDL, HDL), CRP, adiponectin, and leptin. Glucose and lactate were assessed using spectrophotometric methods (11). Insulin and C-peptide were analyzed using radioimmunoassay kits (Siemens Healthcare Diagnostics; Deerfield, IL, USA). Insulin resistance was based on the homeostatic model assessment for insulin resistance (HOMA-IR) equation using fasting glucose and insulin values (12). Lipid profiles (TG, total cholesterol, HDL, and LDL) were analyzed spectrofluorophotometrically (Pointe Scientific; Canton, MI, USA). Leptin, adiponectin, IGF-1, IGFBP-3, and CRP were assessed using sandwich ELISAs (R&D Systems Inc, Minneapolis, MN, USA). IFG-1:IGFBP-3 and leptin:adiponectin molar ratio were calculated as using the following molecular masses: IGF-1: 7.5kDa (13); IGFBP-3: 30.5kDa (13), leptin: 16kDa (14), adiponectin: 30kDa (14).

Body Composition

BMI (kg/m^2) was calculated using weight and height recorded from medical charts. WC (in cm) was measured at the top of the iliac crests and hip circumference (HC, in cm) at the level of greatest gluteal prominence (15). Waist-to-hip ratio was used as a surrogate measure of visceral adiposity.

Single frequency-bioelectrical impedance analysis (BIA-101S, RJL Systems, Clinton TWP, MI, USA) was used to calculate FFM, FM, % body fat, SMM and SMI. Participants lay supine with electrodes on the metacarpal-phalangeal joints on the prone side of the right hand, right wrist, metatarsal-phalangeal joints of the right foot, and right ankle. Reactance and resistance values were generated and used to estimate FFM (16). FFM was used to estimate FM and % body fat. SMM was calculated using the equation described by Janssen et al (17). SMM was divided by height squared (m^2) to determine SMI (kg/m^2).

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 (18). For the 6MWT, participants walked as quickly as possible on a 50m course for 6 minutes and distance travelled was recorded. Hand-grip strength was assessed using a Takei A5001 analogue hand-grip dynamometer (Takei Scientific Instruments Co, Niigata-City, Japan) as previously described (19).

Nutrition Intake

Participants completed a 3-day food diary over 2 weekdays and 1 weekend day during the week of their assessments. 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 supplements taken and whether the recorded eating pattern matched their usual eating patterns. Caloric intake and macronutrient breakdown (% fat, % carbohydrate and % protein) were determined from these records 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.

Statistical Analysis

Values for all results are presented as mean±standard deviation. Statistical analyses were performed on Sigma Plot® version 11.2 (Systat Software Inc.; San Jose, CA, USA). As the data meet 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) using Tukey's post-hoc analysis for pairwise comparisons. Linear regression was used to model the relationship between Gleason score categories 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 significantly different 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 or the multiple component variable were considered in the regression analysis (i.e. glucose and insulin, or HOMA-IR were considered). Statistical significance was identified at p<0.050.

Results

Overall, participants were 66±7years old (range: 53-82years old), with a BMI of 28.2±4.4kg/m^2 (Table 1). Of the 51 patients, 38 patients (75%) were diagnosed with PC, 17 with Gleason 3+3, 14 with Gleason 3+4, 5 with Gleason 4+3 and 2 patients with a Gleason Score >7. Here, we grouped patients with Gleason 4+3 and Gleason 8-10 together (Gleason ≥4+3). Treatment distribution is outlined in Table 2. Patients without cancer were significantly younger than patients with Gleason ≥4+3 (62±7 vs 72±2years, p=0.008; Table 2). As expected, PSA levels were elevated (>4.0ng/mL) (20) across the entire cohort (6.2±2.9ng/mL, Table 2); patients with Gleason ≥4+3 had significantly higher PSA levels compared to the no cancer group (p=0.019, Table 2).

Metabolic syndrome was identified in 32 of 51 (63%) participants based on IDF criteria (5), 21 (66%) of which were diagnosed with cancer (Table 3). All 51 patients had at least one risk factor for metabolic syndrome (Table 3). The medical screening questionnaire revealed that 24 patients were being treated for hypertension, 24 for lipid abnormalities (hypercholesterolemia), and 9 for diabetes or pre-diabetes (Table 3). Interestingly, a large

proportion of participants still had metabolic syndrome, despite receiving medical treatment to manage elements of this syndrome.

Glucose and Insulin Metabolism

Fating glucose and insulin can be seen in Figure 2A and 2B, respectively. 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±1.1ng/mL; Gleason 3+3 1.4±0.6mg/mL; Gleason 3+4: 1.3±0.8ng/mL, p=0.002; Figure 2C), despite the lack of differences in fasting glucose (p=0.101, Figure 2A), lactate (p=0.885, Table 4) and HbA1c values (p=0.834, Table 4). Fasting insulin and HOMA-IR tended to be worse in Gleason ≥4+3 patients compared to the other groups (p=0.087, Figure 2B and p=0.070, Figure 2D, respectively). 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 4). Collectively, these data suggest developing insulin resistance in Gleason ≥4+3 patients compared with the other groups.

Body Composition

As obesity is associated with abnormal insulin signalling (21), we examined the relationship between body composition, insulin secretion and aggressive cancer. Approximately 80% of participants were overweight (BMI 25.0–29.9kg/m^2, n=21) or obese (BMI >30.0kg/m^2, n=20)) (Table 1). On average, WC was 102.8±11.7 cm, indicative of abdominal obesity (IDF cut-point: >94cm; Table 5) (8). Patients with Gleason ≥4+3 had significantly larger WC when compared to patients with Gleason 3+4 (112.4±6.7cm vs 97.5±13.7cm, p=0.028, respectively, Figure 3A). Similarly, Gleason ≥4+3 patients had significantly greater HC than Gleason 3+4 patients (110.1±7.4cm vs 102.0±9.5cm, p=0.034, respectively, Figure 3B). There was a main

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effect for waist-to-hip ratio, though no interactions were identified (p=0.048, Figure 3C), suggesting that abdominal obesity may be a key contributor to PC aggressiveness compared with total adiposity measures (Table 5). There were no significant differences observed in any of the body composition measures assessed by BIA (Table 5). However, patients with Gleason ≥4+3 group tended to have greater BMI (p=0.092), FM (p=0.090), FMI (p=0.087), and % body fat (p=0.058) compared with patients with less aggressive cancer, while the No Cancer group tended to have greater SMM (p=0.080) compared with the cancer patients.

Adipokines, C-reactive Protein, and Lipid Metabolism

Adipokines are signalling molecules linking obesity to insulin resistance (22). Gleason ≥4+3 patients had higher leptin levels compared with Gleason 3+4, but not other groups (p=0.013; Figure 4A). Adiponectin tended to be lower in Gleason ≥4+3 patients compared with the other groups (p=0.069, Figure 4B). Importantly, leptin:adiponectin ratio was highest in Gleason ≥4+3 patients p=0.013, Figure 4C). There were no significant differences observed in CRP between any of the groups (p=0.265, Table 4).

There were no significant differences in the lipid profile (total cholesterol, HDL, LDL, TG) between any of the groups (Table 4). However, only patients with Gleason ≥4+3 had HDL levels (34.6±16.6mg/dL) below the IDF cut point (40 mg/dL) (8).

Functional Assessment, Habitual Physical Activity Levels, and Dietary Intake

Traditional moderators of glucose metabolism, such as functional capacity, habitual physical activity, nutritional intake, and macronutrient distribution, were not significantly different between any of the groups (Table 6).

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 $r^2 = 0.398$. Age, PSA, and leptin:adiponectin ratio were statistically significant in the model (p=0.013, p=0.021 and p=0.027, respectively).

Discussion

This is the first study, to our knowledge, to comprehensively integrate and evaluate the metabolic characteristics of men, prospectively and consecutively recruited following a 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 (20) (measured by WC) and adipokine perturbations (22,23) are associated with insulin resistance. Gleason ≥4+3 patients had significantly greater WC, leptin and leptin:adiponectin ratios compared with other groups. Collectively, these data suggest adiposity-related metabolic sequelae, specifically abdominal adiposity, associate with aggressive localized PC.

Impaired Markers of Glucose Metabolism Were 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 greater insulin secretion in this group. Higher baseline C-peptide concentrations have been associated with PC aggressiveness and increase the likelihood of PC-specific death (24).

Elevated fasting C-peptide concentrations may promote hyperinsulinemia, which aligns with the tendencies for greater fasting insulin concentrations and HOMA-IR in participants with Gleason ≥4+3 compared with the other groups. Hyperinsulinemia is hypothesized to link obesity to PC development, whereby insulin creates a metabolic milieu favourable for cancer growth. Insulin receptors are found on human prostate tumour cells (25), allowing activation of Akt and MAPK pathways, resulting in proliferation and apoptosis inhibition (26). IGF-1 is also proposed to stimulate PC cell growth through the same mechanisms (27). Increased IGFBP-3, the major binding protein of IGF-1, prevents IGF-1 from binding to its receptor on the PC cell, ultimately reducing proliferation (27). We found no differences across Gleason scores for IGF-1, IGFBP-3, and IGF-1:IGFBP-3 ratio; larger sample sizes may be necessary to identify differences across Gleason scores in these measures (28).

Visceral Adiposity, a Feature of Metabolic Syndrome, is Related to Insulin Resistance

The majority of the study participants (63%) had metabolic syndrome, by definition a multifactorial diagnosis; however, central obesity measured by WC is a required criterion for all patients diagnosed with this syndrome (9). Patients with Gleason ≥4+3 had larger WC and waist-to-hip ratio than those with Gleason 3+4. WC is an indirect measure of visceral adiposity and a value >94 cm may be an independent risk factor for PC (4) and a key contributor in numerous disease states including diabetes and cardiovascular disease (22). Visceral adiposity is

associated with increased basal insulin and C-peptide levels (29), supporting the increased C-peptide and insulin levels that we observed here. Visceral adiposity is associated with increased leptin and decreased adiponectin levels (22), suggesting adipokine signalling may link visceral adiposity to PC and secondary disease states.

Here, visceral adiposity was assessed using the indirect measures of waist and hip circumferences, which cannot distinguish between visceral and subcutaneous adipose tissue (30, 31). Employing a more accurate measure of body composition analysis, such as DXA or MRI would have been ideal but access to these modalities was limited in the current study. Despite its indirect nature, waist circumference has been shown to be an important independent health risk predictor for metabolic syndrome, diabetes, cardiovascular disease, and all-cause mortality (30, 31) as well as PC (4) and it has been shown to be as good and potentially better than BMI in predicting morbidities such as diabetes (30, 31).

Adipokines are Associated with Visceral Adiposity and Hyperinsulinemia

Leptin and leptin:adiponectin ratios were significantly higher, while adiponectin concentrations tended to be lower in patients with Gleason ≥4+3. Leptin can stimulate PC cell growth and angiogenesis (25), while adiponectin may have anti-proliferative functions (26). Leptin:adiponectin ratio is emerging as an important predictor of PC risk, with elevated leptin, decreased adiponectin, and higher leptin:adiponectin ratios associated with aggressive PC (27).

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 PC proliferation beyond the independent effects of leptin and adiponectin (22). We observed increased visceral adiposity, leptin, and C-peptide levels, in patients with Gleason ≥4+3

compared with patients with lower Gleason scores. Collectively, these findings suggest a cluster of metabolic disturbances that promote a metabolic environment conducive to aggressive PC development; hence, examining these factors in an integrative nature is important in future work.

Age is an Important Risk Factor for Prostate Cancer

Age is the strongest known risk factor for PC (32) and is associated with more aggressive cancer (32). Therefore, it is unsurprising that in the current study patients with the most aggressive cancers are older than those without cancer and that age was associated with Gleason score in regression analysis. However, age is also associated with insulin resistance (33) and obesity (34). Consequently, some of the differences in C-peptide and central obesity may be explained by the increased age of this group.

Study Considerations

The strengths of our study stem from its prospective design and the inclusion of the no cancer group. Moreover, the absence of cancer was confirmed by a negative extended prostate biopsy (≥16 cores), however, this group may differ from men who have never had a prostate biopsy. Our single-institution cohort study however, is relatively small, and 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.

Conclusions

Overall we revealed a cluster of adiposity-related abnormalities in participants with high Gleason scores, when compared to participants with lower Gleason scores including higher C-peptide concentrations, increased visceral adiposity, lower than normal HDL, increased leptin,

and leptin:adiponectin ratio. These findings suggest that aggressive localized PC is associated with a set of adiposity-driven metabolic perturbations. Further investigation 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.

Acknowledgements

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Figure Legends

Figure 1: Participant Recruitment Flow Diagram. This consort diagram describes the recruitment, enrolment, follow-up and analysis for the current study.

Figure 2: Glucose and Related Hormone Assessments. All represents the average values of all participants in this study. NC represents the patients with No Cancer; 3+3 represents patients in the Gleason 3+3 group, 3+4 represents patients in the Gleason 3+4 group, and ≥4+3 represents patients in the Gleason ≥4+3 group. Different letters indicate significant differences between groups. The ≥4+3 group demonstrated a trend for significance for the insulin and HOMA-IR.

Figure 3: Circumference Assessments. All represents the average values of all participants in this study. NC represents the patients with No Cancer; 3+3 represents patients in the Gleason 3+3 group, 3+4 represents patients in the Gleason 3+4 group, and ≥4+3 represents patients in the Gleason ≥4+3 group. Different letters indicate significant differences between groups. Waist:hip Ratio demonstrated a main effect.

Figure 4: Adipokine Assessments. All represents the average values of all participants in this study. NC represents the patients with No Cancer; 3+3 represents patients in the Gleason 3+3 group, 3+4 represents patients in the Gleason 3+4 group, and ≥4+3 represents patients in the Gleason ≥4+3 group. Different letters indicate significant differences between groups. The ≥4+3 group demonstrated a trend for significance for adiponectin.