THE ASSOCIATION BETWEEN GENETIC VARIANTS, BODY COMPOSITION AND BLOOD PRESSURE IN AFRO-CARIBBEAN MEN FROM TOBAGO

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Prostate cancer is one of the most common malignancies affecting blacks worldwide. Blacks have a higher incidence and prevalence of prostate cancer than Caucasians. Treatment for prostate cancer usually involves androgen deprivation therapy. Even though androgen deprivation therapy has a high efficacy there are many deleterious side effects such as increase in body fat. Consequently, we investigated the association between androgen deprivation therapy for prostate cancer and the rate of change of percent total body fat in a cohort of Afro-Tobagonian men. Case-control analysis of 1691 men in our study indicated that, men with prostate cancer exposed to ADT had higher increases in percent total body fat over time compared to unexposed men with prostate cancer.

Likewise, obesity and hypertension disproportionately affects individuals of African descent. These complex diseases are multi-factorial in origin and are typically controlled by many genes. $ADRB_2$ and AR CAG repeat lengths have been widely studied in the literature and these genotypes were available for study in the Tobago Prostate Study; consequently, we investigated the association between $ADRB_{2,}$ body composition and blood pressure. We also investigated the association between *AR* CAG repeats and body composition.

Cross-sectional analysis of 2,584 men in our cohort indicated that, men with shorter *AR* CAG repeats had higher body fat measurements (DEXA). We found that the direction of the association was opposite what we had anticipated, of men with longer *AR* CAG repeats having

higher body fat measurements. 1,965 men were investigated cross-sectionally to determine the association between $ADRB_2$, body composition and blood pressure. We found no association between ADRB2, DEXA measures of body fat and blood pressure.

In our cohort of Afro-Tobagonian men *AR* CAG repeats and $ADRB_2$ did not show an association with our outcome of interest. Even though we had non-significant findings, other genes should be evaluated to assess if an association exists with body composition and blood pressure in Tobago population. These studies are of public health relevance or importance because they contribute epidemiologic information to the body of scientific information available especially regarding Tobago men.

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PREFACE

I would like to thank the staff at the Tobago Health Studies Office in Scarborough, Tobago for collecting and providing the information used in my dissertation. I would also like to thank my PhD committee, my family (especially my mother Barbara Francis and Dad Valbert Francis), and friends for their encouragement and support throughout the completion of my Ph.D.

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1.0 INTRODUCTION

Prostate cancer is considered one of the most common carcinomas and is the leading cause of cancer death in American men. African American men have the greatest risk for the disease and there is some suggestion that there might be some ethnic variation in prostate cancer risk. [1, 2] Androgen deprivation therapy with GnRH is the most common treatment for metastatic prostate cancer. In addition, androgens are important determinants of body composition in men. Serum Testosterone concentrations have been shown to correlate positively with muscle mass and negatively with fat mass. [1]GnRH agonist treatment in men has been shown to reduce bone mineral density, which is an important determinant of fracture risk. Furthermore, the changes in body composition may contribute to the adverse effects of physical function and potentially their quality of life. [1]

Lean mass, fat mass and body weight have been shown to be associated with high bone mineral density (BMD). Several studies indicate that a decline in lean body mass, or body weight is associated with declines in BMD.[3, 4] It is hypothesized that sex steroid hormone levels (testosterone and estradiol) contribute to low BMD and bone loss in older men and vary according to ethnicities.[5] Variations in BMD are mostly genetic but other factors such as environment, nutrition and physical activity can play an important role[6]. There is still uncertainty regarding the independent risk factors for BMD in men and women. Many of the studies related to BMD have been conducted in women because of bone loss following

menopause1[7] Despite lower osteoporotic risk in African Americans the number of individuals afflicted by this disease is expected to increase exponentially in the next few years.

High blood pressure has been shown to be relatively common in the West Indies [8]. Patrick et al.[9] showed that Tobagonian's have higher blood pressure levels, body bulk and obesity compared to mixed African populations of Jamaica, Guyana, and the European Caucasian populations of rural Wales.[9]In Tobago (2006), hypertension/high blood pressure and its co-morbidies account for approximately 35% of all yearly visits to health care facilities and is considered a significant contributor to morbidity and mortality.[10]

The literature is replete with evidence that genes influence obesity, and body fat distribution[4]. The development of many metabolic diseases such as CVD, prostate cancer, high blood pressure and T2DM is influenced by lifestyle and also genes. Obesity and insulin resistance are key risk factors for these diseases. Metabolic diseases such as T2DM are associated with aberrations in the lipid metabolism that typically cause fat accumulation in the liver and muscle.[11] Even though many of these studies have indicated a genetic basis for these diseases, many of the variations in these diseases are due to genes that are yet unidentified. Furthermore there is little information in the literature regarding genetic factors influencing body fat, and body fat distribution in populations of African decent.

Tobago would serve as an important population to investigate the impact genetics has on various diseases. Since Tobago is comprised mainly of West African ancestry with miniscule European and Native American admixture, the Tobago population may carry a higher prevalence of high risk alleles of African origin compared to more admixed populations.[12] The objective of this study is to determine the association between CAG AR repeat length polymorphism(Xq11-12), androgen deprivation therapy (ADT), β_2-adrenergic receptor polymorphism (5q33.1) and

high blood pressure, body composition, and low bone mineral density in a subset of Tobagonian men in the Tobago Bone Follow-up Study.

1.1 SPECIFIC AIMS

1.1.1 Project 1: β_2-adrenergic receptor (*ADRB2*) genotype, body composition, and blood pressure in African-Caribbean men from Tobago

The Tobago Prostate Cancer Study determined*ADRB2* genotype (single nucleotide polymorphism (SNP) rs1042714), performed dual energy X-ray absorptiometry (DEXA), and measured height, weight, waist circumference, and blood pressure in N=1965 Afro-Caribbean men from Tobago. Research questions include 1) the association between *ADRB2* rs1042714 genotype and obesity and 2) the association between*ADRB2* rs1042714 genotype and blood pressure. Research hypotheses include,

1. Men with one or two copies of a variant *ADRB2* rs1042714 allele have more body fat, expressed as DEXA measured total body fat as a percentage of total body mass, and
2. Men with one or two copies of a variant *ADRB2* rs1042714 allele have higher blood pressure.

1.1.2 Project 2: Androgen receptor (*AR*) genotype and body composition in African-Caribbean men from Tobago

The Tobago Prostate Cancer Study determined*AR* genotype (CAG trinucelotide repeat polymorphism length) and performed dual energy X-ray absorptiometry in N=2,584 Afro-

Caribbean men from Tobago. Research questions include 1) the association between *AR* genotype (expressed as the number of CAG repeats) and obesity. Research hypotheses include,

1. Men with longer *AR* alleles (more CAG repeats) have more body fat, expressed as DEXA measured total body fat as a percentage of total body mass, and

1.1.3 Project 3: To examine the association between androgen deprivation therapy (ADT) for prostate cancer and body composition

The Tobago Prostate Cancer Study performed dual energy X-ray absorptiometry in greater than equal to 40 year-old Afro-Caribbean men from Tobago, before and after androgen deprivation therapy (ADT) for prostate cancer. Research questions include 1) the association between androgen deprivation therapyandtemporal change in body composition. Research hypotheses include,

1. ADT-treated prostate cancer men will experience a gain in DEXA body fat mass (expressed as a percentage of DEXA total body mass) more rapidly than ADT-untreated prostate cancer men and men without prostate cancer

1.2 BACKGROUND

1.2.1 Epidemiology

Prostate cancer is the second leading cause of death in American men. It is estimated 186, 320 men will be diagnosed with prostate cancer and 28, 8660 men will die of cancer of the prostate in 2008. The age adjusted incidence rate is 163.0 per 100, 000 per year and the age adjusted death

rate is 26.7 per 100, 000 men per year. This data is based on diagnosedcases /patients who died in 2001-2005 from 17 SEER locations. [13]

In men with prostate cancer androgen deprivation therapy (ADT) is being widely used in patients with prostate carcinoma. Even though, ADT therapy is effective in controlling prostate cancer, hypogonadism as a result of ADT treatment is associated with many adverse effects for instance: impotence, hot flashes, cardiovascular morbidity, and osteoporosis [14]. Osteoporosis is no longer a disease that only affects women. There is growing concern, regarding men with osteoporosis. In the United States there is approximately 1.5 million men >65 with osteoporosis and another 3.5 million men with osteopenia (low bone density), potentially increasing their risk of fractures. [15]

In the literature there is limited data showing that men with prostate cancer could have preexisting low BMD [16-19] and also showing that ADT causes additional decreases in BMD in men. [17, 18, 20, 21] In addition to the loss in BMD another problem with ADT is the change in body composition. Changes in body composition are adverse side effects of androgen deprivation. [19]

Further more, studies show that body composition is related to other diseases such as diabetes and hypertension[22, 23]an increase in body mass and decreases in muscle mass is typically a result of reduced androgen levels in men.[24-27] It is hypothesized that reduced muscle mass will lead to decreased muscle strength, which would increase the risk of bone fractures in men. Even though, the literature is replete with studies emphasizing the effects of hypogonadism on body composition [28] research on body composition among men with prostate cancer after receiving ADT therapy is rarely discussed.[14]

1.2.2 Anatomy of the Prostate

The prostate gland is an exocrine organ that measures 25cc and is located in the pelvis between the bladder and the urinary sphincter, anterior to the rectum and below the pubis. The prostate is comprised of lobular tubuloalveolar glands that secrete fluids into the prostatic urethra. This fluid is comprised of seminal emissions that are rich in prostate-specific-antigen (PSA). Because of the location of the prostate the treatment of prostate cancer can have significant effects on urinary, sexual and bowel function.[29]

Most cancers of the prostate seem to develop near the capsule of the prostate in the peripheral zone. Prostate cancer is considered a multifocal disease in which tumors are present throughout the gland. The spread of prostate cancer can occur through defects in the capsule in which the neurovascular structures and the ejaculatory ducts enter the gland or possibly in the region of the bladder neck. The cancer can also progress to the seminal vesicles, bladder neck or the levator muscles. Systemic spread of the disease can also occur through the lymphatics to the external iliac, hypogastric, obturator, and presacral nodes. Prostate cancer has also been shown to have a predilection towards bone because of the bidirectional interaction between tumor cells and the surrounding stroma.[29]

A number of hereditary and environmental risk factors have been hypothesized in the risk of prostate cancer. These risk factors include, age race and family history which are the generally accepted risk factors in the development of prostate cancer.[30]

1.2.3 Pathogenesis of the Prostate

Prostate cancer is a heterogeneous disease that is caused by a series of genetic events in the prostate. These genetic events along with environmental risk factors can promote the development of cancer of the prostate.[31, 32]

1.2.3.1 Risk factors

Increasing Age

Aside from being of male sex age is considered one of the most important risk factors for prostate cancer. Prostate cancer is an age-related adult malignancy. Prostate cancer rarely occurs before the age of 40 years but the incidence seems to rapidly increase there after.[32] Bunker et al.,[33] found that prostate cancer rates increased with increasing age in the Tobago population with the highest rates being detected between the ages of 60-69 years (18%) and 70-79 years (28%).

Race and ethnicity

Men of African descent have the highest prostate cancer morbidity and compared to other racial or ethnic groups.[34, 35] Even though the overall morbidity and mortality from prostate cancer has been declining in whites since 1991 possibly due to improvements in diagnosis techniques, screening, surgical and radiologic treatments the rates in African American men are still high; approximately 2.4 times higher than in White men.[34] Furthermore, within the last couple of years, prostate cancer has emerged as the most common cancer in African American men and is considered the second most common cause of cancer related deaths in the western world.[36, 37]

Black men of African descent have high incidence of prostate cancer and men residing in some regions of Caribbean have rates similar to that of US black men. Glover et al.[38] provided some significant data, as prior to his study African American men in the United States were considered to have the highest rates of prostate cancer. The international agency for research in cancer (IARC) in the GlOBOCAN software and database had estimated the incidence rate of prostate cancer in Jamaica to be 42.4 per 100,000 [39]. However, Glovers' retrospective study of

1,000 prostate cancer cases in Kingston between the years of 1989-1994 (by evaluating Jamaican Cancer Registry, hospital, and clinic records) showed that the true prostate cancer incidence in Kingston, Jamaica was 304 per 100,000 which is significantly higher than US blacks and is considered to be the highest incidence in the world. The findings from Glover et al.[38] of high rates of prostate cancer was further supported by Brooks and Wolf [40] who found that prostate cancer is the most prevalent cancer in Jamaican men in St. Andrew and Kingston between the years of 1958-1987. Moreover, in 2001 Hanchard et al.[41] reported that prostate cancer is the main site of cancer in Kingston and St. Andrew based on 1,941 malignant neoplasms.

Bunker et al.[33, 35, 42] reported on information in three studies relevant to Trinidad and Tobago. In that population based prostate cancer study of men 40-79 years screening for prostate cancer was determined via serum prostate-specific antigen (PSA) and digital rectal exam (DRE). This study determined prostate cancer prevalence to be 11% i.e. (277cases out of 2,583 men) [42].These findings were further supported by an earlier study by Bunker et al.,[33] in the same population. In 2002 another study by Bunker et al.[35] compared prostate cancer prevalence in men of West African decent residing in Tobago and Asian-Indian men in Trinidad between the ages of 50-64 years. Afro-Tobagonian men had higher prevalence of prostate cancer (8.3%) compared to Asian-Indian men (2.3%). This difference observed was also true for all age categories: 50-54; 55-69; and 60-64 years.

In a study by Phillips et al.[43] that evaluated cancer incidence and mortality in the Caribbean showed that Puerto Rican men had a high incidence rate of prostate cancer 100.1 per 100,000 and men in Barbados had similar rates of 99.7 per 100, 000. Cuban men had the lowest rate 28.2 per 100, 000, however, Barbados men had the highest mortality rate 68.1 per 100,000 in the Caribbean and Haitian men had the lowest mortality rate of 20.0 per 100, 000.

Migrant studies also show that people of African descent have high rates of prostate cancer. Black men residing in the United Kingdom (UK) are considered an interesting population to examine because that population is mostly comprised of Afro-Caribbean and West African men [34]. Typically immigrants bring with them patterns of disease that characterize their country of origin. However, over time immigrant groups or their progeny usually adopt a pattern similar to that of the host country[43]. Consequently, the UK represents a unique microcosm of people of Afro-Caribbean and West-African populations. *The Prostate Cancer in Ethnic Subgroups* (Process) Study evaluated new prostate cancer diagnosis between January 1, 1999 and December 31, 2000 in the regions of: London Boroughs of Tower Hamlets; Hackney; Newham and the city of London. These parishes are comprised of 600,000 individuals with ethnicities of: white (British, Irish, and other white), black (black Caribbean, black African and other black, white and black African mix, white and black Caribbean mix), Asian (Indian, Pakistani, Bangladeshi, other Asian, white and Asian mix). This study reported 248 European, 91 African-Caribbean, and 20 south Asian prostate cancer cases.[44]

In addition, in a retrospective follow-up study in Process on prostate cancer incidences in North Bristol, South West London, South East London, and North East London from 1997-2001 reported 2,140 cases: 61.4% white, 20% Black Caribbean, 4.8% Black African, and 7% un-coded ethnicity.[2]

Family History

The incidence of prostate cancer is greater/higher in men with a family history of a relative with prostate cancer. This risk increases with the number of affected family members. First-degree relatives of men with prostate cancer have a two-threefold increased risk of developing prostate

cancer. Men with two or more relatives with prostate cancer have a fivefold increased risk of developing this disease.[45]

Studies have shown that familial prostate cancer constitutes approximately 5-10% of all prostate cancers and approximately 50% of all prostate cancers in men younger than 55 years of age.[46]

Diet and lifestyle

Studies have reported that dietary intakes of high saturated fats, red meats, high total consumption, low fruits, low vegetables, low tomato products, low fish and low soy products increase the risk of prostate cancer. Vitamin D, calcium, lycopene, zinc, omega-3 fatty and alpha-linolic fatty acids, selenium, vitamin E, statins, and non-steroidal anti-inflammatory medications have also been shown to affect risk. In addition, certain anthropomorphics and activity have been shown to be linked to prostate cancer.[29]

Likewise, higher circulating insulin like growth factor -1(IGF-1) can affect risk. It has also been shown that ejaculatory frequency maybe protective but the number of sexual partners can affect increase one's risk. The association between obesity and prostate cancer is somewhat controversial however obesity is associated with higher grade prostate cancer which could be caused by lowered testosterone levels.[29]

1.2.4 Clinical risk factors for prostate cancer

Most prostate cancer cases are considered adenoma carcinomas that are derived from glandular epithelial cells. Furthermore, autopsies have shown that middle age individuals have a prevalence of malignant precursor prostatic intraepithelial neoplasia (PIN) and invasive

cancers.[31] When a biopsy shows PIN but not actual cancer further biopsy is typically warranted.[32]

Symptoms

The general symptoms of prostate cancer are urinary frequency, nocturia, and urgency, which are typically caused by the obstruction of the Urethra. However, in some individuals symptoms may come from painful skeletal metastases or anemia caused by bone marrow replacement.[31]

Screening and detection

Various studies have shown that a PSA level >4ng/mL increases the likelihood that prostate cancer will be detected at a prostate biopsy. Programs focused on early detection prostate cancer have reported that approximately 70% of cancer cases can be detected by using a PSA cut-off of 4ng/mL.[47]

PSA test can provide diagnostic lead-time of 5-10 years. Approximately 80% of PSA cancers are biologically significant based on tumor volume and also Gleason grade. The use of PSA, results in earlier detection and diagnosis of organ confined disease.[29] Furthermore, experts assert that the use of PSA and DRE has resulted in the downward spiral of cancer mortality in the last few decades. The use of PSA screening method has resulted in increased number of diagnosed cases and the identification of cancer in the early stages.[29]

Currently, the American Cancer Society recommends annual DRE and PSA test for men 50 years and older who have a life expectancy of over 10 years. For men who are considered high-risk i.e. African American men and men with a family history of prostate cancer screening should commence at 40 years of age.[32, 48]

Digital rectal exam (DRE)

Typically physical findings are limited to the rectal examination. Attention is usually paid to areas of induration and also to determine if there is extension laterally to the pelvic sidewall, superiorly to the seminal vesicles and inferiorly at the apex. If it is determined that there is urinary obstruction, the bladder might be palpable. This examination is subjective and may not correlate cancer volume or extent of disease. Non-the-less, DRE results are an important facet of the algorithms that are used to determine if cancer is confined to the prostate gland or if it has spread to other regional areas.[49-52]

Gleason score

The Gleason score is used to microscopically evaluate prostate tissue and provide careful histologic grading. This is considered one of the most important factors in understanding clinical outcomes and prognosis of this heterogeneous disease.[29]

Gleason grading evaluates the architectural details of cancer cells under a low-to – medium magnification. There are five patterns of growth from well to poorly differentiated.[29] For instance, pattern 1 is the most differentiated with discrete glandular architecture where as pattern 5 is the most undifferentiated, showing loss of glandular architecture. Consequently, the final Gleason score is the sum of the grades of the most common plus the second most common growth patterns. The Gleason score can range from 2(1+1) to 10(5+5).[29] The Gleason score correlates to clinical prognosis and is used to stratify individuals.

1.2.5 Androgens

Testicular androgens influence the development of the normal prostate and also play a key role in neoplasia. Testosterone is the main circulating androgen in the blood. Males who reach

castration levels of testosterone before puberty (eunuchs) do not seem to develop benign prostatic hypertrophy or prostate cancer [53].

Adrenal androgens androstenedione,dehydroepiandrosterone, and dehydroepiandrosterone sulfate can also be converted to testosterone and DHT but they provide a small proportion of circulating androgens hence they do not support the growth and development of the normal prostate. They however, could play an important role in advanced prostate cancer as a residual source of testosterone and DHT and by binding directly to mutant ARs. DHT is considered an extremely potent intracellular mediator both in the internal and the external genitalia [59].

1.2.5.1 Racial differences in sex steroid hormones

Studies have shown that African American men have higher mean circulating testosterone levels and other androgens compared to European men of comparable age. This difference in testosterone levels is higher during young adulthood (10-20%) [54, 55] than in mid–adulthood (30%) [55]. There is also greater age decline in testosterone levels in African American men compared to European men until the age of 40 years (6.7%>than white men age 31-34years; 3.7% by age 35-39; and 0.5% by age 40-50 years) [55]. Epidemiologic studies suggest that testosterone levels decline as part of the natural aging process in men. In addition, to this decline in testosterone there is an age related increase in sex hormone binding globulin (SHBG) which binds to approximately 44% of testosterone. The non-SHBG-bound testosterone fraction seems to decrease with increasing age. Suggested reasons for this decline is, decreased Leydig cell and secretory functions which might contribute to declining serum testosterone levels [56, 57]. Harman et al.[56] suggest that modifiable lifestyle factors that are associated with aging could play a key role in the decline in testosterone levels.

In the CARDIA study, 483 African men and 695 European men aged 24-34 years at baseline were evaluated. In this study African American men were found to have 3% higher mean serum testosterone levels compared to European men even after adjusting for age, body mass index at baseline, and change in BMI after three hormone assessment in 8 years [58]. The decline in testosterone with age was similar between African American and European men. Following the adjustment of baseline waist circumference and change in waist circumference racial difference in testosterone levels were eliminated. It should also be noted that in this study the African American men had smaller waist circumference and they had a greater increase in waist circumference over time compared to European men. In addition there were no racial differences in sex hormone binding globulin and free testosterone. Observations made from the CARDIA study suggested that observed racial variability in hormone levels might not be the result of inherent differences across racial groups but rather differences in certain factors that contribute to hormone levels such as: BMI, waist circumference which are inversely correlated with testosterone. On the other hand, racial differences in body habits could be caused by inherent differences in systemic hormone [59].

Racial differences in androgen variability in utero showed that mean serum testosterone levels were 50% higher in African American mothers in the first trimester compared to age, week of gestation matched European women [60]. Another study observed higher maternal testosterone levels at delivery in African American women even after adjustments for known predictors of maternal testosterone levels. On the other hand, there were no observed differences in cord blood testosterone levels (African American 19.9, European 20.6 ng/dl).[61].

1.2.5.2 **Definition of maleness and androgenecity**

The variations between genders are moderated by sex hormones. This process typically starts in the uterus. A complete insensitivity to testosterone leads to a female phenotype expression. Maleness is described as, "the phenotypical correlation of androgen effects in humans" [62]. Furthermore, maleness is denoted as, the degree of androgenicity. A male phenotype that has not been exposed to adequate androgen will vary according to the time of exposure but the major hallmarks will be during foetal development and also puberty. Moreover, because AR can be found in almost every tissue androgenicity is typically exerted everywhere. The areas that are most affected will be the places with an abundance of androgen receptors such as: prostate, testis, bones, larynx, brain, haematopoetic cells and certain types of hair follicles.[62]

Persons affected by low androgen levels typically present with small prostates, low spematogenesis, low bone density, a high pitched voice, anemia, feminized pattern of secondary hair growth, decreased libido, depression, and poor spatial cognition abilities. The effect of androgen has also been detected in lipid and glucose metabolism, even in fat cell physiology.[63]

Testosterone (T) and its metabolite dihydrotestosterone (DHT) cause gene expression through the androgen receptor (AR). Typically T levels within normal range will saturate existing ARs and their androgenic effects will reach plateau levels depending on the type of tissue [64, 65]. Consequently, it is reasonable to think that within a particular range of saturation, genetically determined differences in androgen receptor activity would be observed, where as in the condition of hypogonadism, androgenicity is dependent on the levels of T and DHT.[62]

1.2.5.3 **Androgen metabolism in the prostate**

The pathway for androgen metabolism involves the two steroid hormones testosterone and dihydrotestosterone, the androgen receptor and important genes in the nucleus that are regulated by androgens [53].

In males testosterone is primarily synthesized by the testes and to a small extent by the adrenal glands. Approximately 45% of circulating testosterone binds to the sex hormone binding globulin (SHBG), 50% binds to albumin and <4% is unbound free testosterone [66]. Testosterone diffuses into the prostate from blood and then binds directly to the AR [53]. Testosterone in the prostate is converted irreversibly to 5α–dihydrotestosterone by the enzyme 5α-reductase type II which is encoded by the SRD5A2 gene[66]. Even though testosterone and 5α–dihydrotestosterone can bind to the androgen receptor; the androgen receptor has a higher affinity for the 5α–dihydrotestosterone than for the testosterone. In addition, the androgen receptor is more transcriptionally active when it is bound to the 5α–dihydrotestosterone. The 5α–dihydrotestosterone –androgen receptor–transcription factor is regulated by translocation to the cell nucleus and binding of androgen receptor co-regulators, co-activators, and co-repressors. Furthermore, the 5α–dihydrotestosterone coregulator complex translocates to the cell nucleus, in which once there it activates the transcription of genes with the help of other hormone responsive elements[67]. The amount of 5α–dihydrotestosterone in the prostate is determined by: testosterone metabolism, metabolism of androstenedione via 5α–androstanedione and 5α–dihydrotestosterone (DHT) inactivation by its reduction to 3α or 3β - androstanediol which is a reversible process. The metabolites of 5α–dihydrotestosterone can also be conjugated into 3α or 3β-androstanediol glucuronide which is also an irreversible process [67]. 3β-androstanediol glucuronide is considered a terminal metabolite of testosterone and is used as a surrogate marker

of tissue androgen levels. 3β-androstanediol glucuronide reflect the activities of 5α-reductase types I and II. Type I is typically expressed in extraprostatic tissue like the skin and type II is expressed in the prostate [68]. Serum concentrations, of 3α-androstanediol glucuronide correlates with 5α-reductase activity in the genital skin.Finasteride a 5α-reductase type II inhibitor show that DHT and 3α-androstanediolglucuronide decrease in men who were treated [69].

1.2.5.4 **Serum testosterone, obesity, age, and tumor grade**

There are many unresolved issues with serum androgen. It is well established that serum androgen decreases with increasing age and increasing age is a major risk factor for prostate cancer along with race and family history [69]. In the literature there is evidence that high levels of serum testosterone is associated low grade prostate tumors but a decreased risk for high grade prostate tumors [70, 71]. There is no evidence showing that total testosterone is associated with a decreased risk of high grade tumors but there is a possible association between free testosterone (unbound testosterone) and an increased risk for low grade tumors [70].

The deleterious effect of obesity also complicates the relationship between androgens and prostatic tumors. Hsing et al. [70] observed that obese men have a decreased risk of low grade and an increased risk for high-grade tumors, which is consistent with other findings that these men also have low serum testosterone levels [72, 73]. This evidence further corroborates the findings that high serum testosterone concentration is associated with increased risk of low-grade prostate cancer and a reduced risk of high-grade prostate cancer. Obesity is associated with increased inflammatory markers, which are associated with a risk of prostate cancer [74]. However, these observations may be modified by other additional modifiers and needs further evaluation.

1.2.5.5 **Androgen deprivation**

The concept that androgen deprivation can affect body composition was first proposed by Charles Edouard Brown-Sequard in 1889 [75] he has been credited by many as the founder of endocrinology because he was the first person to suggest the existence of substances that are secreted by a particular organ but affects others [76].

Male hormones called androgens (testosterone and dihydrotestosterone) is responsible for determining male secondary sex characteristics and is also responsible for stimulating prostate cell growth. Once prostate cells are deprived of androgens both healthy and cancerous they no longer grow and hence eventually die [77].

Androgen deprivation therapy also denoted androgen suppression therapy and hormone therapy uses drugs or surgery to suppress or block male hormones (testosterone and dihydrotestosterone) that cause prostate cell growth. Testosterone is an androgen and androgens are produced by the testis and small amounts are also produced by the adrenal glands located on top of the kidneys. Androgen deprivation therapy blocks the production and the effects of androgen on the body. Androgen deprivation therapy is not considered a cure for prostate cancer but it can control symptoms and also disease progression in individuals. Androgen deprivation therapy is used to treat metastatic cancer and localized prostate cancer depending on PSA levels [77].

In the literature there has been some debate as to when to commence androgen deprivation therapy. According to a report in 2007 by American Cancer Society of Clinical Oncology (ASCO) patients with recurrent, progressive or advanced prostate cancer should delay treatment until patients begin to experience symptoms. However, if therapy is deferred patients should have regular visits with their physicians (every 3-6 months) to monitor their condition.

ASCO also recommends bilateral orchiectomy, or injections with luteinizing hormone-releasing hormone as initial androgen deprivation treatments.[78]ADT treatment is considered palliative care and rarely curative. In the literature there is evidence to suggest that early treatment results in better long-term prognoses for prostate cancer.[29, 32]

1.2.6 Treatment of prostate cancer

1.2.6.1 Orchiectomy

Orchiectomy is the surgical removal of the testicles. This method is considered the most effective way of reducing androgen hormones and is considered a more permanent solution to androgen deprivation. Orchiectomy in addition to radical prostectomy can delay the progression of disease in patients with prostate cancer [79]. Most of the testosterone that is produced by a man is produced by the testis in response to luteinizing hormone produced by the anterior pituitary gland. After bilateral orchiectomy, serum testosterone levels typically falls to castration levels (<10ng/ml) [79].

A reduction in serum testosterone is associated with decreased fat free mass. Mauras et al., reported that when serum testosterone was suppressed by the administration of gonadotropin –releasing hormone in healthy young men it was associated with significant reduction in fat free mass, increased fat mass, and a decrease in fractional muscle protein synthesis. Likewise, an age associated decline in serum testosterone levels was correlated with reduced appendicular muscle mass and reduction in lower extremity strength in Caucasians and African Americans [80, 81].

Current data on hypopituitary men suggested that hypogonadism is associated with abnormalities in body composition such as a reduction in lean body mass and elevation in fat mass[82]. Katznelson et al., reported effects of hypogonadism in men who also had

hypopituitarism and observed a higher percent body mass and abdominal subcutaneous fat in hypogonadal men compared to healthy controls even after controlling for BMI.However visceral fat was not greater in the hypogonadal group compared to the eugonadal groups [83, 84]. Studies have also examined the relationship of androgen in elderly men such as bone mineral density and have found rather weak and inconsistent associations [85, 86].

1.2.6.2 **Prostectomy**

Another procedure that is sometimes recommended for the treatment of prostate cancer is radical prostectomy, which is typically recommended for the treatment of stage A, and B (Whitmore-Jewett system staging system) prostate cancers. This procedure generally requires a surgical cut through the abdomen or the perineal area and is done with general or spinal anesthesia [87].

1.2.6.3 **LHRH agonists**

The main drugs used for suppressing androgens are called luteinizing hormone-releasing hormones (LHRH) agonists. LHRH drugs work by blocking the pituitary gland from producing hormones that stimulate the production of testosterone. These drugs include Leuprolide (leupron, leuprogel) goserelin (zoladex), and buserelin. Side effects from these drugs include hot flashes, nipple and breast tenderness, gynecomastia (breast enlargement) [77, 79].

1.2.6.4 **Antiandrogens**

Anti-androgens are drugs used to block the effects of testosterone. The main anti-androgen drugs are:

1. Flutamide (Eulexin, Drogenil). Flutamide side effects include: diarrhea and liver damage

2. Nilutamide (Nilandron). Nilutamide side effects include reversible interstitial pneumonistis, nausea, alcohol intolerance, and visual disturbances.
3. Bicalutamide (Casodex). Bicalutamide seems to have fewer side effects compared to other anti-androgens
4. Cyproterone combined with estrogen could prevent testosterone surge that typically occurs with LHRH.
5. Other drugs. If patients are not responding to hormonal medications, other drugs that can be tried include estrogen therapy and ketoconazole (Nizoral) which is an anti-fungal drug that blocks testosterone function in men [77].

1.2.6.5 Chemotherapy for hormone resistant cancer

For prostate cancer does not respond to hormone treatment chemotherapy may be use to treat hormone-resistant cancer. Chemotherapy drugs for prostate cancer are: docetaxel (taxotere), mitoxantrone (novantrone), estramustine (Emcyt), andriamycin, paclitaxel and other platinum based drugs such as carboplatin. These drugs are typically combined with other cancer dugs such as 5-fluorouacil, or corticosteroids (e.g. prednisone) [77].

1.2.6.6 Untreated prostate cancer

Information regarding patients who received no therapy for prostate cancer was evaluated in over 3,000 men identified in the Connecticut Tumor Registry. After diagnosis of prostate cancer these men either choose to receive no treatment or were advised by their physicians to receive no treatments. These individuals were followed for a minimum 15 years or until they died. In this study the two major determinants of death because of prostate cancer were: tumor grade and age at diagnosis. This study reported that men with Gleason score of 7 or higher and who were

below the age of 74 years were more likely to die of prostate cancer. On the other hand, men of all ages whose Gleason scores are 6 or lower have a low/minimal risk of dying from prostate cancer. In summary, this study resulted in two important observations namely: Gleason 7 tumors and higher should be considered high grade tumors and that age at diagnosis seems to bear no significant impact on cancer-specific mortality after 15 years in individuals with low grade or high-grade tumors. Age is more of a determinant in men with moderately differentiated tumors. [32, 88]

1.2.7 ADT and body composition

It is well documented that androgen deprivation therapy with GnRH (gonadotropin-releasing hormone) agonist is the mainstay treatment for metastatic prostate cancer. Androgens are extremely important determinants of body composition in men. Studies have shown that GnRH agonist treatment increased weight and percentage fat body mass and decreased percentage lean body mass and muscle size in men [1]. Studies have also indicated that low serum testosterone concentrations are associated with decreased muscle mass and increased fat mass [89]. However, other studies have also shown that testosterone replacement therapy increases lean body mass in men with hypogonadism because of aging, HIV, and other chronic diseases [90-92]. In a prospective study that evaluated 48 weeks of GnRH agonist therapy in men with prostate cancer showed that these men experienced a 2.4% increase in weight, 9.4% increase in fat mass, and 2.7% decrease in lean body mass. This increased fatness resulted primarily from the accumulation of subcutaneous rather than intra-abdominal adipose tissue [1]. This effect on body composition could be related to testosterone deficiency because testosterone has been shown to promote lipolysis in visceral adipose tissue [93].

In addition ADT has been shown to produce other metabolic changes for instance: increased serum insulin levels, total cholesterol, low-density liproprotein, cholesterol, triglycerides and leptin[1, 94, 95]. These abnormalities in lipid and glucose metabolism in addition to increases in body mass suggest that ADT could elevate an individual's cardiovascular risk. For example one study observed that after three months of GnRH agonist treatment participants had increased arterial stiffness in the radial artery and this problem resolved after treatment was discontinued [95]. However, because testosterone and estrogen both have direct and indirect effects on the vascular endothelium, it is not quite clear if the hemodynamic effects are related to deficiencies in one hormone or both [95]. Androgen deprivation therapy is also associated with fatigue, loss of energy, emotional distress and a poorer overall quality of life [96, 97].

1.2.7.1 **ADT and bone loss**

In a study by Greenspan et al. [98] men with prostate cancer who initiated hormonal suppression therapy had significant BMD loss at a various skeletal sites. In this study there was greater bone loss after the first year of androgen deprivation therapy. The observed reduction in BMD following 12 months of acute ADT was 1.5-4.0% depending on the skeletal sites measured in individuals. Moreover, bone loss at the hips was related to the length of time that individuals were on ADT. On the other hand, men with prostate cancer who had not initiated ADT and healthy controls had no significant bone loss over 12 months. This study also observed an increase in percent body fat and a reduction in lean body mass in 12 months after the initiation of ADT. Studies have shown that reduced muscle strength is an independent predictor of falls in the elderly. Consequently reduced muscle mass may contribute a higher risk of fractures in men on ADT[99].

Furthermore in a study that was conducted men on ADT had 47% lower estradiol levels than healthy controls and these levels were still considerably lower than those of men with prostate cancer but had not initiated ADT treatment (31% lower). Treatment of prostate cancer with orchiectomy or GnRH agonist causes testosterone and its metabolite estradiol to fall significantly. These hormones are important determinants of peak bone mass in men [100]. However, estrogen is the dominant hormone that prevents bone resorption in men [101].

Current suggested treatments to prevent ADT related bone losses in men are: Calcium and vitamin D supplement of 1200-1500mg daily. In addition, individuals are encourage to abstain from negative habits that would exacerbate bone loss such as: smoking, excessive alcohol consumption, and a sedentary lifestyle [102]. Another potential option to reduce bone toxicity caused by ADT is the use of bisphosphonates. Bisphosphonates are inhibitors of osteoclastic bone resorption[103].

1.2.7.2 **Definition of osteoporosis**

Osteoporosis is considered a skeletal disorder that is characterized by decreased bone mass and also a deterioration of bone tissue ultimately resulting in fragility of bone and subsequent susceptibility to falls[104]. Studies have shown that there is an inverse relationship between bone mass and fractures. Osteoporotic fracture risk increases continuously as BMD decreases with an approximate 1.5 to 3-fold elevated risk of fracture for every standard deviation fall in BMD[105]. Osteoporotic fractures account for approximately 0.83% of the global burden of diseases worldwide[106] .

According to the World Health Organization, normal, osteopenia, and osteoporosis are based on BMD compared to the mean value in a young person. Normal is defined BMD within 1 standard deviation of the mean of a young adult person. Osteopenia is defined as BMD

between 1.0 and 2.5 standard deviation below the mean of a young adult; and osteoporosis is defined as ≥2.5 standard deviations below the mean of a young adult. These measures are based on DEXA forearm BMD measurements and hip fractures in postmenopausal Caucasian women. However, because men have higher BMD and larger bones than women additional studies are needed in this area[107].

1.2.7.3 Osteoporosis gene polymorphism

Due to the importance of family history and race genome wide linkage scans have been conducted to determine the genes that influence bone mineral density and osteoporosis. Association studies of the genetic effects of certain candidate genes on BMD and /or fractures have focused on the genes directly involved the metabolism of bone, structure, mineral and regulatory/hormonal pathways [108]. Genes that have been involved in association studies are: estrogen receptor (α&β)[109, 110], vitamin D receptor [109-111], PTH receptor type 1[110], interleukin 6 [112-114], insulin like growth factors [115], and alpha 2HS glycoprotein [111].

1.2.7.4 Heritability of bone mass

Genetics does play an important role in the determination of bone mineral density (BMD), and osteoporotic risk. For instance in a comparison of monozygotic twins, dizygotic twins, offspring and parents it was determined that genetic factors play a key role in osteoporosis [116, 117]. Most studies demonstrated a heritability (h^2) of approximately 50-80% for BMD at fracture sites such as the lumbar spine, hips and the radius [3, 118] but fracture heritability is considered more difficult to estimate with estimates of approximately 50% [119, 120]. An individual's susceptibility to fractures depends on factors such as ones propensity to fall, non-skeletal factors, physical environment, and diminished soft tissue cushion. In addition, some heritability of fracture is

independent of bone mineral density [121, 122]. Studies have also shown that SNPs do contribute to variations in BMD but might not contribute to osteoporotic fractures [123, 124].

Furthermore, studies have reported differences in the heritability of BMD in persons of the same and opposite genders [125]. This could possibly be explained by in-utero imprinting expressions of osteoporosis genes. Other studies have found 20% greater heritability of BMD in men compared to women [126, 127] and some did not [128, 129]. A possible explanation for these gender specific heritability differences in BMD are our ability to account for environmental factors for instance estrogen in men [130, 131].

1.2.7.5 **Racial differences with BMD**

Osteoporosis is a serious public health problem that is associated with morbidity and mortality because of the risk of fractures. Fractures are usually associated with pain, limited mobility, and a reduced quality of life. Data suggest that fracture risk is greater among Europeans compared to African Americans living in the United States [132].

The Baltimore Men's Osteoporosis Study examined racial differences in BMD, which is a longitudinal observational cohort study. The study was comprised of 503 ambulatory white and 191 ambulatory African American men. Men with bilateral hip replacement and excess weight of 300 pounds were excluded from the study. Higher BMD at the femoral neck, lumbar spine, and total body of African American men were observed even with the adjustments for potential confounders. Further analysis also showed that older African American men had narrower bone at the proximal femur and higher bone mineral content which is independently associated with greater bone strength [133].

In the Third National Health and Nutrition Survey (NHANES III); osteoporosis was present in 5 and 20 percent of black women and white women age 50 and above at the femoral

neck; 8 and 17 percent at the total femur. However, the prevalence of osteoporosis was 4 and 6 percent in African American and European men at the femoral neck and total femur. In this study prevalence of osteoporosis in men according to race varied depending on if female or male cutoff values were used. Prevalence's tended to be higher based on male cutoffs compared to female cutoffs [15, 132].

Furthermore, in the Men's Osteoporosis study decline in BMD was evaluated in 349 European men and 119 African American men. BMD measurements were taken at the proximal femur and the lumbar spine at approximately 18.8 months apart. The results indicated that the average annual percentage decline in BMD was significantly greater in European men than African American men at the femoral neck and total hip. These observed differences persisted with the adjustment of known confounders: weight, age, and current smoking. In this study current smoking was associated with greater bone loss in blacks and whites even with adjustments of: age, change in weight, and height [132, 134]. Studies have also shown that African Americans have lower rate of bone turnover compared to European Americans [135, 136]. This lower rate of bone turnover≈ lower decline in BMD in African Americans is believed to be the product of the combined effect of: nutrition, genetics, hormones, and lifestyle in adulthood [132].

Bone strength in individuals is determined by a number of factors namely: mass and geometry of the bone, microarchitecture of the trabecular bone, the porosity of the cortical bone, composition of the bone matrix, and the accumulation of micro-damage in the bone. African Americans seem to have greater mineral density compared to their European peers [132]. This observed higher bone strength in African Americans could be the result of: a stronger skeleton during early childhood and adolescence, and slower bone loss in adulthood [132]. Moreover,

studies have shown that African American children had greater bone mass than European children and these results continued into young adulthood in males [137, 138].

1.2.8 Androgen receptor (*AR*)

Androgen receptor (AR) is an intracellular transcription factor that is a member of the steroid nuclear receptor super family [139, 140]. The androgen receptor gene (AR) contains in N-terminal of exon-1 a polymorphic trinucleotide CAG repeat (encodes polyglutamine), whose length regulates androgen receptor action. Included in this region is another trinucleotide repeat GGC which codes for glycine[141]. AR is activated by androgens and then translocated to the nucleus where it binds to DNA sequences called androgen response elements located in regulatory region of androgen dependent genes.Furthermore, the binding of androgen-AR complexes does one of two functions it either represses or activates androgen –regulated proteins [142]. Hence, ARs are very important molecular switches that control transcription of androgen-dependent proteins during embryogenesis until adulthood. The AR gene, located on the X chromosome at Xq11-12 [143], is encoded in eight exons[144] and has three functional domains namely: the transactivation domain (TAD), DNA binding domain and ligand binding domain [145]. Differences in the AR sequence are mostly characterized by polymorphic trinucleotide repeat (CAG) and the normal length of a CAG is approximately 9-37 [146, 147]. The transactivational activity of AR is inversely related to the length of the CAG repeat chain In addition, because the androgen receptor is located on the X-chromosome males have only one copy [148].

The androgen receptor is sometimes mutated in prostate cancer with varying effects on functionality and on activating steroid and non-steriodligands.Evidence for the importance of CAG AR repeat lengths are that: men with 40 or more CAG repeats suffer from Kennedy Syndrome (spinobulbar Ataxia) which is characterized by androgen insensitivity, and

progressive muscle weakness [144, 149]. Men with longer CAG repeats are more likely to have reduced spermatogenesis than men with shorter repeats [150]. One study found that men with prostate cancer had shorter CAG repeats which were associated with being African American and a higher and more aggressive prostatic carcinoma [151]. In addition, shorter repeats are associated with a higher risk of benign prostatic hyperplasia (BPH). BPH is characterized by overgrowth of the tissue of the transition zone and the periurethral area of the prostate [152].

1.2.8.1 Ethnic differences in *AR* CAG repeat length

Within the normal range of the AR polyglutamate, ethnic differences have been reported. For instance, in healthy men of African descent the average number of CAG repeats reported is between 18 and 20 [13, 147] and is somewhat shorter in other African populations [153]. In Caucasians the average number of CAG repeats is 21-22 [13, 147] whereas in East Asians a mean of 22-23 was observed [146, 147, 154].

1.2.8.2 AR CAG repeat length and BMD

Studies have shown that in healthy men, polymorphism of the oestrogen receptor regulate quantity and quality of bone tissue[155] and this is also applicable to CAG repeat polymorphismsfound in the AR gene. In 110 males age 20-50 years an increased number of CAG AR repeats was associated with low bone density[65] and the results of this study was further corroborated by a study conducted inperi-menopausal women [156].

However, results in older men are somewhat conflicting.In a study of 508 Caucasian men 65 and over a negative association between AR CAG repeat length and bone mineral density of the femoral neck was observed [111]. But in this same group, a more pronounced bone loss was

observed at the hip and an increased risk for vertebral fracture was reported in men with longer AR CAG repeat length [111].

In a cohort of 273 Belgian men age 71 and 86 years no influence of AR polymorphism was observed [141]. It is hypothesized that higher androgenization will cause greater peak bone mass[131], and AR polymorphism effects on bone densitywill only be evident in healthy younger men. This difference in AR polymorpisms effects could be mitigated because of age-dependent bone loss in older men caused by confounders that exerted some influence on bone mass [62]. In another study, that evaluated the effects CAG repeat polymorphism on bone density and metabolism, determined that increasing number of CAG repeats in the AR gene influence on bone density and bone metabolism is attenuated in healthy males. In this study CAG repeat polymorphism accounted for 8% of total variation of bone density; which suggests that the number of CAG repeats in the AR is a determinant of bone density in healthy men. Consequently one could speculate that a mild deficiency in testosterone is associated with negative effect on bone density in individuals with long CAG repeats sequence. While persons with shorter CAG triplets show no sign of bone loss for comparable testosterone levels [157]. Hence, longer CAG repeats in the AR gene will lead to lower peak bone density in men. Clearly, more studies are needed to elucidate the relationship between CAG AR and bone mineral density

1.2.8.3 *AR* CAG repeat length and body composition

Testosterone has shown conflicting associations with cardiovascular risk factors. Low levels of testosterone can have unfavorable effect on body composition, insulin sensitivity [57] and haemostatic parameters[158]. Studies have shown that androgen has a lowering effect on good cholesterol (HDL)[159]. Furthermore, studies on the role of testosterone on vascular endothelial

function have shown a negative effect but these results were contradictory and appear to be contingent on endogenous hormone levels and how artificial testosterone was administered[64].

In a study of 110 men age 20-50 years CAG repeat polymorphisms decreases testosterone effect on HDL cholesterol and flow mediated dilation of the brachial artery. A longer polyglutamine stretch in the AR protein is associated with a higher cholesterol levels and arterial vasoreactivity[64]. Similar findings were also reported in patients who suffered from XSBMA (X-linked spino-bulbar muscular atrophy)[160]. It is quite possible that longer CAG repeats in the AR gene could possibly act as a cardio-protective factor but the receptor polymorphism might play a role in determining a person's atherosclerotic risk. It has been reported that in men with longer CAG repeats also had higher body fat and also insulin resistance [62]. Similarly, in patients with XSBMA they had a tendency to develop diabetes mellitus [161].

1.2.8.4 **β2Adrenergic Receptor**

Adrenergic receptors are members of a large super family of cell surface receptors that are involved in signaling via coupling to guanine nucleotide binding proteins (G-proteins). Adrenergic receptors are targets for epinephrine, catecholamines and norepinephrine and are an important component of the sympathetic nervous system for the maintenance of homeostasis and response to disease. Human adrenergic receptors consist of nine subtypes: α_{1A}-, α_{1B}-, α_{1D}AR; α_{2A}-, α_{2B}-, α_{2C}AR; β_1, β_2, and β_3AR [162, 163]. Four of these results in changes in the amino acid sequence: glycine-for-arginine substitution at codon 16 (Arg16Gly), glutamic acid-for-glutamine substitution at codon 27 (Gln27Glu), the methionine-for-valine substitution at codon 34 (Val34Met) and threonine-for-isoleucine substitution at codon 164 (Thr164Ile) [164]. The human β_2-adrenoceptor is located on chromosome 5q31-32 [165].

A variability in a DNA sequence with an allele frequency of >1% in the population is denoted a polymorphism. Polymorphisms may have no effect, or have clinically silent effects, or could modify diseases or alter response to therapy, act as low level risk factors, and have increased prevalence in particular diseases. The most common type of polymorphism is single nucleotide substitution termed SNPs. Within the coding regions of genes, polymorphisms encode different amino acids (nonsynonymous polymorphisms). Polymorphisms occur in the 5' UTR, promoter, 3' UTR and introns[163].

1.2.8.5 β2 adrenergic receptor and hypertension

Studies have shown that the β_2-adrenergic receptor is involved in vascular smooth muscle relaxation and vasodilation in response to the release of epinephrine [85, 166]. For instance, the impairment of β_2-adrenergic receptor-mediated vasodilation in the presence of normal or elevated vasoconstrictor response causes an increased vascular reactivity and also raises blood pressure. Furthermore, blunted β_2-adrenergic receptor-mediated vasodilation has been observed in hypertensive individuals [167-169] indicating that attenuated β_2-adrenergic receptor could be important in the pathogenesis of essential hypertension.

Even though, the mechanism is not fully understood, it is well known that hypertension is typically more severe and associated with higher rates of morbidity and mortality in African Americans compared to Caucasians [170-172].

In addition, it has been denoted that vascular β_2-adrenergic receptor responses were attenuated in blacks considered normotensive[173] implying that an attenuated vasodilator response might play a key role in the development of hypertension in Blacks[174].

Genetic studies indicated that β_2-adrenergic receptor could play a key role in increased hypertension and the risk of hypertension. It was denoted in an association study, that the gene

encoding β_2-adrenergic receptor was associated with the development of essential hypertension [175]. Likewise, salt sensitivity was found to be associated with β_2-adrenergic receptor in African Americans [176].

However, the relationship between β_2-adrenergic receptor polymorphism and hypertension still remains somewhat unclear. For instance, Gly16 polymorphism occurred more frequently in hypertensive Afro-Caribbean's[177]while in blacks from South Africa no association was found between Arg16-Gly and/or Gln27-Glu β_2-adrenergic receptor polymorphism and essential hypertension [95]. However, in a cohort of Germans Arg16 increased the predisposition of hypertension [178].

Consequently, it is evident that the relationship between β_2-adrenergic receptor polymorphism and ethnicity and hypertension is poorly understood and further studies are needed to elucidate this relationship. Clearly, evaluating this relationship in Afro-Caribbean men would contribute significantly to the literature and to current knowledge and understanding of β_2-adrenergic receptor polymorphism in hypertension.

1.2.8.6 **β_2 adrenergic receptor and body composition**

There have been studies showing a putative association between obesity and β_2-adrenergic receptor. The β_2-adrenergic receptor genes have been studied because of their key roles in the regulation of energy metabolism and utilization [179]. The β_2-adrenoceptor plays important roles in the regulation of energy homeostasis, for instance the stimulation of glycogen breakdown and lipid mobilization. β_1 co-exist with β_2 and $\beta_3$adrenoceptors in the white adipose tissue [164, 180].

Gln27Glu variant influences body fat by changing the lypolytic response of adipose tissue to catecholamines. Catecholamines are lipolysis stimulating hormones [180, 181]. Gln27Glu

is associated with a relative risk of obesity≈ 7 and odds ratio of ≈ 10 [179]. The role of β_2-receptor gene polymorphism for obesity has so far been mainly evaluated in women [179]. Women who are homozygous for Glu27 have been found to have 20kg more fat mass compared to controls. Carriers of Glu27 had 50% larger fat cells compared to non-carriers [179]. This polymorphism has also been found to be associated with obesity in the Swedes [182] linked to obesity in the Japanese [183] and is associated with the early onset of obesity in the Danes [184]. Interethnic differences have also been observed in allele frequencies. The Glu27 variant has been found to be more common in European Americans than African Americans [174].

1.2.9 Obesity in the Caribbean

The epidemic of obesity in the Caribbean has become a major health care problem. In the Barbados population between 1968 and 1981 obesity increased from 7.0 to 16.2% in males and 31.0 to 37.9% in females. In women over the age of 40 years the prevalence of obesity increased from 32% to 50%. Furthermore, in Jamaica over a 33 year span (1962-1995) the prevalence of obesity increased from 2% to 25% in men and 30 to 60% in women[185].

Obesity in the Caribbean is a major concern and has been shown to be more prevalent in countries with a higher per capita gross national product (GNP) and it seems to disproportionately affect women. Studies conducted in Jamaica, Barbados, Nigeria, Cameroon, St. Lucia and Maywood have demonstrated that hypertension increases in a manner similar to that of obesity [185]. The prevalence of these chronic diseases are greater in lower income individuals from poorer countries who start living a westernized lifestyle influenced by sedentism, and caloric dense foods[186]. Similar to United States obesity in the Caribbean plays an important role in the pathogenesis of T2DM, hypertension and negative body composition changes. It has also been demonstrated that diabetes mellitus frequently coexists with other

conditions such as abnormal serum cholesterol which is a symptom of the metabolic syndrome[187].

1.2.9.1 Causes of weight gain

Obesity typically occurs when there is an increase in energy consumption compared to energy expenditure [188-190] . Dietary surveys show an increase in caloric intake in the United States of approximately 200kcal/day in the past 20 years [191, 192]. Much of this caloric intake corresponds to the increase consumption of fats, sugars, fast foods, snacks, high caloric beverages, larger food portion sizes and eating outside the home[193]. For instance, meals at fast food restaurants [194], full service restaurants [195], and eating between meals have been shown to cause an increase in body weight [193, 196].

1.2.9.2 Energy output: physical activity

In 2002 the Center for Disease Control (CDC) and Prevention determined that approximately less than 30% of individuals in the United States were engaging in sufficient physical activity. While an additional 30% of persons were active but not sufficiently with the remainder of individuals predominantly sedentary engaging in minimal levels of physical activity [191, 197].

The percentage of the population that reports participating in no leisure time physical activity is greater amongst women compared to men, greater in African Americans, and Hispanics compared to European Americans, higher among older adults compared to younger adults and greater in the less affluent compared to the more affluent in society [198]. It has been shown that individuals with lower levels of education and income engage in less leisure time physical activity [198]. Studies have also demonstrated that individuals who are moderately or

highly active have a longer life expectancy and have more years free of chronic diseases such as diabetes [199].

1.2.10 Distribution of body fat

Obesity related changes are significantly related to an accumulation of fat in the body, and in the literature there is substantial evidence indicating that central obesity is a stronger predictor of morbidity than the accumulation of fat in the lower body [200, 201]. The accumulation of fat in the in the abdominal regions has significant implications for metabolism and insulin sensitivity [201]. These co-morbidities are more related to waist circumference than to BMI. Additionally, visceral or abdominal fat is strongly associated with an elevated risk of cardiovascular disease and type 2 diabetes [202]. Visceral adiposity is considered a risk factor for cardiovascular disease, insulin resistance, metabolic syndrome and T2DM in adults [203, 204].

1.2.10.1 Subcutaneous and visceral fat

Intra-abdominal fat has been shown to be linked to an increased risk of developing debilitating diseases. Adipose tissue that is located in the abdomen is comprised ofboth visceral and subcutaneous fat; and there has been some discrepancy regarding the contribution of these compartments to the development of the metabolic syndrome in overly obese individuals. Studies have shown that there is a positive relationship between abdominal subcutaneous fat and the development of insulin sensitivity [205].

Many researchers report that visceral adipose tissue is a stronger determinant of insulin resistance compared to subcutaneous adipose tissue. Studies have shown that both visceral and subcutaneous fat are related to insulin sensitivity [206]. It has also been shown that the amount of

subcutaneous fat is strongly associated with the level of insulin resistance and increasedlipolytic rates compared to the superficial subcutaneous abdominal fat [207].

1.2.10.2 **Intramuscular fat**

Studies have revealed that the location of fat within the thigh determines disease risk [208].Fat stored in the non-subcutaneous regions (meaning fat located in the muscle or around muscle fibers) was related to insulin resistance in persons considered obese; however, there was no correlation with subcutaneous thigh fat [208]. In addition, a positive association has also been observed between insulin resistance and intramuscular lipid levels [209, 210].

Krssak et al.,[209] has shown that intracellular lipid concentrations were correlated with insulin sensitivity using novel proton nuclear magnetic resonance spectroscopy technique (H NMR) [209]. In addition, Goodpaster et al.,[208] revealed that sub-facial adipose tissue and intermuscular adipose tissue are markers of insulin resistance in obese persons and type II diabetics. However, some but not all studies delineated a relationship between skeletal muscle lipid and insulin sensitivity. One study of older normoglycaemic women found that intracellular lipid content was inversely correlated with insulin sensitivity [210]. Intracellular lipid however was shown to be related to waist hip ratio and fasting plasma concentrations of non-esterified fatty acids (NEFA) but not with glucose tolerance or whole body insulin sensitivity [210]. Another study reported an inverse correlation between muscle triglyceride and insulin sensitivity [211].

Fatty acid concentration of the cell membrane in the skeletal muscle affects insulin sensitivity by altering the physicochemical properties of the membranes, which alters receptor function, ion transport over the membrane, cell energy requirement and cell signaling. In addition, elevated levels of saturated fatty acids in the cell membrane disrupts insulin action by altering insulin receptor binding, altered ability to translocate or insert glucose transporters,

changing phospholipid fatty acids-interaction with function of second messenger (protein kinase C) and reduced ion permeability [212].

1.2.10.3 **Waist circumference (WC) and waist to hip ratio (WHR)**

Waist and hip circumference ratios are important measurements of the abdomen as well as in predicting abdominal obesity [213]. The values of the waist-to-hip circumference ratio (WHR) used to define abdominal obesity are 0.95 for men and 0.80 for women. There is evidence suggesting that waist circumference (WC) is a better clinical measure of abdominal obesity than WHR [214]. A potential limitation of WHR is, it might not change when the hip and waist circumference increase by comparable ratios. Consequently, WHR is typically inaccurate in the event of significant loss of visceral adipose tissue [215].

There have been a few studies that have suggested that WC and WHR predicts abdominal adipose tissue equally well. However, current data are more in favor of using WC as a better measure of abdominal fat [216, 217]. WC predicts percentage body fat which correlates well with the development of insulin resistance and the development of cardiovascular risk factors in African Americans, European Americans and Europeans [218-221]. Moreover, the interpretation of these WC values depends on the race/ethnicity of the group under evaluation. Caribbean populations, such as Jamaica, Nigeria, St. Lucia, Barbados and Cameroon the predictive values of established cut-offs of WC showed poorer sensitivities for abdominal obesity [222].

1.2.10.4 **Body mass index (BMI)**

Body mass index (BMI) is a statistical measurement, which compares a person's weight and height. The BMI is a useful tool to estimate a healthy body weight based on how tall a person is, however; it does not measure the percentage of body fat. This measure of general adiposity is

defined as weight in kilograms (kg) divided by height in meters^2. According, to the WHO a BMI <18.5 kg/m^2 is defined as underweight, 25-29.9 kg/m^2 is considered overweight and ≥30 kg/m^2 is defined as obese [191, 223, 224]. Several studies have shown that abdominal circumference is highly correlated with increased risk of type 2 diabetes than BMI [225]. Although, BMI is one of the most common measures of assessing general obesity in adults, Other measures such as dual X-ray absorptiometry (DEXA), Quantitative computerized tomography (QCT), computed tomography (CT) and peripheral quantitative computerized tomography (pQCT) can provide more accurate measures of body fat or the percentage body fat in an individual [226].

1.2.10.5 **Visceral fat as a pro-inflammatory tissue**

Visceral fat shows evidence of accelerated lipolytic activity with an elevated release of free fatty acids that can severely affect insulin action and glucose diffusion in many tissues [227-230]. These elevated levels in circulating FFAs may also cause an increase in triglyceride reservoirs in the muscle and liver decreasing insulin action and elevating hepatic very low-density protein output [231, 232].

On the other hand, a reduction in visceral adiposity and FFA levels after weight loss has been associated with enhanced insulin sensitivity [233, 234]. Visceral fat has been shown to be responsible for the release of cytokines, such as tumor necrosis factor α (TNF-α) and interleukin 6 (IL-6) [235, 236]. TNF-α inhibits tyrosine kinasephosporylation of the insulin receptor, causing a defect in insulin signaling contributing to insulin resistance and impaired glucose transport [237]. An imbalance of proinflammatory cytokines from adipose tissue can trigger C-reactive protein (CRP) secretion. It has been shown that CRP levels are strongly associated with insulin resistance and adiposity, and elevated levels of CRP has been shown to be correlated with impaired endothelial dysfunction and CAD [238].

1.2.10.6 Race ethnicity and body fat distribution

There have been several studies that have shown that body fat distribution varies according to population. These differences in body fat distribution were found to be associated with elevated triglycerides, high blood pressure, low HDL cholesterol, and high glucose levels. Studies in the United States and South Africa have indicated that visceral fat is smaller in African Americans compared to Europeans matched on BMI. However, it has been noted that in the black populations, individuals are more insulin resistant compared to European populations [239-241].

Abdominal fat contributes different degrees of risk for hypertension depending on the ethnicity of the person. For instance, compared to Europeans, black men and women have a 1.58 and 1.39 greater risk of developing hypertension if their waist circumference values are extremely high [242]. Likewise, the clustering of insulin resistance syndrome associated with abdominal obesity seems to occur more frequently in Black and Hispanic individuals [243]. Depress et al.,[244]reported that with comparable levels of fatness, Europeans appeared to have more abdominal fat compared to African Americans. African American women appear to have higher levels of total body fat but similar abdominal adipose tissues as their European counterparts.

1.2.11 The metabolic syndrome

The term metabolic syndrome is defined as the clustering of cardiovascular risk factors related to hypertension, abdominal obesity, dyslipidemia, and insulin resistance. According to the National Cholesterol Education Program Adult Treatment Panel ATP III guidelines in 2001, metabolic syndrome is the presence of three or more of the five risk factors. Other characteristics of the metabolic syndrome are: hyper-insulinemia, insulin resistance, and higher density of LDL cholesterol. The metabolic syndrome is also associated with elevated levels of inflammatory risk

markers, such as fibrinolysis, plasminogen activator inhibitor-1, oxidative stress, microalbuminuria, irregularity in autonomic regulation, and the activation of the rennin angiotensinaldosterone axis [245].

1.2.11.1 The impact of ethnicity on the metabolic syndrome

Ethnicity has a major impact on metabolic and anthropometric changes as well as co-morbid diseases. A major component of the metabolic syndrome is obesity. In South Africa, obese Black women matched for BMI and body composition were compared to obese urban European Caucasian women and their metabolic differences and β–cell function examined. The Black women had a higher degree of insulin resistance, lower plasma insulin and C-peptide levels, reduced anti-lypolytic activity, higher post absorptive FFA concentrations, lower fasting and 3-h triglyceride concentrations, and lower plasma cortisol concentrations [240, 241, 246-251].

Other studies have shown that black women have less visceral fat and black diabetics have higher fat mass compared to obese black women without diabetes with similar BMIs. These studies also showed that weight loss occurred more swiftly in the glutofemoral regions in black women, but weight loss in the visceral regions occurred in both groups. This weight loss that occurs in the in black women might be the result of greater lypolytic activity in the glutofemoral regions [252, 253]. On the other hand, the visceral fat in obese European women has been shown to be associated with more atherogenic fasting and postprandial lipid profiles [240, 247, 248, 253].

Similar findings were also observed in fit men as that observed in obese women. For example plasma insulin and postprandial glucose tolerance were lower in lean black men compared to European Caucasian men. Lypolytic activity was also elevated in lean black men compared to lean European Caucasian men [253].

1.2.11.2 **Prevalence of the metabolic syndrome**

According, to the NHANES data (1988-1994) the prevalence of the metabolic syndrome in the United States was estimated to be approximately at 23.7 percent or approximately 47 million people have this problem. In the United States the metabolic syndrome is expected to increase exponentially. Prevalence of the metabolic syndrome was defined according to ATP III of the abnormalities: waist circumference greater than 102 cm in men and 88 cm in women; serum triglycerides level of 150 mg/dL (1.69 mmol/L); high density lipoprotein cholesterol level of less than 40 mg/dL in men and 50 mg/dL in women; blood pressure of at least 130/85 mm Hg; or a serum glucose level of 110 mg/dL. The unadjusted and age adjusted prevalence of the metabolic syndrome were 21.8% and 23.7% percent respectively. The prevalence of the metabolic syndrome increased from 6.7% in adult's age 20 years to 29 years to 43.5% and 42% for individuals 60 to 69 years. Mexican Americans had the highest age adjusted prevalence of the metabolic syndrome (31.9%). The age-adjusted prevalence was similar for men (24.0%) and women (23.4%). But African American women had 57% higher prevalence than men [254].

In men the metabolic syndrome (MetSyn) is associated with a fourfold elevated risk for CHD, a twofold risk of CVD and all cause mortality even with adjustments for age, LDL-cholesterol, smoking, and a family history of CHD. Likewise, the metabolic syndrome in women is associated with an increased risk of CHD. Individuals with the metabolic syndrome are five to nine times more likely to develop diabetes. However, in the PROSPER study, the metabolic syndrome and its individual components were not associated with the development of CVD but the presence of the metabolic syndrome at baseline was associated with a future risk of developing diabetes especially in individuals with a BMI >30kg/m2 and a fasting a glucose of >6.1 mmol/L [255]. Similarly, in the BRHS study, the metabolic syndrome was weakly associated

with the development of CVD events but its individual's components such as waist circumference, serum triglycerides, HDL cholesterol, elevated blood pressure, and fasting glucose were associated with an increased risk of developing diabetes [255]. Hence, these results suggest that the metabolic syndrome is strongly associated with the development of diabetes in the middle age and also the elderly but is weakly associated with CVD [255].

In the St. James Coronary Risk Factor study, African Trinidadians had lower cholesterol and LDL cholesterol and higher HDL cholesterol than Asian Trinidadians. It is believed that a large percentage of this population has at-least three maybe four of the characteristics considered the deadly quartet or Raven syndrome X: which are obesity, hypertension, diabetes, glucose intolerance and hyperlipedemia[256-259].

1.2.11.3 **Lifestyle modification**

According to the SMART study regular exercise reduced the risk of developing metabolic syndrome by at least 50% and these results remained significant even without weight loss in individuals. In this cohort insulin resistance was inversely correlated with physical activity on the homeostasis model assessment (HOMA)[260]. Furthermore, exercise reduces adiposity and weight while improving insulin sensitivity.

Lakka et al.,[261] reported that in the HERTAGE study physical activity reduced markers of inflammation for instance C-reactive protein. It is believed that physical activity improves the risk factors for insulin resistance and hypertension by inhibiting the production of cytokines and their potential interaction with monocytes and endothelial cells[261, 262].

Dietary modifications are also helpful in treating insulin resistance and hypertension. For instance, a heart-healthy diet lowers levels of TNF-α [263]. In the Framingham offspring cohort a

diet high in whole grains and fiber reduced the risk of developing insulin resistance and the metabolic syndrome [264].

The ARIC (atherosclerosis risk in communities) study reported that a western diet increases the risk of developing the metabolic syndrome by 18%. This study reported that the consumption of meats, fried foods, and the regular intake of diet sodas was associated with increased risk of the MetSyn[265]. However, diary products were inversely associated with MetSyn both cross-sectionally[266-268] and prospectively [267]. No association was found between the association of the MetSyn and whole grains, refined grains, fruits, vegetables, nuts, coffee and sweetened drinks [265].

Final,the DASH diet, which is considered to be rich in fruits, vegetables, low fat dietary products, sweeteners and reduced consumption of meats, reduced the risk of developing the metabolic syndrome and hypertension [269]. Exercise does provide some protection against the metabolic syndrome and also hypertension. Individuals who lost weight showed a reduction in their mean arterial pressure, plasma renin activity and aldosterone levels [156].

1.2.12 Measurement of body fat distribution

1.2.12.1 Dual X-ray absorptiometry (DEXA)

DEXA scans are primarily used to evaluate bone mineral density, measure total body composition and fat content. The speed of DEXA, its easy use, relatively low costs and lower radiation compared to computed tomography (CT) makes it good a method for use in clinical settings, research, and also in large population studies. The DEXA machine uses two-X-ray beams with differing energy levels. When soft tissue absorption is subtracted out, the BMD can be determined from the absorption of each beam by bone [270, 271].

1.2.12.2 Computed tomography (CT)

Computed Tomography Imaging can be used to quantify a cross sectional area of tissue. CT distinguishes tissue in vivo based on the level of which they attenuate the energy of an X-ray. These attenuation processes depend substantially on their density and are expressed in Hounsfield units (HU), positive for muscle and negative for fat. Adipose tissue is not as dense as water and hence displays attenuation values that are of a negative value. However, muscle is denser than water and consequently would have more positive attenuation values[272]. CT is a very important method for quantification of visceral fat.

1.2.12.3 Quantitative computerized tomography (QCT)

Historically quantitative computerized tomography (QCT) was used to measure volumetric bone density of the spine and hip. QCT also analyzes the cortical and trabecular bone separately [270]. This method has been proven to be quite useful in clinical research settings and also used to evaluate patient progress particularly therapeutic responses to parathyroid hormone therapy. Even though this method has mainly been used to determine bone mineral density of the periphery it can also be used to measure body fat.

1.2.12.4 Peripheral quantitative computerized tomography (pQCT)

Peripheral quantitative computerized tomography (pQCT) is used to measure peripheral sites [272].PQCT has been used to determine body fat in mice. The accuracy of the values obtained from PQCT was compared to chemical extraction in mice. The coefficient variation for determining percentage body fat in mice using PQCT was 3.9% (±1.8SD). PQCT was considered to have good accuracy and also precision in determining percentage body fat and liver fat in animals studies[273]. Ranke et al.,[274]determined that the use of PQCT was a suitable technique to

determine the size, the structure of muscle, fat and bone in pubertal girls with Turner syndrome who were being evaluated on the effects of growth hormone therapy.

1.2.13 Diabetes

1.2.13.1 Prevalence of diabetes in the United States

Current data show that the prevalence of diabetes in the United States has risen 40% from 4.9% in 1990 to 6.9% in 1999 [275]. Type 2 diabetes mellitus is 10 times more common in adults over the age of 65 years compared to those younger than 45 years of age [275].

In addition, T2DM is more common in minority racial groups such as: Native Americans, Hispanics, and African Americans. This disease affects these groups at a rate 2-4 times compared to European American populations. This increased prevalence of T2DM has been reported in adolescence and children, T2DM mellitus tend to occur more frequently than type 1 diabetes [275].

In the United States the estimated lifetime risk of developing T2DM in 2000 was 33% for males and 39% for females [275]. T2DM is also associated with reductions in life expectancy of 11 years in males diagnosed after the age of 40 years [276].

1.2.13.2 Pathogenesis of diabetes

Glucose is considered the body's main source of energy and in most mammals there is a cell membrane protein that facilitates the diffusion of glucose from the blood stream following the digestion of food into the cell. A gradient is maintained facilitating the diffusion of glucose into the cell by phosphorylation of sugar once it enters the cytoplasm lowering intracellular glucose

concentration. There are several proteins that facilitate the transportation of glucose and these isoforms and they are termed GLUT1-5 distinguished by the tissues in which they are found [277].

Insulin is a hormone produced by the endocrine cells of the pancreas (β-cells) and plays an important role in the maintenance of blood sugar levels. An elevation in blood glucose levels triggers the release of insulin, which stimulates the uptake of glucose into target cells such as the skeletal muscle and adipocytes. When insulin levels rise in response to elevated blood glucose levels this hormone acts on target cells and stimulates the translocation of glucose into the cell. Diabetes is caused by a defect in insulin activity[277]. The glucose transporter GLUT4 mediates insulin uptake in adipocytes and also in muscle cells by moving glucose from intracellular storage sites into the plasma membrane [278].

In persons who are not diabetic normal plasma glucose range is between 4 and 7 mM. This maintenance of normal glucose levels is governed by a balance between glucose absorption from the intestines and its production by the liver and its metabolism by the peripheral tissues. As mentioned before insulin increases glucose uptake in muscle and fat, and inhibits hepatic glucose production consequently acting as the regulator of blood glucose concentrations. Likewise, insulin fuels cell growth and differentiation, and enhances the storage of substrates in fat, liver and muscle through the process of lipogenesis, glycogen, and protein synthesis, and also through the inhibition of lypolysis, glycogenolysis, and the breakdown of proteins [279].

T2DM is a non-autoimmune disorder. Patients with T2DM have insulin resistance as well as a significant reduction in insulin response to glucose. Cross-sectional studies have demonstrated that high risk groups have severe reductions in insulin sensitivity and or defects in β-cell function or they may have abnormalities in both path ways [280]. Studies have indicated that older individuals are at an increased risk of developing type II diabetes. Typically because these

patients have impairments in postprandial rather than fasting glucose[281]. It is believed that a significant number of individuals that were originally diagnosed as type 2 diabetics were cryptic type 1 diabetics who later evolved to a type 1 state denoted as anti-β-cell autoimmunity [282].

1.2.13.3 **Obesity and insulin resistance**

Insulin resistance is denoted as, "reduced insulin-mediated glucose uptake in insulin sensitive tissues such as skeletal muscle and liver" [283]. In individuals who are obese, insulin resistance is common and is typically related to central abdominal fat. Much of the fat located in the central abdominal region is called visceral adipose tissue (VAT). VAT is related to insulin resistance and the metabolic syndrome. This fat is mostly comprised of mesenteric and omental fat masses that can be measured through a variety of diagnostic measures such as CTs, MRIs and DEXA scans. Visceral adipose tissue accounts for a small proportion of total fat in the body namely 10% whereas 25% to 50% of abdominal fat is subcutaneous. However, many researchers believe that it is VAT that confers a risk for insulin resistance because it has a higher lipolysis rate than SAT and also because of it close proximity to the portal circulatory system [284].

1.2.13.4 **Adipocytes, insulin resistance and diabetes**

Adipose tissue is considered an extremely active metabolic and endocrine organ that participates in a variety of homeostatic processes that produces hormones such as leptin, adiponectin, resistin, TNFα, and IL-6 which influence insulin sensitivity and plays a key role in the pathogenesis of insulin resistance, diabetes, dyslipidemia, inflammation and atherosclerosis [285-288]. Likewise, adipocyte derived factors contribute to the development of β-cell dysfunction and also type 2 diabetes [289].

Adiponectin is a protein and also an adipose tissue-specific collagen like factor [290] that is secreted by adipocytes and can be found in concentrations of 7 to 12mg/L in healthy individuals.It has been shown that adiponectin strongly correlates with decreased fatty acid blood concentrations and a reduction in BMI and body weight. In addition, adiponectin could possibly be the molecular link between obesity, insulin resistance and could potentially serve as the biomarker for the metabolic syndrome [291].

1.2.13.5 **Insulin resistance**

Insulin resistance is related to diabetes, hypertension and obesity and is characterized by decreases in receptor concentration, kinase activity and also the phosporylation of IRS-1 and IRS-2, PIK activity, glucose transporter translocation and the activity of intracellular enzymes. In persons who suffer from T2DM the activation of the MAP kinase pathways is not diminished potentially leading to some of the harmful effects of chronic hyperinsulinaemia on cell development in the vasculature [279, 292].

Genetic as well as acquired factors can significantly affect insulin sensitivity. Genetic defects in insulin receptor is extremely rare however it can be found in syndromes such as leprechaunism, Rabson Mendenhall, and type A syndrome of insulin resistance.Type 2 diabetes is polygenetic and entails multiple gene polymorphism that encode the proteins involved in insulin signaling, secretion, and also its metabolism [279].

1.2.13.6 **Diabetes in the Caribbean**

Diabetes mellitus is considered one of the most prevalent diseases and a leading cause of death in the Caribbean region and has emerged as a major health concern. Diabetes is characterized by chronic hyperglycemia, and other metabolic disorders for instance, atherosclerosis, damage to

target organs such as the heart, kidneys, eyes, brain, and the peripheral nerves. Obesity seems to play a central role in the development of diabetes and other co-morbid conditions such as hypertension and dyslipidemia. Despite the growing concern regarding diabetes in the Caribbean there are still many undiagnosed individuals in that region [187].

Diabetes mellitus has been shown to be associated with increasing age up to approximately the 6th decade and then eventually leveling off. A denoted reason for this increased prevalence in diabetes mellitus is the aging population in the Caribbean. For example, amongst Barbadian Blacks diabetes was shown to be positively associated with increased age, family history of diabetes, central obesity and a body mass index $\geq$25kg/m [2187, 293].

1.2.13.7 **Economic burden of diabetes**

NIDDM causes economic burden to many societies especially bi-ethnic Caribbean countries such as Trinidad and Tobago [294-296]. This increase in diabetes in the Caribbean seems to be driven by changes in living conditions. For example, diets in the Caribbean have become more like westernized countries high in fat, sugars and low in dietary fibers. Sedentary lifestyle, and labor saving devices have also increased the likelihood of individuals developing diabetes [297] .

1.2.13.8 **Under report of diabetes in the Caribbean**

Diabetes in the Caribbean has a high rate of being under-reported. For instance, in a study of the national death certificates in Jamaica, revealed that there was a 30% under-recognition of diabetes [298]. In Spanish Town, Jamaica the prevalence of diabetes in persons with an average age of 47 years was 9.8% for men and 15.7% in women with an overall prevalence of 13.4% [297]. In Trinidad and Tobago, the prevalence among Indo-Trinidadian men and women 35-69 years of age increased from 11.6% and 18.9% in 1961-62 to 19.5% and 21.6% in 1977. Among Afro-

Trinidadians the rates were 2.5% to 8.2% among men and 5.4% to 14.8% amongst women [294, 299]. This epidemic of diabetes mellitus is expected to continue with a 35% increase in the prevalence from 1995 until 2025 which will be accompanied by growth in population size, which will be largest in developing countries [300].

In Trinidad and Tobago type 2 diabetes accounts for approximately 13.6% of hospital admissions and cost the government an estimated US$ 1.6 million dollars [301]. It is well established that abdominal obesity is associated with insulin resistance and is highly predictive of the development of type 2 diabetes and other cardiovascular diseases [302]. In the Caribbean, there is also a gender dimorphism in which females have a higher prevalence of diabetes and obesity. The influence of lifestyle factors on prevalent diabetes has been succinctly demonstrated by the International Collaborative Study on Hypertension in Blacks in which a gradient of prevalent diabetes cases was delineated ranging from 2.0% in West Africa to 9.0% in the Caribbean and 11% in African Americans [303].

In this region of the world many of the studies on the prevalence of diabetes in the Caribbean were conducted more than 30 years ago [299, 304-306] and since then there have been changes in diagnostic criteria which has significantly limited the ability to compare those data to current data. However, Poon-King et al. conducted a prevalence study in approximately 2400 individuals in July 1961 and found that rates of diabetes were 2.4% in East Indian Trinidadians and 1.4% in African descent Trinidadians [299]. There are no new studies addressing the differences in rates of diabetes in blacks and Indians residing in Trinidad today.

1.2.14 Hypertension

A small percentage of patients have as the main cause of their hypertension renal or adrenal disease approximately 2%-5%. However, in the remaining population there is no single

identifiable cause for this disease called essential hypertension. There are a number of physiologic processes involved in the maintenance of normal blood pressure and their disturbance is possibly a key role in the development of essential hypertension. It is also quite possible that there are a number of inter-related factors involved in the etiology of hypertension. Some of the more intensely studied factors are: salt-intake, obesity, insulin resistance, the rennin-angiotensin system, and sympathetic nervous system[307].

1.2.14.1 **Definition of hypertension**

Hypertension is defined as the "consistent elevation of the systemic arterial blood pressure". Individuals are usually diagnosed as having hypertension after an average of approximately two or more diastolic blood pressure measurements of 90 mm Hg or higher on consecutive visits or when the average systolic blood pressure measurements on consecutive visits is 140 mm Hg or greater [245].

Hypertension is typically caused by an increase in cardiac output, peripheral resistance or a combination of both. Increases in cardiac output can be caused by any condition that could increase the heart rate or stroke volume. On the other hand, peripheral resistance is caused by any condition that increases the viscosity of the blood or decreases vessel diameters for instance arteriolar diameter. Individuals who suffer from hypertension can have a combined systolic and diastolic hypertension or some individuals might have isolated systolic hypertension. Usually most cases of combined systolic and diastolic have no clearly delineated causes and so are termed primary hypertension. Primary hypertension is also called essential hypertension or idiopathic hypertension [308]. Hypertension of unknown origin is termed essential hypertension. This type of hypertension is treatable but not curable and there is a strong hereditary component [309].

1.2.14.2 Characteristics of severe hypertension

- MAP is increased by 40-60%
- In severe stages renal blood flow is decreased by one half of normal
- Blood flow through the kidneys is increased twofold to four fold
- CO is normal
- The kidneys do not excrete adequate salt and water unless the arterial pressure is high [309].

Secondary hypertension is usually caused by altered hemodynamics related to the primary disease such as arteriosclerosis. Secondary hypertension accounts for approximately 5% to 8% of cases.Isolated systolic hypertension is defined as elevated systolic blood pressure accompanied by normal diastolic blood pressure defined as <90 mm Hg. Isolated systolic blood pressure is characterized by increased cardiac output or rigidity of the aorta or both [310].

1.2.14.3 Obesity and Hypertension

The relationship between hypertension and obesity is well documented. Individuals with larger body masses seem to have higher blood pressure and also higher rates of hypertension [311, 312]. The most significant modifiable risk factors for hypertension are overweight and obesity accounting for nearly 66% of the risk in many populations [313]. For instance, in the Nurses Health Study (NHS), data showed that BMI values at 18 years and also midlife were positively correlated with hypertension. In this study, there was a significant association between long-term weight loss after the age of 18 years and a decreased risk of hypertension while weight gain after 18 years significantly increased this risk. This association continued to remain significant following the adjustments for current BMI which implied that BMI and history of weight

changes are independent predictors of hypertension [314]. In the Framingham Study Moore et al.,[315] reported that when modest amounts of weight loss were sustained over an extended period of time it was associated with a 22% to 26% reduced risk of hypertension.

A variety of mechanisms have been postulated to help explain the relationship between obesity and hypertension. One such mechanism states that obesity and overweight causes renal structural changes that lead to tubular re-absorption and also sodium retention [156, 316].This further causes an elevation in arterial pressure which damages nephron function causing obesity, hypertension and renal damage [156].

1.2.14.4 **Environment**

Environmental factors can also play an important role in hypertension. Environmental factors that are implicated in the development of hypertension are salt intake, obesity, occupation, alcohol intake, family size and crowding. These listed factors are important for the development of high blood pressure with increasing age in the more affluent societies compared to the decline in blood pressure noticed in the less affluent societies [317]. The environmental factor of all the above listed that has received the most attention is salt sensitivity. The cause of salt sensitivity in individuals is dependent on a number of factors namely: primary aldosterism, bilateral renal artery stenosis, renal parenchymal disease and low-renin essential hypertension which accounts for approximately half of the patients[317].

1.2.14.5 **Nutrition**

Lifestyle factors can also affect the development of hypertension. These factors include: excessive caloric consumption, excessive sodium consumption, excessive alcohol intake, inadequate potassium, inadequate calcium intake mostly in elderly African Americans,

inadequate physical activity, poor fitness level, and cigarette smoking [317]. Diet related diseases and conditions related to hypertension include: obesity/overweight, diabetes mellitus, and dyslipidemias. In addition, adverse health outcomes that are associated with uncontrolled or poorly controlled hypertension are: cardiovascular disease, coronary heart disease, left ventricular hypertrophy, angina, myocardial infarction, congestive heart failure, nephropathy, cerebrovascular disease, transient ischemic attacks, stroke (CVA), peripheral artery disease (PAD), and retinopathy [317].

1.2.14.6 **Pathophysiology of hypertension**

Although the exact pathogenesis of primary hypertension remains to be elucidated, several hypotheses have been proposed. These include (1) increased blood volume, (2) inappropriate autoregulation, (3) overstimulation of sympathetic neural fibers in the heart and vessels, (4) water and sodium retention by the kidneys, and (5) hormonal inhibition of sodium-potassium transportation to kidneys and blood vessels. Researchers hypothesize that any of these mechanisms or a combination of more than one might be at work in the pathogenesis of primary hypertension [310].

Chronic hypertension can cause changes in the walls of the systemic blood vessels. Extended vasoconstriction along with high pressures within these vessels especially in the arteries and arterioles can cause the vessels to thicken and toughen to handle stress. These arterial smooth muscles further undergo cellular enlargement (hypertrophy) and cellular proliferation (hyperplasia). Later the tunica intima and the tunica media develop fibromuscular thickening that cause significant narrowing of the lumina. The injury of these vessels promotes biochemical mediators of inflammation that increase the permeability of the vascular endothelium.With the increased permeability humoral substances are absorbed into the vessel

walls causing further thickening and smooth muscle contraction [310]. The contraction of the smooth muscle cells is thought to be caused by a rise in the intracellular calcium concentration, which could possibly explain the vasodilatory effect of certain drugs that block calcium channels. Furthermore, this smooth muscle constriction of the arteriolar vessels causes structural changes leading to an irreversible rise in peripheral resistance [307].

In addition, it has been hypothesized that in the early stages of hypertension the peripheral resistance is not raised and that the elevation of the blood pressure is induced by the elevated cardiac output, which is associated with sympathetic over activity. This rise in peripheral arteriolar resistance could be considered a compensatory mechanism to prevent a raise in pressure being transmitted to the capillary bed where it could affect cell homeostasis [307].

1.2.14.7 **Reninangiotensin system**

The rennin angiotensin system is considered to be one of the most important of the endocrine system that regulates blood pressure. Renin is secreted by the juxtaglomerular system of the kidneys in reaction to glomerularunderperfusion or decreased sodium intake. Renin is also emitted in response to stimulation by the sympathetic nervous system [307].

Renin acts on the rennin substrate angiotensinogen converting it to angiotensin I, which is rapidly converted in the lungs by angiotensin converting enzyme to angiotensin II. Angiotensin is a vasoconstrictor and causes a rise in blood pressure. It also stimulates the adrenal gland to release aldosterone which cause a further rise in blood pressure associated with sodium and water retention [307].

1.2.14.8 **Autonomic nervous system**

The stimulation ofthe sympathetic nervous system can induce constriction and dilatation of the arteriolar. The autonomic nervous system plays an important role in the management of blood pressure. The autonomic nervous system also regulates temporary variations in blood pressure that might be related to increases in stress levels and physical activity. It is postulated that hypertension is associated with the interaction of the autonomic nervous system, the rennin-angiotensin system and other factors for instance, sodium and circulating volume [307].

1.2.14.9 **Cardiac output and peripheral resistance**

The ability to maintain a normal blood pressure is dependent on a balance in cardiac output and peripheral vascular resistance. Peripheral resistance is dependent on the walls of the small arterioles, which are comprised of smooth muscle cells. The continued constriction of the smooth muscle can cause structural changes in addition to the thickening of the arteriolar vessel walls possibly mediated by angiotensin leading to an irreversible rise in peripheral resistance. It has been hypothesized that in the very early stages of hypertension elevation of blood pressure is caused by elevated cardiac output that is related to sympathetic over-activity[307].

1.2.14.10 **Sympathetic over-activity**

Individuals who were obese had higher serum catecholamine and muscle sympathetic nervous activity (MSNA) than lean individuals.MSNA was significantly higher in persons with central obesity compared to individuals with peripheral obesity [318]. High resting heart rates and baroreflex dysfunction has been cited in the development of hypertension in the metabolic syndrome [319-321].

Individuals who are obese have an activated rennin angiotensin system, which can bring on hypertension [322]. This angiotensin system and the sympathetic nervous system are linked by positive feedback [323]. Possible agents involved in the stimulation of sympathetic nervous action in the metabolic syndrome are: elevated leptin, insulin resistance, and non-esterfied fatty acids (NEFA) [324]. NEFA raises blood pressure, heart rate, and α1-adrenoceptor vasoreactivity but also reduces baroreflex sensitivity, endothelium-dependent vasodilatation, and vascular compliance [325].

Second, insulin resistance raises plasma leptin levels and it has been delineated that leptin increases sympathetic nervous activity suggesting that leptin-dependent sympathetic nervous activation could contribute to obesity associated hypertension [326]. There is also data suggesting that the metabolic syndrome is related to markers of adrenergic overdrive [327].

1.2.14.11 **Oxidative stress and endothelial dysfunction**

In animal studies involving rats with the metabolic syndrome, following the consumption of high fat and refined sugars these animals had hypertension that was related to oxidative stress, nitric oxide inactivation, down regulation of nitric oxide synthaseisoforms, and endothelial nitric oxide synthase activator [328]. This indicated that oxidative stress and endothelial dysfunction could be related to the development of hypertension in the metabolic syndrome. Moreover, current evidence indicate that oxidative stress, which is elevated in metabolic syndrome is related to sodium retention and sensitivity [329].

In non-diabetics lipid peroxidation was correlated with body mass index and waist circumference suggesting that the accumulation of adipose tissue is correlated to oxidative stress in individuals [330]. Likewise, cross-sectional data from the Framingham offspring Study, showed that systemic oxidative stress is related with insulin resistance [331].

Insulin resistance causes an impairment in phosphatidylinositol 3-kinase (PI3K) which may cause an imbalance in the production of nitric oxide and the secretion of endothelin-1 further leading to endothelial dysfunction [332]. Epidemiological studies show a reciprocal relationship between endothelial dysfunction leading to the development of hypertension and insulin resistance [332].

1.2.14.12 **Inflammatory mediators**

Abnormalities in the inflammatory markers have been reported as being related to the development of hypertension. For instance, in the Women Health Study, a positive relationship existed between elevated serum CRP levels and the development of hypertension [333]. A strong association between inflammation, hypertension, and the metabolic syndrome was likewise proposed by Grundy SM [334].

Second, TNF-α is considered to play a key role in the pathophysiology of hypertension and the metabolic syndrome. TNF-α activates the production of endothelin-1 and angiotensinogen[335, 336] and, TNF-α gene locus is hypothesized to play a role in insulin resistance mediated hypertension [337]. Serum TNF-α has been shown to be positively related to systolic blood pressure and insulin resistance [338] and elevated TNF-α levels has been detected in monocytes from individuals with hypertension [339].

Furthermore, studies have also shown that blood pressure is a significant and independent predictor of IL-6 levels in women [340] and that IL-6 stimulates the central and the sympathetic nervous system which could potentially cause hypertension [341, 342]. In women who were given IL-6 an elevation in heart rate, and serum norepinephrine levels were observed [343]. IL-6 causes an increase in plasma angiotensinogen and angiotensin II leading to the development of hypertension [344].

1.2.15 Hypertension prevalence in Afro-Caribbeans

In a cohort of individuals from the Barbados Eye Study, more than half of the participants had hypertension at baseline with approximately one in two men and three in five women being affected by this disease.In this cohort hypertension increased with age and was similar for men and women between the age of 40-49; however, age specific rates were higher among women at older ages [345]. Age specific hypertension increased from 32.7% in men and 34.0% in women age 40-49 to 63.4% in men and 85.5% in women at age 80 and above with an overall prevalence in this population of 55.4 percent. Additionally, in this study hypertension awareness, treatment and control rates were 62.5, 53.8 and 18.5% respectively [345].

Bobb-Liverpool et al.,[346] reported that, in Jamaica hypertensive women had inadequate knowledge of hypertension, and poor compliance with JNCVI guidelines (Joint National Committee on Prevention, Detection and Evaluation and Treatment of High Blood Pressure).In this population, it was recommended that to improve patient compliance, counseling/education on diet, exercise, medication, and weight management be provided.In Trinidad and Tobago a study that evaluated health behavior patterns of chronic disease patients in relation to diet, weight control, and medication, determined that health professionals did not provide patients with the sufficient knowledge to adopt healthy lifestyles to prevent disease[347].

Following age standardization similarly high rates of hypertension were found in other countries of the African Diaspora that mirror levels of western acculturation. In the French speaking country of the Antilles called Guadeloupe, the prevalence of hypertension was 24.7% for men and 22.1% for women. In this population the major contributing factor was once again obesity especially in the disadvantaged cohort that was evaluated [348].

Similarly findings were confirmed from a combined study of the Birmingham factory screen, Birmingham INTERSALT volunteers and four West-Midlands churches. In this cohort of N=2853, Afro-Caribbean's had a significantly higher systolic blood pressure with higher diastolic blood pressure in the Afro-Caribbean women. Even following the adjustment of age, BMI, smoking, and alcohol intake the odds ratios and 95% confidence interval for being hypertensive were 1.56 (1.14 to 2.13; P=0.005) and 2.40 (1.51 to 3.81; P=0.0002) in Afro-Caribbean men and women respectively [349].

1.2.16 Prevalence of hypertension in Trinidad

In Trinidad the incidence of hypertension and non-insulin dependent diabetes mellitus in African and Indian populations residing in St. James did not differ significantly according to ethnicity. In a cohort comprised of 2,491 men and women age 35 to 69 years of age, incidence rates of hypertension ranged between 33 and 41 per 1000 persons in men and 27 and 32 per 1000 in person years in women [350].

Nichols et al.,[10]reported that blood pressure in Tobagonian adolescent population was found to be significantly higher than children residing in the U.S.A using US reference level cut points for systolic and diastolic blood pressure. The prevalence of elevated diastolic blood pressure was almost twice the expected levels using US cut points. The results of the Tobagonian study indicated that Tobagonian adolescents were heavier than adolescence residing in the United States, United Kingdom and Jamaica. Blood pressure in this study was associated with a family history of hypertension and being overweight.

2.0 PUBLIC HEALTH SIGNIFICANCE

Prostate cancer is one of the most common malignancies and the second leading cause of cancer related deaths in the United States and other countries [351] . African American men have been found to have the highest incidence rates for prostate cancer of all racial and ethnic groups [351]. High rates of prostate cancer have also been observed in Afro-Tobgonian men [35, 42]. Screening detected prostate cancer prevalence rates were 4.9% for individuals aged 50-55, 7.7% for individuals ages 55-59; and 13.3% for individuals aged 60-64 years [35]. Even though the reasons for the large disparity in prostate cancer risk are not fully understood, one possible explanation is androgen levels[351]. A common treatment for prostate cancer is androgen deprivation therapy (ADT) which reduces the levels of androgen in the body but also has many side effects[352]. Consequently, prostate cancer is a major public health concern and especially in persons of African ancestry. Further studies are needed to investigate the effects of treatments for prostate cancer such as ADT in at risk populations.

Obesity is also a major public health concern. Individuals who are considered minority/non-white are more prone to increases in weight and its associated complications[353]. World wide there are approximately a billion adults that are considered overweight and 300 million that are considered obese[353]. In the United States the prevalence of obesity in African Americans between 2003 and 2004 was 76.1%[354]. According to a 2003-2004 health survey that

was conducted in England Black Caribbean men had the highest prevalence rates of obesity compared to Bangladeshi, Chinese, Pakistani, Black African and Indian men[355].

Hypertension is also a disease that affects people of African descent and is of major public health concern. In the United States the prevalence of hypertension is highest amongst black Americans[356]. Individuals of African descent appear to develop high blood pressure much earlier in life, have higher average blood pressures and experience poorer outcomes[357].

Obesity and hypertension are multi-factorial in origin and the development of these diseases requires interplay between genes and the environment[356, 358, 359]. Therefore, to elucidate the relationship between high blood pressure, obesity and candidate genes, we propose to investigate the association between $ADRB_{2,}$ blood pressure, and obesity in Afro-Tobagonian men. In addition, we also propose to investigate the association between *AR* CAG and obesity in Afro-Tobagonian men. We hope that these studies will provide important clues into the etiology of aforementioned diseases. Moreover, we hope that these studies will increase our knowledge in understanding the role race/ethnicity plays as a risk factor, the identification of treatments that target specific populations, and the selection of appropriate therapies.

3.0 SYNTHESIS STATEMENT

This paper attempts to elucidate complex phenotypes such as body weight and blood pressure in a cohort of Afro-Caribbean men who reside on the island of Tobago. Research on obesity and blood pressure has revealed that genetics, diet, environment, behavior, and social norms play important roles in disease etiology. The multi-factorial nature of obesity and blood pressure and the involvement of many genes have made the search for candidate genes exceedingly difficult. These papers seek to further understand the role *ADRB2* and *AR* CAG repeats plays in the development of obesity related phenotypes and blood pressure.

A common treatment for prostate cancer (Prostate cancer) is androgen deprivation therapy (ADT). ADT is typically considered the treatment of choice for metastatic prostate cancer. Androgens are important for physiologic activity of myriad bodily functions. However, ADT administration has several deleterious side effects one of which is altered body composition. This paper also investigates the effect of ADT administration in Prostate cancer individuals on the rate of change of percent total body fat (DEXA) from baseline to follow-up in Afro-Tobagonian men. We hope that these studies will strength our understanding of the association between genes and our primary outcomes and secondly in explaining whether the rate of change of percent total body fat increases or decreases with ADT exposure.

4.0 PAPER 1: ADRB2 GENE VARIANTS, DEXA BODY COMPOSITION, AND HYPERTENSION IN TOBAGO MEN OF AFRICAN DESCENT

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4.1 ABSTRACT

Objective: Classic tissue effects of β2 adrenergic receptor activation include skeletal muscle glycogenolysis and vascular smooth muscle relaxation, factors relevant to obesity and hypertension, respectively. In a population-based study, we examined two common amino acid substitutions in the β2 adrenergic receptor gene (ADRB2) in relation to body composition and blood pressure.

Design and subjects: Cross-sectional analysis of 1965 African descent men living in Tobago and participating in a prostate cancer screening study.

Measurements: Body mass index (BMI), waist circumference, blood pressure, dual energy X-ray absorptiometry (DEXA) body composition, and ADRB2 (Arg16Gly; Gln27Glu) genotype.

Results: Twenty-five percent were obese (BMI ≥ 30 kg/m2) and 50% hypertensive. ADRB2 Arg16Gly and Gln27Glu alleles were in linkage disequilibrium (r^2=0.96). ADRB2 16Gly-containing and 27Glu-containing genotypes were equally frequent in low, medium, and high tertiles of percentage body fat mass (16Gly-containing genotypes: 73.2%, 74.4%, 75.1%, ptrend=0.45; 27Glu-containing genotypes: 27.8%, 24.2%, 26.4%, ptrend=0.58) and in normal blood pressure, pre-hypertensive, and hypertensive men (16Gly-containing genotypes: 73.6%, 72.5%, 74.6%, ptrend=0.55; 27Glu-containing genotypes: 26.1%, 24.6%, 27.1%, ptrend=0.51).

Conclusion: In a high obesity and high hypertension risk population with ancestry in common with African-Americans, genetic variation defined by two common ADRB2 amino acid substitutions was not associated with body composition or hypertension.

Key words: African ancestry, adrenergic β2 receptor, genetic polymorphism, body composition blood pressure, hypertension

4.2 INTRODUCTION

Interacting with diet and physical activity, genetic factors contribute to obesity and hypertension.[1-3] Frequently studied candidate genes include the leptin,[4] catecholamine,[5] and peroxisomeproliferator-activated receptors [6] and genes in the renin-angiotensin system.[7] Stimulating β adrenergic receptor function promotes lipolysis in fat cells.[2, 8] The β2 adrenergic receptor gene (ADRB2) attracts interest because catecholamine activation of the β2 adrenergic receptor regulates skeletal muscle glycogenolysis and vascular smooth muscle relaxation.[9] The ADRB2 single nucleotide polymorphisms most often studied in human populations substitute glycine for arginine at codon 16 (Arg16Gly) and glutamic acid for glutamine at codon 27 (Gln27Glu).[10]

Despite numerous published studies, the connection, if any, between ADRB2 Arg16Gly or Gln27Glu genotype and metabolic phenotypes, including obesity and hypertension, is tentative. Results from early recombinant cell culture experiments,[11] for example, launched a long standing hypothesis asserting that the ADRB2 16Gly variant promoted obesity and hypertension by causing receptor down regulation and physiologic desensitization in response to chronic agonist stimulation.[12] However, subsequent human experiments showed agonist-induced desensitization only in persons with the ADRB2 16Arg variant.[12-14] A 2008 meta-analysis examined obesity risk in relation to ADRB2 codon 16 variation (13 studies with 6825 subjects) and in relation to ADRB2 codon 27 variation (28 studies with 14,450 subjects).[10] Random effects meta-regression analysis showed statistically insignificant obesity risks in

relation to 16Arg-containing genotype (odds ratio 1.02, 95% confidence interval 0.89-1.18) and in relation to 27Glu-containing genotype (odds ratio 1.11, 95% confidence interval 0.98-1.27). However, meta-analysis restricted to Asian (7 studies with 3575 subjects), Pacific Island (1 study with 1020 subjects), and native South American populations (1 study with 149 subjects) showed statistically significant association between 27Glu-containing genotype and obesity (odds ratio 1.46, 95% confidence interval 1.02-2.10). One study [15] with African-American subjects, but not a second,[16] showed association between obesity measures and ADRB2 genotype.

We defined a large population on the Caribbean island of Tobago with ancestry in common with African-Americans, determined ADRB2 Arg16Gly and Gln27Glu genotypes, measured blood pressure, and used dual energy X-ray absorptiometry (DEXA) to estimate body composition. Analyses examined ADRB2 genotypes in relation to body composition and hypertension.

4.3 METHODS AND MATERIAL

Between September 1997 and September 2007, the Tobago Prostate Study used public service announcements, flyers, local health care workers, and word of mouth to solicit 40-79 year-old men in Tobago for participation in a study of periodic prostate cancer screening.[17] The study excluded non-ambulatory, terminally ill, or cognitively impaired men. Participants signed written informed consent. The Institutional Review Boards of the University of Pittsburgh and Tobago Division of Health and Social Services approved the research protocol.

The Tobago Prostate Study enrolled 3837 men. Standardized entry questionnaires provided demographic and basic cancer risk factor information. To support studies of obesity,

hypertension, diabetes, and musculoskeletal health, men enrolling or returning after January 2004, also completed a detailed standardized staff-administered health history questionnaire. We defined, for the current analysis, a race-eligible study group that consisted of 3452 (90.0% of 3837) men who reported having at least three grandparents of African descent.

Procedures included periodic musculoskeletal tests (DEXA and/or quantitative computed tomography) and, for the subset of men who completed the health history questionnaire and accepted musculoskeletal testing, genetic tests. Information from DEXA was available for 2850 (82.6%) and health history questionnaires for 2259 (65.4%) of 3452 race-eligible men and ADRB2 Arg16Gly and/or Gln27Glu genotypes for 1960 of 2133 (91.9%) race-eligible men with both health history questionnaires and DEXA. Data analyses included 1965 (1960 with and 5 without DEXA) race-eligible ADRB2 genotyped men with health history questionnaires. When compared against the group of men not available for the health history questionnaire (n=1193) or not otherwise genotyped (n=294), the 1965 men included in analyses enrolled at younger age (median 52 vs. 56 years, p(Wilcoxon) < 0.0001). Genotyped and non-genotyped race-eligible men available for the health questionnaire had statistically similar body mass index (BMI), waist circumference, hypertension prevalence, and body composition (percent body fat).

ADRB2 Arg16Gly (rs1042713) and Gln27Glu (rs1042714) genotype determinations used a TaqMan allelic discrimination assay performed on an Applied Biosystems (Foster City, CA) 7900HT Fast Real-Time PCR System. Call rates for the ADRB2 Arg16Gly and Gln27Glu polymorphisms were 97.0% and 94.5%, respectively. ADRB2 Arg16Gly, Gln27Glu, and both Arg16Gly and Gln27Glu genotypes were available for 1856 (94.5%), 1871 (95.2%), and 1762 (89.7%) men included in data analyses, respectively. Genotype distributions for ADRB2 Arg16Gly and Gln27Glu satisfied Hardy-Weinberg equilibrium conditions (p>0.05). Allele

frequencies (16Gly 49.6%, standard error 0.8% and 27Glu 14.1%, standard error 0.6%) estimated for the 1965 men included in data analyses agreed with values reported by the International HapMap Project (www.hapmap.org) for the Yoruba population (50.0% and 17.5%, respectively). ADRB2 Arg16Gly and Gln27Glu alleles were in linkage disequilibrium (D'=0.96).

4.3.1 Outcome measurements

Outcome measurements included height (measured without shoes to the nearest 0.1 cm on a wall-mounted stadiometer), weight (measured without shoes to the nearest 0.1 kg on a balance beam scale), BMI (calculated as weight in kg divided by height in meters-squared), and waist circumference (measured in cm at the umbilicus with an inelastic tape measure). Percent body fat was acquired on a Hologic QDR 4500W DEXA operated in array beam mode and analyzed with QDR software version 8.26a (Hologic Inc. Bedford, MA). After seating and resting subjects for 5 minutes, technicians selected an appropriate cuff size and used an automated OMRON HEM705CP sphygmomanometer (Omron Healthcare, Inc., Vernon Hills, IL) to obtain three consecutive blood pressure measurements.

4.3.2 Statistical analysis

Using the second and third measurements, if available, or any two available measurements, otherwise, analyses calculated average values for systolic (SBP) and diastolic blood pressure (DBP). Using these measurements, we classified subjects into three mutually exclusive hypertension categories, hypertension (SBP > 140 mmHg or DBP > 90 mmHg or current antihypertensive medication use), pre-hypertension (SBP 120-139 mmHg or DBP 80-89 mmHg and current antihypertensive medication nonuse), and normal (SBP < 120 mmHg and DBP < 80 mmHg and current antihypertensive medication nonuse). Sixty-six (3.3%) men were not

classified because of missing blood pressure measurements (n=8) and/or missing or inconsistent self-reports of antihypertensive medication use (n=64). BMI classification used World Health Organization cutpoints (underweight <18.5 kg/m2, normal weight 18.5- 24.9 kg/m2, overweight 25-29.9 kg/m2, and obese ≥30 kg/m2).

Statistical analyses (SAS 9.2; SAS Institute Inc., Cary, NC) used conventional methods (chi-square or Fisher exact tests for discrete data and t-tests, ANOVA, or Wilcoxon rank-sum tests for continuous data) to evaluate the significance of group differences. Haplotype-based analyses used the EM algorithm implemented in SAS Genetics (PROC HAPLOTYPE) to estimate group-level haplotype frequencies and to generate subject-level haplotype probability weights.[1] We then used traditional linear models (implemented in SAS PROC GENMOD with normal probability distribution and identity link function) to estimate independent associations between the haplotype probability weights and continuous outcomes (BMI and total body percent body fat) and logistic regression (implemented in SAS PROC LOGISTIC) to estimate independent associations between the haplotype probability weights and binary outcomes (hypertension or pre-hypertension vs. normal blood pressure).

4.4 RESULTS

Study men were mean age 58.9 years (standard deviation (SD) 10.4 years). Over half (50.4%) were hypertensive, 32.5% pre-hypertensive, 44.1% overweight, and 25.5% obese. One in 6 (16.5%) self-reported a physician diagnosis of diabetes.

[1]http://support.sas.com/documentation/cdl/en/geneug/59659/HTML/default/geneug_haplotype_sect021.htm

Genotype frequencies were 25.9% Arg/Arg, 49.1% Arg/Gly, and 25.0% Gly/Gly at codon 16 (n=1856) and 74.0% Gln/Gln, 23.8% Gln/Glu, and 2.2% Glu/Gly at codon 27 (n=1871). The prevalence of obesity (BMI≥30 kg/m2) did not vary according to genotype (27.3% of 480, 24.6% of 908, 25.2% of 465 men with the codon 16 Arg/Arg, Arg/Gly, and Gly/Gly genotype, p=0.5331, and 25.9% of 1382, 26.1% of 445, and 24.4% of 41 men with the codon 27 Gln/Gln, Gln/Glu, and Glu/Glu genotype, p= 0.9728). Height, weight, and waist circumference were slightly higher in men with a 16Arg allele (Table 4-1). Otherwise, weight, waist circumference, BMI, and percent body fat were unrelated to ADRB2 genotype (Table 4-1). In the obese subset, body measurements did not vary in any systematic or meaningful way with respect to ADRB2 genotype (Table 4-1).

ADRB2 16Gly-containing and 27Glu-containing genotypes were equally frequent in low, medium, and high tertiles of percent body fat (Table 4-2; 16Gly-containing genotypes: 73.2%, 74.4%, 75.1%, ptrend=0.45; 27Glu-containing genotypes: 27.8%, 24.2%, 26.4%, ptrend=0.58) and in normal blood pressure, pre-hypertensive, and hypertensive men (Table 4-3; 16Gly-containing genotypes: 73.6%, 72.5%, 74.6%, ptrend=0.55; 27Glu-containing genotypes: 26.1%, 24.6%, 27.1%, ptrend=0.51). In men not taking antihypertensive medication, systolic, diastolic, and mean arterial blood pressure values were unrelated to ADRB2 genotype (data not shown).

Estimated ADRB2 Arg16Gly Gln27Glu haplotype frequencies were Arg-Gln 50.0% (standard error 0.8%), Gly-Gln 35.9% (standard error 0.8%), Gly-Glu 13.8% (standard error 0.6%), and Arg-Glu 0.3% (standard error 0.1%). The Figure 4-1 uses box plots to summarize BMI and percent body fat distributions in men classified according to most probable haplotype combination. In linear models, the less common Gly-Gln and Gly-Gluhaplotypes associated with lower BMI and lower percent body fat (Table 4-4). In obese men, these haplotypes associated

with higher percent body fat and lower waist circumference (Table 4-4). However, none of these associations, including the apparent interaction between haplotype and obesity grouping (data not shown), was statistically significant. Finally, logistic regression did not show statistically significant ADRB2 haplotype associations with either pre-hypertension or hypertension (data not shown).

4.5 DISCUSSION

Mediating fat cell lipolysis [2, 8] and vascular smooth muscle relaxation,[9] ADRB2 gene variants that code for functionally altered receptors could promote weight gain or high blood pressure. In a study of 1965 Tobago men of African ancestry, however, we observed no significant body composition or blood pressure associations with the two most commonly studied ADRB2 gene variants.

Different investigations have reached different conclusions about the significance of ADRB2 genotype in relation to obesity and related phenotypes [10, 18-20]. Variability related to sex may explain inconsistencies in the published literature. Some authors, including Corbalan et al. [21] and GonzálezSánchez et al. [22], suggest that ADRB2 genotype determines not obesity, but obese subtype, perhaps in only one sex group. For example, in a Spanish clinic-based study with 40 men and 199 women, Corbalan et al. [21] compared the ADRB2 Gln27Glu genotypes of subjects with either abdominal obesity (BMI >30 kg/m2 and waist-to-hip ratio >0.85) or normal body mass (BMI <25 kg/m2 and waist-to-hip ratio <0.85). In men, but not women, 27Glu-containing genotypes were more frequent in the group with abdominal obesity. In a Spanish population-based study with 319 white men and 347 white women, GonzálezSánchez et al. [22]

noted a statistically non-significant trend in men (but not women) between ADRB2 Gln27Glu genotype and obesity prevalence (20.0% of 130, 27.7% of 155, and 29.4% of 34 men with the codon 27 Gln/Gln, Gln/Glu, and Glu/Glu genotype, respectively, p= 0.2572). In the obese subset, men with the rare Glu/Glu genotype had significantly higher mean BMI (34.1 kg/m2, n=10) than men with either the Gln/Glu (31.9 kg/m2, n=46, p=0.013) or Gln/Gln genotype (32.0 kg/m2, n=26, p=0.023). To support specific association with abdominal obesity, GonzálezSánchez et al. [22] noted that obese men with the rare Glu/Glu genotype also had significantly higher mean sagittal abdominal diameter (27.8 cm, n=10) than obese men with either the Gln/Glu (24.9 cm, n=46, p=0.037) or Gln/Gln genotype (24.9 cm, n=26, p=0.062). Among obese women, sagittal abdominal diameter was not related to ADRB2 Gln27Gly genotype. In agreement with GonzálezSánchez et al., we observed no statistical association between obesity prevalence and ADRB2 genotype. Among obese Afro-Caribbean men, BMI, waist circumference, and body fat values were unrelated to ADRB2 genotype (Table 4-1). Simply, our results do not support a relationship, specifically in obese men, between ADRB2 genotype and obesity subtype.

Excluding persons treated for high blood lipids, high blood pressure, or diabetes, Meirhaeghe et al. [23] studied a population-based sample of 836 35-64 year-old urban-dwelling persons from northern France (419 men and 417 women, 14.2% obese overall) and observed sex-specific associations between ADRB2 genotype and body composition. Body weight, BMI, waist circumference, hip circumference, and waist-to-hip ratio mean values were significantly higher in men with the codon 16 Arg/Arg genotype than men with either the Arg/Gly or Gly/Gly genotype and significantly higher in men with the codon 27 Glu/Glu genotype than men with either the Gln/Glu or Gln/Gln genotype. In women, differences were not statistically significant. Observing linkage disequilibrium between 16Arg and 27Gln, Meirhaeghe et al. [23] compared,

in men, body composition measures according to combined genotypes at Arg16Gly and Gln27Gly. With the GlyGluhaplotype serving as reference, higher body weight, BMI, and waist-to-hip ratio associated with the ArgGlnhaplotype, not with the GlyGlnhaplotype. In Afro-Caribbean men, body weight and waist circumference were slightly higher in men with a 16Arg allele (Arg/Arg or Arg/Gly genotype) than men without a 16Arg allele (Gly/Gly genotype; Table 1). However, BMI and percent body fat did not vary according to Arg16Gly genotype. Body weight, waist circumference, BMI, and percent body fat were completely independent of Gln27Glu genotype.

Only two studies [15, 16] to date have included a meaningful number of African-Americans. The Insulin Resistance and Atherosclerosis (IRAS) Family Study [15] genotyped 272 African Americans and 720 Hispanic Americans from 18 and 45 families, respectively, and used single-slice computed tomography to measure visceral and subcutaneous fat. Obesity measures associated with Gln27Glu, specifically the Glu/Glu genotype, but not with Arg16Gly genotype. High visceral fat area associated with the Glu/Glu genotype, even after control for BMI. Results for men and women and for African and Hispanic Americans were statistically indistinguishable. The Heritage Family Study included 274 African-Americans (31.8% obese) and 502 whites (19.3% obese) and used under water weighing to measure fat mass, single-slice computed tomography to measure visceral and subcutaneous fat, and skin calipers to measure skin-fold thickness[16]. In white obese (BMI $\geq$30 kg/m2) subjects only, the Heritage Family Study observed lower fat mass in obese white men with 27Glu-containing genotypes and lower fat mass in obese white women with 16Gly-containing genotypes. In African-American subjects, the Heritage Family Study did not report any statistically significant cross-sectional associations between ADRB2 genotype and any body composition measure.

One study of ADRB2 genotype included Afro-Caribbeans recruited from primary care clinics located on St. Vincent [24]. The case group included 136 patients (19.9% men) with high blood pressure (DBP > 95 mmHg or antihypertensive medication use) and family history of hypertension. The control group included 81 patients (46.9% men) with normal blood pressure (DBP < 85 mmHg and antihypertensive medication nonuse) and no family history of hypertension. The 16Gly allele was much more frequent in the case group (84.6% vs. 66.7%; p=0.000014). Our community-based study of 1965 Afro-Caribbean men from Tobago included 331 and 557 persons who satisfied the St. Vincent case and control definitions. In Tobago, 16Gly was also more frequent in cases, 51.5% vs. 48.7%. However, the difference was not statistically significant (p(allele chi-square)=0.2594). In the St. Vincent study, genotype frequencies in both the case and control groups violated Hardy-Weinberg equilibrium. The control frequency (66.7%) of the putative risk allele (16Gly) in St. Vincent was high when compared against reference frequencies in the HapMap Yoruba (50.0%) and Tobago populations (49.5%). Therefore, selection bias and genotyping error plausibly explain the anomalous St. Vincent results.

Our study participants volunteered for prostate cancer screening, in many instances survived a variable duration after study entry, and agreed to extra visits that included measurements, such as DEXA. Therefore, absence of meaningful association between ADRB2 genotype and body composition could occur if these study selection factors preferentially excluded men with certain genotype-phenotype combinations. However, selection bias deriving from factors related to initial study participation can be discounted to the extent that Tobago Prostate Study, as a whole, enrolled a high proportion (60%) of age-eligible men (approximately 5000) living in Tobago. To address possible survival bias, we compared the 1727 and 238 men

who had body composition measurements after study entry and coincident with study entry, respectively, and found statistically similar relationships between ADRB2 genotype and body composition (data not shown). Finally, study procedures captured a reasonable proportion (1965 of 3452, 56.9%) of race-eligible Tobago Prostate Study enrollees.

Results from some studies suggest specific ADRB2 association with regional fat distribution [15, 21-23]. Our primary body composition measures, BMI and DEXA-derived percent body fat, do not distinguish between visceral and subcutaneous fat. However, our data set included waist circumference, an indirect measure of central obesity. Waist circumference and ADRB2 genotype were statistically independent (Table 4-1).

Finally, we evaluated only two ADRB2 genetic variants. These variants change the amino acid sequence of the β2 adrenergic receptor protein. However, these changes are not generally believed to alter agonist binding or signal transduction [12]. The Arg16Gly and Gln27Glu variants may affect function secondarily, perhaps through agonist-induced receptor protein down regulation or linkage disequilibrium with other less common but more influential variants [12].

In a large racially homogenous population of men with black African ancestry and high prevalence of obesity and hypertension, single variant and haplotype-based analyses did not show meaningful or consistent association between ADRB2 Arg16Gly and Gln27Glu variation and phenotypes related to obesity and hypertension.

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Table 4-1: Body composition measures (means ± standard deviations) according to *ADRB2* genotype.

	Arg16Gly				Gln27Glu			
Body composition measure	Arg/Arg	Arg/Gly	Gly/Gly	p-value [1]	Gln/Gln	Gln/Glu	Glu/Glu	p-value [1]
			All men					
n [2]	477–480	905–910	461–465		1379–1383	441–446	41	
Height (cm)	175.7 ± 7.1	175.0 ± 6.9	174.5 ± 6.6	0.0175	174.8 ± 6.8	175.4 ± 7.1	175.5 ± 6.6	0.2534
Weight (kg)	84.9 ± 15.0	84.8 ± 17.1	82.8 ± 15.6	0.0528	84.6 ± 16.2	84.7 ± 17.3	84.0 ± 14.5	0.9733
Waist circumference (cm)	93.4 ± 12.7	93.5 ± 11.3	92.1 ± 11.3	0.0798	93.3 ± 11.6	93.1 ± 11.3	92.2 ± 8.8	0.7911
Body mass index (BMI; kg/m^2)	27.5 ± 4.4	27.7 ± 5.1	27.2 ± 4.7	0.1894	27.7 ± 4.9	27.5 ± 5.1	27.2 ± 3.9	0.6795
Total body fat (%) [3]	20.9 ± 6.4	21.3 ± 6.3	20.7 ± 6.3	0.1963	21.2 ± 6.3	21.0 ± 6.5	21.5 ± 6.0	0.7455
			Obese men (BMI ≥ 30 kg/m^2)					
n [2]	129-131	221-223	116-117		354-358	114-116	10	
Height (cm)	174.9 ± 6.6	175.3 ± 6.2	174.0 ± 6.7	0.2235	174.6 ± 6.3	175.3 ± 7.2	177.5 ± 6.7	0.2709
Weight (kg)	101.2 ± 12.4	104.8 ± 17.2	101.1 ± 12.8	0.0307	103.0 ± 14.7	103.8 ± 17.8	102.0 ± 8.3	0.8557
Waist circumference (cm)	105.5 ± 9.8	106.6 ± 9.5	104.8 ± 8.2	0.2082	106.1 ± 9.6	105.9 ± 8.2	101.6 ± 4.0	0.3093
Body mass index (BMI; kg/m^2)	33.1 ± 3.1	34.1 ± 5.3	33.4 ± 3.3	0.0704	33.8 ± 4.5	33.7 ± 4.9	32.4 ± 1.5	0.6315
Total body fat (%) [3]	25.9 ± 5.2	26.6 ± 4.8	26.1 ± 4.4	0.3392	26.3 ± 4.7	26.6 ± 5.0	25.7 ± 4.0	0.7848

1. Statistical significance (ANOVA) of differences in mean values across genotype categories
2. Sample numbers vary because of missing attribute data
3. Total body (except head) fat mass expressed as a percentage of total body (except head) mass

Table 4-2: *ADRB2* genotype and body composition.

	Percent Total Body Fat [1]						
	Low tertile N=653		Middle tertile N=654		High tertile N=653		
ADRB2 genotype	N	%	N	%	N	%	
Arg16Gly							
Arg/Arg	165	26.8	159	25.6	153	24.9	P_{global}=0.72
Arg/Gly	289	46.9	307	49.4	313	51.0	
Gly/Gly	162	26.3	155	25.0	148	24.1	
Arg/Gly or Gly/Gly	451	73.2	462	74.4	461	75.1	P_{trend}=0.45
Gln27Glu							
Gln/Gln	439	72.2	471	75.8	469	73.6	P_{global}=0.31
Gln/Glu	153	25.2	142	22.9	151	23.7	
Glu/Glu	16	2.6	8	1.3	17	2.7	
Gln/Glu or Glu/Glu	169	27.8	150	24.2	168	26.4	P_{trend}=0.58

1. Percent total body fat (total body (except head) fat mass expressed as a percentage of total body (except head) mass) -- Low tertile: 4.769-18.824; Middle tertile: 18.825-23.819; High tertile: 23.820-44.352

Table 4-3: *ADRB2* genotype and hypertension.

	Normal blood pressure N=324		Pre-hypertensive N=617		Hypertensive N=958		
ADRB2 genotype	N	%	N	%	N	%	
Arg16Gly							
Arg/Arg	81	26.4	160	27.5	229	25.4	P_{global}=0.90
Arg/Gly	146	47.6	281	48.3	445	49.3	
Gly/Gly	80	26.1	141	24.2	229	25.4	
Arg/Gly or Gly/Gly	226	73.6	422	72.5	674	74.6	P_{trend}=0.55
Gln27Glu							
Gln/Gln	224	73.9	447	75.4	667	72.9	P_{global}=0.73
Gln/Glu	75	24.8	134	22.6	228	24.9	
Glu/Glu	4	1.3	12	2.0	20	2.2	
Gln/Glu or Glu/Glu	79	26.1	146	24.6	248	27.1	P_{trend}=0.51

Table 4-4: Estimated difference (Δ; and 95% confidence interval) in body composition measure between men homozygous for a specified haplotyperelative to men homozygous for the Arg-Glnhaplotype.

Body composition measure	n	Gly-Gln			Gly-Glu		
		Δ	95% CI	p-value	Δ	95% CI	p-value
BMI (kg/m^2)	1962	-0.28	-0.97, 0.41	0.4261	-0.52	-1.47, 0.43	0.2828
Total body fat % [1]	1960	-0.16	-1.04, 0.73	0.7290	-0.40	-1.62, 0.82	0.5213
Total body fat % [1], in obese men	497	0.17	-1.14, 1.48	0.7983	0.26	-1.57, 2.09	0.7815
Waist circumference (cm), in obese men	499	-0.57	-3.21, 2.08	0.6751	-2.87	-6.58, 0.83	0.1281

1. Total body (except head) fat mass expressed as a percentage of total body (except head) mass

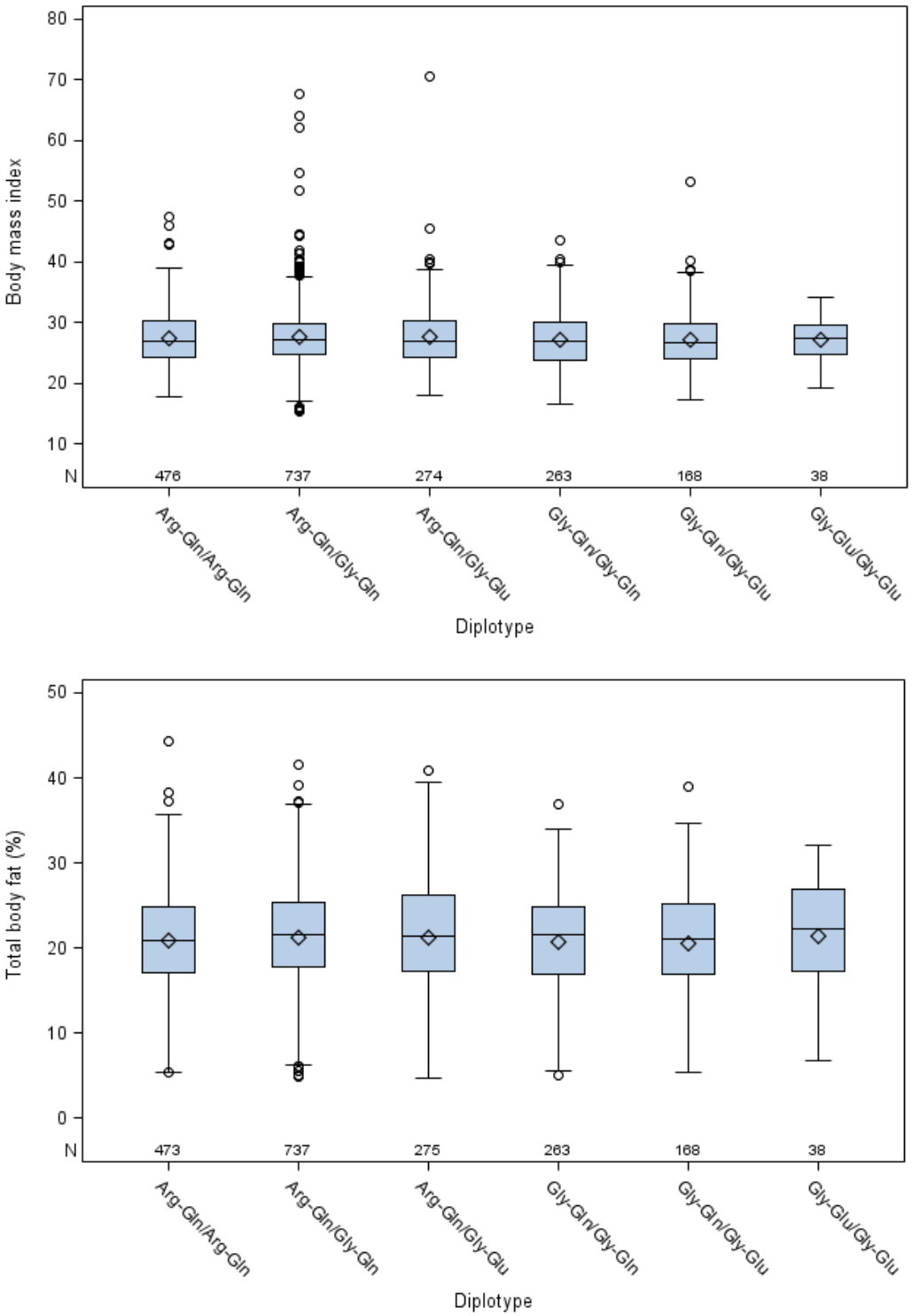

Figure 4-1: Box plots showing body mass index (BMI; upper figure) and DEXA-determined percent body fat (lower figure) in men sub-group according to diplotype.

Figure Footnote: The number (N) in each subgroup appears above the diplotype label. Analyses exclude six men with an ArgGluhaplotype-containing diplotype. The line segment and diamond symbol within each box identify the median and mean, respectively. The lower and upper borders of each box identify the 25th and 75th percentiles, respectively. The lower whisker extends to the minimum observation greater than or equal to the 25th percentile minus 1.5 times the inter-quartile range. The upper whisker extends to the maximum observation less than or equal to the 75th percentile plus 1.5 times the inter-quartile range. Open circle symbols identify individual observations less than the 25th percentile minus 1.5 times the inter-quartile range or greater than the 75th percentile plus 1.5 times the inter-quartile range.

5.0 PAPER 2: ANDROGEN RECEPTOR ARCAG REPEAT LENGTH POLYMORPHISM AND OBESITY IN AFRO-CARIBBEAN MEN: RESULTS FROM THE TOBAGO PROSTATE STUDY.

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5.1 ABSTRACT

Objective: Expansion of *AR* CAG repeat has been shown to lower transcriptional activity causing lower AR protein production and androgen signaling. In a population based study we investigated AR CAG repeat length $(CAG)_n$and its association with body fat parameters in Afro-Caribbean men.

Design and Subjects: Cross-sectional analysis of 2584 Afro-Caribbean men residing on the island of Tobago.

Measurements: Body mass index (BMI), waist circumference, dual energy x-ray absorptiometry (DEXA), AR CAG repeat length genotype.

Results: Mean age was 59 years. *AR* CAG repeats lengths ranged from 9 to 33 with a median of 19. Standardized univariate regression analyses revealed no significant associations between the number of CAG repeatsandpercent total body fat (β=change in percent total body fat per 3.3 standard deviation change in *AR* CAG repeats), (β=-0.052, P =0.690), BMI (β=-0.184, P =0.085), waist circumference (β=-0.236, P =0.352) and weight (β=-0.647, P =0.068).

Conclusion: Longer*AR* CAG repeat length was not associated with higher body fat measurements.

5.2 INTRODUCTION

Obesity is a major health problem and public health concern[361]. Data suggest that genetics in conjunction with environmental factors may play a pivotal role in the development of obesity. Obesity is associated with a plethora of diseases and increases in risk with increasing age[362]. Obesity phenotypes in individuals appear to be dimorphic. Men seem to develop an android shape (distribution of fat around the abdomen) whereas women distribute fat mainly around the hips (gluteofemoral regions) [362, 363].

Exogenous and endogenous testosterone can affect body composition[57]. Testosterone is important in regulating adiposity, insulin resistance and type II diabetes. Men with type II diabetes have lower testosterone (TT) and sex hormone binding globulin (SHBG) compared to normal men[364]. Furthermore, lower than normal testosterone levels seem to determine an individual's future risk of developing the metabolic syndrome and type 2 diabetes[63, 365, 366]. Studies have consistently demonstrated that low levels of testosterone and sex hormone-binding globulin (SHBG) in men are associated with type 2 diabetes, visceral adiposity, and insulin resistance[365, 366].Singh et al, delineated that androgens affect the fat cells ability to store lipids by interrupting or blocking signal transduction pathways that are important in adipocyte function [367]. The actions of androgen in developing obesity are thought to be mediated by the androgen receptor (AR)[368].

The androgen receptor is important for the regulation of androgen in men. Dysfunctional receptors may cause variations in body composition[362]. The AR gene is located on the X-chromosome (Xq11.2-12), highly polymorphic, and comprised of 8 exons[362]. Most

studies have focused on two polymorphic trinucleotide repeats (microsatellite) CAG[2]coding forpolyglutamine and GGN ((GGT)3(GGG)(GGT)2(GGC)n) coding for glycine[351, 362]. CAG repeats range between 8-35 [369] with an average of 20-23 repeats[370]. In vitro studies have demonstrated that CAG length is inversely associated with AR transactivation (increased rate of gene expression triggered by biological processes) [371]. Longer CAG repeats are associated with lower transcriptional activity, Kennedy's disease, androgen insensitivity and infertility[151]. Polymorphisms of the AR gene are associated with prostate cancer, metabolic, and cardiovascular diseases[370] and these polymorphic repeats (CAG) can cause variations in serum hormone levels leading to hormone-dependent diseases[62, 372].

Studies have also shown that the frequency of the *AR* gene CAG repeats varies amongst different racial and ethnic groups[151]. In a population-based study of Afro-Caribbean men we investigated the association of *AR* CAG repeat lengths on body composition parameters. We hypothesize that men with longer *AR* CAG repeats will have more total body fat mass when expressed as percent total body mass.

5.3 MATERIALS AND METHODS

During 1997 through September 2007, the Tobago Prostate Study recruited 40-79 year old men by public service announcements, flyers, local health care workers, and word of mouth to participate in a study assessing prostate cancer screening. Men were excluded if they were found to be non-ambulatory, terminally ill or cognitively impaired. All participants signed a written

[2] http://www.androgendb.mcgill.ca/

informed consent approved by the Institutional Review Boards of the University of Pittsburgh and the Tobago Division of Health and Social Services.

The Tobago Prostate cohort enrolled 3837 Afro-Caribbean men. Demographic and cancer information were provided by standardized baseline questionnaires. To facilitate genetic studies a more detailed staff-administered Health History questionnaire (HH) was used to record information on newly recruited or follow-up men beginning January 2004. Our study population consisted of 3452 (90.0% of 3837) race-eligible Afro-Caribbean men with≥3Afro -Caribbean grandparents.

Procedures consisted of body composition tests (DEXA), and genetic testing. DEXA measurements were obtained on 2850 (82.6%) and health history questionnaires on 2259 (65.4%) of 3452 race eligible men, and *AR* CAG genotyping information on 2816 (81.6) of 3452 race eligible men (2126 with health history information and 690 with no health history information). Two hundred and thirty two (232) individuals were excluded because they did not provide any health history (HH) or DEXA information. Participants with DEXA were pulled from both baseline (566 or 22.9%) and follow-up (1902 or 77.1%). Our final sample size for this study was 2584 (Figure 5-1).

5.3.1 Outcome measurements

Key outcome variables from the Health History Questionnaire included: height (measured with shoes to the nearest 0.1 cm on a wall mounted stadiometer), weight (measured to the nearest 0.1 kg on a beam balance), body mass index (BMI, calculated as weight in kg divided by height in meters-squared), waist circumference (measured in cm at the umbilicus with an inelastic tape measure). Percent total body fatwas ascertained on a Hologic QDR 4500W DEXA operated in array beam mode and analyzed by QDR software version 8.26a (Hologic Inc. Bedford, MA).

5.3.2 Genotyping

High molecular weight genomic DNA was isolated from peripheral blood samples using the salting out procedure. To determine the AR CAG repeat length, we constructed a set of fluorescent dye labeled oligonucleotideprimersthat flanked the CAG repeat region in exon 1: Forward Primer = TET dye-5'-ACCGAGGAGCTTTCCAGAAT-3'; Reverse Primer = 5'-AGAACCATCCTCACCCTGCT-3'. The DNA was amplified by polymerase chain reaction (PCR) to produce fragments which varied in length by the number of CAG repeats. PCR consisted of an initial 4-minute denaturation at 95°C, followed by 37 cycles of denaturation (95°C, 60 seconds), annealing (58°C, 30 seconds), and elongation (72°C, 60 seconds), and a final elongation step (72°C, 10 minutes). Amplified samples were analyzed by automated high-throughput scanning fluorescent detectors that have the ability to detect and separate the TET dye. To determine the exact number of CAGs for each fragment length, we subsequently sequenced a series of PCR products of varying length using standard Sanger sequencing. PCR clean up was performed using Exo-SAP according to manufacturer instructions. Sequencing buffer and a 1:4 dilution of BigDye 3.1 were added and thermocycling performed according to manufacturer recommendations. Removal of unincorporated sequencing reagents was performed using CleanSeq magnetic beads according to manufacturer instructions (Beckman Coulter, Inc; Brea, CA). Sanger sequencing was completed using an Applied Biosystems3730*xl* DNA Analyzer

5.3.3 Statistical analyses

Statistical analyses were carried out using (SAS 9.2; SAS Institute Inc., Cary NC). All continuous descriptive data are expressed as mean±S.D. To determine the association with androgen receptor CAG repeat length correlations were assessed and expressed as Spearman's Rank Correlation coefficient with the associated P-value. *AR* CAG data was standardized using the formula (CAG repeats-mean of CAG)/Standard Deviation of CAG before univariate linear

regression. Univariate linear regression techniques were used to assess the independent association between standardized AR CAG and body fat parameters. Results are expressed using the regression coefficient (β) (beta coefficients are expressed in terms of change in the independent variable per one standard deviation unit change in the number of CAG repeats) and the associated P-value. Stratified regression analyses were also performed according to BMI and age categories using linear regression.

5.4 RESULTS

Table 5-1 summarizes characteristics by (mean±SD) of the study population according to long vs. short repeats. In Table 5-1 we see that the means were the same in the long and short *AR* CAG repeat groups except for BMI which showed that the means were statistically different (p=0.03). These variables used in this analysis were pulled from the Health History Questionnaire. The number of AR CAG repeats ranged from 9-33 with a median of 19 (Figure 5-2). The average age of participants was 59 years. To determine the frequency of long versus short repeats, AR CAG repeat length was split into low (≤22) and high groups (>22) according to Campbell et al[373].Approximately 81.4% or 2103 of (2584) had short repeats and 18.6% or 481 participants had long repeats (Table 5-2).Table 5-3 describes potential confounders (Health History Questionnaire) by long and short repeats. The proportion of men with heart attack (dichotomous variable) was significantly different (P=0.0302) according to long versus short repeats.

In our analysis multiple body fat parameters were explored including: percent trunk fat, percent limb fat, percent arm fat, and percent leg fat. Spearman-Rank order correlation analysis

revealed no significant association between *AR*gene CAG repeat polymorphism as a continuous variable and body fat parameters (Table 5-4). Univariate regression analyses revealed no significant associations between *AR* gene CAG repeat length as standardized continuous variable and body fat parameters, percent total body fat (β=-0.052, P =0.690), BMI (β=-0.184, P =0.085), waist circumference (β=-0.236, P =0.352) and weight (β=-0.647, P =0.068) (Table 5-5). The negative β-coefficient indicates that men with shorter CAG repeats had higher body fat measurements. In addition, comparison of included (2584) and excluded (232) participants revealed that the median number of *AR* CAG repeats was the same for both groups (Table 5-6).

Table 5-8 presents stratified regression analysis by age category. This analysisdid not detect, in any age group, a statistically significant associationbetweenthe number of*AR*CAGrepeatsandpercent total body fat. Table 5-9 presents stratified analysis by BMI category. Likewise, this analysis did not detect, in any BMI category,a statistically significant association between the number of *AR* CAG repeats and percent total body fat. Moreover, the negative association observed between different measures of body fat and *AR* CAG repeats shown in Table 5-10 was not significant even after adjusting for age for all body fat parameters except BMI which was statistically significant with a P=0.04 (Table 5-10). In addition, Table5-7 also shows a significant negative association unadjusted for age (P=0.04). Therefore regardless of how the analyses were performed, age adjusted or not, the associations observed in this cohort were not altered. We also do recognize that different sample sizes were used in the analyses presented so far and that caution should be used in making inferences. Hence, in an effort to show a more complete analysis various non-missing sample sizes were used in analyses shown in Table 5-11. Even in these analyses, a persistent non-significant negative association was observed between AR CAG and percent total body fat. On the other hand BMI continued to

show a statistically significant negative association with *AR* CAG length. In this population having shorter AR CAG means that men may be fatter opposite what was originally hypothesized.

5.5 DISCUSSION

Our study revealed thatlonger AR CAG was not associated with higher body fat parameters. Instead our study revealed that men with shorter *AR* CAG had higher measures of body fat.

Studies elucidating an association between*AR*-CAG repeat length and body composition have produced inconsistent results. For instance, Lapauw et al[374], did not find an association between *AR* CAG repeat length and body composition parameters. This non-significant finding in Belgium men persisted even after the adjustment of potential confounders (age, weight, total testosterone and sex hormone binding globulin (SHBG)). However, Campbell et al[373] reported an association between *AR* CAG repeats and body composition parameters contrary to findings reported in the aforementioned study. In Campbell's study amongst the Ariaal men of northern Kenya AR CAG repeats were positive predictors of height (β=0.420, P<0.05), fat free mass (β=0.383, P<0.05), percent body fat (β=0.329, P<0.05), waist circumference (β=0.385, P<0.05), and suprailliacskinfold (β=0.760, P<0.001). Both Campbell et al[373] and Lapauw et al[374] report a regulatory effect of the *AR* CAG repeats on testosterone and consequently the relationship between testosterone (serum testosterone concentration) and body composition (Lapauw et al[374]β=0.12, P<0.1 modeled an interaction between CAG repeat and testosterone). Possible explanations for our results of no association include: Campbell et al[373] study consisted of a younger cohort of men 20+ compared to our study in which age ranged from 33-91 years.

Other explanations for the divergent results observed may be explained by gene-environment interaction, and differences in study design. Moreover, it has been postulated that *AR* CAG repeats may function as low penetrance alleles that require the presence of additional genetic and environmental factors to cause an increase in disease susceptibility[375]. It is quite possible that AR CAG may be in linkage disequilibrium with other mutations in the AR gene (on the X-chromosome) such as (GGN) that are in close proximity at a particular locus or other unknown genes[370] that may affect body composition in men. Furthermore, obesity is a multifactorial disease that may be affected by other hormones, diet and lifestyle that were not accounted for in the present study.

Non-the-less, the results presented in this paper are of interest because based on our knowledge there have been relatively few studies evaluating the *AR* gene in populations of African descent with a large sample size.

5.5.1 Racial differences in CAG repeat length

The distribution of CAG repeats has been reported to differ amongst different racial and ethnic groups. African Americans have been found to have shorter CAG repeats compared to Caucasians (19.8 vs. 21.9)[151]. Sartor et al[376] reported almost twice as many black men had shorter CAG repeats (<20 vs. >20) compared to non-Hispanic white men (56.92% vs. 28.46%) with mean repeats of 19.0 vs. 21.0. The findings of shorter CAG repeats in previous studies are inline with the findings from our study of a mean of 19.7 and a median of 19.

Limitations of our study include: our study was based on a single time point DEXA measurements where longitudinal data might have provided additional information.

Figure 5-1: Sample selection for paper 2.

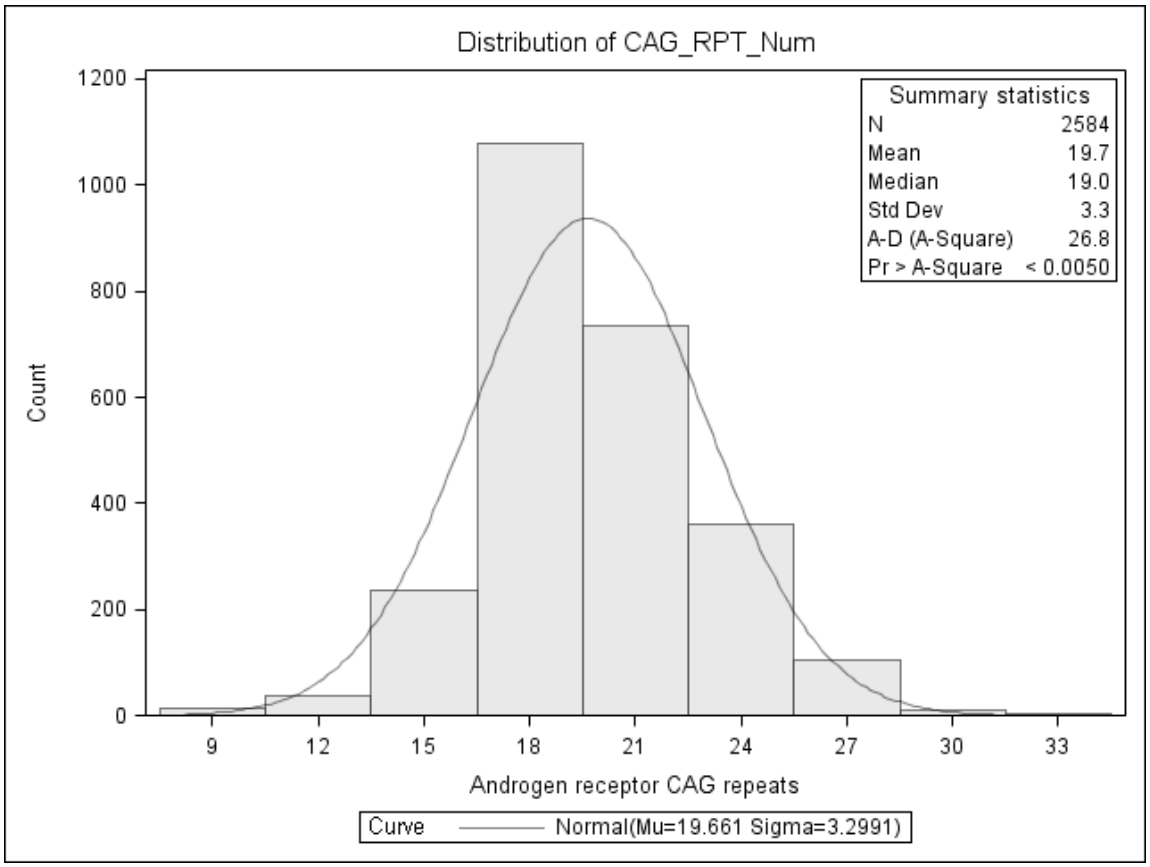

Figure 5-2: Distribution of *AR* CAG repeat length.

Table 5-1: Body composition measures (mean±standard deviations) according to *AR* CAG genotype.

	AR CAG repeat length								
	≤22				>22				
Body composition measure	**n**	**mean**	**±**	**SD**	**n**	**mean**	**±**	**SD**	**P-value [1]**
Age (years)	1733	58.98	±	10.49	393	58.94	±	10.25	0.95
Age at 2nd DEXA (years)	2013	59.20	±	10.94	455	59.28	±	10.63	0.78
Weight (kg)	1726	84.85	±	16.71	392	83.15	±	15.03	0.09
Body mass index (kg/m^2)	1724	27.68	±	5.04	392	27.10	±	4.48	0.03
Waist circumference (cm)	1717	93.40	±	11.77	393	92.66	±	11.40	0.23
Percent total body fat (%)	2013	21.06	±	6.42	455	20.71	±	6.67	0.31

1. Wilcoxon-Mann-Whitney test
Data presented as mean±S.D
Data restricted to individuals with non-missing*AR* CAG repeat length genotype and ≥3 Afro-Caribbean grandparents
Percent total body fat calculated as total fat 100*mass/total body mass.

Table 5-2:Proportion of men with long vs. short *AR* CAG repeats (N=2584).

***AR* CAG Repeats**	**Men (N)**	**Men (%)**
<=22	2103	81.4
>22	481	18.6

Table 5-3: Potential confounders.

		AR CAG repeat length (n=2584)				
		<=22 (n=2103)		>22 (n=481)		
Attribute		**N**	**%**	**N**	**%**	**P-value**
Demographic						
Education	Primary only	1287	75.2	293	75.7	0.9627
	Post primary	368	21.5	82	21.2	
	University graduate	57	3.3	12	3.1	
Marital status	Married	1197	69.6	261	67.3	0.3450
	Widowed	101	5.9	19	4.9	
	Divorced	96	5.6	18	4.6	
	Separated	88	5.1	27	7.0	
	Never married	239	13.9	63	16.2	
Risk factors						
Smoking status	Current smoker	162	9.4	49	12.5	0.1452
	Ex-smoker	393	22.7	92	23.4	
	Never smoker	1175	67.9	252	64.1	
Alcohol (drinks/wk)	None	679	39.3	158	40.2	0.3072
	<1	735	42.5	156	39.7	
	1-3	144	8.3	26	6.6	
	4-7	71	4.1	22	5.6	
	8-14	56	3.2	16	4.1	
	>14	44	2.5	15	3.8	
Physical activity (walking outside)	Never	52	3.0	9	2.3	0.457
	Seldom (1-2 days/wk)	196	11.4	44	11.3	
	Sometimes (3-4 days/wk)	413	23.9	81	20.8	
	Often (5-7 days/wk)	1065	61.7	255	65.6	
Health history						
Diabetes	Yes	287	16.6	60	15.4	0.5582
	No	1442	83.4	330	84.6	
Heart attack	Yes	14	0.8	8	2.0	0.0302
	No	1716	99.2	385	98.0	

Chi-square test

Table 5-4: Spearman rank correlations between *AR* CAG repeats and body composition measures.

	Weight	Waist circum-ference	Body mass index	Total body fat	Trunk body fat	Arm body fat	Leg body fat	Limb body fat
CAG repeat length	-0.04	-0.02	-0.04	-0.01	-0.02	0.00	0.00	0.00
Weight		0.83*	0.89*	0.59*	0.60*	0.58*	0.51*	0.55*
Waist circumference			0.84*	0.76*	0.76*	0.73*	0.65*	0.70*
Body mass index				0.68*	0.68*	0.68*	0.58*	0.63*
Total body fat					0.97*	0.92*	0.92*	0.96*
Trunk body fat						0.86*	0.81*	0.86*
Arm body fat							0.83*	0.92*
Leg body fat								0.98*

Entries correlation coefficients. *P<0.001.
Data based on restrictions to individuals with CAG repeat length genotyping information, and≥3 afro -Caribbean grandparents.
The number of observations used to estimate individual correlations range between 1998 to 2468

Table 5-5: Results from standardized univariate linear regression models (n=2584).

Attribute	β	SE	t-value	P-value
Age (years)	0.155	0.219	0.71	0.48
Body weight (kg)	-0.647	0.354	-1.830	0.068
Waist circumference (cm)	-0.236	0.253	-0.930	0.352
Body mass index (kg/m^2)	-0.184	0.107	-1.720	0.085
Percent total body fat (%)	-0.052	0.130	-0.400	0.690
Percent trunk fat (%)	-0.109	0.141	-0.780	0.438
Percent arm fat (%)	0.003	0.152	0.020	0.984
Percent leg fat (%)	0.010	0.124	0.080	0.935
Percent limb fat (%)	0.005	0.128	0.040	0.969

Entries are standardized linear regression coefficients

Data presented are participants who are race eligible (>=3 Afro-Caribbean grandparents) with AR CAG genotyping

Table 5-6:A comparison of *AR*CAGlength between excludedmen (men excluded from analysis because of missing DEXA and missing HH questionnaire) and included men (men included in analysis of association between *AR* CAG and body composition).

Study	N	Q_{25}	Median	Q_{75}	P-value
Excluded	232	18	19	22	0.45
Included	2584	17	19	22	

Q_{25}, 25th Percentile; Q_{75}, 75^{th} percentile

Table 5-7: Unadjusted *AR* CAG repeat length category (<=22 vs. >22 repeats) associations with age, weight, body mass index (BMI), percent total body fat, percent trunk fat, percent arm fat, percent leg fat, percent limb fat (n=2584).

Attribute	β	SD	t-value	P-value
Age(years)	0.07	0.56	0.13	0.90
Weight (kg)	-1.70	0.92	-1.85	0.06
Average standing height (cm)	0.06	0.38	0.17	0.87
Waist circumference (cm)	-0.73	0.65	-1.12	0.26
Body Mass Index (kg/m^2)	-0.58	0.28	-2.10	0.04
Percent trunk fat (%)	-0.27	0.36	-0.73	0.46
Percent arm fat (%)	-0.41	0.39	-1.04	0.30
Percent leg fat (%)	-0.44	0.32	-1.39	0.16
Percent limb fat (%)	-0.44	0.33	-1.35	0.18
Percent total body fat (%)	-0.35	0.34	-1.04	0.30

Entries linear regression coefficients

Data presented are participants who are race eligible (>=3 Afro-Caribbean grandparents) with AR CAG genotyping and DEXA

Table 5-8: Association between *AR* CAG repeat and percent total body fat, by age.

Age category (years)	N	β(%TBF/CAG repeat)	P-Value
33-50	652	-0.08	0.32
51-57	570	0.09	0.29
58-66	572	-0.04	0.59
67-92	674	-0.05	0.55

Age in years
%TBF; change in percent total body fat
CAG repeat; change in CAG repeat length
Estimated regression coefficients

Table 5-9: Association between *AR* CAG repeat and percent total body fat, by BMI category.

BMI category	N	β(%TBFAT/CAG Repeat)	P-Value
Underweight	29	-0.04	0.83
Normal weight	609	-0.01	0.83
Overweight or obese	1478	0.04	0.33

%TBF; change in percent total body fat
CAG repeat; change in CAG repeat length
Estimated regression coefficients

Table 5-10:Age-adjusted associations between *AR* CAG repeat length category (<=22 vs. >22 repeats) and eight different measures of body composition (n=2584).

	Dependent variables							
	PcnTBFat		Weight		Waist circumference		Body mass index	
	β	*P*	β	*P*	β	*P*	β	*P*
CAG repeat[a] <=22 vs.>22	-0.36	0.28	-1.71	0.056	-0.73	0.26	-0.58	0.034
	Dependent variables							
	PcnTarmFat		PcnTlimbFat		PcnTlegFat		PcntTrunkFat	
	β	*P*	β	*P*	β	*P*	β	*P*
CAG repeat[a] <=22 vs.>22	-0.42	0.28	-0.45	0.16	-0.45	0.15	-0.27	0.45

[a]Adjusted for age

BMI, body mass index; PcnTBfat, percent total body fat; PcnTarmFat, percent arm fat; PcnTlimbFat, percent limb fat; PcntlegFat, percent leg fat; PcnTtrunkFat, percent trunk fat;

Table 5-11: *AR* CAG repeat length associations with age, BMI, and percent total body fat, in study groups defined differently with respect to the availability of BMI and percent total body fat information.

Table 5-11a: TBFat% not missing AND BMI not missing at the visit with the non-missing DEXA, n=2330.

		Rank order correlation with *AR* CAG repeat number		Average difference in the dependent variable, comparing men with long (23-33 repeats) vs. short (9-22 repeats) *AR* CAG			
Parameters	n	r	p-value	β	S.E.	t-value	p-value
Age at DEXA visit (years)	2330	0.02	0.40	0.09	0.58	0.16	0.87
BMI at DEXA visit (kg/m^2)	2330	-0.05	0.02	-0.54	0.25	-2.14	0.03
TBFat%	2330	-0.01	0.73	-0.37	0.35	-1.07	0.29

Age, BMI, and TBFat% pulled from Visit 1 and 3 in 435 and 1895, respectively
TBF%; percent total body fat

Table 5-11b: TBFat% not missing, n=2468.

		Rank order correlation with *AR* CAG repeat number		Average difference in the dependent variable, comparing men with long (23-33 repeats) vs. short (9-22 repeats) *AR* CAG			
Parameters	n	r	p-value	β	S.E.	t-value	p-value
Age at DEXA visit (years)	2468	0.01	0.52	0.07	0.56	0.13	0.90
BMI at DEXA visit (kg/m^2)	2330	-0.05	0.02	-0.54	0.25	-2.14	0.03
TBFat%	2468	-0.01	0.68	-0.35	0.34	-1.04	0.30

Age, BMI, and TBFat% pulled from Visit 1 and 3 in 566 and 1902, respectively
TBF%; percent total body fat

Table 5-11c: BMI not missing, n=2466.

		Rank order correlation with *AR* CAG repeat number		Average difference in the dependent variable, comparing men with long (23-33 repeats) vs. short (9-22 repeats) *AR* CAG			
Parameters	n	r	p-value	β	S.E.	t-value	p-value
Age at BMI visit (years)	2466	0.02	0.37	-0.04	0.55	-0.08	0.94
BMI (kg/m^2)	2466	-0.05	0.01	-0.62	0.25	-2.44	0.01
TBFat%	2355	-0.01	0.63	-0.43	0.34	-1.25	0.21

BMI pulled from Visit 3, if not missing, from Visit 1, otherwise
Age and BMI pulled from Visit 1 and 3 in 350 and 2116, respectively
TBFat% (available in n=2355) pulled from Visit 1 and 3 in 455 and 1900, respectively
TBF%; percent total body fat

Table 5-11d: TBFat% not missing OR BMI not missing, n=2585.

		Rank order correlation with *AR* CAG repeat number		Average difference in the dependent variable, comparing men with long (23-33 repeats) vs. short (9-22 repeats) *AR* CAG			
Parameters	n	r	p-value	β	S.E.	t-value	p-value
Age at DEXA visit (years)	2468	0.01	0.52	0.07	0.56	0.13	0.90
BMI at Visit 3 (kg/m^2)	2116	-0.04	0.08	-0.58	0.28	-2.10	0.04
TBFat%	2468	-0.01	0.68	-0.35	0.34	-1.04	0.30

N=2468 with non-missing TBFat% and 2116 with non-missing Visit 3 BMI.
Age and TBFat% pulled from Visit 1 and 3 in 566 and 1902, respectively
TBF%; percent total body fat

6.0 PAPER 3: EFFECTS OF ANDROGEN DEPRIVATION THERAPY ON CHANGE IN BODY COMPOSITION IN AFRO-CARIBBEAN MEN: TOBAGO PROSTATE STUDY

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6.1 ABSTRACT

Objective: A common treatment for prostate cancer is androgen deprivation therapy (ADT). ADT affects the body by lowering the concentrations of androgen. This can have adverse side effects of which changes in body composition is a major concern. These changes may influence risk for other chronic diseases

Design and Subjects: Case-control Study analysis of 1691 Afro-Caribbean men residing in Tobago and participating in a prostate cancer screening study. Controls included participants without a history of prostate cancer and individuals with prostate cancer unexposed to ADT.

Measurements: Body mass index (BMI), waist circumference, total fat estimated by dual energy x-ray absorptiometry (DEXA).

Results: Percent total body fat increased over time (mean ±S.E.) (0.57±0.08) compared in men with prostate cancer treated with ADT compared to men with prostate cancer unexposed to ADT (0.29±0.07) with a p-value for change (0.28±0.11, P=0.0111). The change in men with prostate cancer unexposed to ADT did not differ from controls with prostate cancer

Conclusion: Men with prostate cancer exposed to ADT had greater gains in percent total body fat over time compared to men unexposed to ADT.

6.2 INTRODUCTION

Prostate cancer is known as one of the most common malignancies affecting black men in the United States and other countries around the world[351]. There are considerable variations in prostate cancer incidence across racial/ethnic groups with people of African descent having the highest rates, whites having an intermediate rate and Asians the lowest [53, 55, 351]. Even though this disparity remains to be clearly elucidated, possible explanations for the overt population differences include androgen levels, and dietary factors [351, 377].

The production of androgen is regulated by the hypothalamic–pituitary-gonadal axis. Gonadotropin releasing hormone is secreted by the hypothalamus stimulating the pituitary gland to secrete luteinizing hormone, which acts on the Leydig cells of the testis to produce androgen. Testosterone is the main androgen circulating in males. However, Dihydrotestosterone (DHT) is considered the most potent androgen and the main androgen found in tissue[351]. Testosterone moves from the blood into the prostate where it binds to the Androgen receptor (AR)[53]. Testosterone is also converted to DHT by the enzyme 5α -reductase types II which is encoded by the SRD5A2 gene.DHT binds to the Androgen receptor in addition to its co-regulators (AIB1/SRC3) and co-repressors (DAX1 and SHP) (the androgen receptor has a higher affinity for DHT) in the cytoplasm and is then translocated into the nucleus where transcription begins [378].Androgens are steroid hormones that influence the development of male secondary sex characteristics. In men androgen are developed in the testes and the adrenal glands and are metabolized in the prostate and skin[351].Testosterone and DHT exact their androgenic effects through the androgen receptor[351].

There is evidence suggesting that serum levels of testosterone are associated with the development of prostate cancer. A high level of serum testosterone is associated with an

increased risk of low-grade prostate tumors and a decreased risk of low-grade prostate cancer. Whilst low levels of serum testosterone is associated with an increased risk of high grade prostate cancer and reduced risk of low grade prostate cancer[378].

A common treatment of prostate cancer is androgen deprivation therapy (ADT). ADT involves the suppression of gonadal androgen in the body. This can be accomplished through a variety of ways some of which are orchiectomy, and gonadotropin-releasing hormone. ADT has numerous side effects such as sexual side effects, hot flashes, gynecomastia, changes in body composition, metabolism, osteoporosis, the cardiovascular system, cognition problems and an overall decline in one's quality of life [379]. This paper investigates the association between ADT treatment for prostate cancer and rate of change in percent total body fat in Afro-Caribbean men from Tobago.

6.3 MATERIALS AND METHODS

6.3.1 Study sample

From 1997 until September 2007, the Tobago Prostate Study recruited 40 to 79 year old men to a population-based prostate cancer screening study. The men were recruited by public service announcements, flyers, local health care workers, and by word of mouth to participate in a study evaluating prostate cancer screening. Men were excluded from the study if they were determined to be non-ambulatory, terminally ill, or cognitively impaired. All eligible participants signed a written informed consent approved by the institutional review boards of the University of Pittsburgh and the Tobago Division of Health and Social Services.

The Tobago Prostate study enrolled 3837 Afro-Caribbean men (Five hundred and thirty one men were excluded because 485 were new enrollees and did not have a baseline DEXA and 46 were less than 40 years of age). Demographic and cancer information was provided by staff-administered standardized baseline questionnaire and a more detailed follow-up questionnaire (Health History Questionnaire). The Health History questionnaire was used to record information on newly recruited and follow-up men beginning January 2004. Our study consisted of 3452 (90% 0f 3837) race eligible Afro-Caribbean men with ≥3 Afro-Caribbean grandparents.

Procedures consisted of body composition measurements (DEXA). Dexa measurements were obtained on 2850 (82.6%) and health history (HH) questionnaires on 2259 (65.4%) of 3452 race eligible men. Our final sample size was 1691 (Figure 6-1). Men with prostate cancer were categorized as unexposed to ADT, and ADT exposed based on questions from questionnaires. ADT constituted either treatment with gonadotropin releasing hormone (GnRH) agonists, luteinizing releasing hormone (LHRH), anti-androgens, orchiectomy, or any combination of the aforementioned.

6.3.2 Outcome measurements

Important outcome variables included height (measured with shoes to the nearest 0.1 cm on a wall mounted stadiometer), weight (measured to the nearest 0.1 kg on a beam balance), body mass index (BMI- calculated as weight in kg divided by height in meters-squared), waist circumference (measured in centimeters at the umbilicus with an inelastic tape measure). These key anthropometric measures were ascertained from the HH questionnaire. Percent total body fat was ascertained on a hologic QDR4500W DEXA operated in array beam mode and analyzed by QDR software version 8.26a (Hologic Inc. Bedford, MA). Prostate specific antigen test prior to year 2000 was the Abbot AxSYM PSA assay (Abbott Laboratories, Abbott Park, IL, USA)

and after 2000 the PSA assay was the Advia (Siemen's) Centaur (Siemens Healthcare Diagnostic Advia Centaur, Deerfield, IL).

6.3.3 Statistical Analyses

All statistical analyses were performed using SAS® software, version 9.2 (SAS Institute, Inc., Cary, NC, USA). Descriptive statistics were used to characterize the six groups in the study sample. Three hundred individuals and two were determined to have prostate cancer and these individuals were placed in particular groups depending on when they were diagnosed with prostate cancer. 143 individuals were diagnosed with prostate cancer before initial DEXA (80 unexposed to ADT and 60 ADT exposed), 98 were diagnosed with prostate cancer between initial and repeat DEXA (59 unexposed to ADT and 39 ADT exposed), and 61 were diagnosed with prostate cancer after repeat DEXA (59 unexposed to ADT, and 2 ADT exposed). Analysis of variance (one-way-ANOVA) was used to compare the mean difference among the groups. Adjusted comparisons controlled for baseline percent body fat measurements, ageand PSA as potential confounders.

6.4 RESULTS

Of the individuals enrolled in the study, 143 individuals were diagnosed with prostate cancer before initial DEXA (80 unexposed to ADT and 60 ADT exposed), 98 were diagnosed with prostate cancer between initial and repeat DEXA (59 unexposed to ADT and 39 ADT exposed), and 61 were diagnosed with prostate cancer after repeat DEXA (59 unexposed to ADT, and 2 ADT exposed). The 2 ADT exposed individuals were excluded from the study because this

group was extremely small to use in making any reliable conclusions. Our control group consisted of, 1391 individuals without a history of prostate cancer and unexposed to ADT (21 individuals indicated that they were ADT exposed on questionnaires but they had no history of prostate cancer; these individuals were excluded (Figure 6-1). One person was also excluded from the final analysis because they did not have all the relevant repeat DEXA measurements). The majority of the study samples were Afro-Caribbean andparticipants were more likely to be married and have at least a primary school level education. Table 6-1 shows the mean and standard deviation for a number of clinical characteristics. Men had significantly different age (P=0.0001), percent total body fat (P=0.0076) and Prostate specific antigen (P=0.0001) across groups. Mean body mass index was not significantly different across groups (P=0.6691).

Table 6-2 shows the mean change in percent total body fat in men according to prostate cancer status and ADT exposure. In men exposed and unexposed to ADT who had their prostate cancer diagnosed before the 1st DEXA had significant mean difference in percent total body fat unadjusted (P=0.0056), adjusted for age (P=0.0036) and adjusted for age and 1st DEXA percent total body fat (P=0.0021).Similar findings were also observed in men exposed and unexposed to ADT with prostate cancer diagnosed between the 1st and 2nd DEXA scans; unadjusted (P=0.0041), adjusted for age (P=0.0032) and adjusted for age and 1st DEXA percent total body fat (P=0.0021). Men with prostate cancer diagnosed after repeat (2nd) DEXA and unexposed to ADT had similar mean difference in percent total body fat (prostate cancer diagnosed after 2nd DEXA (0.82±0.31), compared to men without prostate cancer (0.80±0.07). Finally Table 6-3 shows the rate of change of percent body fat in the six groups. Overall table 4 indicates that the rate of change of percent total body fat was greater in persons exposed to ADT than in persons unexposed the ADT. In the prostate cancer diagnosed after 2nd DEXA and Healthy controls the

mean rate of change of percent total body fat was similar 0.17±0.09 and 0.18±0.02. The difference in the two mean rate of change of percent total body fat 0±0.09, p=0.9626.

Table 6-4 shows the median years between initial and repeat DEXA, which was approximately 4.5 years.Table6-5 shows the frequency of the type of androgen deprivation therapy used. Antiandrogen, GnrH, and LHRH was the treatment of choice in this cohort. Antiandrogen, GnrH, and LHRH was used in 74.6% (47) in prostate cancer diagnosed after 1st DEXA and 76.9% (30) in the group prostate cancer diagnosed between the 1st and 2nd DEXA. Nineteen percent (12) persons had orchiectomy only, in the group prostate cancer diagnosed after 1st DEXA and 12.8% (5) persons had orchiectomy in the group prostate cancer diagnosed between 1st and 2nd DEXA. Four (6.3% and 10.3% respectively) had combined treatments in both groups.

6.5 DISCUSSION

Androgen suppression or androgen ablation or androgen deprivation is chemical treatment to suppress or block the production or action of the male sex hormone to treat prostate cancer in men. Two common methods by which this can be done is by removing the testicles or by taking anti-androgens [380]. There are several different types of anti-androgen drugs available. In our study evaluating the effect of ADT on percent total body fat we determined that percent total body fat increased in men with prostate cancer exposed to ADT. In comparison men without prostate cancer and men with prostate cancer who did not initiate ADT therapy had much lower increases in percent body fat.

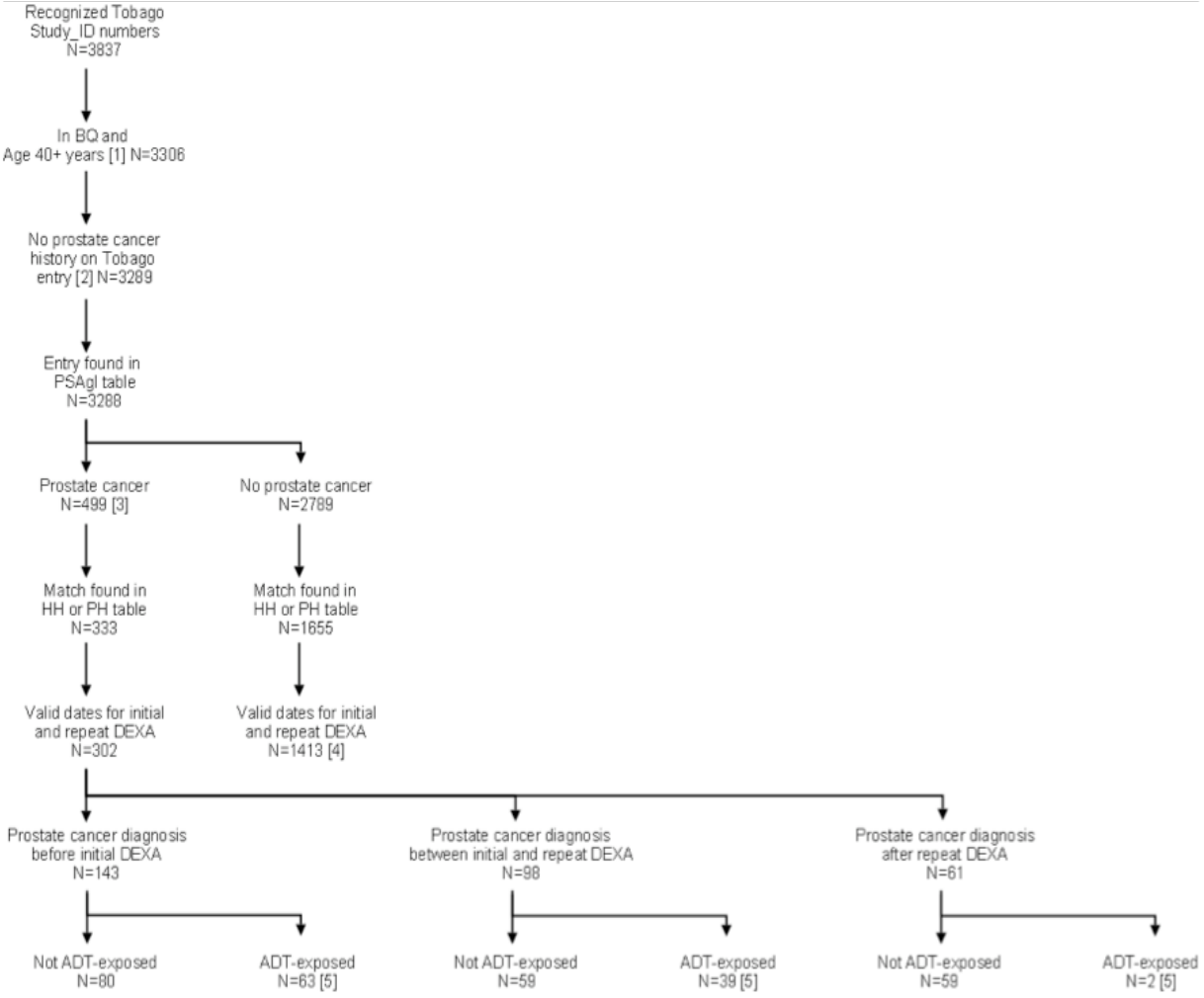
Androgens are considered important determinants of body composition in men. ADT has been shown to increase serum triglycerides and cholesterol[380]. A reduction in muscle mass in men leads to reduced strength and increased risk of falls and fractures (sarcopenia)[14]. The replacement of androgen in the body reverses the effects of androgen deprivation[28]. In men with prostate cancer the use of androgen antagonist affects the body by reducing physical function[380].

Our results support an increase in percent total body fat measured by DEXA following ADT administration in Afro-Caribbean men with prostate cancer.However, there were several limitations with our study. First we were unable to determine the duration of ADT treatment. It is possible that men who had a longer duration of ADT gained more fat. Hence, our study even though we found an increase in percent body fat may have underestimated the response. We also encountered possible information bias. There were 21 individuals who did not have prostate cancer but reported that they were taking ADT. ADT exposure variable was determined from self-report and was not independently verified. These individuals were of course excluded from the study. There was also the issue of channeling bias; beyond PSA clinical indications for ADT treatment may have confounded observed associations between ADT and changes in percent total body fat. Moreover, some individuals with prostate cancer were prescribed ADT while others were not. One can only speculate about possible reasons why some individuals received ADT treatment and others did not such as the cases were more advanced or had metastasized, or maybe it was not affordable. Another limitation was selection bias; the requirement that participants have a second DEXA may have excluded some men according to ADT exposure and outcome. Finally we had small sample sizes for the various groups.

Nonetheless, our study had important strengths. It prospectively observed body composition changes following ADT administration. Two different control groups were used for

comparison (healthy controls and prostate cancer individuals unexposed to ADT). Finally we used dual-energy absorptiometry machine to determine percent total body fat measurements.

In summary, the results of our study should be interpreted with caution because of the small sample size. Non-the-less, in men with prostate cancer percent total body fat increased over time, on average, more so in men exposed than in men unexposed to ADT.



1. Age according to BRTHDATE and INDATE on Wave 1 Tobago BQ.
2. No 31 cancer code entered in CANCER_1, CANCER_2, or CANCER_3 fields of Wave 1 Tobago BQ.
3. GLEASDX>0 in PSAgl table.
4. Includes N=21 (1.5%) with ADT exposure. See Footnote [5].
5. RMTE=Yes ("Have you had your testicles removed (castration, orchiectomy) for prostate cancer?") on HH table or TESTR=Yes on PH table ("Since your last Tobago Prostate Survey visit, have you had your testicles removed?") or MDPRO=Yes on HH table ("Have you ever taken Lucrin (Lucopride), Zolodex, Androcur, Stilbesterol, or Casodex?") or MDFLU=Yes on HH table ("Have you ever taken Flutamide?") or MDCAR=Yes on HH table ("Have you ever taken Proscar or Finasteride?") or MEDIP=Yes on PH table ("Since your last Tobago Prostate Survey visit, have you taken any medication(s) for your prostate?"

Figure 6-1: Sample selection for Paper 3.

Table 6-1: Baseline body composition characteristics (mean ±S.D.).

Parameter	Prostate cancer before 1st DEXA		Prostate cancer diagnosis between1st and 2nd DEXA		Prostate cancer after 2nd DEXA	No Prostate cancer	P-Value (across groups)
	ADT (n=63)	No ADT (n=80)	ADT (n=39)	No ADT (n=59)	No ADT (n=59)	No ADT (n=1391)	
Age (years)	67.9±6.9	63.58±8.7	64.5±6.97	61.76±9.0	58.3±7.85	53.71±9.9	0.0001
BMI (kg/m2)	26.89±4.3	28.2±4.1	27.3±4.15	27.2±3.03	27.42±4.02	27.57±4.2	0.6691
TBF (%)	22.75±5.7	21.7±6.8	21.26±5.76	20.52±5.1	20.01±6.3	20.17±6.2	0.0076
PSA (ng/ml)	23.64±34.6	9.69±18.35	10.14±10.52	15.54±45.34	8.92±24.61	2.21±10.69	0.0001

Abbreviations: TBF (%); Percent total body fat; ADT; Androgen deprivation therapy
PSA; Prostate specific antigen
Age used in this table was the age at 1st DEXA

Table 6-2:Change in percent total body fat (ΔTBFat%) by prostate cancer status and androgen deprivation exposure.

	Androgen deprivation treatment								
	Yes			No					
		ΔTBFat%			ΔTBFat%		Δ meanTBFat%		
Categories	N	Mean	S.E.	N	Mean	S.E.	Delta	S.E.	p-value
Prostate cancer diagnosed before 1st DEXA scan									
Unadjusted	63	2.56	0.43	80	1.25	0.39	1.30	0.47	0.0056
Age-adjusted	63	2.50	0.44	80	1.30	0.39	1.37	0.47	0.0036
Age and 1st DEXA scan TBFat%-adjusted	63	2.85	0.36	80	1.43	0.31	1.42	0.46	0.0021
Age, psa, and 1st DEXA scan TBFat%-adjusted	63	2.75	0.36	80	1.40	0.31	1.35	0.46	0.0038
Prostate cancer diagnosed between 1st and 2nd DEXA scans									
Unadjusted	39	3.16	0.45	59	1.50	0.37	1.66	0.58	0.0041
Age-adjusted	39	3.08	0.45	59	1.55	0.37	1.70	0.58	0.0032
Age and 1st DEXA scan TBFat%-adjusted	39	3.29	0.44	59	1.55	0.36	1.74	0.56	0.0021
Age, psa, and 1st DEXA scan TBFat%-adjusted	39	3.28	0.44	59	1.49	0.36	1.78	0.57	0.0017
Prostate cancer diagnosed after 2nd DEXA scan									
Unadjusted				59	0.82	0.31			
No prostate cancer									
Unadjusted				1391	0.80	0.07			

Legend:

ΔTBFat% = 2nd DEXA scan TBFat% – 1st DEXA scan TBFat%

Delta = mean TBFat% in the group that received androgen deprevation treatment – mean TBFat% in the group that did not receive androgen deprivation Treatment

PSA; prostate specific antigen

Values for comparison groups, no prostate cancer and prostate cancer diagnosed after 2ndDEXA (ΔmeanTBF%, 0.0153), (S.E., 0.37076) and (P-value=0.9672)

Table 6-3:Mean rate of change of percent total body fat (ΔTBFat%) by prostate cancer and androgen deprivation.

	Androgen deprivation treatment								
	Yes			No					
		rate of ΔTBFat%			rate of ΔTBFat%		Delta		
Categories	N	Mean	SE	N	Mean	SE	Mean	S.E.	p-value
Prostate cancer diagnosed before 1st DEXA scan									
Unadjusted	63	0.57	0.08	80	0.29	0.07	0.28	0.11	0.0111
Age-adjusted	63	0.61	0.09	80	0.31	0.07	0.30	0.11	0.0077
Age and 1st DEXA scan TBF%-adjusted	63	0.63	0.08	80	0.32	0.07	0.31	0.11	0.0047
Age, psa and 1st DEXA scan TBF%-adjusted	63	0.61	0.09	80	0.32	0.07	0.29	0.11	0.0084
Prostate cancer diagnosed between 1st and 2nd DEXA scans									
Unadjusted	39	0.68	0.11	59	0.33	0.09	0.35	0.14	0.0094
Age-adjusted	39	0.71	0.11	59	0.35	0.09	0.36	0.14	0.0077
Age and 1st DEXA scan TBF%-adjusted	39	0.71	0.10	59	0.34	0.08	0.37	0.13	0.0054
Age, psa and 1st DEXA scan TBF%-adjusted	39	0.70	0.11	59	0.32	0.09	0.38	0.13	0.0047
Prostate cancer diagnosed after 2nd DEXA scan									
Unadjusted				59	0.17	0.09			
No prostate cancer									
Unadjusted				1391	0.18	0.02			

Legend:
rate of ΔTBFat% =(2nd DEXA scan TBFat% – 1st DEXA scan TBFat%)/ years between 1st and 2nd DEXA
Delta = mean rate ΔTBFat% in the group that received androgen deprevation treatment -mean rate ΔTBFat% in the group that did not receive androgen deprivation treatment
PSA; prostate specific antigen
Values for comparison groups, no prostate cancer and prostate cancer diagnosed after 2ndDEXA (rate of ΔmeanTBF%, 0.00), (S.E., 0.09) and (P-value=0.9626)

Table 6-4:Tobago Prostate Study, subject mean age, median interval between sequential DEXA scans, median interval between prostate cancer diagnosis and first DEXA, and median interval between prostate cancer diagnosis and second DEXA, according to prostate cancer status, timing of prostate cancer diagnosis with respect to sequential DEXA, and prostate cancer treatment status (ADT vs. no ADT).

		Age Tobago entry (years)		Age at initial DEXA (years)		Years between initial and repeat DEXA			Years from diagnosis to initial DEXA			Years from diagnosis to repeat DEXA		
Category	N	Mean	S.D.	Mean	S.D.	Median	Q_{25}	Q_{75}	Median	Q_{25}	Q_{75}	Median	Q_{25}	Q_{75}
No prostate cancer	1391	52.55	9.69	53.71	9.92	4.45	3.95	4.77						
Prostate cancer before initial DEXA, no ADT	80	62.28	8.62	63.59	8.77	4.50	4.05	4.76	0.71	0.38	1.11	5.25	4.72	5.53
Prostate cancer before initial DEXA, ADT	63	66.64	6.85	67.89	6.91	4.36	4.08	4.88	0.88	0.42	1.50	5.45	5.07	6.26
Prostate cancer between initial and repeat DEXA, no ADT	59	60.88	9.15	61.76	9.01	4.65	4.33	5.04	-1.04	-1.78	-0.27	3.73	2.95	4.50
Prostate cancer between initial and repeat DEXA, ADT	39	57.15	7.69	64.49	6.97	4.56	4.31	4.91	-0.84	-1.89	-0.21	3.37	2.51	4.82
Prostate cancer after repeat DEXA	59	52.55	9.69	58.31	7.86	4.61	4.24	4.94	-5.62	-6.75	-5.03	-0.76	-2.19	-0.27

Legend:

Q_{25}; 25 percentile; Q_{75}; 75 percentile

Table 6-5:Type of androgen deprivation,in men reporting an ADT exposure.

Type of androgen deprivation	Prostate cancer before 1st DEXA and ADT exposed (N=63)		Prostate cancer diagnosis between 1st and 2nd DEXA and ADT exposed (N=39)	
	N	%	N	%
Medical treatment only (anti-androgen or LHRH agonist)	47	74.6	30	76.9
Surgical treatment (orchiectomy) only	12	19.0	5	12.8
Medical and surgical treatment	4	6.3	4	10.3

Legend:
ADT, androgen deprivation therapy
LHRH, luteinizing hormone releasing hormone

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