THE LONGITUDINAL EFFECT OF IMPAIRED KIDNEY FUNCTION ON BONE MINERAL DENSITY AND THE ASSOCIATION OF BODY COMPOSITION ON BIOMARKERS OF KIDNEY FUNCTION AMONG AFRO-CARIBBEAN MEN OF WEST AFRICAN ANCESTRY

by

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ABSTRACT

Background: Chronic kidney disease (CKD) is a rising global health problem. African Americans bear a greater proportion of CKD burden compared to Caucasians. Little is known about the relationship of CKD with bone loss and body composition distribution with biomarkers of CKD in blacks.

Objective: The prevalence of CKD among Tobago black, African American and Caucasian men, aged 40 years and older were determined and compared. The risk factors of CKD, the association of body composition with biomarkers of CKD and the effect of CKD on longitudinal bone loss were examined among Tobago black males.

Methods: Tobago men were recruited from Tobago Island in 2004-2007. Counterparts from U.S were obtained from the National Health and Nutrition Examination Survey (NHANES) 2003-2006. Standardized serum creatinine, cystatin C and urinary albumin were measured using Jaffè reaction, Dade Behring nephelometer and fluorescent immunoassay respectively. Longitudinal Bone Mineral Density changes in trochanter, femoral neck and total hip from 2004/2007-2012 were measured using Dual X-Ray Absorptiomertry (DXA). Body composition was measured using DXA and Peripheral Quantitative Computed Tomography (PQCT). Covariates were assessed from questionnaires in 2004-2007.

Results: The prevalence of CKD was 19.7%, 23.4% and 19.7% in Tobago black, African American and Caucasian men respectively. Age, hypertension and diabetes were significantly associated with CKD in Tobago men. Lean body mass and calf muscle area were positively associated with serum creatinine. All adiposity measures were positively associated with cystatin C, but not with calf muscle area. There was consistent greater decline in BMD across quartiles of ACR, serum creatinine and cystatin C in trochanter, femoral neck and total hip bones. The rate of bone loss in Tobago men was similar to that in Caucasian men.

Public Health Significance: The biomarkers used for assessing CKD (serum creatinine and cystatin C) are influenced by body composition. Future CKD screening among blacks with high lean or muscle mass should include cystatin C assessment due to the influence of muscle mass on serum creatinine.

CKD is associated with bone loss. Proper management of bone minerals and DXA screenings are necessary in order to reduce bone loss among individuals with CKD.

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PREFACE

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1.0 INTRODUCTION

Chronic kidney disease (CKD) is a major health problem posing serious public health challenge. The U.S Center for Disease Prevention and Control (CDC) report from 1999-2004 indicates that CKD affects over 10% of Americans 20 years or older and the incidence of CKD has continued to increase since 1994 [1]. The rising CKD incidence is paralleled by a rising elderly population, obesity, cardiovascular disease (CVD) and diabetes in U.S. As the population ages and the number of people with CKD risk factors increases such as diabetes and hypertension, the incidence of CKD is expected to continue to rise in the coming years.

CKD is associated with progression to End Stage Renal Disease (ESRD), which in turn is associated with several health complications including bone loss, anemia and CVD. The conversion rate of CKD to ESRD requiring renal replacement therapy is also on the rise. In U.S, the annual incidence of ESRD is 354 persons per million. Approximately 595,000 ESRD patients are on dialysis at an annual cost of approximately $48 billion [2]. The mortality rate associated with ESRD is about 152 per 1,000 patient years, and this rate is expected to double in the next decade [2]. African Americans and other racial minorities are disproportionately affected by ESRD. During the period 1994-2004, the age adjusted incidence of ESRD declined from 63.5 to 55.0 per million among African Americans and from 25.2 to 22.8 per million among Caucasians [1] due to improved treatment and increased awareness of ESRD. Despite the decline, African Americans are four times more likely to develop ESRD compared to Caucasians [2].

Suggested reasons for CKD/ESRD disparity between African Americans and Caucasians includes heritability of susceptible genes of kidney disease and differences in modifiable risk factors [1]. The role of susceptible genes of CKD/ESRD will be the consideration of future studies. In the meantime, it is important to focus on the role of modifiable risk factors including hypertension, diabetes, smoking, alcohol, obesity and low socioeconomic status in the development of CKD/ESRD and observed racial disparities. Much of the work on ESRD/CKD disparities has been focused on African Americans and Caucasians. Given the ancestral similarities between blacks of West African descent and African Americans, it is important to also examine and compare the role of modifiable risk factors of ESRD/CKD among blacks of West African descent. Therefore, the goal of our project is to determine and compare the prevalence of CKD among African Americans, Caucasians and populations of West African descent and identify modifiable risk factors affecting CKD among populations of West African descent. Furthermore, this study will attempt to evaluate the association of several body composition measures with kidney function biomarkers and the effect of worsening kidney function on bone loss among populations of West African descent.

1.1 BACKGROUND

A recent report indicates that worldwide, over 2 million people with ESRD are kept alive or are being treated with renal replacement therapy and the number is expected to double in the next decade [3-5]. Compared to U.S, publications of prevalence and incidence rates of ESRD for some countries show that the prevalence of ESRD is 800 Persons per million[6] in Europe. The U.K, data for 2008, shows that the prevalence of ESRD, is 774 persons per million [7]. African

countries lack reliable statistics for ESRD. This is probably a reflection of poor data collection and insufficient data to characterize ESRD in Africa. However, some experts suggests an ESRD prevalence of approximately 150 persons per million in Africa [6]. There are also no reliable estimates of ESRD prevalence for the Caribbean as a whole. Although, a renal registry (The Caribbean Renal Registry) was set up in 2009, this registry is not fully functional and is inadequate to characterize ESRD in the Caribbean region at present. However, Barton E.N et al, found ESRD prevalence rate of 316 persons per million in Jamaica [8]. A study found that ESRD prevalence rate was 2.53% among Chinese adult 35-75years [9]. Table 1 shows unadjusted prevalence rates of ESRD for select countries. Japan and Taiwan have some of the world's largest prevalence rates of ESRD, while Russia has the lowest prevalence rate. This may be due to much better treatment enabling so many more survival on dialysis in Japan and Taiwan, contrary to Russia where low rates may be due to poor access to treatment and poor survival, even with treatments.

Table 1. Prevalence of ESRD (Per Million Populations) for Select Countries/Region, 2009

Country	Prevalence Rate	Country	Prevalence Rate
Brazil	481	Russia	173
Argentina	634	Finland	780
U.S.A	1811	Romania	502
Canada	1119	Croatia	930
Japan	2205	UK	793
Taiwan	2447	France	1094

Source: USRDS Annual Data Report 2011[2].

Taken together, CKD/ESRD presents a global challenge. Current population distribution points to an increasing problem that is fast becoming an epidemic. A majority of the new ESRD cases is expected to occur in the less developed countries, including countries in Africa and the Caribbean where kidney transplant and dialysis services are either rudimentary, totally lacking or

if present are unaffordable. In addition, ESRD and ESRD complications are a financial burden to less developed countries already overburdened by communicable and non communicable diseases. The distribution of CKD/ESRD worldwide, underscores a need for action. Concerted preventive strategies are needed to slow progression form CKD to ESRD in order to address complications and health consequences of ESRD. The nonprofit organization of Kidney Disease Improving Global Outcomes (KDIGO), established in 2003 and the U.S National Kidney Foundation's Kidney Disease Outcome Quality Initiative (KDOQI) established in 1997 have been creating clinical practice guidelines to improve clinical outcome in chronic kidney disease worldwide using evidence based approach.

2.0 KIDNEY DISEASE

Kidney disease is a multi-etiologic disorder which may lead to short-term acute kidney injury (AKI) or persistent long term CKD. Kidney diseases affect kidney function and accompanied with a wide spectrum of prognosis. The diseases of the kidney are collectively known as glomeruli diseases and two major categories: 1, Glomerulonephritis; and 2, Glomerulosclerosis. Glomerulonephritis results in inflammation of the glomerular filtration membrane while Glomerulosclerosis results in scarring and hardening of blood vessels of the kidney. Several diseases may lead to either one or both types of glomerular diseases including, autoimmune disease, systemic lupus erythematosus, Good-pasture syndrome, IgA nephropathy, hereditary nephritis-Alport syndrome, infection-related glomerular disease, glomerulosclerosis, diabetic nephropathy, focal segmental glomerulosclerosis, CVD(hypertension), membranous nephropathy and minimal change disease [10].

AKI and CKD are directly related. Many AKI's will resolve without further damage to kidney, whereas in some individuals, AKI may progress to CKD. Whenever AKI or CKD occurs, the filtration capacity of the kidney is affected, followed by loss of kidney physiological function and declining glomerular filtration.

2.1 ACUTE KIDNEY INJURY (AKI)

AKI is an inflammatory disease [11, 12], leading to sudden onset of impaired kidney function: oliguria (low urine volume) or anuria (no urine production). The occurrence of AKI leads to high blood concentrations of nitrogenous compound including urea and creatinine, a condition known as azotemia [13].The causes of AKI are multifactorial and are classified into three etiological sources: pre-renal azotemia, intra-renal azotemia and post-renal azotemia[13]. In pre-renal azotemia, the events leading to kidney injury occurs before the kidney. Among the causes are cardio-renal system disturbances including heart failure and congestive heart diseases, hepatic-renal syndrome and renal-pulmonary disturbances. In intra-renal azotemia, the events leading to kidney injury arises within the kidney and they include polycystic kidney disease, glomerular nephritis and tubular necrosis. In post-renal azotemia, the events leading to kidney injury are found below or after the kidney and they include urinary tract infection, infection from kidney stone formation, tubular obstruction and back-leaking of solute. AKI may involve one or a combination of these sources.

Individuals with AKI have a significant risk of developing CKD [14] especially among those with history of dialysis [15]. An estimated 10-25% of those with history of AKI develop CKD within few months of having AKI [16]. Age, glomerular filtration rate, high levels of serum albumin, diabetes mellitus and severity of AKI are some factors that determines whether an individual with a history of AKI will eventually develop CKD [17]. Conversely, individuals with CKD are also susceptible to AKI. This condition is referred to as acute-on-chronic kidney condition. Study show that acute-on-chronic CKD is associated with severe adverse health outcomes and increased risk of mortality [14].

The mechanism underlying AKI is not fully understood. However, the widely accepted mechanism involves renal tubular cell injury resulting from renal ischemia [13]. In this model, hypovolemia (low blood volume), activates the Renin Angiotensin System (RAS), followed by vasoconstriction of renal vessels (afferent and efferent capillaries vessels). In turn, reduced renal blood flow and renal under-perfusion ensues, leading to ischemia, oxidative stress, and release of reactive oxygen species, such as O_2 and OH radicals, hydrogen peroxide, hypo-chloric acid and ozone radicals. Cell injury and tissue damage is mediated by release of inflammatory mediators (Tumor Necrosis Factors, Chemokine's, Cytokines and Interleukins) and eventual AKI. Persistent occurrence of renal ischemia may lead to epithelial and endothelial tissue damage, tubular dysfunction, histological changes, severe loss of kidney function marked by reduced glomerular filtration rate and rise in serum creatinine. Further damage may lead to leakage of small amounts of protein molecules including albumin into urine, a condition known as micro albuminuria. As glomeruli damage occurs further, larger amounts protein molecules will leak into urine also known as macroalbuminuria or proteinuria. Proteinuria represents a more extensive damage of kidney glomerulus marking an advanced stage of kidney function loss. Proteinuria is associated with significant health problems including fluid and electrolyte imbalance, acid base disorder, uremic syndrome, impairment of red blood cell formation and hemostasis, impaired immune response, increased risk of infection and death [18].

2.1.1 SYSTEM OF AKI CLASSIFICATION

The first national multidisciplinary consensus to classify AKI was developed in 2004, by the Acute Dialysis Quality Initiation (ADOQI). The RIFLE acronym was developed referring to

Risk, **I**mpairment, **F**ailure, **L**oss of function and **E**nd stage, for characterizing AKI severity, diagnosis and treatment as shown in Table 2.

Table 2. AKI Stages by RIFFLE System of Classification

RIFFLE	GFR Criteria	Urine Output Criteria
Risk	Increased Serum Creatinine X1.5, or GFR decrease >25%	UO < 0.5ml/kg/hr×6hr
Injury	Increased Serum Creatinine X2, or GFR decrease >50%	UO < 0.5ml/kg/hr×12hr
Failure	Increased Serum Creatinine X3, GFR decrease >75% or Serum Creatinine>4mg/dl	UO < 0.3ml/kg/hr×24hr or anuria×12hr
Loss	Persistent ARF: Complete Loss of Kidney Function >4wks	
ESRD	End Stage Kidney Disease >3 Months	

Source: KDIGO Clinical Practice Guideline for Acute Kidney Injury *http://www.kidney-nternational.org*: UO: Urine Output, ESRD: End Stage Renal Disease.

Because RIFLE classification did not take into account, baseline serum creatinine changes occurring within a short time period, subsequent modification by the Acute Kidney Injury Network (AKIN) resulted in creation of another AKI classification to recognize small changes in serum creatinine occurring within 48 hours shown in Table 3.

Table 3 AKI Stages by AKIN System of Classification

Stage	Serum Creatinine	Urine Output
1	1.5-1.9 times baseline, or ≥0.3mg/dl increase	<0.5ml/kg/h for 6-12hrs
2	2.0-2.9 times baseline	<0.5 ml/kg/h for ≥12hrs
3	3.0 times baseline or Increase in serum creatinine≥4.0mg/dl, or Initiation of renal therapy or in patients less than 18 yrs., decrease in eGFR to <35ml/min/1.73m^2	0.3ml/kg/h ≥24 hrs., or Anuria for≥12hrs

Source: KDIGO Clinical Practice Guideline for Acute Kidney Injury http://www.kidney-international.org

The current system of AKI classification combines both RIFLE and AKIN systems to generate a composite system which now includes changes in urine output and serum creatinine

for determining AKI severity. Based on RIFLE/AKIN system, AKI is defined as increase in serum creatinine by 0.3mg/dl within 48 hours or increase in serum creatinine to 1.5 times the baseline within 7 days or presence of urine volume less than 0.5ml/kg/h for 6 hours[19]. The RIFLE/AKIN system has been validated and is found to be more accurate in staging and diagnosing AKI compared to either RIFFLE or AKIN system alone [20, 21].

However, because serum creatinine is affected by several factors including age, muscle mass, gender and ethnicity, there is growing concern in the use of serum creatinine for staging and classification of AKI. Recent developments have identified Interluekin-6 (Il-6), Cystatin C and Neutrophil Gelatinase-Associated Lipocalin (NGAL) as alternative biomarkers of AKI. However, as at present, none have been standardized and validated for population use.

2.2 CHRONIC KIDNEY DISEASE (CKD)

In U.S, according to the National Kidney Foundation Kidney Disease Outcome Quality Initiative (KDOQI), CKD is defined as presence of kidney damage and estimated glomerular filtration rate (eGFR) less than 60ml/min/1.73m^2 occurring for at least three months irrespective of etiological origin[22]. CKD is progressive and irreversible and is characterized by kidney fibrosis [14]. Because of the pathophysiology of renal disease, CKD does not manifest in signs and symptoms until in the later stages when treatment may be less effective. In the majority of cases, early kidney function impairment is often missed leaving an individual unaware of impending kidney failure. CKD may eventually progress to ESRD and is associated with several health complications including bone mineral density loss, anemia, depression and mental health,

CVD and CVD deaths. The risk factors of CKD include hypertension, diabetes, obesity and persistent AKI lasting for at least three months.

2.2.1 SYSTEM OF CKD CLASSIFICATION

In 2002, the Kidney Disease Outcome Quality Initiative (KDOQI) classified CKD into five stages on the basis of eGFR shown in Table 4[23].

Table 4. Stages of Kidney and Associated Risk of CVD

CKD Stage	eGFR (ml/min/1.73m^2)	Characteristics
1	Greater or equal to 90	Some renal damage
2	60-90	Mildly decreased eGFR with some Kidney damage
3	30-59	Moderate decreased eGFR
4	15-29	Severely decreased eGFR
5	Less than 15	Kidney failure (may require dialysis or transplant)

In the year 2004, Kidney Disease Initiative on Global Outcome (KDIGO) adopted the five-stage classification with little modification. However, in October 2009, two changes, allowing determination of prognosis were made. First, stage three was split into moderate and mild/severe stages. Second, albumin creatinine ratio (ACR) was included in the system of classification. By the new classification system, a composite ranking system was developed with color chart as shown in (Figure 1) for guiding risk of CKD outcome. Each color represents a combination of eGFR and ACR that is associated with risk of CKD. The direction of increasing risk or worsening outcome is indicated by going from green to red. Accordingly, individuals with normal eGFR may be at high risk of worsening outcome due to increasing ACR resulting from inapparent kidney damage. Also, because of the limitation of albuminuria as a non-specific biomarker of CKD, the combination eGFR and ACR in Figure 1, allows for appropriate staging

and diagnosis of CKD in populations of non-CKD induced albuminuria including diabetic and CVD populations.

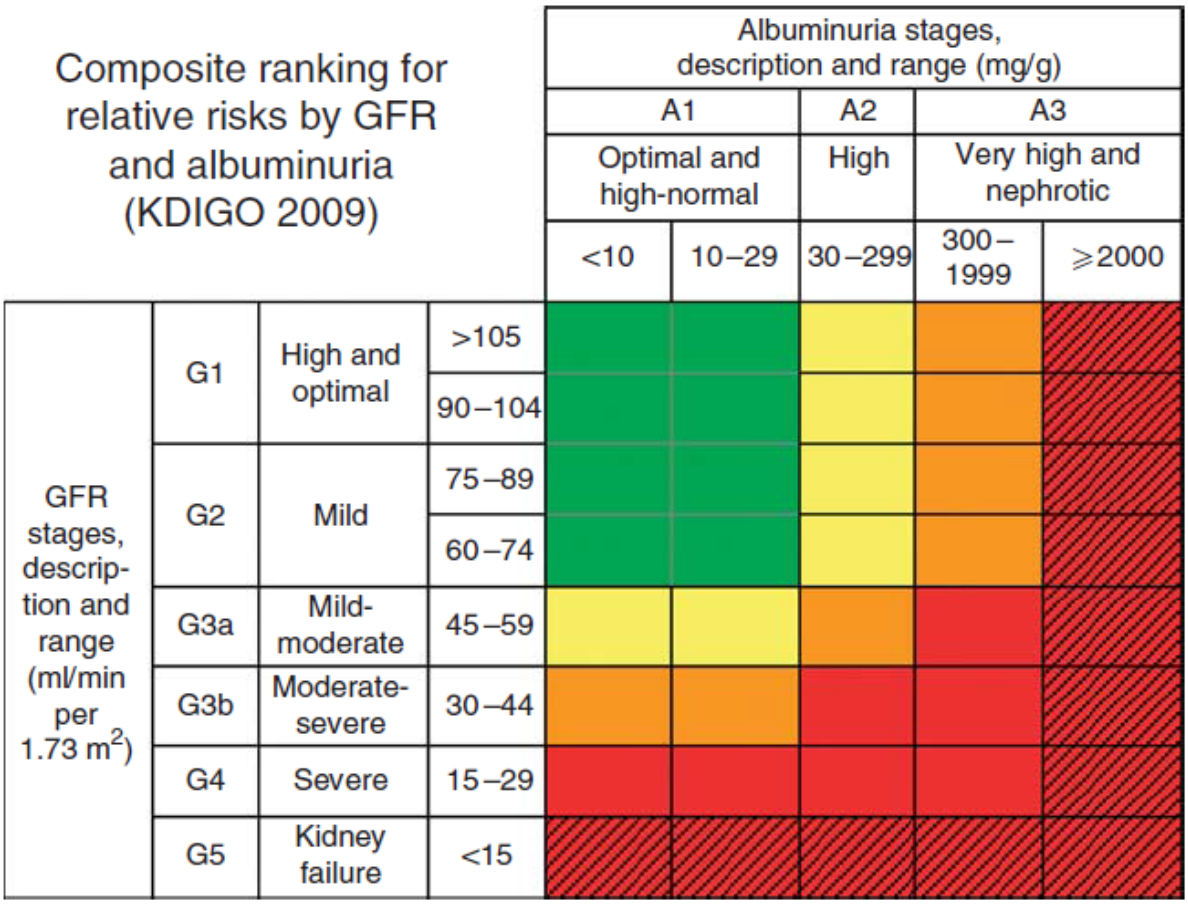

Figure 1. Composite Ranking for Relative Risk of CVD
CKD was defined by glomerular filtration rate (GFR) and Albuminuria by the Kidney Disease: Improving Global Outcomes (KDIGO) 2009. Source: Levey et al.[24]

3.0 ESTIMATING GLOMERULAR FILTRATION RATE (EGFR)

The filtering capacity of the glomeruli determines kidney function. Whenever that capacity is impaired, toxins are suspended in circulation and glomerular damage occurs, leading to leakage of small protein molecules including albumin into urine. The gold standard for assessing the filtering capacity of kidney is by measuring the rate at which Iothalamate or Inulin is cleared from the blood plasma. Iothalamate/Inulin clearance tests are costly, cumbersome and not widely recommended for population screening. Other methods for assessing kidney function rely on 24-hour creatinine clearance test. This method is also cumbersome, and because it requires 24 hour urine collection, variability in urine collection renders the results inaccurate. Nowadays, estimating equations, based on serum creatinine and demographic characteristics have found practical use in clinics and research for estimating glomerular filtration rate (eGFR), expressed in units of (ml/minute/1.73m^2). The KDOQI clinical guidelines recommend routine annual assessment of eGFR for determining kidney function among at risk populations [25].

The equations for estimating eGFR include: Cockcroft–Gault (CG)[26], Modification of Diet in Renal Disease (MDRD)[27] and Chronic Kidney Disease –Epidemiology Collaboration (CKD-EPI) equations [28, 29].

3.1 COCKROFT-GAULT (C-G)

Prior to 1999 there were several equations for estimating glomerular filtration capacity of kidney. Among them is Cockcroft-Gault (C-G) equation which received the most attention, whereby eGFR was expressed as:

eGFR (ml/min)= [(140 - Age) × Weight / (0.814 × Serum. Creatinine (μmol/L)] × (0.85 if female).

C-G equation was validated and found to have a correlation coefficient of (0.8) with gold standard Iothalamate test. However, the use of C-G equation soon became unpopular due to requirement of weight in the formula. Nevertheless, some clinical laboratories still make use of C-G equation to estimate glomerular filtration rate [26]. Studies show that compared to MDRD and CKD-EPI equations, C-G is least accurate in estimating glomerular filtration rate. However, it has been shown that C-G compared to other equations, is more accurate for estimating glomerular filtration rates among the elderly and those with low BMI's [30].

3.2 MODIFICATION DIET IN RENAL DISEASE (MDRD)

Levey et al, in 1999 developed the MDRD equation. In the early stage, MDRD made use of 6 variables: serum creatinine, age, gender, ethnicity, urea and albumin concentrations to estimate eGFR [27]. The utility of this equation was limited due to urea and albumin requirements in the formula, which restricted its use as a practical clinical diagnostic tool. In 2000, Levey et al, modified the equation to include only four variables: serum creatinine, age, gender and race [31] whereby glomerular filtration rate was expressed as:

eGFR (ml/min/1.73m^2) = 186 (Serum Creatinine (µmol/l) × 0.011312) $^{-1.154}$ × (age)$^{-0.203}$ × (0.762 if female) × (1.212 if African/American Black).

New developments in analytical techniques led to calibration of serum and plasma creatinine to an international standard using an isotope dilution mass spectrometry (IDMS) reference. Therefore, if serum creatinine measurement is standardized against IDMS, then the constant 186 is replaced by 175 [32].

The ease of MDRD use and the non-requirement of body weight, unlike C-G, gave it a worldwide appeal and acceptance. However, MDRD suffers from several limitations. Although, MDRD has been validated with Iothalamate test, validation was performed among a small number of U.S. Caucasians and African Americans between ages 18-70 with eGFR less than 60(ml/min/1.73m^2), therefore, it may not work well in other population groups. MDRD is sensitive to low eGFR and performs best with eGFR between 15-30, but not as sensitive to GFR greater than 60(ml/min/1.73m^2) [33].Therefore, MDRD may overestimate CKD stages 3-5 and underestimate those with higher eGFR in a population.

3.3 CHRONIC KIDNEY DISEASE-EPIDEMIOLOGY COLLABORATION (CKD-EPI)

In 2009, Levey et al tackled the problem of CKD stage 3-5 overestimation of MDRD by modifying MDRD to the CKD-EPI equation [29]. CKD-EPI reflects the effect of gender and age-decline on eGFR especially among the elderly who may have low eGFR but do not show any evidence of impaired kidney function. MDRD was modified to reflect changes at creatinine values < 60 µmol/L and > 60 µmol/L for males and females of different races.

eGFR= 141 × min(Scr/κ, 1)α × max(Scr/κ, 1)-1.209 × 0.993Age × 1.018 [if female] _ 1.159 [if black], where Scr is serum creatinine, κ is 0.7 for females and 0.9 for males, α is -0.329 for females and -0.411 for males, min indicates the minimum of Scr/κor 1, and max indicates the maximum of Scr/κ or 1 [29]

CKD-EPI was found to yield lower prevalence of CKD compared to the MDRD equation, which has a tendency to overestimate the prevalence of stages 3-5. Levey et al, suggested replacement of MDRD equation with CKD-EPI. In U.S, although there are a few laboratories that have converted to CKD-EPI, MDRD is still universally applied for estimating eGFR.

3.4 OTHER MEASURES OF KIDNEY FUNCTION

In epidemiological and clinical studies, serum creatinine based equations are the most widely used biomarkers for estimating glomerular filtration rate. However, serum creatinine is influenced by age, gender, race, nutrition (meat consumption) and body composition. Serum creatinine levels increase with age. Individuals with high BMI or high lean muscle mass resulting from exercise or weight training have a disproportionately higher serum creatinine compared to those with low BMI and/or lean mass[34, 35]. Serum creatinine is slow in responding to kidney damage. It is estimated that about 20% of kidney function damage would have occurred before serum creatinine rises to a significant level [36]. Studies show that creatinine is produced by several tissues and organs of the body at different rates in different individuals. The variability of serum creatinine makes comparison of GFR among individuals difficult. To overcome these problems, alternative measure of kidney function makes use of

cystatin C biomarker. Cystatin-C is now beginning to gain popularity in epidemiological and clinical studies [37]. A recent validated cystatin-C based equation estimates glomerular filtration rate as:

$$eGFR_{cys} = 76.7 \times S_{cys}^{-1.18} \quad [37].$$

3.4.1 CYSTATIN C

Cystatin C is a low molecular weight (13 kilodalton) biochemical compound belonging to the human cystatin family. The cystatin family comprises of 11 homologues (A, B, C, D, E, F, S, SA, SN and, low and high molecular weight KININOGEN) grouped into three subfamilies; cystatin C belongs to the second family. Cystatin C is mainly found in extracellular fluid and its function is to inhibit cysteine protease. It is produced by all nucleated cells in the body and is freely filtered at a constant rate in the glomerulus. As cystatin C passes through glomerulus, about 99% is completely catabolized in the proximal tubule leaving only a tiny fraction for reuptake [38]. These properties make cystatin C an attractive candidate as a biomarker of kidney function, unlike creatinine for which serum concentration is influenced, among other factors, by diet(meat consumption), muscle mass production and, tubular production and reabsorption [39].

A meta-analyses comparing cystatin C and creatinine show overall, cystatin C has a higher correlation with a standard Iothamalate test, compared to serum creatinine [40]. Although, earlier report suggests that serum cystatin C is independent of body composition, a recent study now find that lean body mass is a predictor of cystatin C [41]. Also large doses of glucocorticoids [42] and hyperthyroidism have been shown to increase serum cystatin C [43]. Therefore, in applying cystatin C to estimate GFR, these factors need to be considered. The greatest disadvantage of cystatin C in estimating kidney function is due to lack of

standardization. Because several different assay methods are used with different formulas, it is challenging comparing cystatin C based eGFR across different assay platforms.

3.4.2 ALBUMIN CREATININE RATIO (ACR)

Extensive damage of the kidney glomerulus is associated with an advanced stage of kidney function loss and is often marked by proteinuria. Proteinuria is characterized by leaking of protein into urine especially of albumin. Proteinuria is not only found among patients with renal impairment, it has also been implicated in diabetes and cardiovascular disease conditions [44] . Proteinuria is measured by a 24-hour urine collection. However, such method has become less useful, due to the inconvenience of a 24-hour timed urine collection [45]. Proteinuria is measured by quantifying urine Albumin to Creatinine Ratio (ACR). A normal ACR is less than 30mg/g. ACR greater than 30mg/g but less than 300mg/g is defined as microalbuminuria and ACR greater than 300mg/g is referred to as macroalbuminuria. Micro and macro albuminuria represent more extensive kidney damage. The essence of ACR is to accurately diagnose and stage CKD alongside an eGFR estimating equation as shown in Figure 2.

ACR measure is not without controversy. It is argued that the use of ACR in CKD staging is flawed and misleading because the correction factor in the denominator (urine creatinine), is dependent on muscle mass and does not correct for body surface area BSA [45]. Also, it is well known that the quantity of protein and creatinine excretion depends on time of the day. Woman and the elderly generally have lower creatinine production compared to men and the young respectively [46]. These variations limit comparisons across individuals. In spite of these controversies, the National Kidney Disease Education Program Current guidelines recommend ACR as a surrogate for collection of timed urine samples [47].

3.5 C-G, MDRD, CKD-EPI AND CYSTATIN C COMPARED

Several studies comparing eGFR estimating equations with cystatin C show that cystatin C is superior to MDRD, C-G and CKD-EPI equations. In a study to compare cystatin C with the C-G equation, cystatin C based eGFR showed a higher correlation with the gold standard compared to C-G equation [48]. In the Multi-Ethnic Study of Atherosclerosis (MESA) and the Cardiovascular Health Study (CHS), to assess risks for complications, Paralta et al, also showed that cystatin C identified persons with CKD having the highest risk for complications much more frequently compared to CKD-EPI equation [49]. Thus, cystatin C is seen as a better biomarker for estimating kidney function compared to the estimating equations.

4.0 CKD AND CARDIOVASCULAR DISEASE

The interrelationship of CVD and CKD has received a lot of attention in recent time. CVD is any disease that involves the heart and blood vessels. The common CVD's are coronary heart disease, cerebrovascular disease (stroke) and hypertension. Others include arrhythmias, heart failure and heart valve problems [50]. Estimates compiled between 1997-2009 show that among adults 18 years and older, the prevalence rate of heart diseases remained the same from 1997 to 2009 while the prevalence rate of hypertension increased from 191 to 249 cases per 1000 in the same period[51]. Among the racial groups reported, whites had the highest prevalence rate of heart disease followed by blacks and Native Americans.

In addition to being the leading cause of death in the U.S general population [52], CVD is a leading cause of death among patients with CKD [53]. Recent studies have reported a rising CVD mortality with rising CKD in the U.S population [54]. The rising trend is further compounded by increasing number of elderly population, and the rising incidence of diabetes, hypertension and obesity, which are risk factors associated with both CKD and CVD. A study also showed that a majority of CKD cases are more likely to die from CVD than progress to renal failure [55], and, among those who progress to renal failure, about half will eventually develop CVD or die from CVD [56]. Evidence from several studies also suggests CVD is increasing among patients with earlier stages of CKD [53, 55-58].

CKD is an independent risk factor for CVD. In a recent pooled study comprising data from four community-based longitudinal studies(Atherosclerosis Risk in Communities Study, Cardiovascular Health Study, Framingham Heart Study, and Framingham Offspring Study), after adjusting for confounding variables, CKD was found to be an independent predictor of CVD. In addition, blacks with CKD had significantly higher risk of CVD outcome compared to whites with CKD [59].

Conversely, CVD is also an independent predictor of CKD. In a study to evaluate the relationship between the levels of kidney function on all-cause mortality Al-Ahmad et al, showed that worsening renal function was associated with patients with CVD [60]. Similar result was reported among men hospitalized for heart disease, who subsequently developed CKD with decreasing kidney function [61] [62].

In light of these findings, it is evident that the relationship between CVD and CKD is bidirectional. Moreover, it has been shown that acute or chronic changes in one organ(heart or kidney) can lead to acute or chronic changes in the other [57]. CVD and CKD not only share common risk factors which includes atherosclerosis, diabetes mellitus, dyslipidemia, renal vascular disease, anemia, and hypertension [63], interactions among these risk factors may also exist[64]. The consequence of the interrelatedness of CVD and CKD is to create a vicious cycle that further complicates morbidity and mortality outcomes.

In 2008, the Acute Disease Outcome Quality Initiative (ADOQI) introduced a classification system to characterize the joint relationship between the cardiac and renal system, also known as the cardio-renal syndrome (CRS). CRS is classified into 5 stages, each with specific primary and secondary causes as shown in Table 6.

Table 5.Cardio-Renal Syndrome Classification and Definition

CRS	Definition	Primary Event	Secondary Event
Acute cardio-renal (type 1)	Acute worsening of heart function (AHF–ACS) leading to kidney injury and/or dysfunction	Acute heart failure (AHF) or acute coronary syndrome (ACS) or cardiogenic shock	AKI
Chronic cardio-renal (type 2)	Chronic abnormalities in heart function (CHF-CHD) leading to kidney injury or dysfunction	Chronic heart disease (LV remodeling and dysfunction, diastolic dysfunction, chronic abnormalities in cardiac function, cardiomyopathy)	CKD
Acute reno-cardiac (type 3)	Acute worsening of kidney function (AKI) leading to heart injury and/or dysfunction	AKI	AHF, ACS, arrhythmias, shock
Chronic reno-cardiac (type 4)	Chronic kidney disease (CKD) leading to heart injury, disease and/or dysfunction	CKD	CHD (LV remodeling and dysfunction, diastolic dysfunction, abnormalities in cardiac function), AHF, ACS
Secondary CRS (type 5)	Systemic conditions leading to simultaneous injury and/or dysfunction of heart and kidney	Systemic disease (sepsis, amyloidosis, etc.)	AHF, ACS, AKI, CHD, CKD

Adapted from Ronco et al.,*(Open Access)* [55].

The mechanism involved in CRS begins with a failing heart, leading to reduction of cardiac output. In attempt to compensate for reduction in cardiac output, the heart expands and enlarges. That is why cardiac hypertrophy is a common feature among patients with CRS, occurring at the advanced stage of CRS [58]. Reduced cardiac output in turns leads to renal under perfusion accompanied with release of rennin. Renin leads to the release of angiotensin II, arginine vasopressin (AVP), aldosterone, and activation of sympathetic nervous system

(collectively referred to as the RAAS system: Renin Angiotensin and Aldosterone System). Activation of the RAAS system, in turn results in vasoconstriction and compensatory increase in systemic demand of increased cardiac output and increased renal perfusion. In the heart organ, the resultant effect is cardiac hypertrophy and the stretching of the heart muscles [58]. In the kidney, trans-membrane secretion of proteins is vital components for the normal functioning kidney. These proteins are produced by the endoplasmic reticulum ER [57]. The presence of cardiac hypertrophy stimulates the production of proteins by ER. Continuous and prolonged stimulation of ER may result in ER stress which further exacerbates cardiac hypertrophy, leading to reduction in cardiac output and subsequent congestive heart failure [58] and protein secretion.

5.0 CKD AND DIABETES

An estimated 360 million people worldwide have diabetes [65]. In 2004, 3.4 million people worldwide died from diabetes complications [65]. According to the World Health Organization (WHO), it is projected that deaths from diabetes will double by the year 2030 [65]. In U.S, diabetes is the seventh leading cause of death. About 25.8 million adults representing 8.3% of the U.S population have diabetes [66]. Approximately 18.8 million are diagnosed and 7 million undiagnosed. In 2005–2008, based on fasting glucose or hemoglobin A1C levels, 35% of U.S. adults aged 20 years or older had pre-diabetes [66]. Diabetes is increasingly recognized as a public health problem associated with increased mortality and co-morbid conditions including excess adiposity, dyslipidemia, CVD, stroke, limb amputation, blindness and kidney failure [66-68]. It has been established that diabetes is the leading cause of kidney failure among adults in U.S [66]. In fact, in 2008, diabetes accounted for 44% of new cases of kidney failure among adults in the U.S population [66]. According to the USRDS, in 2008, approximately 48.2% of patients with diabetic nephropathy were CKD stages 1 or 2, whilst 49.4% were stages 3 to 5 [68]. Even among those with undiagnosed or pre-diabetes, an estimated 17.7% have some level of kidney impairment [69]. As the number of people with risk factors contributing to diabetes rises in the general population, such as obesity and over nutrition [70], it is expected that the number of diabetic cases will continue to rise, and the incidence of diabetic nephropathy will likely increase as a consequence. Even though the prevalence of diabetes is high in U.S, African

Americans are disproportionately affected [71]. In 2010, 18.7% of all non-Hispanic blacks aged 20 years or older had either diagnosed or undiagnosed diabetes compared to 10.2% among non-Hispanic whites [72]. This trend correlates with high prevalence of diabetic complications in these populations. African Americans with diabetes are more likely, compared to other ethnic groups, to develop diabetic nephropathy [73]. In 2006, the rate of initiation of treatment for end-stage renal disease related to diabetes was 2.2 times among blacks compared to whites [74]. In a study by Young et al. African Americans with diabetes were more likely than whites to develop micro vascular complications, such as renal disease compared to whites [75]. Similar observation was made by Karter AJ et al, who reported significant differences in ESRD among ethnic minorities compared to whites [76]. Environment, lack of social and economic resources, access to healthcare and poor disease management are common among minorities and may be the reason among others, for the observed racial disparities. In 1986, the Office of Minority Health (OMH) within the National Institute of Health (NIH) was established to address poor health outcomes among minorities and bridge the disparity gap between minorities and their white counterparts [77].

A hallmark of diabetes is the presence of albuminuria, defined as protein creatinine ratio greater than 30mg/g. Presence of micro-albuminuria is an early indicator of proteinuria. While many with micro-albuminuria will revert back to norm-albuminuria spontaneously [78], approximately 30% of people with micro-albuminuria go on to develop proteinuria[79]. Albuminuria is regarded as a general marker of vascular dysfunction throughout the body, and a predictor of both micro-vascular disease such as diabetes nephropathy and retinopathy, and macro-vascular dysfunction such as CVD as suggested by Steno hypothesis [80]. Among several

causative factors, diabetes, hypertension, dyslipidemia and inflammatory mediators have been identified as the leading causes of albuminuria [80].

Not all diabetes is associated with albuminuria. But if present, albuminuria is a marker of diabetic nephropathy and also of progression to kidney failure [81-83]. The effect of diabetes on diabetic nephropathy depends on the diabetes type, and whether albuminuria is present or absent. Among type I diabetes patient with albuminuria, about 80% will progress to diabetic nephropathy whilst among Type II diabetic patients with albuminuria, about 20-40% will progress to diabetic nephropathy [82, 84, 85]. Although progression is influenced by many risk factors, hypertension is by far the most important factor influencing progression to kidney failure. In a study by Nielson et al, among patients with type 1 diabetes who have hypertension, approximately half of the patients developed kidney failure within a decade [86, 87]. That is why current clinical guidelines recommend the lowering of blood pressure to below 130/80 among diabetic patients [88]. Other factors that determine progression to kidney failure among diabetic patients include uncontrolled glucose levels, cholesterol, activated inflammatory mediators and a patient's genetic predisposition to kidney failure [89, 90].

It has also been established that diabetes is an independent predictor of CVD. Diabetic patients experience higher rate of CVD complications compared to non-diabetic [91]. According to National Cholesterol Evaluation Program (NCEP) report, diabetes is a risk equivalent of CHD, meaning that 20 out of 100 individuals with diabetes will develop CHD or have a recurrent CHD within 10 years [92]. In addition, several studies have shown that many patients with diabetes have generalized arteriosclerosis manifesting in vascular stiffness [80, 93, 94]. The combined effect of CKD and diabetes confers a higher risk of CVD outcomes and CVD mortality among patients with both diabetes and CKD, than among patients with diabetes or CKD alone. The risk

of CVD increases as the GFR declines among patients with diabetes. In a cross-sectional study to determine risk of CVD events among patients with diabetes, it was shown that the risk of CVD events was approximately 40% higher among those with CKD compared to those without CKD [69]. This finding suggests an interaction between diabetes and CKD with respect to CVD events. In a similar study, Mann FHE et al, reported a significant increase in CVD events resulting from interaction between CKD risk factors and diabetes [71]. In addition, the risk of CVD mortality among diabetic patients with reduced eGFR was further increased in the presence of uncontrolled high blood glucose, high blood pressure, high cholesterol mediated by inflammatory markers, which enhanced the degree of susceptibility to developing advanced diabetes nephropathy. Thus diabetes confers excess risk on CKD resulting in CVD events.

The mechanisms involved in diabetic nephropathy are multiple and complex. Distinct stages in the mechanism involve structural, hemodynamic and metabolic changes leading to diabetic nephropathy. Studies have established that diabetes and CKD are micro vascular diseases [95, 96]. It is generally believed that the process begins with early hemodynamic changes resulting in glomerular hyper-perfusion and hyper -filtration [97]. Hyper-filtration has been reported in as many as 66% of type 1 and 50% of type 2 patients with diabetes [98, 99]. In addition, most small arteries depend on myogenic response to auto regulate the diameter of blood vessels and blood flow. In diabetic patients, there is a loss of myogenic response resulting in dysfunctional auto regulation and blood flow. The loss of myogenic function is exacerbated by mild increases in blood pressure resulting in the frequent vascular disease often observed in diabetic patients and in patients with diabetic nephropathy [100, 101].Consequently, the loss of auto-regulation in small arteries leads to damage of the glomerular filtration barrier. Concurrent rise in blood pressure leads to elevation of glomerular capillary pressure causing hyper filtration

and permeability of protein across the membranes. With time, concomitant hyper filtration and high glomerular capillary pressure leads to capillary endothelium damage, fibrotic changes in connective tissues characteristic of diabetic nephropathy, glomerular damage, albuminuria and declining kidney function [102].

The complex relationships between CKD, diabetes, CVD and albuminuria are modeled in Figure 2. Three disease combinations observed in this model are: Cardio-Renal diseases (CVD & CKD), Diabetic Nephropathy (Diabetes & CKD) and Cardio-Diabetic diseases (CVD & Diabetes). The first two are well established, while the last have not been well documented. While CKD and CVD are bidirectionaly linked, there is no evidence suggesting a causal link from CKD to diabetes, although, the reverse is a well-established causal link as shown by one directional arrow from diabetes to CKD in Figure 2. The role of albuminuria in the triad is complex and controversial. Not all cardio-renal, diabetic-nephropathy, CKD, diabetes or CVD patients develop albuminuria [79, 103, 104]. However, studies have shown that whenever albuminuria occurs it is associated with increased risk of CVD and CVD mortality, diabetic nephropathy, decline in renal function, renal histological damage and increased risk of progression to ESRD [105, 106]. In view of these complexities, it is not clear whether albuminuria is a biomarker or a mediator in the causal pathways of the diseases in the triad. The biological plausibility of albuminuria as a mediator is rationalized through a suggestion that albuminuria is a pro-inflammatory molecule. Therefore, when reabsorbed by the renal tubule, albuminuria induces production of profibrotic- cytokines leading, to tubule-interstitial fibrosis and glomerulosclerosis [107]. Further studies are needed to validate this hypothesis. Meanwhile, much less controversial is the etiology of albuminuria, which many believes occurs as a result of generalized endothelial and vascular dysfunction [80].

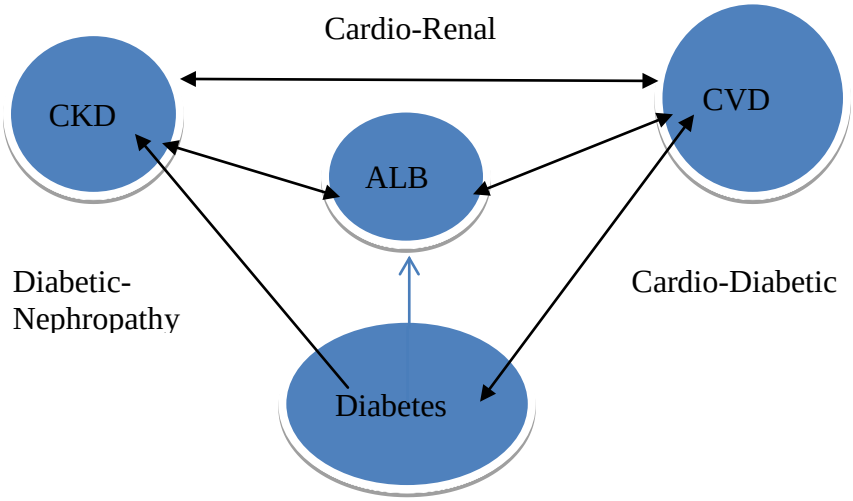

Figure 2. Model Linking Diabetes, CKD/DN, CVD and MA
Chronic Kidney Disease; DN, Diabetic Nephropathy; CVD, Cardiovascular Disease; ALB, Albuminuria.

6.0 CKD AND OBESITY

Excess body fat is a considerable cause of morbidity and mortality. A common measure used for determining and classifying excess body fat is Body Mass Index (BMI), obtained by dividing an individual's weight in (kg) by height in (m) squared. A BMI of 25 and less than 30 is defined as overweight whereas a BMI of 30 or greater is defined as obese [51, 108, 109].Worldwide in 2008, an estimated 1.4 billion adults and 40 million children were overweight[109]. Overweight and obesity are the fifth leading risks of death in human population [109]. Once common in developed western countries, overweight and obesity are now on the rise in low and middle income counties, and are associated with significant public health problems [109]. In U.S, obesity is an epidemic [51, 108]. One in three adults and one in six children and adolescents are obese [110]. The prevalence of obesity has more than doubled since 1980, in particular among young adolescents [108, 111] due mostly to changes in dietary habit and sedentary lifestyle. Prior forecast had noted that by the year 2030, the prevalence of obesity may reach 30% among children and adolescents and 50% among adults. But, a recent 2012 Center for Disease Prevention and Control (CDC) report indicates that the prevalence of obesity is leveling off at 17% among children and adolescent and 36% among adults [111].

The health consequence of obesity is enormous. Obesity has been found to reduce life expectancy by 6 years in men and 7 years in women [112]. It is associated with sleep apnea,

certain malignancies such as renal cell carcinoma, nephrolithiasis (kidney stone), hyperlipidemia, insulin resistance, diabetes, hypertension and cardiovascular disease (CVD) [113].

Early hints of the association between obesity and kidney function were introduced in the early seventies. In 1974, Weisinger et al, described four patients with massive obesity and nephrotic syndromes. Proteinuria was noted among these patients, and tended to decrease with dietary weight loss. Mesangial glomerulopathy (a form of kidney disease affecting the glomerulus) was noted among two of the patients. The study concluded that the observed proteinuria was as a result of renal venous hypertension [114]. Subsequent studies reported occurrence of glomerulosclerosis (scarring of the glomerulus, which are of two types: primary and secondary) with proteinuria among obese patients, which increased with increasing BMI [115, 116].

In recent years, several studies have examined the rising trend in CKD with the increasing prevalence of obesity [117, 118]. In 2004, Iseki et al found that BMI was associated with an increased risk of ESRD in men in the general population in Okinawa [119] after controlling for age, sex, systolic blood pressure, and proteinuria. Similar findings were made by Ejarbald et al, who showed that overweight at age 20 was associated with a three-fold excess risk for chronic renal failure [120], however, hypertension and diabetes were not adjusted for. Obesity has not only been noted as a cause of renal disease, but also to its deterioration [116]. Hsu et al found that high BMI was independently associated with ESRD and the risk of ESRD increased with rising BMI [121]. In all, there is growing evidence suggesting that obesity is a significant risk factor in CKD and progression to ESRD.

The term Obesity Related Glomerulopathy (ORG) refers to glomerular changes associated with obesity. ORG is characterized by proteinuria and kidney failure complications

[114]. The severity of ORG outcome is further amplified in the presence of hypertension or diabetes, which are known risk factors for CKD and CVD. Recent epidemiological studies show that the prevalence of ORG is on the rise, the trend of ORG parallels the rising trend in obesity and is an emerging epidemic [122].

Closely related to obesity are a group of risk factors collectively termed metabolic syndrome (MetS): diabetes, hypertension, high triglyceride, low HDL and abdominal obesity. According to the Adult Treatment Panel (ATP) of the National Cholesterol Evaluation Program (NCEP) guideline, a diagnosis of MetS is made when a combination of three or more of these risk factors are present [123] [124]. It has been well established that MetS is more likely to develop among obese individuals compared to non-obese. It has also been established that occurrence of these factors together increases the risk of CVD and diabetes. Many studies have now shown that MetS is also directly associated with CKD [125]. Each component of MetS is an independent predictor of CKD and the higher the number of MetS components, the greater the risk of CKD, thus, suggesting that MetS is a mediator between obesity and kidney failure [125].

Because a majority of people who are obese also have hypertension and or diabetes, it is not clear whether obesity and MetS can initiate CKD independent of hypertension and diabetes. In a study by Bonnet et al it was shown that among patients with IgA nephropathy, excess body weight was predictive of chronic renal failure [126]. In another to study to evaluate the outcome among recipients of kidneys from 37 living donors with obesity, it was reported that glomerular filtration rate was lower among patients who had organs from obese donors compared to non-obese donors [127].In the prevention of renal and vascular disease (PREVEND) study, among 7676 participants without diabetes, central obesity was associated with decreased kidney

function in obese and normal weights [128]. These findings suggest not only the association of obesity with CKD but also in initiating CKD.

Obesity itself is the result of excess calorie intake. Adipose tissues growth is a common feature of obesity. Not only does adipose tissues provide insulation in cold and heat and padding of organs, the locations and distribution have also been shown to predict health outcomes including CVD, diabetes and hypertension, which are known risk factors for CKD. The body fats located around internal organs in the trunk region called visceral fat(also referred as central obesity or central adiposity) has been shown to correlate well with MetS, while the fats located beneath the skin called subcutaneous fat (also known as peripheral fat) tend to have opposite effect [129]. Given the association of MetS with CKD, it is likely that central adiposity is also associated with CKD. Indeed, a number of studies have shown increasing risk of CKD with rising central obesity [120, 128, 130]. In the Framingham studies, it was shown that overweight and obesity is associated with increased odds of CKD, but after adjusting for CVD risk factors the effect disappeared [131]. This may have been due to use of BMI in excess-fat classification. BMI measures general-obesity but not abdominal adiposity which is assessed by waist circumference or waist hip ratio. It has also been shown that, because BMI is affected by muscle mass, central or abdominal obesity is a better predictor of health outcomes compared to BMI. It may also be that, CVD is a mediator in the relationship between obesity and CKD. Further studies are needed in this area.

Paradoxically, excess adipose tissue may be protective of patients with ESRD. Studies aimed at determining the protective effect of obesity among CKD patients undergoing dialysis are beginning to emerge [132, 133]. Kalanter et al, found that higher BMI (up to 45) and higher serum creatinine concentration were incrementally and independently associated with greater

survival, even after adjustment for nutritional status and inflammation [134]. This paradoxical relationship between obesity and CKD survival, termed “reverse epidemiology” is not fully understood. One explanation attributes it to Protein Energy Malnutrition (PEM), in the sense that, obese individuals with CKD undergoing dialysis suffer from malnutrition due to loss of protein. Therefore, high BMI compensates for the metabolic demands among these patients [132]. It has also been speculated that greater survival may be due to protective effect of inflammation which tend to be unusually common among obese individuals [133].While this theory may be plausible, the proponents failed to proffer possible mechanisms of action. However, large studies are needed to better understand the relationship and mechanism linking obesity to longer survival among ESRD patients.

Another common feature among overweight/obese individuals is dyslipidemia. Dyslipidemia is characterized by hyper-triglyceridemia, elevated level of very low density lipoprotein (VLDL), high plasma concentration of lipoprotein, accumulation of oxidized lipids and lipoproteins, low plasma HDL cholesterol concentration and impaired HDL maturation and function [135]. In obesity, the three common types of dyslipidemia are high triglycerides, low HDL and high LDL, which are the result of insulin resistance, caused in particular by central obesity [136]. Studies have shown that among patients with kidney failure, the level of triglycerides, HDL and LDL varies. The pattern of lipid profile among chronic kidney disease patients with or without proteinuria is shown in Table 7.

Table 6. Lipid/Lipoprotein Profile in CKD Patients and Proteinuria

Serum Lipid	CKD Patients	
	Heavy Proteinuria	Minimal Proteinuria
Triglyceride	↑	↑
Total cholesterol	↑	↔,↓
LDL-Cholesterol	↑	↔,↓ or ↑
Small dense LDL	↑	↑
IDL Cholesterol	↑	↑
HDL Cholesterol	↓	↓
apoA-1, apoA-11	↓	↓
apoC-111	↑	↑

Adapted from Vaziri et al., *(Open Access)* [135]. CKD, Chronic kidney disease; LDL, low density lipoprotein; IDL, intermediate density lipoprotein; HDL, high density lipoprotein; apo, apolipoprotein.

The role of dyslipidemia in arteriosclerosis formation begins when oxidized LDL Cholesterol is engulfed by macrophages leading to foam cell formation and subsequently forming atherosclerosis. The net effect is endothelial and epithelial tissue damage resulting in impaired glomerular filtration[135]. HDL plays a major role in mitigating this process by limiting LDL-Cholesterol oxidation by retrieving surplus cholesterol from vascular tissues for disposal in the liver through a process commonly known as reverse cholesterol transport.

The mechanism linking obesity with CKD is complex and not clear. It is important to note that in addition to being a fat storage site, adipose tissue is an endocrine organ. As such, adipose tissue is involved in the release of pro-inflammatory cytokines including leptin, interluekin-6, C - reactive protein and tumor necrotic factor, and anti-inflammatory factor including adiponectin. These pro and anti inflammatory factors regulate the activity of adipose tissue and its systemic effect. The inflammatory process begins with invasion of adipose tissue with macrophages which result in the release of cytokines and free fatty acids [135]. While cytokines tend to promote glomerulus kidney damage through expansion of the mesangial cells and destruction of podocytes, adiponectin acts in opposite manner to attenuate the effect of

cytokines by regulating the actions of reactive oxygen species (ROS) [137]. In addition to this mechanism, animal and human models have also shown that obesity is associated with renal hyperperfusion, hyperfiltration and hypertrophy [138, 139]. Adrenergic and rennin-angiotensin-aldosterone systems as well as glucocorticoids are stimulated in the process, resulting in afferent arteriolar dilatation and efferent arteriolar vasoconstriction due to stimulation of the rennin-angiotensin system [140]. These in turn lead to increased renal tubular reabsorption, volume expansion and increased blood pressure and glomerular damage.

In all, the constellations of inflammation, oxidative stress, hemodynamic changes, lipid disorders coupled with metabolic abnormalities of insulin resistance, diabetes mellitus and hypertension [141] together, are largely responsible for the atherosclerosis process resulting in endothelial dysfunction, renal cell damage leading to CKD and kidney failure in obesity [142].

7.0 CKD-MINERAL BONE DISORDER (CKD-MBD)

Bone and mineral disorder affects a substantial number of people in the U.S population, in particular dialysis patients[143]. The first awareness about bone disorders in dialysis patient was reported by Pendras and Erickson in 1966 [144]. Since then, there is increasing body of evidence showing that CKD is associated with bone and mineral disorders. Levin et al., showed that patients with CKD stage 3-5 developed bone mineral disorders compared to controls without CKD [145]. In a similar study, Melamed et al., reported a high prevalence of bone mineral abnormalities including calcium, phosphorous and parathyroid hormone PTH, among stage 5 renal patients [146]. In U.S, the incidence of bone disorders is on the increase, and it is paralleled by a rising number of CKD patients and a rising elderly population who also have a high risk of CKD [147].

Before 2005, bone disorders ensuing from renal disease were collectively termed renal dystrophy. In 2005, a KDIGO conference understudied the effect of renal system on bone and recommended a new system of classification. Currently, renal dystrophy refers to the various bone pathological and histological disorders, including osteitis fibrosa, osteomalacia, osteoporosis and adynamic (osteodystrophy characterized by reduced osteoblast and osteoclast activities) bone diseases that may be associated with the renal system, while Chronic Kidney Disease – Mineral & Bone Disorder Disease (CKD-MBD) refers to broader effect of CKD on bone, which includes mineral disorders, bone disorders and cardiovascular disease [148].

7.1 CKD-MINERAL BONE DISORDER-MINERAL DISORDERS

Animal and human models have shown that the effect of CKD on bone is largely mediated by imbalances in serum minerals and certain biochemical compounds. The majority of these imbalances begin from CKD stage 3 when the eGFR drops below 60ml/min/1.73/m^2 and gradually worsens as eGFR falls below 15ml/min/1.73/m^2 [149]. In children, minerals and biochemical imbalances have been reported as early as stage 2 of CKD [150]. The mineral and biochemical regulators involved in bone metabolism include mainly calcium, phosphorous, vitamin D and parathyroid hormone (PTH). Others include alkaline phosphatase (ALP), vitamin D receptors (VDR) and most recently, fibroblast growth factor -twenty three (FGF-23). These regulators are all interrelated and highly integrated. Whenever any event alters the level of one of the components, the levels of the others adjust to maintain constant balance and homeostasis. Also, there is increasing evidence suggesting that each factor alone, may independently predict the risk of mortality in CKD patients, For instance a study found that increased ALP independently predicted the risk of mortality among stage 5, CKD patients [151, 152].

7.1.1 CALCIUM

Calcium is predominantly stored in the bone and regulated by PTH. About 99% of calcium is stored in bone matrix while only 1% circulates in the extracellular compartment as either ionized (the active form) or un-ionized (less active form bound to albumin) calcium [153]. Approximately half of serum calcium is in the active, ionized form, while the remaining half is

bound to albumin. To correct for albumin-bound calcium, 0.8mg/dl is added to every 1 mg/dl decrease in serum albumin below 4mg/dl [153].

Serum calcium is absorbed from intestine or released from bone. Vitamin D aids in intestinal absorption of calcium. CKD is associated with vitamin D deficiency [154, 155] which in turn is associated with low serum calcium, increased serum phosphorous and elevated PTH, leading to bone calcium resorption.

7.1.2 PHOSPHOROUS

Phosphorous is a vital component of cellular membranes and nucleic acid, and essential for many cellular functions. Phosphorous is also a component of bone matrix. Like calcium, approximately 85% of phosphorus is stored in intercellular compartments of bone [156]. Serum phosphorous is obtained from bone and through intestinal absorption, regulated by vitamin D. Low serum calcium and vitamin D results in elevation of serum phosphorous (also called hyperphosphatemia). Elevated serum phosphorous in turn, is associated with release of PTH. Because of the impaired capacity of the kidney to filter phosphorous in individuals with CKD and ESRD , hyperphosphatemia is worsened, leading to further elevation of PTH [156]. High levels of phosphorous and PTH are associated with high risk of mortality among CKD and dialysis patients [157]. It has also been established that hyperphosphatemia is associated with precipitation of calcium along soft tissues including arterial vessels, which increases the risk of vascular calcification among CKD patients [158].

7.1.3 VITAMIN D

Vitamin D is a compound name of two related forms of vitamins: vitamin D2, and vitamin D3. The metabolism of each is initiated in the liver, then, converted by a complex biochemical reaction in the skin to 25-hydroxyl vitamin D (also known as calcidiol). In the kidney, calcidiol is converted to 1-25-hydroxyl vitamin D (calcitriol: active form of vitamin D) by the action of alpha-hydroxylase enzyme [36].The first effect in onset of CKD, when eGFR falls below 60ml/min/1.72m^2, is a deficiency of vitamin D[36]. This occurs as a result of reduction or absence of kidney alpha-hydroxylase which catalyzes the final hydroxylation step of calcidiol to calcitriol (Vitamin D).

Because vitamin D is required for intestinal calcium absorption, deficiency of Vitamin D results in calcium depletion which in turn increases serum PTH, thereby leading to bone calcium resorption. In addition to aiding intestinal absorption of phosphorous and calcium, vitamin D also controls the release of PTH through the mediating action of vitamin D receptors (VDR), located in the parathyroid gland. To achieve this, vitamin D forms a complex with VDR. The (calcitriol/VDR- complex) effectively blocks transcription of PTH genes, inhibiting PTH production, while at the same time, increasing intestinal calcium absorption [159, 160]. The role of VDR has been confirmed in several studies, Cozollino M. et al. showed that, administering VDR activators (a vitamin D analog, given to patients who have low or lacks VDR e.g. paricalcitol) resulted in increased serum vitamin D and reduced serum PTH among CKD patients [161]. A similar study also showed that VDR activator among CKD patients with secondary hyperparathyroidism (SHPT) resulted in lower serum PTH and better health outcomes [162].

7.1.4 PARATHYROID HORMONE (PTH)

PTH is produced by parathyroid gland. Elevated serum PTH is common among CKD patients and has been shown to be associated with CVD mortality [163, 164] and bone disorders [165, 166]. Serum PTH is regulated by calcium, phosphorous and vitamin D. In fact, among bone minerals, serum calcium is the main, minute -by- minute regulator of PTH. In early stages of CKD, the parathyroid gland becomes hyperactive resulting in excess PTH production, a condition known as hyperparathyroidism [167]. Continual rise in serum PTH results in secondary and even tertiary hyperparathyroidism, characterized by parathyroid hyperplasia and, elevated serum phosphorous and calcium [168].Studies have shown that, secondary and tertiary hyperparathyroidism are associated with increased risk of vascular calcification and CVD mortality among CKD patients [169-171].

7.1.5 FIBROBLAST GROWTH FACTOR-23 (FGF-23)

FGF-23 has received considerable attention as a regulator of vitamin D and phosphorous, and therefore of PTH and bone mass. Several studies have shown a correlation between increasing stages of CKD and increasing serum FGF-23 [172-174]. The mechanism underlying the action of FGF-23 is not fully understood. But, animal models show that in early stages of impaired kidney function, high serum FGF-23 regulates phosphorous by promoting elimination of phosphorous in the urine and blocking resorption of phosphorous from the tubules[175, 176]. However, in the later stages of impaired kidney function, increased serum FGF-23 decreases the level of alpha hydroxylase, the enzyme responsible for converting 25-Hvdroxyl vitamin D to calcitriol [176]. Deficiency of vitamin D in turn results in impaired intestinal phosphorous and

calcium absorption and increased serum PTH production, which further reduces serum phosphorous [177].

Thus, by its action, FGF-23 appeared to be protective in early stages of CKD, by triggering an adaptive response for maintenance of phosphate balance. The extent to which FGF-23 maintains mineral homeostasis is limited in early CKD. A study found that, FGF-23 was no longer responsive to mineral imbalances in patients with hyperparathyroidism with advanced stage of CKD and on dialysis or have been on long time dialysis[178].The reason for FGF-23 unresponsiveness at advanced stage of CKD is not known and warrants further investigation. However, some theory suggests that it may be due to declining levels of *Klotho* (a FGF- 23 co-receptor aiding FGF-23 expression) or to fewer functional nephrons for filtering phosphorous [178]. But, none of these mechanisms have been confirmed in separate studies.

7.1.6 ALKALINE PHOSPHATASES (ALP)

A majority of ALP is found in liver and bone. The action of ALP is tissue specific. There are two types of ALP (a-ALP and b_ALP). b-ALP enzyme is specific to bone. A product of the action of ALP is phosphate ions released from phosphate containing compounds. High b-ALP results in elevated PTH and b-ALP is an indicator of bone mineral disorders. ALP along with PTH is applied in clinical practice to monitor the rate of bone turnovers. A recent study found that ALP, was associated with a significant increase in the hazard of all-cause mortality among hemodialysis (HD) patients with secondary hyperparathyroidism [179].

7.2 CKD-MINERAL BONE DISORDER: BONE DISORDERS

CKD-MBD bone disorder is a condition emanating from mineral and biochemical imbalances of calcium, phosphorous, vitamin D and PTH. Low serum calcium or vitamin D and high phosphorous promotes release of PTH and hyperparathyroidism. High circulating PTH in turn triggers bone resorption of calcium. Persistent occurrence of bone resorption leads to bone loss and ultimately affects rate of bone turnover, volume and mineralization. Bone disorder is further worsened among CKD and ESRD patients, who are unable to produce vitamin D and filter high serum phosphorous due to impaired kidney function [36, 145].

Bone disorder is associated with several complications including fracture, infection, bone-pain, retarded growth in children, abnormal height and brittle bones (also known as renal rickets). The most common complication of bone is fracture, and is associated with increased mortality. The factors determining bone fracture includes previous fracture, age, gender and stage of CKD [180-184]. Danese et al, showed that the risk of a new fracture was seven times higher among patients with CKD stage 5 who had previous fracture compared to those without fracture [185]. In a similar study, Mittalhenkle et al., showed that mortality from fracture was 2.15 times higher among CKD patients compared to controls and it is independent of age and gender [186].

Bone is composed of cortical and trabecular bone. Trabecular bone is porous and spongy compared to hard cortical bone. The forearm is made up of cortical bones while the spine and femur are made up of 42% and 25% trabecular bone respectively [187]. These findings are important, because studies have shown that CKD patients with elevated PTH tend to have bone composition made up of greater quantity of trabecular bone than of cortical bone[188], which may be influencing bone metabolism among individuals with CKD.

7.3 CKD-MINERAL BONE DISORDER: CARDIOVASCULAR DISORDERS

In general, calcification, which means deposition of calcium, is a highly regulated process involving the bone and teeth. However, calcification outside of these structures (extraosseous calcification) often occurs. Among many sites, vascular tissues are the most common sites, especially the arteries. Arterial calcifications are involved in formation of atherosclerotic plaques, in turn associated with increased risk of CVD and CVD deaths [189]. In dialysis patients, arterial calcification is a common feature [190, 191]. There is increasing evidence suggesting arterial calcification occurs in earlier stages of CKD as well. In fact, a pooled study of about twenty five reports containing over 4,000 participants, showed that about 47-83% of patients in stage 3-5 CKD have cardiovascular calcification [192].

The pathophysiologic effect of vascular calcification leads to stiffening of vascular walls. In CKD patients, the media and intima layers of arteries are affected, resulting in restricted flow, increased pulse wave velocity and elevated pulse pressures.

The mechanism involved in calcification of arterial wall among CKD patients was originally thought to be a passive processes involving calcium ion precipitation from serum calcium super-saturation as well as increased calcium phosphate product (Ca × P). Recent studies have shown that vascular calcification is also an active process dependent on some biochemical promoters and inhibitors, the most common promoter being phosphorous. Others include osteopontin, osteocalcin, bone morphorgenic protein (BMP), sialoprotein, type 1 collagen and ALP. The main inhibitors are Fetuin A, and Osteoprogetrin (OPG) [193-198]. In CKD patients, inhibitors are down regulated while the promoters are up regulated [194]. In an *ex vivo* experimental study, it was discovered that up-regulation of biochemical inducers of osteogenic

cells caused differentiation of vascular smooth muscles into Osteoblast-like cells, which in turn promoted calcification of arterial walls[194].

The role of bone in vascular calcification has received considerable attention. Vascular calcification is associated with bone demineralization and loss among dialysis patients [199]. Recent studies among dialysis patient also show that vascular calcification was related to bone loss and osteoporotic fracture [200]. These findings suggest that bone loss is associated with vascular calcification. Further studies are needed to determine if indeed there is a causal link between bone loss and vascular calcification among CKD patients independent of other risk factors including age.

8.0 CKD AND HEREDITY

CKD/ESRD results from diverse biological processes in interplay with environmental and behavioral factors. However, certain aspects of CKD/ESRD are hereditary. One of the commonest and earliest known genetic disorders of kidney is polycystic kidney diseases (PKD). The heritability of PKD was first documented in 1957 by Dalgaard OZ[201], since then, several family studies have increasingly reported the role of hereditary in kidney disease and declining kidney function. Ferguson et al. demonstrated that individuals with family history of CKD had a significant risk of being on dialysis [202]. Similar findings were made by Seaquist et al, who also showed that diabetic nephropathy occurred in family clusters [203]. Modern genetic techniques and approaches, including Linkage Studies, Mapping by Admixture Locus Disequilibrium (MALD), Association Studies, and Genome –Wide Association Studies (GWAS) are increasingly applied to further understand the genetic bases of kidney diseases and impaired kidney function in the general population.

Inherited kidney diseases arise from mutated genes occurring at one or several sites in the genome and subsequently transmitted to offspring. While some genes, such as PKD genes, are expressed by simple Mendelian transmission from parents to offspring, others involve complex interaction with other genes, environment and lifestyles. Mendelian and complex inherited kidney diseases are rare and have low penetrance in human population. Nonetheless, several

candidate genes, and single nucleotide polymorphism (SNP's) have been implicated in the spectrum of kidney diseases affecting humans [204].

Much of kidney diseases disproportionately affect blacks compared to whites. The prevalence of kidney disease is approximately four times higher among African Americans compared to European Americans [205]. Despite controlling for social and environmental factors, African Americans presents with far greater risk of CKD and ESRD compared to European Americans. The reason for the apparent disparity is not fully clear, but there is a high possibility that it may be due to genetic differences in expression of kidney diseases among blacks and whites. Indeed, studies have shown that candidate genes and SNP's of kidney disease are clustered in the African American population. Freedman et al, showed that familial history of CKD and ESRD was associated with nine fold increased risk of CKD/ESRD among those with family history compared to matched controls without family history of CKD/ESRD[206]. Lei HH et al., also reported a familial aggregation of CKD independent of race [207], however, Spray BJ et al, found that familial aggregation was not as strong among whites as in blacks [208]. In all, there is strong evidence supporting familial aggregation of CKD which tend to be stronger in blacks than in whites.

It has been suggested that African Americans and Caucasians may not share similar pathophysiology or etiology for CKD/ESRD. Evidence of this assertion was hinted by Appel LJ et al., showing that despite beneficial effect of ACE therapy in slowing hypertensive CKD progression in the general population, a majority of African Americans continued to progress to CKD/ESRD [209]. Bleyer AJ et al., also reported that, arteriosclerosis formation was more strongly associated with ESRD among whites compared to blacks [210]. These findings suggest that two different mechanisms that are race-dependent may be involved in CKD/ESRD,

explained by genetic differences among blacks and whites. However, additional studies are needed to confirm this hypothesis.

8.1 PKD GENES

According to the National Kidney Foundation, PKD is the most common life-threatening genetic disease[211]. Two genes are associated with PKD: PKD1 and PKD2. PKD1 is located on chromosome 16 [204], while PKD2 is located on chromosome 4. PKD affects approximately 12.5 million people of all race and gender worldwide including approximately 600,000 people in U.S [211]. PKD is characterized by cyst formation in kidney and other organs, progressive decline in kidney function and kidney failure. It is estimated that approximately 5-8% of all ESRD cases requiring dialysis are due to PKD [201].

There are two types of PKD: Autosomal Recessive PKD (ARPKD) and Autosomal Dominant PKD (ADPKD). ADPKD is more common and occurs among adults. Approximately 85% of PKD is due to ADPKD, while 15% of PKD is due to ARPKD[211]. ADPKD and ARPKD are passed from parents to offspring in a simple Mendelian fashion. If one parent is affected with ADPKD, the offspring have a 50% chance of developing PKD whereas in ARPKD, if both parents are carriers of ARPKD genes, their offspring will have a 25% chance of developing PKD [211].

8.2 SINGLE NUCLEOTIDE POLYMORPHISMS (SNP)

In 2008, Kao WH et al., and Kopp JB et al, showed that MYH9 candidate gene is associated with kidney disease [212, 213]. To date, several other candidate genes and Single Nucleotide Polymorphisms (SNP) have been linked or associated with genes linked to kidney disease and declining function. These SNP's includes Uromodulin (UMOD), Shroom Family Member 3 (SHROOM3), Spermatogenesis Associated 5-Like 1 - Glycine Amidinotransferase (SPATA5L-GATM), Jagged 1 (JAG1), Stanniocalcin 1 (STC1), Cystitis-C (CST) , Engulfment and Cell Motility Protein 1 (ELMO1), PVT1, CNDP1, CNDP2,ENOS, ACTN4 , Chromogranin-A Gene Polymorphisms (*CHGA*), EPO, SOD1, CARS, NPHSI, NPHS2, PLCEI, TRPC6, TCF7L2, MMP1, UCP2 [214] and most recently, APOL1 gene [215]. A majority of these SNP's have not been replicated in large population studies. Therefore, their degree and strength of association with CKD and ESRD have not been validated.

SPATA5L-GATM and CST are involved in creatinine and cystatin-C synthesis respectively. They do not play a role in kidney function decline. CHGA is associated with ESRD among African Americans. Table 8 shows select genes and SNP’s associated with diabetic nephropathy, non-diabetic nephropathy and renal function.

Table 7. Genes Associated With Diabetic, Non-diabetic Nephropathy and Renal Function

Diabetic Nephropathy	Non- Diabetic Nephropathies	Kidney Function Regulation
EPO	MYH9	UMOD
SOD1	NPHS1	SHROOM3,STC1
CARS	CHGA	TCF7L2
PVT1	NPHS2	MMP1 and UCP2
ELMO	PLCE1	
CNDP1,CNDP2	ACTN4	
eNOS	TRPC6	

Source: Divers J. et al. [204].

8.2.1 MYH9 GENE

MYH9 gene, located on chromosome 22, is the first candidate gene linked to kidney function. MYH9 polymorphism is found mostly among non-diabetic nephropathy patients. In fact, MYH9 polymorphism is responsible for approximately 70% of the non-diabetic ERSD among blacks. The major risk haplotype of MYH9 is E1, present in approximately 60% of African Americans, 36% of whom carry two copies of the homozygous haplotype (E1)[212, 213]. In contrast, only about of 4% of whites carry MYH9 gene [216]. Future studies should aim at comparing the distribution of MYH9 gene among Tobago and U.S black males, in particular non-diabetic nephropathy patients.

Evidence shows that haplotype E1 may be involved in gene-environment interaction. For example, while about 4-5% of the patients with homozygous condition will develop nephropathy, about 20% will develop nephropathy in the presence of HIV and two E1 haplotypes, suggesting a possible gene-environment interaction of HIV and MYH9. About 14 MYH9 SNP's have been identified and all are associated with non-diabetic ESRD. The spectrum of kidney diseases resulting from MYH9 includes Focal Segmented Glomerulo-Sclerosis, HIV – associated-FSGS and hypertensive nephropathy. When MYH9 genes are expressed, it results in production of non-muscle myosin heavy chain 9 proteins, which are found in almost all extracellular compartments. The function of this protein is to maintain cell function and structure. The effects of the non-muscle myosin heavy chain 9 proteins in the kidney are not fully understood, but it has been suggested that it disrupts and depletes podocyte formation in the glomerulus [214] leading to declining kidney function.

8.2.2 UMOD GENE

UMOD gene encodes the most abundant protein in urine. Its mechanism of action is not fully understood, but it has been shown that UMOD gene is significantly associated with declining kidney function among all types of patient including diabetic and non-diabetic CKD patients [214].

8.2.3 APOL1 GENE

APOL1 gene is relatively newly studied, common, highly penetrant, and found in humans and a few primates [217]. APOL1 gene is located on chromosome 22 near the MYH9 gene. MYH9 has been shown to exert strong effect in FSGS, hypertensive-ESRD and HIV-nephropathy [218, 219]. Therefore, the proximity of APOL1 genes to MYH9 gene, suggests that FSGS, hypertensive-ESRD and HIV- nephropathy may share similar etiology, mediated by APOL1 and MYH9 gene. APOL1 gene is made up of two variant alleles: G1 and G2. G1 is formed by two base substitutions, and G2 is formed by deletion of two amino acid residues which ultimately alters sequence of APOL1 amino acids. Both alleles are recessively inherited, and have been found to be more common in the African American population compared to other racial groups. Studies are beginning to emerge suggesting that APOL1 gene may be partly responsible for the high rate of kidney disease among African Americans. Indeed, approximately 50% of African Americans have been shown to have either G1 or G2 alleles alone, while 10-12% is reported to have both G1 and G2 alleles.

The origin of APOL1 has been examined. It is widely believed APOL1gene arose from adaptive mechanism against Trypanosome Brucei (bacteria that causes sleeping sickness,

common in Africa). Protection from T. Brucei in nature is achieved by the lysing action of APOL1 on T. Brucei. However, in the course of evolution, a new resistant strain, T. Bruce Rhodesianse, emerged (TBR). TBR is resistant to the lysing action of APOL1 and over time, G1 and G2 variants of APOL1 gene emerged to confer selective advantage and protection against TBR [220, 221]. The likelihood of the presence of APLO1 gene variant depends on ancestry. while it has been established that APOL1 gene variants are present in large populations of West Africans, those in east Africa, such as Ethiopia, show no evidence of APOL 1 gene variants [222].

The mechanism involving APOL1 in kidney function decline is not understood. APOL 1 has been linked to cell apoptosis, autophagy, ion-channel function and lipid biology. Therefore, it is probable APOL1 action may be mediated by one or combinations of these pathways. Further studies are needed to verify this hypothesis.

Discoveries of candidate genes, SNP's of kidney diseases and their plausible biological pathways are still on-going. The task is challenging and may require many years. However, with the advent of new methodological approaches and improvement in existing ones, susceptible genes of kidney disease and function will be discovered. As such, new preventive measures focusing on susceptible genes may be applicable in reducing kidney diseases worldwide, in particular among blacks.

9.0 CKD AND OTHER HEALTH OUTCOMES

CKD is a continuum and in many cases, it is accompanied with co morbid conditions with considerable burden on patients. CKD co morbidity includes anemia, depression, infection, anxiety, dementia and physical disability. Some occur before the stage of kidney failure and others occur after kidney failure and initiation of dialysis. Of considerable importance are behavioral risk factors including diet, cigarette smoking and alcohol abuse in kidney disease initiation and complications.

9.1 CKD AND ANEMIA

Anemia is characterized by imbalance in hemoglobin level. Anemia occurs when the rate of hemoglobin or red blood cell depletion outpace its production in the bone marrow leading to hemoglobin deficiency. According to WHO, anemia is blood hemoglobin less than 12g/dl in non-pregnant women and 13g/dl in men [223]. The National Kidney Foundation (NKF) defines anemia as hemoglobin less than 12 g/dl and 13.5g/dl in females and males respectively[224]. Anemia is associated with several health outcomes including increased mortality, weakness, fatigue, physical and mental impairment, dyspnea and impaired cognition [225-228].

The prevalence of anemia tends to increase with increasing age especially among men. By WHO criteria, an estimated 11% of men and 10.2% of women 65 years or older, have anemia

in the U.S. population [229]. Other factors affecting hemoglobin deficiency includes altitude, smoking, pregnancy and race [223]. Blacks have higher prevalence of anemia compared to whites. Guralnik et al., found that among blacks, the prevalence of anemia is 27.5 % in men and 28% in women, whereas among whites the prevalence of anemia is 9.2 % in men and 8.7% in women [229]. Other studies have also demonstrated anemia prevalence rate of about 3 to 4 times higher among blacks compared to whites[230, 231].

Anemia is a complication of CKD [229]. There are no clear cut eGFR values that are associated with increased risk of anemia among CKD patients. However, according to KDIGO, anemia in CKD is diagnosed in adults greater than 15 years old when hemoglobin levels falls below 13.0g/dl in males and 12.0g/dl in females[232]. Anemia further complicates the strongly coupled relationship between CKD and CVD and CKD and diabetes. In fact, a study found that individuals with CKD and CVD have increased mortality and CKD progression in the presence of anemia compared to those without anemia [233, 234]. Another study also showed that anemia increases the risk of mortality and worsening of CKD among diabetic-CKD patients [235, 236]. An estimated one in five diabetic-CKD patients have anemia and the risk of mortality increases with increasing stage of CKD [237-239].

The causes of anemia are multifactorial and they include nutrient deficiency, chronic disease and inflammation. In CKD, the contributing factors are iron and erythropoietin deficiency. Both factors are known to have anti-apoptosis and anti-inflammatory properties [240]. Iron-deficiency is common among individuals with CKD. A study reported iron deficiencies among 50% of CKD patient in stages 2-5 of CKD [241]. The mechanism linking CKD to anemia is not fully understood, but involves inflammation, micro-vascular damage of the bone marrow and release of cytokines, leading to erythropoietin unresponsiveness and iron

deficiency [242, 243] among individuals with kidney failure. Further loss of proteins, iron and erythropoietin due to kidney damage leads to further iron and erythropoietin deficiency and anemia [244].

Replenishment of iron is accomplished by administering erythropoietin stimulating agent (ESA). However, studies on the effect of ESA have generated mixed result. While some found benefits others reported toxic effects [245, 246]. In light of these findings, NKD recommend hemoglobin target range not exceeding 13.0g/dl for optimal performance of ESA [25].

9.2 CKD DEPRESSION AND MENTAL HEALTH

The psychological state of individuals with CKD has been studied extensively. Merrill FE and associates found that low eGFR was associated with cognitive function impairment and that higher creatinine was associated with lower cognitive function even after adjusting for demography, CVD and Stroke [247]. A similar study also found a graded inverse relationship between CKD stages and cognitive function outcome measures [248]. Several other studies have shown similar inverse relationship between CKD and cognitive functions [249-251]. The effect of CKD on mental health is so profound, that the term “psycho-nephropathy” was introduced by Levy et al to underscore impact of CKD on mental health [252].

Mental health is a complex condition with different phenotypes. The most common mental health condition among individuals with CKD or ESRD is depression and anxiety [253, 254]. In U.S, the precise estimate of individuals with CKD and mental depression is unknown. Several studies have found a range between 30-46% [255-257]. Kutner et al, Taskapan et al and Cukor et al, each found prevalence rates of 45, 30 and 45.7 % of depression respectively among

ESRD patients. In a systematic review of 59 studies, Murtahgh FE et al, showed the prevalence of anxiety ranged between 12-52% [258]. Experts believe the rate of depression and anxiety may be much higher in U.S. due to under diagnosis resulting from barriers preventing access to healthcare and treatment [259].

There are no data showing prevalence of anxiety and depression in the milder stages of CKD. However, Hailpern SM et al, showed that moderate CKD was associated with functional cognitive abilities relating to learning, concentration and visual attention [249]. In a similar study, Seliger SL et al, also showed that moderate renal impairment was associated with dementia even among men in good health with moderate CKD [260].

In addition to the existing mental health burden on individuals with CKD, studies show that many are also faced with several social behavioral problems, including suicide, substance abuse, low quality of life and adherence to therapy [261-264]. While the mechanisms for these behavioral issues are not fully understood, it is plausible that the degree of hope among individuals with CKD may be related to depressive and anxiety moods impacting mental health. Other theories suggest neuropathological changes in the brain caused by oxidative stress, inflammation and micro vascular disease [251, 265], similar to the mechanism involved in atherosclerosis. But further research is needed to validate these hypotheses.

9.3 CKD, PHYSICAL DISABILITY AND MUSCLE WASTING

Muscle wasting is a significant cause of disability in diseases including chronic kidney disease. Occurrence of muscle wasting leads to many complications including sedentary lifestyle, decreased quality of living which further promotes and complicates CKD and CVD

mortality. Under normal circumstance, the skeletal muscle is in a state of homeostasis and therefore maintains a balance of muscle mass according to the Schoenheimer theory [266]. However, when catabolic conditions exist, far greater amount of muscle proteins are broken down to amino acids resulting in lower muscle mass[267]. In CKD patients, the systems that have been identified to play a significant role in protein breakdown in muscles include the Cathespin and the calcium-dependent Calpain, and Ubiquitin-Proteasome Systems (UPS). The UPS is by far the most active system involved in muscle wasting among CKD patients [268].

9.4 CKD AND INFECTIONS

CKD often coexists with other conditions including diabetes, proteinuria, physical and functional impairments, amputation and cognitive function. In the clinical settings, many who are receiving dialysis have vascular access [269], which are sites of possible pathogen entry. During dialysis, blood comes in contact with equipment's that are reused among several dialysis patients who also have a high rate of contact with hospitals and clinics [53]. This increases the chances of nosocomial infection. In addition, studies have shown that CKD patients have a lower rate of response to vaccines and declining levels of antibody compared to non-CKD patients [270]. Very little is known about bacteria, viral and fungal infections in the early stages of CKD. However, among patients with ESRD, studies have shown that, urinary tract infection, pneumonia, sepsis (from abscess formation) are the leading causes of hospitalization and mortality [271]. Pneumonia and septicemia are associated with 10 and 100 fold increases in mortality respectively [272, 273] and are responsible for longer length of hospital stay compared to the general population [274]. The risk factors predisposing CKD patients to infections include

age, immunosuppressive therapy, anemia, uremia and malnutrition [269, 275, 276]. These factors singly or in combination, results in alterations of the white blood cells, monocyte and lymphocytes, leading to impaired response to infection [277, 278].

The role of Hepatitis C virus in CKD has received considerable attention in recent time. A study found that HCV positive patients have a 40% higher likelihood of developing renal insufficiency compared to sero-negative patients [279]. The chances of CKD progression to ESRD are higher in HCV patients due to other co-morbid illnesses [280]. Little is known about the prevalence of Hepatitis C in early stages of CKD, but, Hepatitis C is common in ESRD patients on dialysis. In U.S, an estimated 14% of ESRD patients are reported to have Hepatitis C [281], in particular stage 5 CKD patients on dialysis. In general, approximately 60-85% [282] of those with Hepatitis C will develop the chronic form of Hepatitis C and about 5-25% [283] will develop cirrhosis, associated with increased risk of liver cancer. The degree of susceptibility to infections depends on several risk factors including age, immunosuppression, obesity, alcohol consumption, coexisting illnesses, malnutrition and nephritic syndrome [269, 275, 276, 284-286].

Although it is a common knowledge that a majority of Hepatitis C infections in ESRD patients are acquired from contaminated equipment and blood transfusion in hospitals and clinics[287, 288], there is increasing evidence suggesting Hepatitis C may be an initiator of CKD [289]. The most common Hepatitis C related infection among CKD patients is Membranoproliferative Glomerular Nephritis (MPGN)[290]. MPGN is mediated by cryoglobulin, a protein that precipitates other serum proteins, and it is invoked as an immune response to Hepatitis C infection in the body [289, 291]. Because of persistent Hepatitis C infection, cryoglobulin are deposited in mensangial cells and capillary vessels of the kidney. Not

all patients with cryoglobulin will develop MPGN [292]. Only about one third of patients with cryoglobulin will develop MPGN. The selective action of cryoglobulin is not fully understood. Cryoglobulin also affects other tissues including the skin and nervous system [292]. The selective action of cryoglobulin in MPGN formation may be due to selective preference for the skin or nervous system tissues. Further studies are needed to confirm this theory.

Other forms of CKD that have been associated with Hepatitis C include IgA nephropathies, post infectious glomerulonephritis, membranous nephropathy, thrombotic nephropathy, focal and segmented glomerulosclerosis, fibrilary and immunotactoid glomerulopathy[[293].

9.5 CKD, SMOKING AND ALCOHOL

9.5.1 TOBBACO SMOKING

According to the report of the Surgeon General, smoking harms nearly every organ of the body including the kidneys[294]. Smoking is the leading preventable cause of disease and mortality in U.S, resulting in about 440,000 deaths annually [294]. Tobacco smoke is known to contain over 4000 chemicals and 400 toxins, in particular cadmium and lead which are easily accumulated in the kidney and are toxic to kidney at low dosages[295].

The first evidence of impact of smoking on CKD risk was observed in 1978 by Dales and associates [296]. Dales et al, observed proteinuria, appeared in heavy smokers compared to light smokers. Since then, several studies have confirmed this finding. In the Prevention of Renal and Vascular End Stage Disease (PREVEND) study, Pinto-Sietsma et al showed that rate of urine

albumin excretion was associated with number of cigarette smoked, even after adjusting for potential confounders[297]. Similar result was shown by Halimi et al, who also observed irreversible kidney damage as a result of tobacco smoke [298]. In another study, Beyer et al showed a correlation between the number of cigarette consumed and progression of CKD [299].

Diabetes and hypertension are established risk factors of CKD. In view of the health effects of smoking which includes hypertension, diabetes [300], it is plausible diabetes, hypertension and cigarette smoking may be interacting to further elevate risk of CKD and CKD progression. Indeed, studies show cigarette smoking hastens the risk of CKD and CKD progression among diabetic and hypertensive patients faster, compared to non-smokers [301, 302]. In addition, not only is smoking associated with acceleration of micro-albumin to proteinuria [303], the rate of CKD progression to ESRD is associated with the number of cigarette smoked [304].

The degree of susceptibility to CKD from cigarette smoking may also be dependent on the genetic make-up and response to the effect of cigarette smoking. The traditional risk factors which include smoking were found to contribute to arteriolar nephrosclerosis among European Americans more than any other racial group, suggesting genetics may predispose some groups to a greater adverse effect of cigarette smoking compared to others [210], but further studies are needed to confirm this hypothesis among individuals with CKD.

The mechanism of cigarette smoking in CKD initiation or progression is multiple, complex and involves many systems working together to cause hemodynamic and non-hemodynamic changes in the kidneys. One such mechanism involves nicotinic acetylcholine receptors which are expressed on the mensangial cells. Activation of these receptors by

concentrations of cigarette smoking leads to release of Tumor Growth Factor –Beta (TGF-β_1) and cell proliferation. TGF in turn leads to scaring of nephrons and tissue fibrosis [305].

9.5.2 ALCOHOL

The role of alcohol in CKD is not fully known. But, some studies have shown light-to-moderate alcohol consumption is beneficial in reducing CVD and CVD mortality by providing cardiovascular protection, preventing arthrosclerosis and plaque formation and improving levels of HDL [306-310]. It is not clear whether similar benefit is conferred to individuals with CKD/ESRD. While some studies found none or positive benefits of alcohol, others reported a negative effects of alcohol with respect to CKD initiation and progression. Perneger et al and Schaeffner et al, reported small to moderate alcohol consumption resulted in a protective effect against CKD, compared to those who had fewer alcohol [311, 312]. In the Honolulu study, alcohol intake was reported to have protective effect on renal arteries by significantly reducing hyalinization [313]. Conversely, Muthukumar et al reported that the risk of renal dysfunction was significantly increased among heavy drinkers compared to light drinkers [314]. Shankar et al went further to show not only was heavy drinking associated with CKD, individuals who are both smokers and heavy drinkers had substantially higher risk of CKD [315].

Taken together, there is growing evidence in support of protective effect of light to moderate alcohol consumption on CKD [316]. In addition, KDOQI guideline recommend moderate alcohol intake not more than two drinks per day for men and not more than one drink per day for women[25]. It is believed this protection is mediated by HDL cholesterol which confers vascular protection by preventing formation of atherosclerosis[317].

10.0 SPECIFIC AIMS

The incidence of CKD has been on the rise since the past decade[1]. African Americans have a higher prevalence of CKD compared to whites. A recent report indicates that the risk of CKD is four times higher among African Americans compared to whites[2]. Data also suggests an increasing trend in CKD prevalence among other populations of African descent [318]. Much of the risk for CKD is attributable to conventional risk factors such as hypertension and diabetes. However, these and several other modifiable risk factors of CKD/ESRD have not been well studied in populations of West African descent.

Few studies have examined the relationship between body compositions with biomarkers of CKD. A majority of the studies that have examined this relationship based body composition on anthropometrics including BMI and waist circumference (WC). These measures do not take into consideration body build and lean mass. Studies using Dual X-ray Absorptiometry (DXA) and Peripheral Quantitative Computed Tomography (PQCT) to determine body compositions are not common. This may be due to concerns for radiation exposure, time and cost considerations.

Previous study using data from the Osteoporotic Fracture in Men Study (MroS) among older Caucasian men with mild to moderate CKD show a significant association between worsening kidney function and increasing bone loss [319]. However, this finding may not be generalizable to older black men with CKD. Moreover, it is not certain if rate of bone loss among older Caucasians and blacks with CKD is similar.

With these considerations in mind we propose to determine and compare the prevalence of CKD in populations of older Tobago black, African American and Caucasian men and to assess the risk factors associated with CKD among older Tobago black men. In addition, we will address the association of body composition with biomarkers of kidney function and the relationship of worsening kidney function with bone loss among Tobago black men. This project is an ancillary study, built upon an existing longitudinal cohort studies of bone loss and the Tobago Health studies.

10.1 SPECIFIC AIM 1

Determine the prevalence of CKD among Tobago black males.

Compare prevalence with U.S black and white males, of NHANES of 2003-2006.

Identify modifiable risk factors of CKD among Tobago black males.

Hypothesis: We hypothesize that the prevalence of CKD will be higher among Tobago black men compared to U.S black and white men. We also suspect that other modifiable risk factors including hypertension, diabetes, markers of obesity and body composition, level of education, alcohol consumption and cigarette smoking will be strongly associated with CKD among Tobago black males independent of age.

10.2 SPECIFIC AIM 2

Determine the association of body composition with markers of kidney function among Tobago black males.

Hypothesis: We posit that body composition measures including regional and general adiposities determined by DXA/PQCT are associated with serum creatinine and cystatin C among Tobago black males, independent of age, hypertension, diabetes and standing height.

10.3 SPECIFIC AIM 3

Determine whether low kidney function predicts bone loss among Afro-Caribbean black males.

Hypothesis: We hypothesize that increasing quartiles of serum creatinine, cystatin C and albuminuria are each associated with greater bone loss among Tobago black males. The rate of bone is loss is similar between Tobago black men and their Caucasian counterparts.

11.0 THE PREVALENCE AND RISK FACTORS ASSOCIATED WITH CKD AMONG TOBAGO BLACK MALES 40 YEARS AND OLDER, 2004-2007

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11.1 ABSTRACT

Introduction: Chronic kidney disease (CKD) is a leading cause of morbidity and mortality. CKD is associated with progression to End Stage Renal Disease (ESRD) and several health complications. Early diagnosis, intervention and identification of risk factors are important steps in minimizing complications associated with CKD. African Americans and other racial minorities bear a greater proportion of CKD burden compared to whites. **Objectives:** Prior studies on CKD have been largely focused on African Americans and Caucasians. We extended study to include Afro-Caribbean men. We compared the prevalence of CKD, by criteria based on Modification Diet in Renal Disease (MDRD) estimated Glomerular Filtration Rate (eGFR) and albuminuria, between Afro-Caribbean men on the Island of Tobago and their U.S African American and Caucasian counterparts. The risk factors for CKD in Tobago men were determined. **Methods:** 975 Tobago black males aged 40 years and older were included in analyses. Data for U.S men was obtained from the National Health and Nutrition Examination Survey (NHANES) of the 2003-2006 cycles. It included 538 African American and 1592 Caucasian men 40years and older. Standardized serum creatinine measurements were performed among Tobago and NHANES study participants, using Jaffè reaction to calculate MDRD-eGFR. Urinary albumin was measured among Tobago and NHANES study participants using fluorescent immunoassay. The prevalence of CKD defined as MDRD-eGFR <60ml/min/1.73m^2 or ACR ≥30mg/g was determined and age-standardized using U.S census 2000. Age-adjusted associations of potential risk factors for CKD were determined for Tobago men, using logistic

regression. **Results:** The prevalence of CKD in Tobago men was 19.7%, compared to 23.4% and 19.7% in African American and Caucasian men respectively. Hypertension, diabetes and measures of obesity including BMI and waist circumference were significantly associated with CKD independent of age in Tobago men. **Conclusion:** The prevalence of CKD in Tobago men was lower compared to African American men, but, comparable to Caucasian men. Hypertension, diabetes and obesity were significant predictors of CKD risk independent of age in Tobago men.

11.2 INTRODUCTION

Chronic kidney disease (CKD) is a growing health problem in the U.S, and a leading cause of morbidity and mortality [1]. In the decade following 1994, CKD increased from 15.5% to 16.8% among Americans 20 years or older[1]. A Center for Disease Prevention and Control (CDC) report shows that CKD incidence is growing rapidly, in particular among people 65 years and older, and people with cardiovascular disease (CVD), hypertension, diabetes and obesity[320]. It is likely that this trend will continue as the aging population and the number of people with risk factors for CKD increases in the U.S population [131].

CKD is associated with progression to renal failure, which in turn is associated with several health complications including CVD, bone loss and anemia. Approximately 530,000 renal failure patients are on dialysis at an annual cost of approximately $40 billion [2]. Early diagnosis and intervention as well as identification of CKD risk factors are important steps in reducing these burdens. However, many people may not even be aware of the presence of early kidney function disorder until in later stage, when treatment is difficult. African Americans and

other racial minority groups are more affected by CKD than whites. In 1994-2004, the age adjusted incidence of renal failure was 55.0 per million among African Americans compared to 22.8 per million among Caucasians [1] [2]. Much of the racial disparity in CKD is attributable to genetic susceptibility [320] and modifiable risk factors of CKD including diabetes, obesity, hypertension, lifestyle and socioeconomic factors. A relatively inexpensive method of determining CKD is by measuring serum creatinine and using Modification Diet in Renal Disease (MDRD) equation to estimate glomerular filtration rate. However, the performance of serum creatinine is controversial. Serum creatinine is influenced by age, race, gender and muscle mass [321]. Although, the effects of race, age and gender differences are corrected in MDRD equation, the accuracy of MDRD-eGFR alone to detect early kidney damage is uncertain. The nonprofit organization of the Kidney Disease Improving Global Outcomes (KDIGO) recommendations include addition of albuminuria test to eGFR for CKD screening [322].

Several prior studies on CKD have been largely focused on African Americans and Caucasians. To explore racial/ethnic differences in CKD, it is important to extend research to include other racial/ethnic groups such as Afro-Caribbean. Given the ancestral similarities between Afro-Caribbean blacks and African Americans, we sought to compare the prevalence of CKD, based on MDRD-eGFR and albuminuria [322] in Afro-Caribbean men on the Island of Tobago to their U.S African American and Caucasian counterparts. Furthermore, the risk factors for CKD among Afro-Caribbean men on the Island of Tobago will be determined.

11.3 DESIGN AND METHODS

11.3.1 Study Population

Our study participants are black males 40 years or older from the Caribbean Island of Tobago. Tobago Island is one of the Islands that make up Trinidad and Tobago, located in the southern Caribbean. In 2000, the estimated population of Tobago was 54,084[323]. The island measures 42 km by 10 km and occupies a land area of 300 square kilometers. In 1997-2003, approximately 3094 men were enrolled into the Tobago Prostate Cancer Screening Study. Enrollment into the study was accomplished largely through word of mouth. To be eligible, participants had to be non-institutionalized, 40 years or older, not terminally ill and must be able to give informed consents. After initial participation, all men were invited for a follow -up visit in 2004-2007. During this visit, approximately 2,025 participants returned. The data for this report include anthropometric measures, demographic variables and medical history which were collected by staff-administered questionnaires in the 2004/2007 visit. Informed consent was obtained and the study was approved by the Institutional Review Boards of the University of Pittsburgh and the Tobago Division of Health and Social Services.

The comparison groups were U.S African American and Caucasian males, 40 years or older. Data for U.S men were obtained from National Health and Nutrition Examinations (NHANES), a probability sample of the U.S population [51]. NHANES 2003-2006 cycles were used to determine the prevalence of CKD among approximately 538 African American and 1592 Caucasian men 40 years and older.

11.3.2 Laboratory Measurements

The present study includes approximately 1000 samples from the 2004/2007 visit. Blood samples were drawn from participants by venipuncture after a 12-hour fast. Drawn blood was allowed to sit for at least 20 min to clot before centrifugation. Samples were locally stored at -20^{o}C before being shipped with dry ice to the Heinz Nutrition Laboratory, where they were stored at -80^{o}C. Serum creatinine was quantitatively measured by a kinetic determination based on the Jaffè reaction [324]. For Jaffe reaction, the Sigma Diagnostics (St. Louis, Montana) creatinine kit was used. Briefly, frozen samples collected in 2004-2007 were thawed. Serum was diluted 1:26 with picric acid/sodium hydroxide solution, incubated at 30^{o}C for four minutes and read at 500 nm. Serum controls and duplicate samples were run with each assay standardized to IDMS-traceable creatinine value[325].The coefficient of variation between runs was 6.0%[326]. Similar standardized serum creatinine measurement was performed in NHANES 2003-2006 cycles[51]. Glomerular filtration rate was estimated by using MDRD equation to calculate MDRD-eGFR[27, 61], based on the following equation: MDRD-eGFR (ml/min/1.73m^2) = 175 * (Standard Serum Creatinine (mg/dl)) $^{-1.154}$ * (Age)$^{-0.203}$ * (0.742 if Female) * (1.212 if African American/ Black) [37, 327]. The MDRD estimating equation was chosen because of its general application in many clinical and epidemiological studies for determining kidney function

Albumin was measured using a turbidimetric procedure on the Olympus AU400 with reagents provided by Olympus America, Inc. (Center Valley, PA). Briefly, 10 µl of urine was incubated with goat anti-human albumin antibody for 5 minutes at room temperature. The resulting turbidity was measured at 340/800 nm. The procedure was linear from 0.5 – 30 mg/dL. Blanks, calibrators and control pools were run simultaneously with all samples. The intra-and interassay coefficients of variation were below 2.5% and 5.1%, respectively[326]. Urinary

albumin was divided by urinary creatinine to calculate the albumin-creatinine ratio (ACR). Similar computation was performed in NHANES 2003[51].

Body composition measures including percent whole body lean and fat were determined with Dual X-ray Absorptiometry (DXA). The Hologic QDR-4500W (Hologic Inc., Bedford, MA) was used to measure whole body lean and total body mass by following standard scan procedures for patient positioning. Each scan was read and analyzed using QDR software Version 8.26a. The coefficient of variations between scans was $\leq 1.16\%$.

This study was based on 975 Tobago black, 538 African American and 1592 Caucasian males with both serum creatinine and ACR measures. MDRD-eGFR less than 60ml/min/1.73m^2 or ACR greater than 30mg/g was defined as CKD [24]. The prevalence of CKD in Tobago black, African American and Caucasian men were determined by using this definition[322].

11.3.3 Covariates

Hypertension was measured by taking blood pressure after five minutes of seated rest using an automated blood pressure machine (Omron model HEM-705CP, Illinois). The average of the second and third measurements was taken to calculate systolic and diastolic pressures. Hypertensions was defined as systolic pressure equal or greater than 140mmHg or diastolic pressure equal or greater than 90mmHg, or self-report of diagnosis by a doctor or other health professional and taking antihypertensive medications. Cardiovascular disease included diagnosis of congestive heart failure, arrhythmias, angina pectoris, valvular problems or taking cardiovascular prescription medications for cholesterol and lipid treatments. Diabetes was assessed by measuring fasting blood glucose. Diabetes was defined as fasting blood glucose >126mg/dl, or self-report of diagnosis by a doctor or other health professional and taking anti-

diabetic medications. BMI was measured by determining a participant's weight in kilograms and height in meters and then dividing the weight by square of the height. Underweight was BMI < 18.5 kg/m^2 , normal weight was BMI (18.5 – 24.9) kg/m^2 , overweight was BMI(25.0 – 29.9) kg/m^2 and obese was BMI > 30.0 kg/m^2. Cigarette smoking was defined as current smoker. Alcohol consumption was defined as taking more than three drinks per week. Physical activity was defined as taking a walk outside home or yard for any reason including fun walk, exercise, walking to work, store or church for at least 5 days in a week. Education was categorized into three levels as none or completion of primary school, completion of high school/advanced-level and college/graduate or associate. All covariates were assessed in 2004-2007.

11.3.4 Statistical Analyses

In this study, only male subjects aged 40 years or older, who identified as Tobagonian blacks from the Tobago Health Studies, and African Americans or Caucasians from the NHANES 2003-2006 cycles were included. Observations with missing measurements for MDRD-eGFR and ACR were excluded. The characteristics of the remaining 975 Tobago black, 538 African American and 1592 Caucasian men were examined. The means and standard deviations of continuous and percentages of categorical independent variables were examined. The prevalence rates of CKD by age group were determined in Tobago black, African American and Caucasian males. The prevalence rates across all racial/ethnic groups were direct-age-standardized using the U.S. census 2000 population data.

The relationships between potential risk factors and CKD were examined among Tobago men using logistic regression adjusting for age. A prediction model for the probability of CKD among Tobago men was developed using forward step-wise multiple logistic regression.

Interactions between main effect risk factors were examined for significance. Models were checked for adequacy, assumptions violations, multi co-linearity and parsimony. A receiver operating characteristic curve was performed to determine the sensitivity of the final prediction model. Statistical significance was determined at alpha level of 0.05. All analyses were performed using SAS Version 9.3 TSIMI software, SAS Inc.

11.4 RESULTS

The characteristics of Tobago black males are displayed in (Table 9). The average age was 59.1 years (SD: ±10.5). The mean serum creatinine was 1.2mg/dl (SD: ±0.5) with a mean MDRD-eGFR of 77.7ml/min/1.73m^2 (SD: ±17.5). The median ACR was 3.3mg/g. The mean waist circumference was 92.7cm (SD: ±11.6) and about 70% were either obese or overweight by BMI categorization. In spite of this high categorization of overweight and obesity, 80.1% (SD: ±5.7) of the body mass was lean mass. The mean scaled body lean mass and fat were 2.1(SD: ±0.2) and 0.5(SD: ±0.2) respectively. About 65% reported being physically active, 51.3% had hypertension, 22.0% had diabetes and 5.9% had cardiovascular disease. The lipids profile show that the mean total cholesterol was 204.9mg/dl (SD: ±44.2), the mean triglycerides was 111.9mg/dl (SD: ±58.2), while the mean HDL and LDL were 50.1mg/dl (SD: ±13.4) and 132.4mg/dl (SD: ±40.5) respectively. Approximately 11 % reported as current smokers and 11.7% self identified as alcohol drinkers of more than three drinks per week. Nearly 75% had either no education or completed primary education, while 17.4% had secondary school (O/A level) and 7.9% had college/associate degree.

Table 8. Population Characteristics of Tobago Black Males 40 Years and Older, 2004-2007

Characteristics	N=975	%	Mean(±SD) Median(variance)
Age, (yr)	975		59.1 ±10.5
40-49	205	21.1	
50-59	345	35.4	
60-69	237	24.3	
>70	187	19.2	
BMI, (kg/m^2)	973		27.3±4.5
Underweight (<18.5)	15	1.5	
Normal (18.5-24.9)	286	29.4	
Overweight (25-29.9)	440	45.2	
Obese (>30)	232	23.8	
Waist Circumference, (Cm)	972		92.7±11.6
Scaled Body Lean Mass*	821		2.1±0.2
Scaled Body Fat*	821		0.5 ±0.2
Serum Creatinine (mg/dl)	975		1.2 ±0.5
MDRD eGFR ($ml/min/1.73m^2$)	975		77.7 ±18.8
MDRD eGFR<60($ml/min/1.73m^2$)	137	14.1	
ACR, (Median)	975		3.3(18535)
Albuminuria (ACR≥30mg/g), %	101	10.4	
Hypertension, (%)	500	51.3	
Diabetes, (%)	214	22.0	
CVD, (%)	57	5.9	
Total Cholesterol, (mg/dl)	853		204.9±44.2
Triglycerides, (mg/dl)	853		111.9±58.2
HDL, (mg/dl)	853		50.1±13.4
LDL, (mg/dl)	853		132.4±40.5
Current Smokers, (%)	111	11.4	
Alcohol Drinkers (> 3 Drinks Per Week); %	114	11.7	
Physical Activity, (%)	628	64.7	
Education, (%)			
Completed Primary School/No Education	721	74.7	
Completed Secondary School (O/A Level)	168	17.4	
College(University /Associate)	76	7.9	

Abbreviations: **Albuminuria**, ACR≥30mg/g; **BMI,** body mass index; **CVD**, cardiovascular disease; **Diabetes**, self-reported and taking diabetes medication or fasting glucose > 126mg/dl; **eGFR**, estimated glomerular filtration rate; **Hypertension**, systolic pressure≥140, or diastolic pressure ≥90, or taking medication and self-reported; **SD**, standard deviation; **MDRD,** modification diet in renal disease; **Physical activity**, taking a walk outside home or yard for any reason including fun walk, exercise, walking to work, store or church for at least 5 days in a week; *, whole body lean mass and fat were scaled by dividing each measure with square of the standing height.

The age adjusted prevalence of albuminuria defined as ACR≥ 30mg/g and low kidney function defined as eGFR less than 60ml/min/1.73m^2 among Tobago black, African American and Caucasian males is shown in (Table 10). African American had the highest prevalence of albuminuria across all age groups followed by Caucasians. Albuminuria was almost 2 to 3 times as common in African Americans compared to Tobago blacks or Caucasians except in the >70 years group. This group showed smaller difference, but still higher prevalence rates among African Americans compared to Tobago blacks and Caucasians (>70years old: TB=20.3, AA=27.4, CC=25.6)%.

The prevalence of low kidney function was comparable in African Americans and Tobago blacks, but was higher when compared to Caucasians in all age groups except in the >70 years group. In this group, low kidney function was twice as common in Tobago blacks compared to African Americans but was comparable in Caucasians (>70years old: TB=40.1, AA=19.8, CC=39.3)%.

The overall age standardized prevalence of impaired kidney function defined as MDRD eGFR<60ml/min/1.73m^2 was higher among Tobago men(13.5%) compared to African American men(9.0%), while the overall age adjusted prevalence of albuminuria defined as ACR≥30mg/g was higher among African American men(18.7%) compared to Tobago men (9.6%). Albuminuria was almost twice as common among African American men compared to Tobago men, while impaired kidney function was one and half times as common in Tobago men compared to African American men.

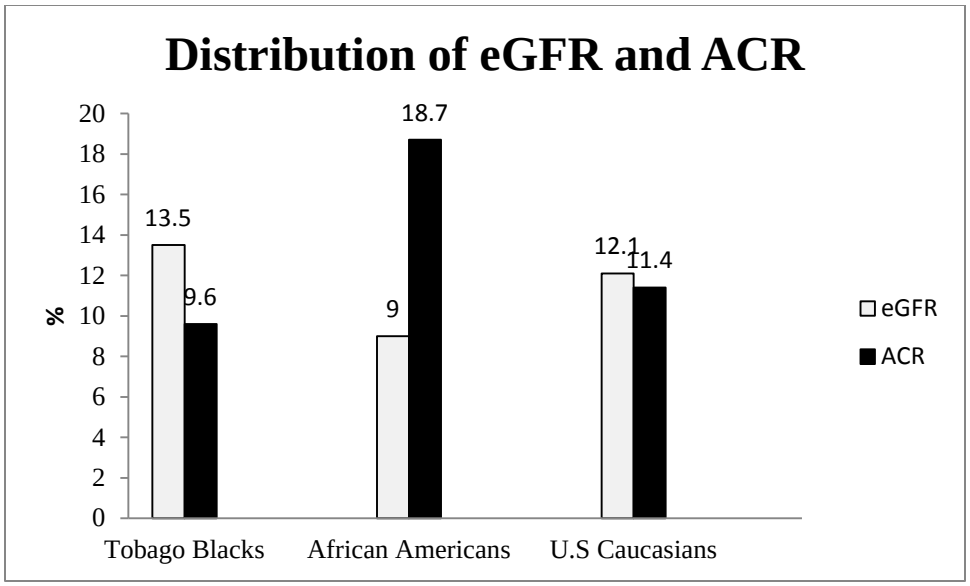

Figure 3. Age Standardized Distribution of Low eGFR and Albuminuria
Low eGFR was based on eGFR < 60ml/min/1.73m2 and Albuminuria defined as ACR ≥ 30 mg/g in Tobago black, African American and Caucasian males.

Table 9. Age Adjusted Prevalence of Albuminuria and MDRD-eGFR <60ml/min/1.73m^2 Among Tobago Blacks, U.S African American and Caucasian Males 40 Years and Older, 2004/2007

		GFR <60ml/min/1.73m^2						Albuminuria: ACR>30mg/g					
		TB		AA		CC		TB		AA		CC	
Age	STD	PGFR	AAR	Rate	AAR	Rate	AAR	PAL	AAR	Rate	AAR	Rate	AAR
40-49	42285	1.95	82456	2.41	101907	1.68	71039	2.93	123895	10.24	432998	3.64	153917
50-59	30531	5.22	159372	4.07	124261.	4.57	139527	9.86	301036	15.45	471704	8.23	251270
60-69	20064	16.88	338680	16.78	336674	11.44	229532	9.7	194621	30.77	617369	14.71	295141
>70	25300	40.11	1014783	19.81	501193	39.27	993531	20.32	514096	27.36	692208	25.62	648186
	118180		1595291		1064035		1433629		1133648		2214280		1348515
	OAAR		13.49		9		12.1		9.59		18.74		11.41

Abbreviations: **ACR**, albumin creatinine ratio; **AA**, African Americans; **TB**, Tobago black males; **CC**, Caucasians; **CKD**, chronic kidney disease; **eGFR**, estimated glomerular filtration rate; **MDRD**, modification diet in renal disease; AAR, age specific number of people with CKD; **OAAR**, overall age adjusted rate; **STD**, U.S census 2000 population data in thousand; PGFR, age specific prevalence of GFR<60ml/min/1.73m^2; PAL**,** age specific prevalence of albuminuria.

The overall age adjusted prevalence rate of CKD in Tobago blacks and Caucasians were surprisingly similar at 19.7%, compared to African Americans at 23.4 % (Table 11).

Table 10. Age Standardized* Prevalence Rate of CKD Using Combined Criteria (Albuminuria and eGFR) Among Tobago Black, African American and Caucasian Males, Aged 40 Years and Older, 2004/2007

	Tobago N=974				African Americans N=538				U.S. Caucasians N=1592				
Age(yr)	A	B	C: (A/B) %	D: C×M 100	E	F	G: (E/F) %	H: G×M 100	I	J	K: (I/J) %	L: K×M 100	M
40-49	9	205	4.4	1860.5	19	166	11.5	4862.8	19	357	5.3	2241.1	42285
50-59	45	345	13.0	3969.0	22	123	17.9	5465.0	39	328	11.9	3633.2	30531
60-69	55	237	23.2	4654.8	52	143	36.4	7303.3	70	306	22.9	4594.7	20064
>70	95	187	50.8	12852.4	42	106	39.6	10018.8	305	601	50.8	12852.4	25300
Total	204	974		23336.7	135	538		27674.9	433	1592		23321.4	118180
				23336.7 / 118180				27649.9 / 118180				23321.4 / 118180	
				19.7				23.4				19.7	

Abbreviations: **A**, number of CKD in Tobago blacks sampled; **B**, age-specific number of Tobago blacks sampled; **C**, age-specific rate of CKD among Tobago blacks; **D**, age-specific number of Tobago blacks with CKD; **E,** number of CKD in U.S blacks sampled; **F,** age-specific number of U.S blacks sampled; **G**, age-specific rate of CKD in U.S blacks; **H,** age-specific number of African Americans with CKD; **I**, number of CKD in U.S Caucasians sampled; **J**, age-specific number of U.S Caucasians sampled; **K**, age-specific rate of CKD in U.S Caucasians; **L,** age-specific number of Caucasians with CKD; **M**, U.S census 2000 population data in thousand; **ACR**, albumin creatinine ratio; **eGFR**, estimated glomerular filtration rate; **CKD,** chronic kidney disease, defined as MDRD-eGFR<60ml/min/1.73m^2 or ACR≥30mg/g; *****, direct age standardized using U.S census 2000 population data.

The association of potential risk factors for CKD after adjusting for age is shown in (Table 12). Hypertension (OR= 2.4, P <0.0001) and diabetes (OR= 1.8, P=0.0002) were each associated with increased risk of CKD. CVD (OR=1.70, P=0.0754) was associated with increased risk of CKD but this association was not significant. Each SD of scaled body fat (OR=1.3, 0.0085) increased risk of CKD, and scaled body lean mass (OR=1.3, P=0.0038) increased the risk of CKD. One SD of waist circumference (OR=1.2, P=0.051) was marginally associated with increased risk of CKD. Obesity (OR=1.6, P=0.0437) was accompanied with increased risk of CKD. Among the lipid markers examined, only HDL (OR=0.80, P=0.0308) was found to be associated with a decreased risk of CKD. Smoking and alcohol were not significantly associated with risk of CKD.

Table 11. Age Adjusted Associations of Risk Factors with CKD Defined as MDRD-eGFR< 60ml/min/1.73m^2 or ACR≥30mg/g Among Tobago Black Males 40 Years and Older, 2004/2007

Exposure			(N=975)		
Exposure variable	Mean	1 STD	OR	95% CI	P-Value
Age(years)[S*]	59.1	10.5	2.7	2.29-3.26	<0.0001
WC, (cm)[S]	92.7	11.6	1.2	1.00-1.42	0.0511
Scaled T Lean Mass[S]	2.1	0.2	1.3	1.09-1.56	0.0038
Scaled T Body Fat[S]	0.5	0.2	1.3	1.06-1.52	0.0085
Lipids Profile[S]					
Total Cholesterol	204.9	44.2	1.0	0.84-1.20	0.9768
Triglycerides	111.9	58.2	1.1	0.92-1.31	0.2946
HDL	50.1	13.4	0.8	0.67-0.98	0.0308
LDL	132.4	40.5	1.0	0.87-1.25	0.6296
Hypertension(Yes)			2.4	1.69-3.50	<0.0001
No			Reference		
Diabetes(Yes)			1.8	1.23-2.57	0.0022
No			Reference		
Smoking(Yes)			0.7	0.34-1.25	0.1970
No			Reference		
Alcohol(Yes)			0.6	0.34-1.18	0.1485
No			Reference		
Education					
Pry/No Education			1.1	0.53-2.10	0.8840
High Sch. O/A Level			1.4	0.64-3.04	0.4053
College Education			Reference		
BMI					
Obese			1.6	1.01-2.61	0.0437
Overweight			1.3	0.90-2.01	0.1423
Normal			Reference		
CVD (Yes)			1.7	0.94-3.24	0.0754
No			Reference		
Physical Activities (Yes)			1.2	0.84-1.72	0.3049
No			Reference		

Abbreviations: **Alcohol**, current alcohol drinker of greater than three drinks per week ; **BMI**, body mass index**; CVD**, cardiovascular disease; **Diabetes**, self-reported and taking diabetes medication or fasting Glucose > 126mg/dl; **eGFR**, estimated glomerular filtration rate; **HDL**, high density lipoproteins; **Hypertension**, systolic pressure≥140, or diastolic pressure ≥90 or taking medication and self –reported; **LDL**, low density lipoproteins; **OR**, odds ratio ; **Physical activities,** taking a walk outside home or yard for any reason including fun walk, exercise, walking to work, store or church for at least 5 days in a week; **Smoking**, current smoker; **S**, continuous variables was standardized to mean=0 and standard deviation=1; **STD**, standard deviation. * , Unadjusted for age**.**

The relationships of hypertension and diabetes with albuminuria defined as ACR≥30mg/g and impaired kidney function defined as MDRD eGFR<60ml/min/1.73m^2 in Tobago black males is shown in (Table13). The presence of hypertension was associated with 170% and 120% increased risk of albuminuria and impaired kidney function respectively, independent of age and diabetes status. In contrast, the presence of diabetes was associated with 120% increased risk of albuminuria independent of age and hypertension and only 20% increased risk of impaired kidney function but was not significant. This suggests that hypertension considerably influences both kidney damage (albuminuria) and impaired glomerular filtration (kidney function), while the influence of diabetes was mainly on kidney damage (albuminuria) and less so on impaired glomerular filtration (kidney function).

Table 12. The Risk Associated with Albuminuria and Kidney Function by Hypertension and Diabetes Among Tobago Blacks 40 Years and Older, 2004/2007

	Albuminuria (ACR≥30mg/g)			Kidney Function (eGFR<60ml/min/1.73m^2)		
Exposure	OR	95% CI	P-Value	OR	95%CI	P-Value
Hypertension(yes)*	2.9	1.79-4.83	<0.0001	2.2	1.42-3.46	0.0005
Hypertension(yes)ǂ	2.7	1.67-4.52	<0.0001	2.2	1.39-3.41	0.0006
No	Reference			Reference		
Diabetes(yes)*	2.4	1.53-3.68	0.0001	1.3	0.84-2.00	0.2364^{NS}
Diabetes(yes)α	2.2	1.39-3.37	0.0007	1.2	0.78-1.86	0.4001^{NS}
No	Reference			Reference		

Abbreviations: **ACR**, albumin creatinine ratio; **CI**, confidence interval; **Diabetes**, was defined as self- reported and taking diabetes medication or fasting glucose > 126mg/dl **Hypertension**, was defined as systolic pressure ≥140mmHg, or diastolic pressure ≥ 90mmHg or taking medication and self-reported; **OR**, odds ratio; **NS,** Non-statistical significance P >0.05;
*, adjusted for age;
ǂ, adjusted for diabetes and age;
α, adjusted for hypertension and age.

The prediction model for the probability of CKD occurrence among Tobago black males is shown in (Table 12). The probability of CKD was predicted by age, hypertension and diabetes according to the equation P(CKD)= -7.10 +Age(0.08)+ Hypertension(0.81)+ Diabetes(0.42). The Hosmer Lemeshow goodness of fit test was 0.5429 and the sensitivity analyses (Figure 4) show that approximately 79% of CKD in Tobago black males was predicted by this equation. No significant interactions were found among the main effect risk factors.

Table 13. Prediction Model for Risk of CKD Defined Using Combined Criteria (MDRD-eGFR< 60ml/min/1.73m^2 & Albuminuria ACR≥30mg/g) Among Tobago Black Males 40 years and Older, 2004/2007

	Model HL=0.5429		
Exposure	OR	P-Value	Beta Coefficient
Intercept	-	<0.0001	-7.10
Age	1.09	<0.0001	0.08
Hypertension	2.25	<0.0001	0.81
Diabetes	1.52	0.0417	0.42
Wald P-Val		<.0001	

Abbreviations: **CKD**, chronic kidney disease; HL, Hosmer & Lemeshow; Goodness-of-Fit; OR, odds ratio.

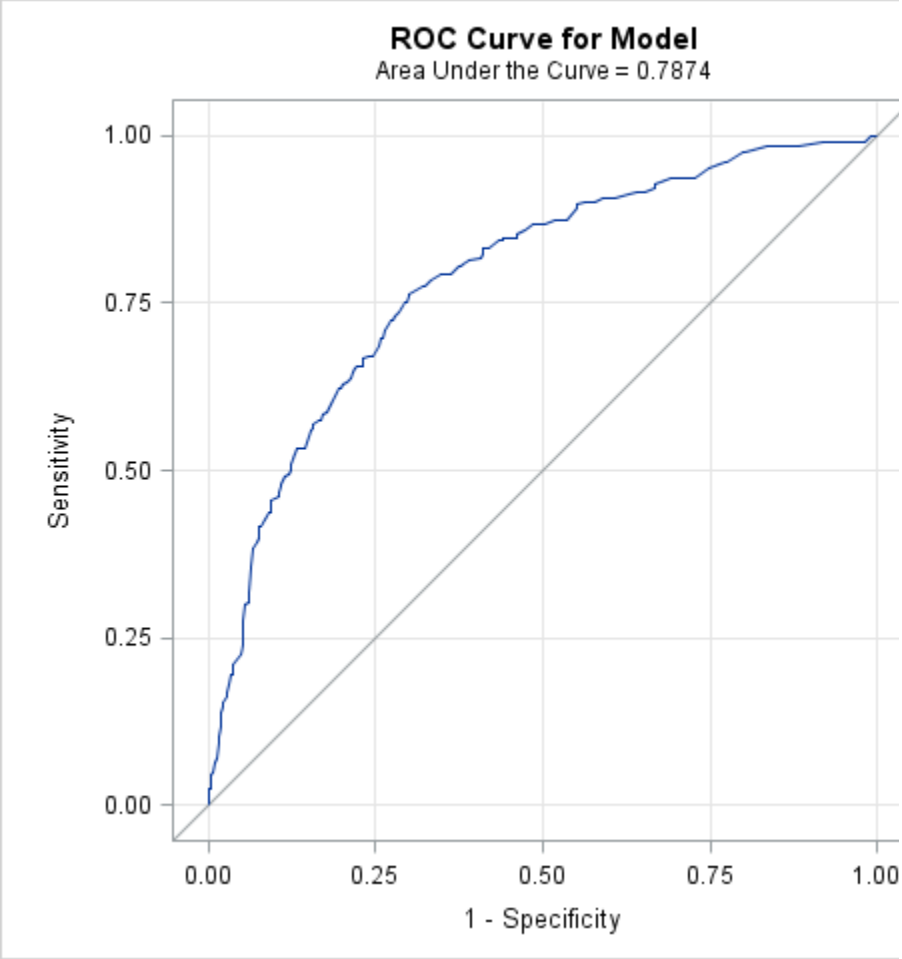

Figure 4. ROC for The Probability of CKD Among Tobago Males 40Years and Older
Area under the curve is 78.74%, indicating that 78.74% of chronic kidney disease defined as albuminuria(ACR≥30mg/g) or MDRD-estimated glomerular filtration rate (≤60ml/min/1.73m^2 is predicted by model;
Prediction model P (CKD) = -7.10 +Age (0.08) + Hypertension (0.81) + Diabetes (0.42); Abbreviations: ROC, receiver operation curve; ACR, albumin creatinine ratio.

11.5 DISCUSSION

In this study, the prevalence of CKD (Pre-dialysis) defined as eGFR less than 60ml/min/1.73m^2 or having albuminuria defined as ACR≥30mg/g was 19.7% among Tobago

black males aged 40 and older. Few studies have attempted to determine the prevalence of CKD in Caribbean countries [8, 328]. Barton et al reported unadjusted prevalence rate of 327 per million population in Jamaica[8]. Soyibo et al, reported from the Caribbean Renal Registry, a total of 564 cases of CKD/ESRD in Trinidad and Tobago [328]. Because these investigators did not provide a definition of CKD, it was not certain if these numbers represented those with earlier stages of CKD or ESRD receiving dialysis or both. It is important to emphasize that the Caribbean Renal Registry was set-up in 2004, to track ESRD in Caribbean countries and data collection still presents a challenge [318, 328].

The age-standardized prevalence of CKD was lower among Tobago black males (19.7%) compared to African American males (23.4%), but was the same for Caucasian males (19.7%). Given the ancestral similarities between Tobago blacks and African Americans, the lower prevalence rate of CKD in Tobago black compared to African American males was unexpected. The higher prevalence rate of CKD among African American men may be due to higher incidence rate in African Americans, compared to Tobago men. Further analyses showed that albuminuria was twice as common among African American men compared to Tobago men. In addition it was also observed that albuminuria was twice as common as low eGFR among African Americans. These findings suggest that, not only was albuminuria behind much of the racial ethnic differences between Tobago black and African American men, it was also responsible for the observed high prevalence of CKD among African Americans. This later observation confirms report by Muntner et al, showing that the high incidence of CKD among African Americans was attributed to albuminuria [329]. In contrast, the higher prevalence of low eGFR compared to albuminuria among Tobago men may also imply that low eGFR may be the major culprit of CKD among Tobago men. Therefore, given the results of these findings we

report that the driving mechanism for much of the CKD among African American males was albuminuria whereas among Tobago black males it was low eGFR. But additional studies are needed to confirm these theories.

The conventional risk factors associated with CKD include hypertension and diabetes [328]. In this study, among Tobago men, hypertension and diabetes were significantly associated with increased risk of CKD independent of age. But further analyses also revealed that the relative contribution of hypertension and diabetes each to the risk of albuminuria and low eGFR varied. After adjusting for important confounders, the risk of albuminuria was 42% higher with hypertension than with diabetes and the risk of low eGFR was 500% higher with hypertension than with diabetes. These findings confirm that hypertension is by far the major risk factor driving a majority of the CKD cases among Tobago black males, in particular through reduction of eGFR. Our findings support reports showing hypertension was more prevalent than diabetes and is the cause of most CKD cases in a majority of Caribbean countries [318, 328, 330]. The important role of hypertension and diabetes in CKD was further highlighted in the prediction equation (Table 14), which along with age predicted approximately 79% of the probability of CKD among Tobago black males 40 years and older (Figure 4). This prediction model also shows that the effect of hypertension on probability of CKD was twice compared to diabetes.

In contrast, a recent United State Renal Data System (USRDS) report showing that, 44% and 28% of new cases of CKD/ESRD had primary causes of diabetes and hypertension respectively[331], suggests that diabetes is the leading risk factor for CKD/ESRD among U.S men, in particular among African Americans. Given that albuminuria is an important clinical marker for patients with diabetes[332], this also confirms our finding showing that albuminuria is the driving mechanism behind much of CKD among African Americans.

Our result showed that CVD was associated with 70% increased risk of developing CKD independent of age, but this association was borderline significant. This non-significant association may have been due to the low prevalence of CVD which was less than 6% among Tobago males. This, in turn, may be due to "healthy subject effect", as the participants who responded in this study were ambulatory and generally in good health. However, the role of CVD in CKD has been well documented in other populations as a major risk factor for CKD [55, 60-62]. Not only do CVD and CKD share common risk factors such as age, diabetes, dyslipidemia and hypertension, these risk factors also interact with one another to elevate the risk of CKD morbidity and mortality [52, 53, 55, 56, 63, 64]. Although, we did not find any significant interactions between CKD risk factors and CVD , nonetheless, the occurrence of CKD in almost half of the participants who reported having CVD (not shown), suggest an important connection between CVD in CKD in Tobago black males.

Obesity was found to be significantly associated with increased risk of CKD independent of age. The relationship of BMI with CKD has been documented in other populations. Our result is consistent with several prior studies showing that BMI is negatively associated with kidney function. Iseki et al., showed that higher BMI was associated with increased risk of CKD in men in the general population in Okinawa after controlling for important confounders [119]. In a similar study, Ejarbald et al., reported that being overweight was associated with a three-fold excess risk for CKD [120]. Hsu et al., also found that high BMI was independently associated with CKD and the risk of ESRD increased with rising BMI [121]. There is overwhelming evidence suggesting that obesity is a significant risk factor in CKD progression and deterioration [116]. The mechanism linking obesity with CKD is not clear. However, some believe it involves the release of pro-inflammatory cytokines and free fatty acids by adipose tissues as a result of

invasion by macrophages [135]. Cytokines, in turn, regulate the activities of adipose tissue and its systemic effect, thereby, causing kidney damage through expansion of the mesangial cells and destruction of podocytes [137].

Our study has several strengths. This is a population based study with a large sample size. Participants were a representative sample of Tobago black males 40 years and older. The comparison of CKD prevalence in Tobago blacks with African American and Caucasian males was performed to underscore the relative difference in CKD distribution among different racial/ethnic groups. Our study also has some limitations. This was an observational cross-sectional study and direct measurement of glomerular filtration rate was not possible. Therefore, we relied on estimates of glomerular filtration by using MDRD equation. In addition, only one measurement of serum creatinine and ACR were taken in 2004-2007. The use of one measurement to determine kidney function and albuminuria hence CKD, may be misleading and the MDRD equation is known to underestimate high eGFR and overestimates low eGFR. This may have led to misclassification in the actual CKD prevalence. Self reported responses including smoking, alcohol and education were not verifiable. Future direction should focus on environmental factors impacting CKD and their mechanisms of action among Tobago black males.

11.5.1 Conclusion

In this study, based on MDRD eGFR in combination with ACR, the prevalence of CKD was lower among Tobago black males at 19.7% compared to African American males at 23.4% but was the same as U.S white males at 19.7%. The observed prevalence rate difference between African American and Tobago black men was mainly due to higher prevalence of albuminuria

among African Americans. Hypertension, diabetes and obesity were associated with increased risk of CKD independent of age among Tobago black males. However, hypertension was the major risk factor and low eGFR was the mechanism driving CKD among Tobago black males 40 years and older. In conclusion, Tobago black males could significantly reduce the risk of CKD, by eliminating or modifying risk factors leading to diabetes, obesity and in particular hypertension. These can be achieved through regular screening and control of hypertension, proper dieting, lower salt and fat consumption, increased physical activity, weight control and early CKD screening.

12.0 THE ASSOCIATION OF BODY COMPOSITION WITH SERUM CREATININE AND CYSTATIN C, AMONG TOBAGO BLACK MALES, 2004-2007

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12.1 ABSTRACT

Introduction: Chronic kidney disease (CKD) is a leading cause of morbidity and mortality in the U.S. Serum creatinine and cystatin C are biomarkers of CKD but are influenced by body composition . The association of body composition with serum creatinine and cystatin C has not been elucidated among blacks. **Objective**: We sought to examine the relation of body composition with serum creatinine and cystatin C among older Afro-Caribbean males. **Methods:** approximately 1000 non-institutionalized and non-terminally ill males aged 40 years and older were recruited from Caribbean Island of Tobago. Anthropometric measures, peripheral quantitative computed tomography (pQCT) of a cross-section of the lower leg at the mid-calf, and dual X-Ray absorptiometry (DXA) scan of general and regional body compositions were performed. Serum creatinine and cystatin C were measured by Jaffè reaction and Dade Behring nephelometer respectively. Albumin creatinine ratio (ACR) was measured by fluorescent immunoassay. The Modified Diet in Renal Disease (MDRD) equation was used to estimate glomerular filtration rate and combined with ACR to determine CKD status. The relationships of body composition measures with serum creatinine and cystatin C were examined using robust regression and controlled for important confounders. **Results:** BMI was significantly associated with increase in serum creatinine. BMI and waist circumference were related to cystatin C. DXA lean body mass and calf muscle area were positively associated with serum creatinine. Cystatin C was positively associated with general and regional adiposities, but not with calf muscle area. **Discussion/Conclusion:** Body composition is related to serum creatinine and

cystatin C, therefore, should be considered when interpreting serum creatinine and cystatin C. Serum creatinine and cystatin C may have prognostic value in diseases related to body composition such as obesity related Cardiovascular Disease (CVD) and muscle related diseases among older Tobago black males.

12.2 INTRODUCTION

Chronic Kidney Disease (CKD) is a leading cause of morbidity and mortality in U.S [54]. The prevalence of CKD has been on the increase since the past decade due to rising incidence of diabetes, hypertension and obesity [54]. CKD is determined by measuring glomerular filtration rate, using gold standard procedures such as iothalamate or inulin clearance tests. The complexities and cost associated with these tests limits their practical use and clinical application. Alternative procedures use plasma clearance of serum creatinine and cystatin C to estimate glomerular filtration rate. Because of influence by extra renal sources, the utility of serum creatinine and cystatin C for determining glomerular filtration rate is controversial [333]. Serum creatinine is a byproduct of muscle metabolism. The influence on serum creatinine by age, gender, muscle mass, protein consumption and body composition make serum creatinine less sensitive as a biomarker of renal function[34, 35]. An alternative biomarker to serum creatinine that has gained some acceptance in recent time is Cystatin C. Cystatin C is a low molecular weight compound produced by all nucleated cells at a constant rate and filtered in the glomerulus. Cystatin C is completely catabolized in the proximal tubules and unlike creatinine, is not reabsorbed back into circulation. Studies show that cystatin C is a better and more sensitive marker of kidney function with higher precision for detecting CKD compared to serum

creatinine [48, 334-337]. However, studies have also shown that serum cystatin C is influenced by adiposity, inflammatory biomarkers, large doses of glucocorticoids use, hyperthyroid state and lean mass [41-43]. The resulting influence from several sources makes serum creatinine and cystatin C less sensitive biomarkers of kidney function. Although the estimating equation of the Modification of Diet in Renal Disease (MDRD) Study was developed to adjust serum creatinine-estimated eGFR for demographic variables [31], the utility of MDRD equation has not been validated in many populations and the influence of muscle mass and extra renal sources have not been fully addressed in this equation. Therefore, the competence of MDRD in assessing kidney function is controversial[33] .

The influence of body composition on serum creatinine and cystatin C suggests, not only should body composition be considered when evaluating serum creatinine and cystatin C, but that serum creatinine and cystatin C may also have prognostic values in diseases related to body composition. The majority of prior studies assessing the relationship between body composition and serum creatinine and cystatin C were based on anthropometric measures such as waist circumference (WC) and body mass index (BMI) [121, 338, 339]. Muntner et al., showed that BMI was associated with increasing serum cystatin C [340]. Steven et al, also reported that body anthropometrics including, height, weight, BMI and waist circumference were associated with increasing cystatin C [333]. Anthropometric measures are widely accepted correlates of body composition and their ease of measurement and low cost makes them attractive for use. However, they fail to take into account muscle mass and do not discriminate between general and regional adiposities. Moreover, despite the important role of body composition on serum creatinine and cystatin C, no study has been conducted in the black population to evaluate this relationship. Therefore, the purpose of this study is to examine the relationship of body

composition with serum creatinine and cystatin C among Afro-Caribbean males of West African ancestry. Anthropometrics, Dual X-ray Absorptiometry (DXA) and Peripheral Quantitative Computed Tomography (PQCT) measures of general and regional adiposities will be examined in relation to concentrations of serum creatinine and cystatin C, while controlling for important confounders.

12.3 DESIGN AND METHODS

12.3.1 Study Population

Approximately 3094 unscreened men from the island of Tobago located in southern Caribbean were enrolled into the Tobago Prostate Cancer Survey between 1998 and 2003. Recruitment was done by public announcement and word of mouth. To be eligible, participants had to be non-institutionalized, 40 years or older, non-terminally ill and must be able to give informed consent. The racial composition of recruits were 97% Africans; 2% East Indian; less than 1% Caucasians, and less than 1% of other race[341]. The current study is based on the follow-up visit, in 2004-2007, at which anthropometric measures, demographic variables, medical history, PQCT of the lower leg in the mid-calf region, and DXA scan of whole and regions of the body were performed to assess general and regional adiposities on approximately 2,025 participants. The current study includes a subsample of approximately 1000 participants aged 40 years or older. Informed consent was obtained and study was approved by the Institutional Review Boards of University of Pittsburgh and the Tobago Division of Health and Social Services.

12.3.2 Baseline Questionnaire

Questionnaires were administered to participants by experienced interviewers in 2004-2007. Information about age, socioeconomic status, lifestyle, habits including cigarette smoking, alcohol consumption, level of education, past medical history, prescription and non-prescription medication use, history of diabetes and hypertension were ascertained.

12.3.3 Laboratory Measurements

Blood samples were collected in 2004-2007 from participants in the morning after a 12-hour overnight fast, by venipuncture. Samples were allowed to sit at room temperature for at least 20 minutes to clot before centrifugation. Serum and urine samples collected were frozen at -20°C locally, before been shipped with dry ice to Heinz laboratory, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania, where they were stored at -80°C.

Jaffè reaction was used to measure serum creatinine in the laboratory [324]. The Jaffe reaction, Sigma Diagnostics (St. Louis, Montana) creatinine kit was used. Briefly, frozen samples were thawed. The resulting serum was diluted one part in twenty six parts with picric acid/sodium hydroxide solution, incubated at 30°C for four minutes and read at 500 nm. Serum controls and duplicate samples were run with each assay. Each assay was standardized to IDMS-traceable creatinine values [325]. The coefficient of variation between runs was 6.0%[326].

Serum cystatin C was measured at the University of Vermont. Thawed samples were tested using a BNII nephelometer (Dade Behring, Inc, Deerfield, Ill) with a particle-enhanced immunonephelometric assay (N Latex cystatin C, Dade Behring, Inc). The assay range was from 14.6 - 549.0 nmol/L (0.195 - 7.330 mg/L). The reference range for young, healthy persons was

reported to be 40 - 71nmol/L (0.53 - 0.95 mg/L). The intra-assay coefficient of variation ranged from 2.0% - 2.8%, and the inter-assay coefficient of variation ranged from 2.3% - 3.1% [342] .

Urinary albumin was measured using a turbidimetric procedure on the Olympus AU400 with reagents provided by Olympus America, Inc. (Center Valley, PA). Ten microliter of urine was incubated with goat anti-human albumin antibody for 5 minutes at room temperature. The resulting turbidity was measured at 340/800 nm. The procedure was linear from (0.5 to 30) mg/dL. Blanks, calibrators and control pools were run simultaneously with all samples. The intra-and interassay coefficients of variation were below 2.5% and 5.1%, respectively[326]. In this study, kidney function was determined by using serum creatinine in the MDRD equation to estimate glomerular filtration rate (MDRD eGFR)[32]. CKD was defined as having ACR greater than 30mg/g or MDRD eGFR less than 60ml/min/1.73m^2.

12.3.4 Adiposity Measurements

DXA and PQCT technologies were used to determine body composition measures including general and regional adiposity distributions.

Regional and body adiposity were measured using a Hologic QDR-4500W DXA densitometer (Hologic Inc., Bedford, MA). During DXA measurements, standardized procedures for participant positioning and scan analyses were followed according to the manufacturer's recommended protocol. The precision of DXA measurements was assessed in 12 subjects. The coefficient of variations was $\leq$ 1.16% and all test-retest correlations were above 0.99. To maintain longitudinal quality assurance of the scanner during the course of the scanning, a phantom was scanned daily. All scans were analyzed with QDR software version 8.26a [343].

An XCT-2000 scanner (Stratec Medizintechnik, Pforzheim, Germany) was used to measure subcutaneous, intermuscular and total adiposities, and muscle area of the lower leg in the mid-calf. Assessment of the scanned region was performed using the scanner "soft tissue algorithm" to differentiate between tissue densities. Fats, muscles and bones were measured with mineral equivalent densities of 0, 80, and 1200 mg/cm^{3,} respectively. By calibrating the scanner to these densities, the scanner was able to detect shift in densities from one tissue type to another and separate the fat, muscle and bone tissues accordingly. Image processing was performed by Stratec analyses software (Version 5.5) [343].

12.3.5 Covariates

Covariates were selected based on literature findings of the risk factors associated with body composition measures and univariate analyses of the correlates of serum creatinine and cystatin C. Hypertension was measured by taking blood pressure after five minutes of seated rest using an automated blood pressure machine (Omron model HEM-705CP, Illinois).The average of the second and third measurements was taken to calculate systolic and diastolic pressures. Hypertensions was defined as systolic pressure equal or greater than 140 mmHg or diastolic pressure equal or greater than 90mmHg or self-report of diagnosis by a doctor or other health professional and taking antihypertensive medications. Diabetes was defined as fasting glucose >126mg/dl, or self-report of diagnosis by a doctor or other health professional and taking anti-diabetic medications. Cardiovascular disease was assessed by questionnaire in 2004-2007. It included diagnosis of congestive heart failure, arrhythmias, angina pectoris, valvular disease or taking cardiovascular prescription medications for cholesterol and lipid treatment. Body Mass Index (BMI) was measured by determining a participant’s weight in kilograms and height in

meters and then dividing the weight by square of the height. Cigarette smoking was defined as current smoker. Alcohol consumption was defined as taking at least three drinks per week. Physical activity was defined as taking a walk outside home or yard for any reason including fun walk, exercise, walking to work, store or church for at least 5 days in a week.

12.3.6 Statistical Analyses

In this study, participants with missing data for serum creatinine and cystatin C were excluded from analyses. The characteristics of the remaining 1000 study participants were examined. The means and standard deviations of continuous, and percentages of categorical variables were determined. Univariate analyses were performed to examine the correlates of serum creatinine and cystatin C. The relationships between serum creatinine or cystatin C with anthropometric measures of waist circumference and BMI were examined using robust regression and controlling for important confounders. Robust regression was used to deal with normality assumption violations and outliers. The results were presented as changes in serum creatinine or cystatin C for each standard deviation of waist circumference or BMI or other body composition measure. Also, the relationships between serum creatinine or cystatin C with DXA-measured general and regional adiposities and with PQCT-measured adiposities were examined using robust regression and controlling for important confounders. Statistical significance was based on alpha of 0.05. Statistical analyses were performed using SAS Version 9.3 TSIMI software, SAS Inc.

12.4 RESULTS

The characteristics of study participants are displayed in (Table 15). The mean age of participants was 61.8 years (SD: ±11.1). The mean waist circumference was 92.8cm (SD: ±11.8). Approximately 70% were either overweight or obese. The mean whole body adiposity was relatively low at 16.8kg, representing 19.8% of total body mass, while the mean whole body lean mass was 63.6kg representing 80.4% of total body mass. The mean adiposity for the trunk was 8.2kg. The mean muscle area of the lower leg at the calf region was 7523.9mm^2. The total areal adiposity at this region (the lower leg at the calf) was 1864.9 mm^2 (SD: ±844.5), comprising of 1359.6mm^2 and 340.0 mm^2 of subcutaneous and intermuscular adiposities respectively. The mean serum creatinine was 1.3mg/dl (SD: ±0.4), and serum cystatin C mean was 0.8mg/L (SD: ±0.3). Approximately 55% of participants had hypertension, 25% had diabetes, while 5.4% had cardiovascular disease. About 12% reported as current smokers, 9% reported consuming more than three alcohols per week and 61.4% were physically active.

Table 14. Characteristics of Tobago Black Males, 40 Years and Older, 2004-2007

Exposure	N=1000	%	Mean(SD)
Age (Years)	994		61.8(11.1)
40-49	174	17.5	
50-59	261	26.3	
60-70	289	29.1	
>70	270	27.2	
BMI(kg/m^2)	998		27.3(4.7)
Underweight (<18.5)	17	1.7	
Normal (18.5-24.9)	292	29.6	
Overweight (25-29.9)	442	44.9	
Obese (>30)	234	23.8	
Waist Circumference(Cm)	996		92.8 (11.8)
DXA Body Composition			
Whole Body Mass,(Kg)	743		80.4(13.6)
Whole Body Lean Mass,(kg)	743		63.6(8.6)
Whole Body Adiposity (kg)	743		16.8(6.8)
Trunk Adiposity,(kg)	743		8.2(3.7)
Peripheral PQCT of Lower Skeletal Muscle Composition			
Subcutaneous Adiposity(mm^2)	945		1359.6(692.3)
Intermuscular Adiposity(mm^2)	945		340.0(432.5)
Total Calf Adiposity(mm^2)	947		1864.9(844.5)
Muscle Area(mm^2)	947		7523.9(1388.9)
Serum Creatinine, mg/dl	1000		1.3(0.4)
Serum Cystatin C, mg/L	1000		0.8(0.3)
Hypertension, (%)	548	54.8	
Diabetes, (%)	247	24.7	
CVD, (%)	54	5.4	
Current Smoker, (%)	87	11.7	
Physical Activity, (%)	611	61.4	
Alcohol Drinker, (%)	170	9.0	

Abbreviations: **Alcohol Drinker, c**urrent alcohol drinker of more than three drinks in a week**; BMI,** body mass index; **CVD**, cardiovascular disease; **Diabetes**, self reported and taking diabetes medication or fasting glucose > 126mg/dl; **DXA**, dual X-ray absorptiometry; **HDL**, high density lipoprotein; **Hypertension**; systolic pressure

≥140mmHg, or diastolic pressure ≥90mmHg or self reported and taking medication; **LDL**, low density lipoprotein; **PQCT**, peripheral quantitative computed tomography; **Physical activity,** taking a walk outside home or yard for any reason including fun walk, exercise, walking to work, store or church for at least 5 days in a week ;**Smoking**, current smoker; **SD**, standard deviation**.**

The age adjusted correlates of serum creatinine and cystatin C are displayed in (Table 16). BMI, waist circumference, DXA whole body and trunk adiposities, and whole body total and lean mass, PQCT calf subcutaneous adiposity and muscle area, hypertension and smoking were significant correlates of serum creatinine ($p<0.05$). BMI, waist, DXA whole body and trunk adiposities, and whole body total mass, PQCT calf intermuscular, subcutaneous and total calf adiposities, hypertension and alcohol were significant correlates of serum cystatin C ($p<0.05$).

Table 15. Univariate Analyses of Correlates of Serum Creatinine and Cystatin C, Among Tobago Black Males 40 Years and Older, 2004-2007

Exposure	Serum Creatinine(mg/dl)			Serum Cystatin C(mg/L)		
	Effect Change per STD	Unadjusted P-Value	Age-Adjusted p-value	Effect Change per STD	Unadjusted P-Value	Age-Adjusted p-value
Age(years)	0.0697	<0.0001	N/A	0.0744	<0.0001	N/A
BMI, (kg/m^2)	0.0165	0.0160	**0.0004**	0.0145	0.0019	**<0.0001**
WC, (cm)	0.0262	0.0001	**0.0008**	0.0288	<0.0001	**<0.0001**
Standing Height, (cm)	-0.0164	0.0161	0.3335	-0.0248	<0.0001	0.7975
DXA Body Composition						
Whole Body Adiposity,(Kg)	0.0170	0.0443	**0.0261**	0.0235	<0.0001	**<0.0001**
Trunk Adiposity, (Kg)	0.0207	0.0145	**0.0063**	0.0220	0.0001	**<0.0001**
Whole Body Lean Mass,(kg)	-0.0070	0.4072	**0.0170**	-0.0190	0.0010	0.0552
Whole Body Total Mass,(kg)	0.0038	0.6575	**0.0110**	0	0.9936	**0.0004**
Peripheral PQCT of Lower Skeletal Muscle Composition						
Intermuscular Adiposity,	0.0228	0.0012	0.4720	0.0308	<0.0001	**0.0002**
Subcutaneous Adiposity,	0.0026	0.7105	**0.0387**	0.0057	0.2485	**<0.0001**
Total Calf Adiposity,	0.0093	0.1845	0.0514	0.0192	<0.0001	**<0.001**
Muscle Area,(mm^2)	-0.0121	0.0847	**<0.0001**	-0.0353	<0.0001	0.6099
Hypertension[NS]	0.0831	<0.0001	**0.0004**	0.0682	<0.0001	**0.0050**
Diabetes[NS]	0.0261	0.0993	0.3900	0.0398	0.0003	0.8072
CVD[NS]	0.0108	0.7221	0.4332	0.0855	<0.0001	0.1172
Current Smokers[NS]	-0.0986	<0.0001	**0.0015**	-0.0192	0.2409	0.7166
Alcohol Drinkers[NS]	-0.0175	0.4639	0.9216	-0.0613	0.0002	**0.0041**

Abbreviations: **BMI,** body mass index; **CVD**, cardiovascular disease; **HDL**, high density lipoprotein; **LDL**, low density lipoprotein; **NS**, categorical variables were not standardized, but, continuous variables were standardized to mean=0 and standard deviation=1; **SD**, standard deviation; **WC**, waist circumference. **Bolded Figures** are important correlates of serum creatinine and cystatin C.

Association of Adiposity Measures with Biomarkers of Kidney Function is shown in (Table 17).

After adjusting for age, diabetes and hypertension, similar highly significant positive associations were observed between waist circumference (0.0190mg/dl, p=0.0044) and BMI (0.0201mg/dl, P=0.0027) with serum creatinine. Waist Circumference (0.0228mg/L, p<0.0001) and BMI (0.0189mg/L, p<0.0001) were also highly associated with cystatin C.

For DXA/PQCT measures of adiposities, after adjusting for age, hypertension, diabetes and standing height, only trunk (0.0175mg/dl, P=0.0395) and subcutaneous (0.0135mg/dl, p=0.0485) adiposities were significantly associated with serum creatinine. All DXA/PQCT adiposity measures, had high significant and positive associations with serum cystatin C (p<0.05)

Association of Muscle Measures with Biomarkers of Kidney Function is shown in (Table 17).

Muscle area (0.0284mg/dl, P= 0.0003) was significantly associated with serum creatinine. Lean mass was only associated with serum creatinine after adjusting for age hypertension and diabetes (0. 0205, mg/dl, 0.0170). On further adjusting for standing height the association was no longer significant. As expected, muscle area (0.0014mg/L, p=0.7797) was not associated with serum cystatin C. We also found unexpectedly, a positive association between lean mass (0.0140mg/L, p=0 .0266) and serum cystatin.

Table 16. The Association of Body Anthropometrics, General and Regional Adiposity with Serum Creatinine and Cystatin C as Continuous Variable

			Serum Creatinine(mg/dl)		Serum Cystatin C(mg/L)	
Exposure	Mean	1 STD	Change per STD of Exposure	p-value	Change per STD of Exposure	p-value
Adiposity Measures						
Anthro-Waist Circumference, (Cm)	92.8	11.8	0.0219[a]	0.0008	0.0235[a]	<.0001
			0.0190[b]	**0.0044**	0.0228[b]	**<.0001**
Anthro-BMI, (Kg/m^2)	27.3	4.7	0.0233[a]	0.0004	0.0199[a]	<.0001
			0.0201[b]	**0.0027**	0.0189[b]	**<.0001**
DXA-Whole Body Adiposity, (kg)	16.8	6.8	0.0179[a]	0.0261	0.0219[a]	<.0001
			0.0134[ba]	0.1120	0.0321[ba]	**0.0011**
DXA-Trunk Adiposity, (kg)	8.2	3.7	0.0220[a]	0.0063	0.0213[a]	<0.0001
			0.0175[ba]	**0.0395**	0.0211[ba]	**<0.0001**
PQCT-Subcutaneous Adiposity, (mm^2)	1359.6	692.3	0.0141[a]	0.0387	0.0174[a]	<.0001
			0.0135[ba]	**0.0485**	0.0169[ba]	**<.0001**
PQCT-Intermuscular Adiposity, (mm^2)	340.0	432.5	0.0049[a]	0.4720	0.0156[a]	0.0002
			0.0043[ba]	0.5298	0.0156[ba]	**0.0002**
PQCT-Total Calf Adiposity, (mm^2)	1864.9	844.5	0.0130[a]	0.0514	0.0209[a]	<.0001
			0.0120[ba]	0.0720	0.0204[ba]	**<.0001**
Muscle Measures						
DXA-Whole Body Lean Mass, (kg)	63.6	8.6	0.0205[a]	0.0170	0.0099[a]	0.0552
			0.0169[ba]	0.1110	0.0140[ba]	**0.0266**
PQCT-Muscle Area, (mm^2)	7523.9	1388.9	0.0307[a]	<0.0001	0.0025[a]	0.6099
			0.0284[ba]	**0.0003**	0.0014[ba]	0.7797

Abbreviations: **BMI**, body mass index; **DXA**, dual X-Ray absorptiometry;; **PQCT**, peripheral quantitative computer tomography; **STD**, standard deviation, exposure variables were standardized to mean=0 and standard deviation=1; **Anthro-,** anthropometric measure; **a**, adjusted for age; **b**, adjusted for age, hypertension and diabetes; **ba,** adjusted for age, hypertension, diabetes and standing height; **Bolded Figures**, were statistically significant associations P<0.05.

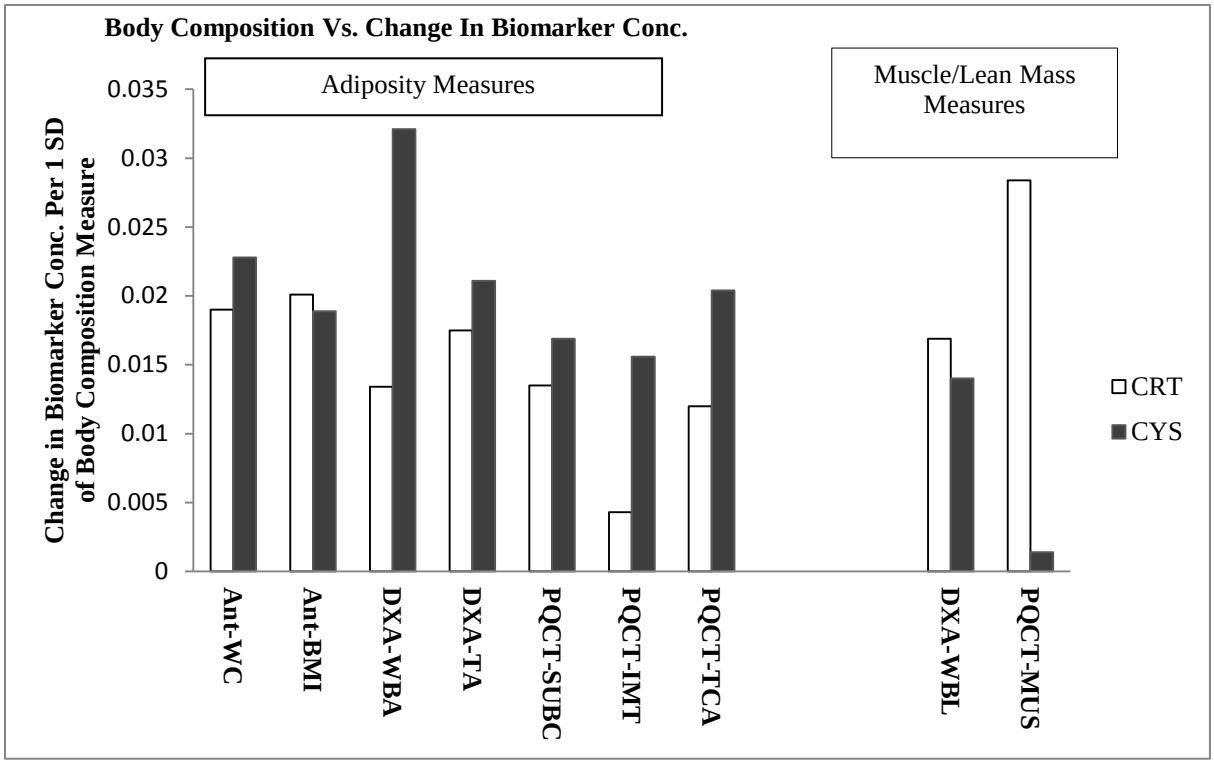

Figure 5. Changes in Serum Concentration of Kidney Function Biomarkers per One SD of Body Composition Measure Among All Participants
Adjusted for age, diabetes, hypertension and standing height. Abbreviations: Ant, Anthropometric measures; WC, waist circumference; BMI, body mass index; TA, total lower leg adiposity in the mid-calf; SUBC, subcutaneous adiposity; IMT; intermuscular adiposity; WBL; whole body lean mass; MUS, muscle area; DXA, DXA measures; PQCT, PQCT measures

We further tested the relationships between body composition measures with serum creatinine and cystatin C in the absence of CKD and age related impaired kidney function. This

was achieved by excluding participants having eGFR less than 60ml/min/1.73m^2 or ACR greater than 30mg/g and also participants who were greater than 70 years old.

The Association of Adiposity Measures with Biomarkers of Kidney Function Among Participants <70years, eGFR < 60ml/min/1.73m^2 or ACR >30mg/g is Shown in (Table 18).

For anthropometric measures of adiposity, on adjusting for age, hypertension and diabetes, only BMI (0.0195kg/m^2, p=0.0027) was positively associated with serum creatinine. Waist circumference and BMI were positively associated with cystatin C (p<0.05).

For DXA/PQCT measures of adiposities, after adjusting for age, diabetes, hypertension and standing height, only subcutaneous adiposity (0.0227mg/dl, p=0.0005) was positively associated with serum creatinine. All DXA/PQCT adiposity measures were significantly associated with serum cystatin C (p<0.0001).

The Association of Muscle Measures with Biomarkers of Kidney Function Among Participants <70years. eGFR <60ml/min/1.73m^2 or ACR >30mg/g is Shown in(Table 18).

Whole body lean mass (0.0188mg/dl, p=0.0591) was marginally associated with serum creatinine. Muscle area (0.0272mg/dl, p<0.0001) was related to serum creatinine.

Whole body lean mass (0.0214mg/L, p=0.0009) was also unexpectedly associated with serum cystatin C. As expected muscle area (0.0074, mg/L, p=0.1116) was not related to serum cystatin C.

Table 17. The Association of Body Anthropometrics, General and Regional Adiposity with Serum Creatinine and Cystatin C as Continuous Variable Among Participants <70years old with eGFR > 60ml/min/1.73m^2 or ACR < 30mg/g

			Serum Creatinine N=671		Serum Cystatin C N=671	
Exposures	Mean	1 STD	SCr Change(mg/L) Per 1 STD	p-val	SCy Change(mg/dl) Per 1 STD	p-val
Adiposity Measures						
Anthro-WC(cm)	92.4	11.8	0.0102[a] 0.0093[b]	0.1078 0.1555	0.0208[a] 0.0218[b]	<0.0001 **<0.0001**
Anthro-BMI(kg/m^2)	27.5	5.0	0.0197[a] 0.0195[b]	0.0018 **0.0027**	0.0213[a] 0.0222[b]	<0.0001 **<0.0001**
DXA-Whole Body Adiposity(kg)	16.8	7.0	0.0072[a] 0.0066[ba]	0.3667 0.4323	0.0188[a] 0.0197[ba]	0.0003 **0.0003**
DXA-Trunk Adiposity(kg)	8.2	3.7	0.0076[a] 0.0074[ba]	0.3427 0.3815	0.0179[a] 0.0195[ba]	0.0006 **0.0004**
PQCT-Subcutaneous(mm^2)	1432.8	705.9	0.0231[a] 0.0227[ba]	0.0004 **0.0005**	0.0195[a] 0.0194[ba]	<0.0001 **<0.0001**
PQCT-Intermuscular(mm^2)	275.3	328.1	-0.0031[a] -0.0024[ba]	0.6355 0.7149	0.0177[a] 0.0189[ba]	**<0.0001** **<0.0001**
PQCT-Total Calf Adiposity(mm^2)	1874.6	824.0	0.0190[a] 0.0188[ba]	0.0033 0.0036	0.0237[a] 0.0237[ba]	<0.0001 <0.0001
Muscle Measures						
DXA-Whole Body Lean Mass(kg)	65.6	8.5	0.0185[a] 0.0188[ba]	0.0222 0.0591	0.0159[a] 0.0214[ba]	0.0025 **0.0009**
PQCT-Muscle Area(mm^2)	7872.4	1286.5	0.0275[a] 0.0272[ba]	<0.0001 **<0.0001**	0.0076[a] 0.0074[ba]	0.0963 0.1116

Abbreviations: **BMI**, body mass index; **DXA**, dual X-Ray absorptiometry; **PQCT**, peripheral quantitative computer tomography; **STD**, standard deviation, exposure variables were standardized to mean=0 and standard deviation=1; **Anthro-,** anthropometric measure; **a**, adjusted for age; **b**, adjusted for age, hypertension, diabetes; **ba** , adjusted for age, hypertension, diabetes and standing height; **Bolded Figures**, were statistically significant associations P<0.05.

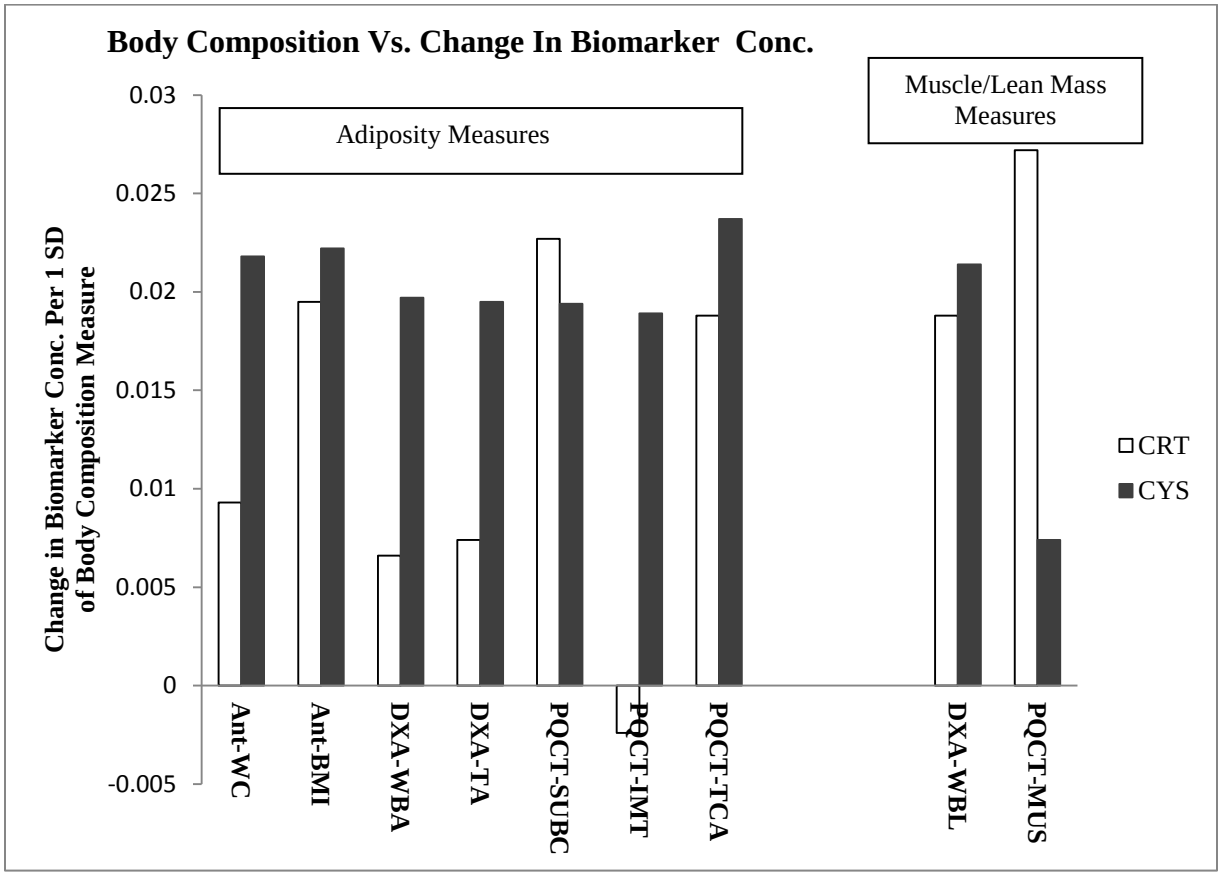

Figure 6. Changes in Serum Concentration of Kidney Function Biomarkers Per One SD of Body Composition Measure Among Participants Without CKD
Adjusted for age, diabetes, hypertension and standing height. Abbreviations: Ant, Anthropometric measures; WC, waist circumference; BMI, body mass index; TA, total lower leg adiposity in the mid-calf; SUBC, subcutaneous adiposity; IMT; intermuscular adiposity; WBL; whole body lean mass; MUS, muscle area; DXA, DXA measures; PQCT, PQCT measures.

12.5 DISCUSSION

The objective of this study was to examine the association of several body composition measures with serum creatinine and cystatin C, among older Afro-Caribbean males. We found that after adjusting for age, diabetes and hypertension, BMI was significantly associated with serum creatinine. BMI and WC were also highly associated with cystatin C. Our result extends

prior studies showing that rising serum creatinine or cystatin C levels are influenced by anthropometric measures such as BMI and WC [340, 344]. The positive association between BMI and serum creatinine may have been due to high muscle mass component. It can also be argued that the association of BMI and WC may have also been due to adiposity components.

The failure of anthropometric measures to distinguish between body adiposity and muscle mass, and its inability to discriminate between general and regional adiposity distributions, makes BMI and WC less ideal as surrogates for body composition. For these reasons, DXA and PQCT scan, which discriminate between general and regional adiposities and also quantify lean mass and adiposity separately, were used to assess several body composition measures.

In general, we observed a positive association between measures of adiposity and cystatin C and positive association between measures of muscle mass and creatinine, which was independent of age, hypertension, diabetes and standing height. Similar results were observed regardless of the CKD status. This consistent association suggests that rising serum concentrations of creatinine or cystatin C are not only the result of renal impairment, but are in fact also due to body compositions. Our results extends reports from previous studies showing that serum concentrations of creatinine and cystatin C are influenced by muscle mass and regional adiposities [41, 333, 344, 345].

It is emphasized here that even though the magnitude of association between various body compositions with biomarkers of kidney function was highly significant, the effect change was very small in the order of (10^{-2}). Therefore, the clinical relevance of this small change in serum creatinine and cystatin C by body composition is not certain. Nonetheless, our findings highlight the important role of body composition on biomarkers of kidney function.

In view of this, the contribution of body composition measures should be considered when interpreting serum concentrations of creatinine and cystatin C. The MDRD equation is widely used for estimating kidney function for determining CKD. But the utility of MDRD in determining CKD among blacks is highly questionable [33]. Although, MDRD is standardized using demographic characteristics including age, gender and race, it still does not account for the effect of lean or muscle mass for accurate estimation of glomerular filtration rate. To this end, the use of MDRD GFR for an estimating equation in a population with high mean percent lean body mass as in Tobago men (80% -Percent lean body mass) without correction factor for lean and muscle mass may not be accurate. The use of cystatin C as an alternate biomarker of kidney function may also be inadequate as it is influenced by body adiposity. However, given the high muscle mass (80% of body mass) and relatively low adiposity (20% of body mass) body composition of Tobago black males, it is our opinion that cystatin C should be the biomarker of choice for assessing kidney function in this population.

The mechanism linking body composition such as muscle and lean mass with rising serum creatinine is fairly well known. Serum creatinine is a breakdown product of creatinine phosphate in muscle tissues. The highest concentration of creatinine is found in the skeletal muscle, and as such, the rate of serum creatinine production is dependent on muscle or lean mass. Synthesis of creatinine begins in the liver where guandinoacetate, which is produced from glycine and arginine, is methylated by adenosyl methionine and transported to muscle and other energy demanding tissues. In the muscle, methylated guandinoacetate undergoes phosphorylation to become phosphocreatinine. Depending on the energy need of the muscle, phosphocreatinine is subsequently broken down to creatinine by the action of creatinine kinase [346]. The body's balance of creatinine is maintained by generation from muscle cells and

clearance by kidney. However, in the presence of kidney disease, serum creatinine concentration tends to rise and the rate of clearance may represent renal impairment.

The mechanism relating adiposity and cystatin C is complex and poorly understood. However, the elevation of Cystatin C in obesity suggests possible secretion by large adipose tissues and macrophages [347]. Additional study is needed to further understand the mechanism linking adiposity to higher levels of serum cystatin C.

In this study, the association of body compositions with serum creatinine and cystatin C suggests that cystatin C and serum creatinine may have prognostic values. In obesity related CVD [348, 349], hypertension, dyslipidemia (LDL and HDL cholesterol) and diabetes are indirect covariates that have been used previously to link obesity with CVD. Given, the finding of the relationship between obesity measures with serum creatinine and cystatin C, the expression of serum creatinine may have important prognostic values in people with obesity related CVD. Also, given the relationship between muscle and lean mass with serum creatinine, serum creatinine may also be important in predicting muscle related diseases among Tobago men. Taken together, our findings show that body compositions are small but important extra-renal source of circulating serum creatinine and cystatin C.

The strength of our population-based study includes a large sample size of 1000 men to detect the effects of body compositions on serum creatinine and cystatin C. Adjustments were made for important confounding variables and risk factors via modeling. PQCT and DXA scanning were used to determine body's general and regional adiposity distributions. PQCT procedures use modern technologies to assess three instead of two dimensional measurements of regional adiposities. The advantage is that, instead of area measurements as in DXA, volume measurements are performed which improves accuracy and precision.

Our study has some limitations. This was an observational cross-sectional study. Temporality could not be ascertained. Therefore, true cause and effect could not be established. In addition, only one cross sectional measurement of serum creatinine and cystatin C were taken. All participants in this study are blacks. Therefore, our findings may not be generalizable to other racial/ethnic groups.

12.5.1 Conclusion

In this study, after controlling for important confounders, our findings suggest that serum concentrations of circulating creatinine or cystatin C are influenced by body compositions. Therefore, the effect of body composition on these biomarkers of kidney function should be considered when interpreting serum creatinine and cystatin C.

13.0 ASSOCIATION OF INCREASING QUARTILES OF CKD BIOMARKERS WITH LONGITUDINAL BONE MINERAL DENSITY (BMD) AMONG TOBAGO BLACK MALES 40 YEARS AND OLDER, FROM 2004/2007 TO 2012

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13.1 ABSTRACT

Introduction: Chronic Kidney Disease (CKD) affects over 20 million Americans 20 years or older. One of the complications associated with CKD is bone mineral disorder. The rate of bone loss is mediated by highly integrated and interrelated biochemical regulators, involving mainly calcium, phosphorous, vitamin D and parathyroid hormone (PTH). Blacks have higher bone mass compared to whites. However, it is not certain whether blacks and whites with CKD, lose bone at similar rate. **Objective:** We sought to determine the relationship between markers of CKD, serum creatinine and cystatin C, with bone loss among Afro-Caribbean males, 40years and older, of similar ancestry as African Americans. We compared our findings to a MrOS longitudinal bone study among U.S Caucasian men with CKD. **Methods:** Approximately 1,425 Afro- Caribbean males from the Caribbean Island of Tobago were included in our analyses. In 2004/2007, questionnaires were administered to ascertain demographic information, medical history and risk factors. Dual X-ray Absorptiometry (DXA) of total hip and its sub-region (trochanter and femoral bone) were measured in 2004-2007 and at follow-up visits in 2012. Serum creatinine was measured by Jaffè reaction. Serum cystatin C was measured using Dade Behring nephelometry. After a mean follow-up of 6 years, the relationship of annual percent BMD change with makers of CKD was analyzed using quartiles of serum creatinine and cystatin C after controlling for important confounders. Annual percent bone loss was compared with published bone loss data in Caucasian men in the MrOS Study. **Result:** There was a consistent decline in annual percent BMD across quartiles of Albumin Creatinine Ratio (ACR) and serum

creatinine and cystatin C in trochanter, femoral neck and total hip bones. The rate of bone loss was similar to that in Caucasian men. **Conclusion**: Higher levels of each of the three biomarkers of CKD predicted higher bone loss in Tobago men over six years follow-up.

13.2 INTRODUCTION

Chronic Kidney Disease (CKD) is a growing health problem in U.S, affecting over 20 million Americans aged 20 years or older [350]. A Center for Disease Prevention and Control(CDC) report indicates that the prevalence of CKD rose from 12.3% to 14% in the past decade [331]. One of the complications associated with CKD is bone mineral disorder. According to the Surgeon General's report on bone health and osteoporosis, an estimated 44 million Americans over 50 years have osteoporosis or osteopenia[351]. The first awareness about the relationship between bone disorders and CKD was reported among dialysis patients by Pendras and Erickson in 1966 [144]. Since then, there is increasing evidence suggesting that early CKD stages, preceding dialysis, may also be associated with bone and mineral disorders [145] [146]. Bone disorders from renal disease were previously termed renal dystrophy. However, a 1995 Kidney Disease Improving Global Outcomes (KDIGO) consensus modified the system of CKD classification to recognize the systemic component of bone disorders. The term renal dystrophy now refers to bone pathological and histological disorders, including osteitis fibrosa, osteomalacia, osteoporosis and adynamic bone disease, whereas Chronic Kidney Disease – Mineral & Bone Disorder Disease (CKD-MBD) refers to bone disorders arising from CKD and the broader effect of CKD on bone, such as mineral disorders [148]. In order to understand the

mechanism of CKD -MBD, it is important to first understand the bone architecture. There are two types of bone: the trabecular bone which is soft, less dense and found principally in the ends of long bones and in vertebrae; the cortical bone which is hard, dense and found mostly in the shaft of long bones, and in the outer shell covering around the trabecular bones at the end of joints or vertebrae [352]. The rate of bone loss is mediated by highly integrated and interrelated biochemical regulators, involving mainly calcium, phosphorous, vitamin D and parathyroid hormone (PTH). Low serum calcium or vitamin D and high phosphorous promotes the release of PTH and hyperparathyroidism. High circulating PTH in turn triggers bone resorption which ultimately affects the rate of bone turnover, volume and mineralization. This condition is further worsened in CKD and ESRD patients unable to synthesize vitamin D and excrete phosphorous [36, 145]. Biochemical imbalances may begin from CKD stage 3 when the eGFR drops below 60ml/min/1.73/m^2 and gradually worsens as eGFR falls below 15ml/min/1.73/m^2 [149]. In children, minerals and biochemical imbalances have been reported as early as CKD stage 2 [150].

Although, cortical bones are metabolically less active compared to the trabecular bone, studies show that bone loss occurs more in cortical than trabecular bone due to elevated PTH which tends to have a protective effect on trabecular bone and deleterious effect on cortical bone [353-355]. The strength of cortical or trabecular bone is largely determined by bone mass, which is usually in a state of balance by the bone forming osteoblast and bone resorption osteoclast cells. Imbalances may be caused by many disease conditions including CKD, resulting in bone resorption taking more precedence over formation. Age, race and certain medications are known risk factors affecting the rate of bone resorption [356, 357].

The social and economic cost, including lifestyle changes, loss of functionality, increased mortality and risk of fracture associated with bone loss, makes CKD-BMD a major public health burden [180-184]. The risk of fracture is seven times higher among patients with CKD stage 5 and among CKD patients with previous fracture compared to those without fracture [185, 186]. Bone loss is also associated with CVD and CVD mortality through arterial calcification, a highly regulated process involving the bone and teeth. In CKD patients, extra-osseous calcification occurs in vascular tissue especially in the arteries, resulting in stiffening of arterial walls including the media and intima layers, and formation of atherosclerotic plaques [189-191]. A study found that 47-83% of patients with CKD stage 3-5 have cardiovascular calcification and many die from CVD causes [192].

Previous studies have shown that blacks have higher bone mass compared to whites [358, 359]. Nonetheless, it is not certain whether blacks and whites with CKD, lose bone at the same rate. Much of the study on bone loss has been focused on Caucasians and women, and few on ethnic minorities. Given the limited information on ethnic minorities and high prevalence of CKD among African Americans at (19.9%) compared to Caucasians at (16.1 %), we sought to determine the relationship between CKD, or low kidney function, with bone loss among Afro-Caribbean males, forty years or older of similar ancestry as African Americans. This is also in keeping with the Surgeon General report recommending expansion of research on bone loss to include racial/ethnic minorities [351]. In addition, we will compare the results of our findings to a longitudinal study among U.S Caucasian men with CKD to ascertain racial/ethnic differences in the rate of bone loss among blacks and whites.

13.3 DESIGN AND METHODS

13.3.1 Study Population

In 2000, the estimated population of Tobago was 54,084 [323]. In 1997- 2003, men from the Caribbean Island of Tobago were enrolled into the Tobago Prostate Cancer Survey [360]. Recruitment was mostly accomplished through word of mouth throughout the island. Men who were institutionalized, terminally ill or unable to give informed consent were excluded and men who are 40 years or older were included. The racial composition of recruited participants was 97% African, 2% East Indian and less than 0.5% Caucasian. Questionnaires were administered to participants by experienced interviewers.

Among the initial 3094 men in 1997-2003, information about demography, medical history, risk factors, socioeconomic status, lifestyle, habits including cigarette smoking, alcohol consumption, level of education, past medical history, prescription and non prescription medication use, history of diabetes and hypertension were recorded. After initial participation, all men were invited for a follow -up visit in 2004-2007. At this visit, anthropometric measures, medical history, risk factor profile and Dual X-ray Absorptiometry (DXA) scan of the hip were taken. Urine and blood samples were collected from participants and stored at -80^{0}C in the Heinz laboratory, located at the Graduate School of Public Health, University of Pittsburgh, Pennsylvania U.S. Approximately 1700 participants from 2004-2007 are currently being followed and measurements of anthropometrics and DXA scan of the hip, and whole body are, presently ongoing in 2012. The current study includes a subsample of approximately 1492 Tobago black males followed for an average of 6 years who have complete bone measures from 2004-2007 to 2012. Informed consents were obtained from participants and study was approved

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

13.3.2 Bone Mineral Density Measurements

Hologic QDR-4500W densitometer (Hologic Inc., Bedford, MA) DXA machine was used to measure areal BMD g/cm^2 of total hip and its sub regions: femoral neck and trochanter. Standard procedures for participant positioning and scan analyses were followed according to the manufacturer's recommended protocol. Scans were analyzed with QDR software version 8.26a. The short-term in vivo precision of the DXA measurements was assessed in 12 subjects. The coefficient of variations was less or equal to 1.16% and all test-retest correlations were above 0.99. A phantom was scanned daily and reviewed to maintain longitudinal quality assurance of the scanner during the course of the study [361]. In this study, annual percent BMD change was calculated by dividing the percent BMD change from 2004/2007 to 2012, by the number of years of follow-up.

13.3.3 Laboratory Measurements

Serum creatinine was quantitatively measured in samples collected at the 2004-2007 visit using Jaffè reaction[324] and Sigma Diagnostics (St. Louis, MO) creatinine kit. Serum was diluted 1:26 with picric acid/sodium hydroxide solution, incubated at 30^{o}C for four minutes and read at 500 nm. Serum controls and duplicate samples were run with each assay. Serum creatinine assays were standardized to the national IDMS-traceable to obtain creatinine value[325]. The coefficient of variation between runs was 6.0%[362]. Quartiles of serum

creatinine were created by using the following cut points: first, <0.7mg/dl; second, (0.7 to <1.10) mg/dl; third, (1.10 to 1.5) mg/dl; fourth >1.5mg/dl.

Serum cystatin C was measured at the University of Vermont from frozen samples collected in 2004-2007 using BNII nephelometer (Dade Behring, Inc, Deerfield, Ill) with a particle-enhanced immunonephelometric assay (N Latex Cystatin C, Dade Behring, Inc). The assay range was from 14.6 to 549.0 nmol/L (0.195 to 7.330 mg/L). The reference range for young, healthy persons was reported to be 40 to 71nmol/L (0.53 to 0.95 mg/L). The intra-assay coefficient of variation ranged from 2.0% to 2.8%, and the inter-assay coefficient of variation ranged from 2.3% to 3.1% [342].Quartiles of serum cystatin C was created according to the following cut points: first quartile, cystatin C <0.60mg/L; second quartile, cystatin C (0.60 to <0.85)mg/L; third quartile, cystatin C (0.85 to 1.11)mg/L; fourth quartile, cystatin C >1.11mg/L.

Urinary albumin was measured using a turbidimetric procedure on the Olympus AU400 with reagents provided by Olympus America, Inc. (Center Valley, PA). Briefly, 10 µl of urine was incubated with goat anti-human albumin antibody for 5 minutes at room temperature. The resulting turbidity was measured at 340/800 nm. Blanks, calibrators and control pools were run simultaneously with all samples. The intra-and interassay coefficients of variation were below 2.5% and 5.1%, respectively[326]. Urinary creatinine was measure using the Jaffe reaction as described above. Categories of the albumin/creatinine ratio (ACR) were created as follows: first, <30mg/g; second, (30 to <135) mg/g; third, (>135 to 300) mg/g; fourth, >300mg/g.

13.3.4 Covariates

All covariates were assessed or measured from the 2004/2007 visit. Hypertension was measured by taking three blood pressure measurements after five minutes of seated rest using an

automate blood pressure machine (Omron model HEM-705CP, Illinois).The average of the second and third measurements was taken to calculate systolic and diastolic pressures. Hypertension was defined as systolic pressure equal or greater than 140mmHg or diastolic pressure equal or greater than 90mmHg or self reported diagnosis by a doctor or other health professional and anti-hypertension medication use. Cardiovascular disease was assessed from questionnaire, and included diagnosis of congestive heart failure, arrhythmias, angina pectoris, valvular disease or taking cardiovascular prescription medications for cholesterol and lipid treatments. Diabetes was defined as fasting glucose >126mg/dl or self-reported diagnosis by a doctor or other health professional and taking anti-diabetic medications. BMI was measured by determining a participant's weight in kilograms and height in meters and then dividing the weight by height squared. Cigarette smoking was self reported as current smoker. Alcohol consumption was defined as self reported current drinker of at least 3 drinks per week. Physical activity was defined as taking a walk outside home or yard for any reason including, walking to store, work, church, fun walk and exercise for at least five days in a week. Education, vitamin D and calcium supplements use were self reported.

13.3.5 Statistical Analyses

Only participants who identified as black with measurements for BMD change from 2004-2007 to 2012 were included. The characteristics of study participants were described using means, standard deviations and percentages as appropriate. The primary outcome of interest is annual percent BMD change in each bone site examined (trochanter, femoral and total hip bone) and the predictor is each quartile of ACR, serum creatinine and cystatin C. Unadjusted and age-adjusted univariate analyses were performed to examine the correlates of annual percent BMD

change each of trochanter, femoral neck and total hip bone. The association between annual percent BMD change, each, in trochanter, femoral neck and total hip bone from 2004-2007 to 2012 follow-up visits, with quartile of ACR, serum creatinine and cystatin C, was examined using robust regression. Robust regression was used to deal with possible normality violations and extreme data point outliers. In each analyses, least square means (LSMEANS) was used to calculate mean annual percent BMD change across quartiles of ACR, serum creatinine and cystatin C. Four models were developed for each bone site: First model, was unadjusted; second model, was adjusted for age; third model, was adjusted for age, baseline BMD of the respective bone site and percent weight change from 2004/2007 to 2012; fourth model, was the full model, which included model three in addition to other important covariates of the respective bone site being examined. A p-value ≤ 0.1 was used as cut point for including covariates from univariate analyses. Correlated covariates were examined and only one was chosen based on the degree of relevance to the primary outcome of interest. A sub analyses involving a subsample of 219 Tobago men aged 65 years and older was performed. The association of increasing cystatin C quartiles with annual percent total hip BMD change was examined and compared with Osteoporotic Fractures in Men study (MrOS)[319], involving Caucasian males 65years and older. Categories for serum cystatin C was defined similarly as in MrOS as follows: Q1,(<0.97); Q2,(0.98-1.10); Q3,(1.11-1.25); Q4($\geq$1.26). Test for linear trend was performed. Statistical significance was based on alpha value of 0.05. Statistical analyses were performed using (SAS, Version 9.3, SAS Institute, Cary, NC).

13.4 RESULTS

The average follow-up for all participants was 6 years. The characteristics of study participants are shown in (Table 19). The mean age was 57.0 years (SD: ± 9.2).The mean BMI was 27.6kg/m^2 (SD: ± 4.5) with average waist circumference of 93.2cm (SD: ± 11.2) and mean whole body lean mass of 65.2kg (SD: ± 8.8). Mean percent change in body weight was 0.0037 %(SD: ±7.2). Mean weekly calcium supplement intake was 3372.1mg (SD: ± 1621.3). Approximately 14% reported vitamin D supplement use. The median ACR measure was 2.9 mg/g. The mean serum creatinine and cystatin C were 1.2mg/dl (SD: ±0.4) and 0.8mg/L (SD:±0.2) respectively. Approximately 48% had hypertension, 18.7% had diabetes and 3.5% reported CVD. About 13% reported as current smokers, 10.7% reported alcohol consumption of more than three drinks per week and 64.5% reported being physically active. The mean percent annual BMD change from 2004/2007 to 2012 for femoral, trochanter and total hip bone were -0.4 (SD: ± 0.8), -0.2 (SD; ± 0.9) and -0.1 (SD: ± 0.6) % respectively. The mean BMD at baseline for femoral, trochanter and total hip were 1.0 (SD: ± 0.2), 0.9 (SD: ± 0.1) and 1.2 (SD: ± 0.2) mg/cm^2 respectively.

Table 18. Population Characteristics of Tobago Males 40 Years and Older, 2004/2007

Characteristics	N=1425	%	Mean(SD) *Median(Variance)*
Age(yr)	1404		57.0(9.2)
40-49	344	24.5	
50-59	548	39.1	
60-69	346	24.7	
>70	165	11.8	
BMI(kg/m^2)	1422		27.6(4.5)
WC (Cm)	1417		93.2(11.2)
Baseline(2004-7) BMD ,(g/cm^2)			
Femoral Neck Bone	1425		1.0(0.2)
Trochanter Bone	1425		0.9(0.1)
Total Hip Bone	1425		1.2(0.2)
Annual BMD Change (2004-7 to 2012), %			
Femoral Neck Bone	1425		-0.4(0.8)
Trochanter Bone	1425		-0.2(0.9)
Total Hip Bone	1425		-0.1(0.6)
Weekly Calcium Supplement Intake(mg)	1407		3372.1(1621.3)
Body Weight (V3) (Kg)	1422		85.0(15.0)
% Change in Body Weight from V3-v5	1402		0.0037(7.2))
ACR, (mg/g), *Median(Variance)*	643		2.9(23922)
Serum creatinine(mg/dl)	1338		1.2(0.4)
MDRD eGFR <60ml/min/$1.73m^2$)	1334		80.6(37.0)
Serum Cystatin C(mg/L)	592		0.8(0.2)
Physical Activity	904	64.5	
Vitamin D supplement Use (At least one time per week)	193	14.2	
Hypertension	683	48.0	
Diabetes	266	18.7	
CVD	49	3.5	
Current Smokers	144	13.0	
Alcohol Drinkers (> three drinks per week)	150	10.7	
Education			
Primary/No Education	1004	72.1	
High School O/A Level	285	20.5	
College/Associate	103	7.4	

Abbreviations: **Alcohol,** current alcohol drinker of more than three drinks per week; **BMD**, bone mineral density; **CVD**, cardiovascular disease; **Diabetes**, self-reported and taking diabetes medication or fasting glucose > 126mg/dl; **Hypertension**; systolic pressure ≥140mmHg, or diastolic pressure ≥90mmHg or taking medication and self-reported; **Physical Activity**, taking a walk outside home or yard for any reason including fun walk, exercise, walking to work, store or church for at least 5 days in a week **SD,** standard deviation; **Smoking**, current smokers; **WC**, waist circumference .

Correlates of trochanter, femoral and total hip annual percent BMD change after adjusting for age are shown in (Table 20).

Trochanter Bone

For trochanter bone, BMI, waist circumference, percent change in body weight, physical activity, vitamin D supplement intake, diabetes and CVD were each associated with annual percent BMD change in trochanter bone ($p \leq 0.1$).

Femoral Bone

For femoral bone, waist circumference, percent change in body weight, physical activity, vitamin D supplement intake, hypertension, diabetes and CVD were each associated with annual percent BMD change in femoral bone ($p \leq 0.1$).

Total Hip Bone

BMI, waist circumference, percent change in body weight, vitamin D supplement intake, diabetes, CVD and smoking, were each related to annual percent BMD change in total hip bone ($p \leq 0.1$).

Table 19. The Univariate Analyses of Correlates of Percent Annual BMD Change In Trochanter, Femoral Neck and Total Hip Among Tobago Black Males 40 Years and Older, 2004/2007

	Trochanter			Femoral Neck			Total Hip		
Exposure	AC	P-Val	P-Val*	AC	P-Val	P-Val*	AC	P-Val	P-Val*
Age(yr)^{S}	-0.2411	<0.0001	N/A	-0.1292	<0.0001	N/A	-0.1459	<0.0001	N/A
BMI(kg/m^2) ^{S}	0.0903	0.0001	**0.0010**	0.0298	0.1379	0.3287	0.0760	<0.0001	**0.0001**
WC (Cm)	0.0852	0.0003	**<0.0001**	0.0385	0.0554	**0.0309**	0.0755	<0.0001	**<0.0001**
Baseline(V3)BMD (g/cm^2)^{S}	0.0606	0.0101	**0.0868**	0.0182	0.3644	0.4706	0.0670	<0.0001	**0.0058**
Weekly Calcium Intake(mg)^{S}	0.0199	0.4032	0.8219	0.0196	0.3327	0.6444	0.0183	0.2506	0.6657
Whole Body Lean Mass(Kg)^{S}	0.0586	0.0251	0.8110	0.0228	0.3114	0.4915	0.0564	0.0013	0.3635
% Change in Body Weight (V3-V5)	0.1376	<0.0001	**<0.0001**	0.1447	<0.0001	**<0.0001**	0.1239	<0.0001	**<0.0001**
Serum creatinine(mg/dl)^{S}	-0.0860	0.0004	**0.0092**	-0.0459	0.0251	0.1383	-0.0622	0.0001	**0.0051**
ACR, (mg/g)^{S}	-0.0689	0.0784	0.1074	-0.0195	0.5277	0.6546	-0.0570	0.0165	**0.0249**
Cystatin C,(mg/L)^{S}	-0.1341	0.0020	**0.0088**	-0.0599	0.0848	0.3277	-0.1010	0.0001	**0.0053**
Physical Activity (walk >5 times in past 7 days)	0.1023	0.0401	**0.0591**	0.0596	0.0236	**0.0323**	0.0599	0.0737	0.1164
Vitamin D supplement Use	0.0977	0.1606	**0.0397**	0.0927	0.1155	**0.0493**	0.0643	0.1670	**0.0361**
Hypertension	-0.1348	0.0043	0.4004	-0.1043	0.0095	**0.060**	-0.1198	0.0008	0.2942
Diabetes	-0.2728	<0.0001	**0.0004**	-0.2359	<0.0001	**0.0001**	-0.1950	<0.0001	**0.0002**
CVD	-0.4007	0.0020	**0.0217**	-0.3429	0.0019	**0.0139**	-0.2476	0.0044	**0.0559**
Current Smoker	-0.0711	0.3209	0.1152	-0.0496	0.4465	0.2226	-0.0979	0.0604	**0.0121**
Alcohol Drinker (> three drinks per week)	0.1226	0.1096	0.1965	0.0870	0.1846	0.2310	0.0879	0.0893	0.1695

Abbreviations: **ACR,** albumin creatinine ratio; **BMI**, body mass index; **BMD**, bone mineral density; **CVD**, cardiovascular disease; **AC**, percent annual BMD change in % per standard deviation of continuous exposure variable denoted by **S** ; **P-Val*,** age-adjusted P-Value; **S**, standardized to mean=0 and standard deviation=1; **WC**, waist circumference; % Change in

Weight , percent change in weight from V3(2003-2007 to V5(2012). **Bolded Figures,** were considered significant and included in full model if P-Val was ≤0.100.

Association of Quartiles of ACR with Mean Percent Annual BMD Change

The relationships of trochanter, femoral neck and total hip annual percent BMD change each, with quartiles of ACR were further examined in (Table 21). In general, there was greater decline in annual percent BMD across increasing quartiles of ACR in all the bone sites (Figure 7).

Trochanter Bone

A significant linear trend was observed with greater bone loss across increasing quartiles of ACR in the unadjusted model (annual percent BMD decline across ACR quartiles; -0.19, -0.48,-0.58,-0.85. P-vale for trend=0.0482). The result was similar after adjusting for age (P for trend =0.0068). Further adjusting with baseline trochanter BMD and percent change in body weight still showed greater bone loss across increasing quartiles of ACR, but the linear trend was no longer significant(P=0.0644). Similar result was also observed in the full model adjusting for age, percent change in body weight, baseline trochanter BMD, physical activity, vitamin D supplement use, diabetes and CVD.

Femoral Bone

We observed greater femoral neck bone loss across increasing quartiles of ACR (annual percent BMD decline across ACR quartiles: -0.38,-0.56,-0.58,-0.83; P for trend=0.1036), without significant linear trend in the unadjusted model. Similar results were obtained in model 2, adjusting for age; model 3, adjusting for age baseline femoral neck BMD and percent change in body weight; and in the full; adjusting for age, percent change in body weight, baseline femoral neck BMD, waist circumference, hypertension, diabetes, physical activity and CVD .

Total Hip Bone

The unadjusted analyses show a significant linear trend in increasing total hip bone loss across increasing quartiles of ACR (annual percent BMD decline across ACR quartiles: -0.10, -0.23,-0.20,-0.73, P for trend=0.0034). Similar result was also found after adjusting for age (P for trend=0.0044). Although there was attenuation of total hip bone loss after controlling for age, baseline total hip BMD and percent change in body weight, nonetheless, the linear trend still remained significant (P for trend=0.0043). Similar attenuation, but with significant linear trend was also observed in the full model adjusting for age, percent change in body weight, baseline total hip BMD, BMI, vitamin D supplement use, diabetes, CVD and smoking (P for trend=0.0089).

Table 20. Longitudinal Association of Quartiles of ACR With Percent Annual BMD Change From 2004/2007 to 2012 in Trochanter, Femoral Neck and Total Hip Bone, Among Tobago Black Males 40 Years and Older

Bone	Annual Percent Bone Mineral Density Change (95% CI)				
	Q1 <30mg/g	Q2 (30 to -135)mg/g	Q3 (136 - 300)mg/g	Q4 >300mg/g	P-Val. For Trend
Trochanter Bone					
1 Unadjusted	-0.19(-0.27,-0.11)	-0.48(-0.81,-0.16)	-0.58(-1.16,0.1)	-0.85(-1.50,-0.20)	0.0482
2 Age	-0.19(-0.27,-0.11)	-0.42(-0.74,-0.10)	-0.49(-1.07,0.09)	-0.81(-1.44,-0.17)	0.0068
3 Age+%WBLM+TBMD	-0.18(-0.26,-0.10)	-0.42(-0.75,-0.10)	-0.41(-0.98,0.17)	-0.83(-1.00,-0.17)	0.0644
4 Full Model[t]	-0.17(-0.25,-0.10)	-0.43(-0.75,-0.11)	-0.46(-1.04,0.11)	-0.75(-1.39,-0.11)	0.0938
Femoral Neck Bone					
1 Unadjusted	-0.38(-0.44,-0.31)	-0.56(-0.82,-0.31)	-0.58(-1.05,-0.13)	-0.83(-1.33,-0.32)	0.1036
2 Age	-0.38(-0.45,-0.32)	-0.49(-0.75,-0.24)	-0.51(-09.6,-0.06)	-0.79(-1.29,-0.29)	0.1267
3 Age+%WBLM+FBMD	-0.39(-0.45,-0.33)	-0.49(-0.74,-0.24)	-0.41(-0.86,-0.04)	-0.82(-1.31,-0.33)	0.1303
4 Full Model[t]	-0.39(-0.45,-0.33)	-0.48(-0.73,-0.23)	-0.37(-0.82,0.08)	-0.76(-1.26,-0.26)	0.2138
Total Hip Bone					
1 Unadjusted	-0.10(-0.14,-0.05)	-0.23(-0.43,-0.04)	-0.20(-056, 0.15)	-0.73(-1.12,-0.34)	0.0034
2 Age	-0.10(-0.15,-0.05)	-0.18(-0.37,0.01)	-0.13(-0.48,0.21)	-0.70(-1.08,-0.32)	0.0044
3 Age+%WC+HBMD	-0.09(-0.14,-0.05)	-0.19(-0.37,0.00)	-0.06(-0.40,0.28)	-0.72(-1.09,-0.34)	0.0043
4 Full Model[h]	-0.12(-0.17,-0.07)	-0.18(-0.37,0.02)	-0.03(-0.39,0.34)	-0.77(-1.19,-0.35)	0.0089

Abbreviations: **Full model[t],** adjusted for age, percent change in body weight from V3(2003/2007) to V5(2012), baseline trochanter BMD, physical activity, vitamin D supplement use, diabetes and CVD; **Full model[f]**, adjusted for age , percent change in body weight from V3(2003/2007 to V5(2012), baseline femoral neck BMD, waist circumference, hypertension, diabetes, physical activity and CVD; **Full model[h],** adjusted for age, percent change in body weight from V3(2003/2007) to V5(2012), baseline total hip BMD, BMI, vitamin D supplement use, diabetes, CVD and smoking; **TBMD**, trochanter baseline BMD; **FBMD**, femoral neck baseline BMD**; HBMD**, total hip bone baseline BMD; **Q1-4**, quartiles of Albumin Creatinine Ratio(ACR).

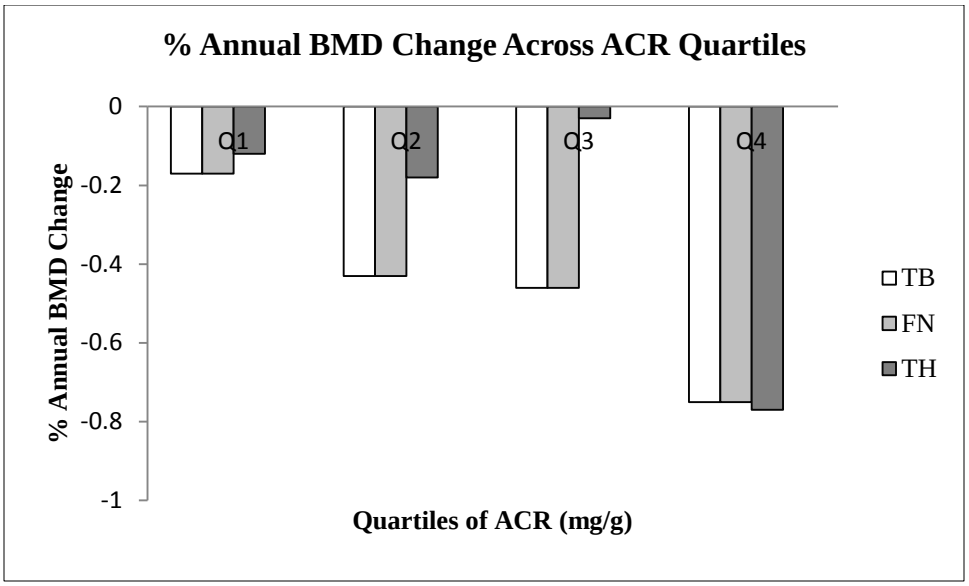

Figure 7. Multivariable Adjusted Percent Annual BMD Change Across Bone Sites with ACR
Abbreviations: TB,trochanter bone; FN, femoral-neck bone; TH, total hip bone; ACR, albumin creatinine ratio; BMD, bone mineral density; Q1-4, Categories of ACR; Q1, <30mg/g; Q2, (30-135)mg/g; Q3, (136-300)mg/g; Q4, >300mg/g; Q2 +Q3, micro-albuminuria; Q4, macro-albuminuria.

Association of Quartiles of Serum Creatinine with Mean Annual Percent BMD Change

The relationships of trochanter, femoral neck and total hip annual percent BMD change each with quartiles of serum creatinine were examined in (Table 22). There was no significant trend in all the bone sites examined across increasing quartiles of serum creatinine (Figure 8).

Trochanter Bone

There was no significant linear trend in annual percent BMD change of trochanter bone across increasing quartiles of serum creatinine in the unadjusted model(annual percent BMD decline across serum creatinine quartiles: 0.16, -0.16,-0.24, -0.64, P for trend=0.1073). Similar results were found for model 2, adjusting for age; model 3, adjusting for age, baseline trochanter BMD and percent change in body weight; and in the full model, adjusting for age, percent change in body weight, baseline trochanter BMD, physical activity, vitamin D supplement intake, diabetes and CVD.

Femoral Neck Bone

There was increasing bone loss at the femoral neck across increasing quartiles of serum creatinine in the unadjusted model (annual percent BMD decline across serum creatinine quartiles:-0.36, -0.37, -0.40, -0.66, P for trend=0.5030). Similar results were found after adjusting for age. On further adjustment with, baseline femoral BMD and percent change in body weight and in the full model adjusting for age, percent change in body weight, baseline femoral neck BMD, waist circumference, hypertension, diabetes, physical activity and CVD , similar results were found.

Total Hip Bone

In the unadjusted model, we observed slight increase in mean annual percent total hip bone in the first quartile of serum creatinine. However, the higher quartiles of serum creatinine were associated with increasing bone loss (annual percent BMD decline across serum creatinine quartiles: 0.11, -0.08, -0.12, -0.43 P for trend=0.1117) with no significant linear trend. We also observed similar result after adjusting for age, and after further adjusting for baseline total hip BMD and percent change in body weight and, in the full model adjusting for age, percent change in body weight, baseline total hip BMD, BMI, vitamin D supplement intake, diabetes, CVD and smoking.

Table 21. Longitudinal Association of Quartiles of Serum Creatinine With Percent Annual BMD Change From 2004-7 to 2012 in Trochanter, Femoral Neck and Total Hip Bone, Among Tobago Black Males 40 Years and Older

Bone	Annual Percent Bone Mineral Density Change (95% CI)				
	Q1 <0.70mg/dl	Q2 (0.70- 1.10)mg/dl	Q3 (1.11- 1.50)mg/dl	Q4 >1.50mg/dl	P-Val For Trend
Trochanter Bone					
1 Unadjusted	0.16(-0.84,1.16)	-0.16(-0.24,-0.08)	-0.24(-0.30,-0.17)	-0.64(-0.81,-0.48)	0.1073
2 Age	0.13(-0.85,1.12)	-0.19(-0.27,0.11)	-0.24(-0.30,-0.18)	-0.49(-0.66,-0.32)	0.2085
3 Age+%WC+TBMD	0.15(-0.83,1.13)	-0.18(-0.26,-0.10)	-0.24(-0.30,-0.18)	-0.48(-0.65,-0.31)	0.1980
4 Full Model[t]	0.23(-0.74,1.21)	-0.18(-0.27,-0.10)	-0.24(-0.30,-0.18)	-0.47(-0.64,-0.30)	0.1536
Femoral Neck Bone					
1 Unadjusted	-0.36(-1.23,0.46)	-0.37(-0.44,-0.30)	-0.40(-0.45,-0.35)	-0.66(-0.45,-0.53)	0.5030
2 Age	-0.40(-1.24,0.44)	-0.39(-0.46,-0.32)	-0.40(-0.45,-0.35)	-0.56(-0.70,-0.41)	0.7073
3 Age+%WC+FBMD	-0.34(-1.18,0.49)	-0.39(-0.46,-0.32)	-0.40(-0.45,-0.35)	-0.55(-0.70,-0.41)	0.6207
4 Full Model[f]	-0.31(-1.13,0.52)	-0.39(-0.46,-0.32)	-0.40(-0.45,-0.35)	-0.55(-0.69,-0.40)	0.5632
Total Hip Bone					
1 Unadjusted	0.11(-0.55,0.77)	-0.08(-0.14,-0.03)	-0.12(-0.16,-0.08)	-0.43(-0.53,-0.32)	0.1117
2 Age	0.09(-0.56,0.73)	-0.11(-0.17,-0.06)	-0.12(-0.16,-0.08)	-0.30(-0.41,-0.19)	0.2411
3 Age+%WC+HBMD	0.12(-0.52,0.76)	-0.12(-0.16,-0.05)	-0.13(-0.16,-0.09)	-0.30 (-0.41,-0.19)	0.2007
4 Full Model[h]	0.19(-0.56,0.93)	-0.11(-0.16,-0.04)	-0.15(-0.19,-0.11)	-0.31(-0.44,-0.18)	0.1826

Abbreviations: **Full model[t],** adjusted for age, percent change in body weight from V3(2003/2007 to V5(2012), baseline trochanter BMD, physical activity, vitamin D supplement use, diabetes and CVD; **Full model[f]**, adjusted for age , percent change in body weight from V3(2003/2007 to V5(2012), baseline femoral neck BMD, waist circumference, hypertension, diabetes, physical activity and CVD; **Full model[h],** adjusted for age, percent change in body weight from V3(2003-2007 to V5(2012), baseline total hip BMD, BMI, vitamin D supplement use, diabetes, CVD and smoking; **TBMD**, trochanter baseline BMD; **FBMD**, femoral neck baseline BMD**; HBMD**, total hip bone baseline BMD; **Q1-4**, quartiles of serum creatinine.

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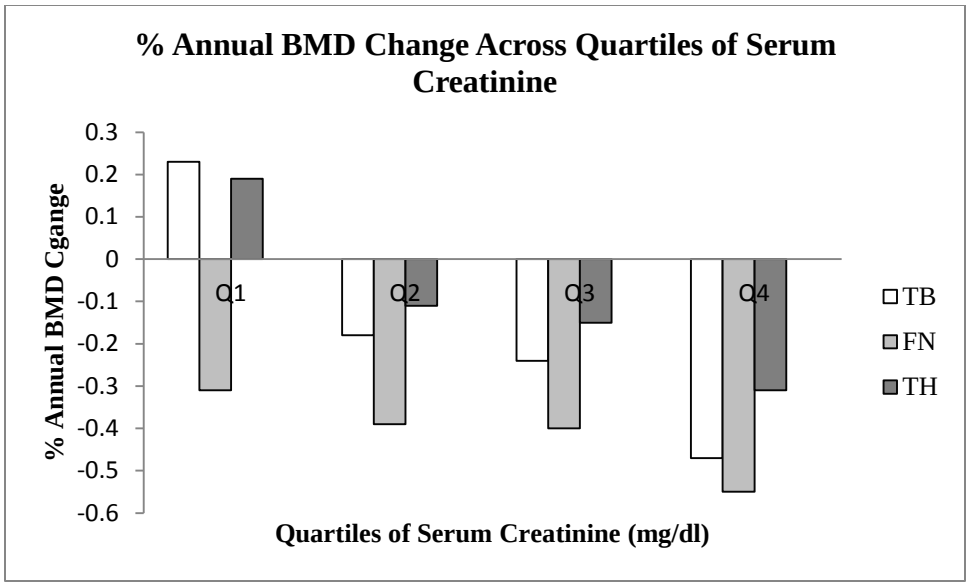

Figure 8. Multivariable Adjusted Percent Annual BMD Change Across Bone Sites With (Serum Creatinine)
Abbreviations: TB, trochanter bone; FN, femoral-neck bone; TH, total hip bone; BMD, bone mineral density; Q1-4, quartiles of serum creatinine; Q1, 0.7mg/dl; Q2, (0.7-1.1)mg/dl; Q3,(1.11-1.50)mg/dl; Q4, >1.50mg/dl.

Quartile of serum cystatin C with mean annual percent BMD change

The relationships of trochanter, femoral neck and total hip mean annual percents BMD change each with increasing quartiles of serum cystatin C is shown in (Table 23). We observed a significant linear trend and greater bone loss, in all the bone sites examined across increasing quartiles of serum cystatin C (Figure 9).

Trochanter Bone

The unadjusted model show significant linear trend with increasing bone loss across increasing quartiles of serum cystatin C (annual percent BMD decline across serum cystatin C quartiles: -0.28, -0.34, -0.51, -0.96 P for trend=0.0031). Similar results were observed after adjusting for age and further adjusting for baseline trochanter BMD and percent change in body weight. Similar result was also found in the full model adjusting for age, percent change in body

weight, baseline trochanter BMD, physical activity, vitamin D supplement intake, diabetes and CVD.

Femoral Neck Bone

There was greater bone loss across increasing quartiles of serum cystatin C (annual percent BMD decline across serum cystatin C quartiles: -0.36, -0.46, -0.54, -0.76 P for trend=0.0340). On adjusting for age, increasing serum cystatin was still associated with greater bone loss; however, the linear trend did not persist. We also observed similar result on further adjusting with baseline femoral neck bone BMD and percent change in body weight, and also in the full model adjusting for age, percent change in body weight, baseline femoral neck BMD, waist circumference, hypertension, diabetes, physical activity and CVD.

Total Hip Bone

There was increasing total hip bone loss across increasing quartiles of serum cystatin C (-0.10, -0.20, -0.31, -0.65 P=0.0001). This bone loss persisted after adjusting for age and after further adjusting for baseline total hip BMD and percent change in body weight. We also observed that on adjusting for age, percent change in body weight, baseline total hip BMD, BMI, vitamin D supplement intake, diabetes, CVD and smoking in the saturated model, the association still persisted.

Table 22. Longitudinal Association of Quartiles of Serum Cystatin C With Annual Percent BMD Change (APBMDC) From 2004-7 to 2012 in Trochanter, Femoral Neck and Total Hip Bone, Among Tobago Black Males 40 Years and Older

	Annual Percent Bone Mineral Density Change (95% CI)				
	Q1 <0.6mg/L	Q2 (0.6 -0.85)mg/L	Q3 (0.86 - 1.11)mg/L	Q4 >1.11mg/L	P-Val For Trend
Trochanter Bone					
1 Unadjusted	-0.28(-0.53,-0.03)	-0.34(-0.44,-0.24)	-0.51(-0.72,-0.30)	-0.96(-1.37,-0.54)	0.0031
2 Age	-0.30(-0.55,-0.04)	-0.34(-0.44,0.23)	-0.49(-0.71,-0.26)	-0.93(-1.35,-0.51)	0.0095
3Age+%WC+TBMD	-0.31(-0.56,-0.05)	-0.33(-0.44,-0.23)	-0.48(-0.70,-0.26)	-0.92(-1.34,-0.50)	0.0120
4 Full Model[t]	-0.23(-0.49,-0.02)	-0.33(-0.44,-0.22)	-0.51(-0.74,-0.28)	-0.93(-1.35,-0.50)	0.0051
Femoral Neck Bone					
1 Unadjusted	-0.36(-0.56,-0.16)	-0.46(-0.55,-0.38)	-0.54(-0.71,-0.38)	-0.76(-1.09,-0.43)	0.0340
2 Age	-0.39(-0.59,-0.19)	-0.47(-0.55,-0.39)	-0.50(-0.68,-0.32)	-0.72(-1.05,-0.38)	0.1100
3 Age+%WC+FBMD	-0.46(-0.66,-0.13)	-0.46(-0.55,-0.38)	-0.51(-0.69,-0.33)	-0.71(-1.04,-0.37)	0.1349
4 Full Model[f]	-0.41(-0.61,-0.21)	-0.46(-0.55,-0.36)	-0.54(-0.72,-0.36)	-0.69(-1.04,-0.35)	0.0972
Total Hip Bone					
1 Unadjusted	-0.10(-0.25,0.05)	-0.20(-0.26,-0.13)	-0.31(-0.43,-0.18)	-0.65(-0.90,-0.40)	0.0001
2 Age	-0.13(-0.29,0.02)	-0.20(-0.26,-0.14)	-0.26(-0.40,-0.13)	-0.61(-0.86,-0.35)	0.0019
3Age+%WC+HBMD	-0.14(-0.29,-0.01)	-0.20(-0.26,-0.13)	-0.27(-0.40,-0.14)	-0.60(-0.85,-0.34)	0.0022
4 Full Model[h]	-0.15(-0.32,-0.02)	-0.24(-0.31,-0.17)	-0.27(-0.41,-0.12)	-0.53(-0.81,-0.24)	0.0301

Abbreviations: **Full model[t],** adjusted for age, percent change in body weight from V3(2003/2007 to V5(2012), baseline trochanter BMD, physical activity, vitamin D supplement use, diabetes and CVD; **Full model[f]**, adjusted for age , percent change in body weight from V3(2003-2007 to V5(2012), baseline femoral neck BMD, waist circumference, hypertension, diabetes, physical activity and CVD; **Full model[h],** adjusted for age, percent change in weight from V3(2003/2007 to V5(2012), baseline total hip BMD, BMI, vitamin D supplement use, diabetes, CVD and smoking; **TBMD**, trochanter baseline BMD; **FBMD**, femoral neck baseline BMD**; HBMD**, total hip bone baseline BMD; **Q1-4**, quartiles of serum cystatin C; **%WC**, percent weight change from 2004/2007 to 2012.

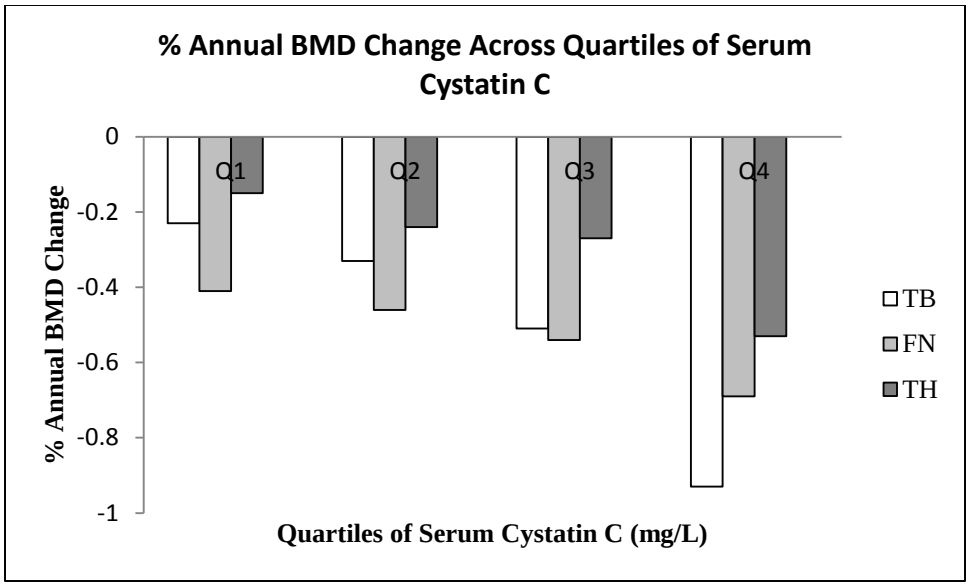

Figure 9. Multivariable Adjusted Percent Annual BMD Change Across Bone Sites With Serum Cystatin C

Abbreviations: TB, trochanter bone; FN, femoral-neck bone; TH, total hip bone; BMD, bone mineral density; Q1-4, quartiles of serum cystatin C . Q1, <0.mg/L; Q2, (0.6-0.85) mg/dl; Q3, (0.86-1.11) mg/L; Q4,>1.11mg/L**.**

Tobago Black from Tobago Health Study (THS) vs. Caucasian Men from The Osteoporotic Fracture in Men Studies (MrOS)

Figure 10 shows the comparison of rate of total hip bone loss across increasing quartiles of cystatin C in MrOS defined as : Q1, 0.97; Q2, (0.98-1.10)mg/L; Q3, (1.11-1.25)mg/L; Q4,>1.26mg/L and THS quartiles defined as: Q1, <0.mg/L; Q2, (0.6-0.85) mg/dl; Q3, (0.86-1.11)mg/L; Q4,>1.11mg/L. The quartiles cut points are lower in THS compared to MrOS men. There was increasing loss of total hip bone across increasing quartiles of cystatin C in THS and MrOS men. The highest quartiles were associated with highest bone loss in THS and MrOS men. These quartiles represent the worst kidney function. However, because the range of quartiles was lower in THS compared to MrOS men, bone loss was much lower in each quartile of cystatin C in THS compared to MrOS men.

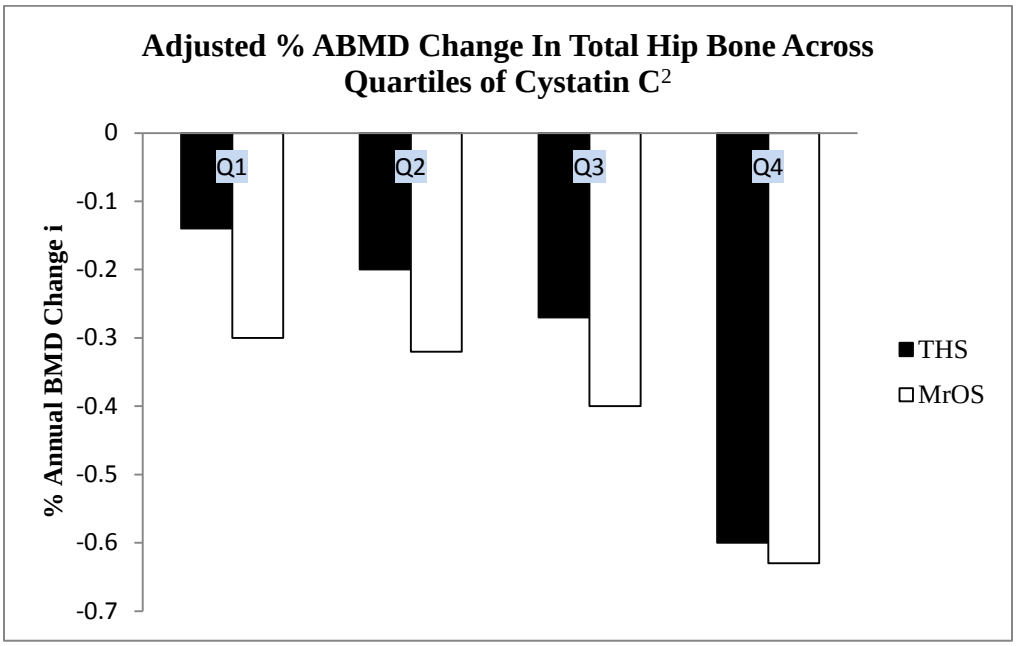

Figure 10. Comparison of Annual Percent BMD Change in Total Hip Bone Among THS(Black) and MrOS(White Males Across Increasing Quartiles of Cystatin C

[2]Cystatin C quartiles was defined in THS men as: Q1, <0.mg/L; Q2, (0.6-0.85) mg/dl; Q3, (0.86-1.11)mg/L; Q4,>1.11mg/L and in MrOS men as: Q1, 0.97; Q2, (0.98-1.10)mg/L; Q3, (1.11-1.25)mg/L; Q4,>1.26mg/L. MrOS, osteoporotic fracture in men study; THS, Tobago health study

Table 23.The Univariate Analyses of Correlates of Annual Percent BMD Change In Total Hip Bone Among Tobago Black Males 65 Years and Older, Mean Age=70.7(SD: ±4.6), Mean MDRD eGFR=67.7ml/min/1.73m^2 (SD: ±15.5) 2004-2007

	Total Hip Bone Percent Annual BMD Change		
Exposure	AC	p-val	p-val*
Age(yr)^{S}	-0.0611	0.1754	N/A
BMI(kg/m^2) ^{S}	0.0832	0.0646	0.1000
WC (Cm)	0.0677	0.1340	0.1675
Baseline(V3)BMD (g/cm^2)^{S}	0.0781	0.0829	0.1371
Weekly Calcium Intake(mg)^{S}	0.0708	0.1163	0.1261
Whole Body Lean Mass(Kg)^{S}	0.0270	0.5721	0.6672
% Weight Change from V3-V5	0.2958	0.0296	**0.0336**
Serum creatinine(mg/dl)^{S}	-0.1092	0.0192	**0.0315**
ACR, (mg/g)^{S}	-0.2406	0.0005	**0.0004**
Cystatin C,(mg/L)^{S}	-0.1415	0.0083	**0.0167**
Physical Activity (walk >5 times in past 7 days)	0.2073	0.0283	**0.0330**
Vitamin D supplement Use	0.1582	0.1977	0.2248
Hypertension	-0.1520	0.1109	0.1521
Diabetes	-0.1297	0.1941	0.1606
CVD	-0.1144	0.5259	0.5187
Current Smoker	-0.0315	0.8458	0.8510
Alcohol Drinker (> three drinks per week)	0.0025	0.9879	0.9821

Abbreviations: **ACR,** albumin creatinine ratio; **BMI**, body mass index; **BMD**, bone mineral density; **CVD**, cardiovascular disease; **AC**, percent annual BMD change per standard deviation of continuous exposure variables denoted marked s ; **p-val*,** age-adjusted p-value; **S**, variables standardized to mean=0 and standard deviation=1; **WC**, waist circumference.

We performed a sub analysis to compare the rate of total hip bone loss between THS and MrOS men [319], across similarly defined increasing categories of cystatin C. This was achieved by selecting THS men 65 years and older and categorizing cystatin C according to MrOS study.

Univariate analysis was performed to determine important covariates of total hip bone among this subgroup of THS men (Table 24). The characteristics of participants in both groups are summarized in Table 25. As shown in Table 25, THS and MrOS men have similar characteristics. The result of sub-analyses to determine rate of total hip bone loss across increasing quartiles of serum cystatin C among THS men is shown in Table 26. After adjusting for age, percent change in weight and baseline total hip BMD, we observed significant greater increase in total hip bone loss across increasing categories of serum cystatin C (annual percent BMD decline across serum cystatin C categories: -0.29, -0.24, -0.50, -0.79 p for trend=0.0285) Figure 10. For MrOS study, after similarly adjusting for age, percent change in body weight and baseline total hip BMD, the investigators also reported significant greater increase in total hip bone loss across increasing quartiles of serum cystatin C (annual percent BMD decline across serum cystatin C quartiles: -0.30,-0.32,-0.40,-0.63 p for trend=0.002) [319] Figure 10.

Table 24. Comparison of MrOS and THS Study Participants

Characteristics	MrOS (N=404)	THS (N=219)
Population	White	Black
Mean Age (SD)	72.3 SD±5.0	70.7 SD:± 4.6
Mean Cystatin C (mg/L)	1.15 SD±0.25	0.85 SD±0.21
Mean MDRD eGFR(ml/min/1.73m^2)	73.8, range 27.0-117.0 (Mild to Moderate eGFR)	67.7, range 27.9-116.7 (Mild to Moderate eGFR)
Mean Follow-Up years	4.5	6
Gender	Male	Male
Bone Site	Total Hip Bone	Total Hip Bone
Biomarker	Cystatin C	Cystatin C
Quartiles(Q1-4)	<0.97,(0.98-1.10), (1.11-1.25), >1.26	<0.97,(0.98-1.10), (1.11-1.25), >1.26
Adjusted	Age, % weight change, baseline total hip BMD	Age, % weight change, baseline total hip BMD
p for Trend	0.002	0.0285
Outcome	-0.30,-0.32,-0.40,-0.63	-0.29, -0.24, -0.50, -0.79

Table 25. Longitudinal Association of Quartiles of Serum Cystatin C With Annual Percent BMD Change From 2004/7 to 2012 in Total Hip, Among Tobago Black Males 65 Years and Older, Mean Age=70.7, Mean MDRD eGFR=67.77ml/min/1.73m^2

	Annual Percent Bone Mineral Density Change (95% CI)				
Total Hip Bone Model	Q1 <0.97mg/L N=178	Q2 (0.98 -1.10)mg/L N=22	Q3 (1.11 - 1.25)mg/L N=7	Q4 >1.26mg/L N=12	P-Val For Trend
1. Unadjusted	-0.28(-0.40,-0.16)	-0.27(-0.60,0.06)	-0.51(-1.10,0.08)	-0.84(-1.29,-0.39)	0.0153
2. Age	-0.28(-0.40,-0.16)	-0.25(-0.59,0.08)	-0.49(-1.08,0.10)	-0.81(1.26,-0.36)	0.0216
3. Age + %Weight Change + Baseline Total Hip BMD	-0.29(-0.40,-0.17)	-0.24(-0.58,0.10)	-0.50(-1.10,0.09)	-0.79(-1.24,-0.33)	0.0285
4. Full Model*	-0.28(-0.40,-0.16)	-0.29(-0.63,0.05)	-0.51(-1.11,0.08)	-0.78(-1.23,-0.32)	0.0346

Abbreviations: *** Full model**; adjusted for Age , %WBLM, baseline total hip BMD, BMI, physical activity; **%Weight Change,** percent weight change from V3(2004/2007) to V5(2012); BMI, body mass index; **BMD**, bone mineral density; **MDRD**, modification diet in renal disease; **eGFR**, estimated glomerular filtration rate.

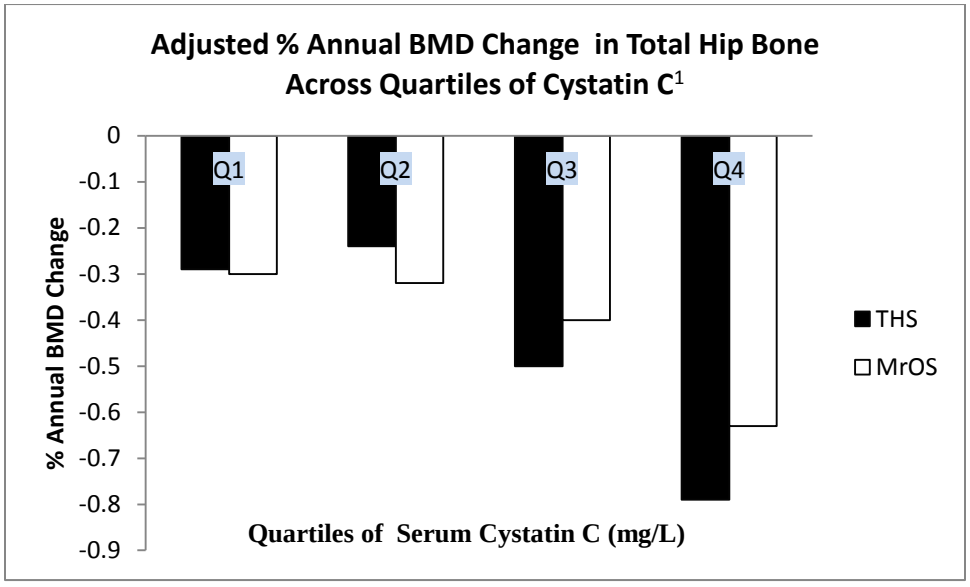

Figure 11. Comparison of Annual Percent BMD Change in Total Hip Bone Among THS(Black) and MrOS (White) Males Across Increasing MrOS Quartiles of Cystatin C
Comparison of annual bone mineral density changes in total hip bone among THS(black) and MrOs (white) males participants across increasing quartiles of cystatin C. [1]Cystatin C quartiles was defined in THS and MrOS men as: Q1, 0.97mg/L; Q2, (0.98-1.10)mg/L; Q3, (1.11-1.25)mg/L; Q4,>1.26mg/L. MrOS, osteoporotic fracture in men study; THS; Tobago health study.

13.5 DISCUSSION

The goal of this study was to examine the longitudinal association of BMD changes across quartiles of kidney function biomarkers among Afro-Caribbean males forty years or older of West African ancestry. Over approximately six years period of follow-up, we found that increasing quartiles of kidney function biomarkers (ACR, serum creatinine and cystatin C) were associated with higher bone loss across all the bone sites examined. The highest loss was observed in the highest quartile of each biomarker considered which corresponds to quartile of the poorest kidney function. The association with bone loss persisted even after controlling for important confounders. The results of our analyses support the inference from prior studies showing that worsening kidney function is associated with longitudinal bone loss after controlling for important confounders. In a study among white participants aged 50 years and older of both gender followed for five years, Jamal S.A et al, found greater bone loss across increasing quartiles of worsening kidney function at the total hip and femoral neck after adjusting for age, weight, and sex [363]. Jassal and colleagues also reported a significant annual bone loss at the hip bone among CKD participants [364, 365]. In the cardiovascular health study, which examined bone loss among black and white participants , 65 years and older with CKD, Fried LF et al reported progressive bone loss across increasing quartiles of serum cystatin C after adjusting for potential confounders [366]. Thus, there is overwhelming evidence suggesting that poor kidney function regardless of the kidney function biomarker used for examination was associated with bone loss.

The mechanism underlying bone loss with worsening kidney function has been widely studied. It is generally believed that bone loss begins from biochemical and hormonal imbalance as kidney function declines [154-156, 158, 199, 200, 355]. The body stores about 99% of

calcium in the bone matrix. In CKD, as the kidney becomes increasingly unable to excrete phosphorous and produce sufficient vitamin D required for intestinal absorption of calcium, the elevation of parathyroid hormone (PTH) follows. Calcium bone resorption begins when PTH levels exceed 30mg/dl. The long term consequence of bone calcium resorption includes bone demineralization and vascular calcification. When demineralization occurs in the cortical bone, it in turn leads to cortical thinning and porosity, and eventual cortical trabecularization. It has been shown that CKD patients with elevated PTH tends to have bone composition made up of greater quantity of the softer trabecular bone than of the harder cortical bone [188]. These conditions are further worsened as the kidney filtration rate drops below 15mL/min/1.73m^2, especially among dialysis patients [154-156, 158, 199, 200, 355].

There are conflicting findings on bone loss among different racial ethnic groups. Tracy and associates showed that longitudinal bone loss occurs at a slower rate among older black males than whites males in the U.S general population [367]. Sheu et al, reported that the rate of bone decline with age was similar among Tobago men of African ancestry and U.S Caucasians [368]. In spite of these disparate findings, it is widely known that higher peak bone mass formation occurs among blacks compared to whites in the general population [356, 368, 369]. However, it is not certain if bone loss occurs at similar rate among blacks and whites with compromised kidney function. Our study did not include white participants. Therefore, it was impossible to perform direct racial comparison with Caucasians. However, we compared our result with previous longitudinal studies with Caucasian participants. In a study by Ishani et al involving 94% Caucasian older men (mean age=72.3 SD±5.0; mean MDRD eGFR=73.8, range 27.0-117.0, N=404) enrolled in the Osteoporotic Fractures in Men (MrOS) followed for four and half years [319], the mean annualized percent change in total hip bone across increasing quartiles

of serum cystatin C was (-0.30%, -0.32%, -0.40%, -0.63% p for trend=0.002) after adjusting for age, percent change weight change and baseline total hip BMD. In a sub analyses performed on a subsample of Tobago men 65years and older with similar characteristics as MrOS men (mean age=70.7 SD:± 4.6, mean MDRD eGFR=67.7, range 27.9-116.7, N=219), we observed mean annual percent loss in total hip bone across similarly defined categories of cystatin C (-0.29,-0.24,-0.50,-0.79, p for trend=0.0285) after similarly adjusting for age, percent body weight change and baseline total hip BMD (Figure 10). Compared to Caucasians, bone loss was generally similar in the lower quartiles of serum cystatin C, whereas in the higher quartiles, which are characterized by a relatively poorer kidney function, Tobago men had slightly higher bone loss compared to Caucasians. Sheu et al, in a prior study had shown that the rate of bone decline was similar among Tobago men of African ancestry and U.S Caucasians [368]. We further report that in general, the rate of bone loss was similar in Tobago black and U.S Caucasian men with CKD.

In spite of prior studies showing higher peak bone mass formation and slower rate of bone loss among blacks compared to whites in the general population [356, 368, 369], and in spite of higher yearly sunlight exposure by Tobago compared to U.S Caucasian men, which is vital for vitamin D synthesis and protection against BMD loss, our result showing similar rates of bone loss in Tobago black and Caucasian males with CKD suggests that the impact of CKD on bone loss may not be amenable to the effects of higher peak bone mass and vitamin D synthesis in the Tobago population. Future studies should focus on risk factors that are associated or contributing to risk of bone loss in Tobago men.

The following are the strengths of our study. Ours is a prospective, population based study. Important confounding factors were controlled and the sample size used in our analyses

had sufficient power to detect bone loss from baseline and follow-up visits. In this study, the effect of kidney function with bone loss was examined in the context of three biomarkers. Although, serum creatinine is the most widely used marker of kidney function, the disadvantages are that it is affected by age, muscle density and mass, and protein diet. It is also less sensitive to declining kidney function and therefore has less precision [321]. On the other hand, the advantage of Cystatin C lies in its capacity to detect minor kidney function impairment. Several studies have found Cystatin C a superior measure of kidney function and a better estimator of early stages of CKD than creatinine [40, 48, 49].

This study has several limitations. Because the Island of Tobago is tropical, the yearly sunlight exposure for vitamin D production may have been higher among Tobago men compared to U.S men. In this study, we determined only baseline kidney function biomarkers. Changes in kidney function, environment, dieting and lifestyle over time may have influenced bone loss among Tobago and U.S men differently. Finally, we did not account for possible cohort or period effects which may have altered bone loss or kidney function over time in our study.

13.5.1 Conclusion

Poor kidney function as measured by ACR, serum creatinine and cystatin c are associated with longitudinal bone loss in the trochanter, femoral neck and total hip bone among Tobago black males irrespective of biomarker used. Comparison with U.S Caucasians shows that the rate of bone loss in Tobago black and U.S Caucasian men was similar among participants with poor kidney function. For prevention, there is a need for proper management of calcium, vitamin D, PTH and phosphorus among CKD individuals in order to reduce bone loss and associated mortality.

14.0 GENERAL DISCUSSION

14.1 SUMMARY OF FINDINGS

Chronic kidney disease (CKD) is a major health problem with enormous public health consequence. CKD incidence is rising globally and many attribute this rise to increasing elderly population, obesity, hypertension, cardiovascular disease (CVD) and diabetes. CKD is accompanied with several co-morbid conditions including anemia, depression, infection, anxiety, dementia, physical disability and bone loss resulting in untold personal and economic burden on sufferers and the community.

African Americans and other racial minorities present with far greater risk of CKD compared to other racial groups, yet fewer studies have been conducted among these groups. In this project, we extend studies to include blacks recruited from the island of Tobago in southern Caribbean in order to better understand CKD in the black population. It has been suggested that heritability of susceptible genes of kidney disease is partially responsible for the existing racial disparities in CKD. Equally relevant in CKD, is the role of modifiable risk factors in ethnic disparities and progression to ESRD. The unexpected increase in CKD in recent decade is also partially attributed to the characteristics of CKD. In many quarters, CKD has been described as the “the silent killer". This is because, CKD does not manifest in signs and symptoms until in the later stages when treatment is less effective. In the majority of cases, early kidney function

impairment is often missed leaving an individual unaware of impending kidney failure. Whenever the filtering capacity of kidney is impaired through glomerular damage, toxins are suspended in circulation and further damage may lead to albuminuria. Because of the impracticability of the gold standard procedures for detecting early onset of CKD, estimating equations such as MDRD, based on serum creatinine and demographic characteristics, have found practical use in clinics and research for screenings. However, the limitations of MDRD prevent its use alone to identify CKD. These limitations includes lack of sensitivity to eGFR greater than 60[30] and validation on a wide range of population groups. For these reasons and others, KDIGO recommendation includes addition of ACR to determine CKD. It was our intention in this project to characterize the prevalence of CKD using MDRD and ACR, and to identify modifiable risk factors of CKD among Tobago black men. In this project, we found that prevalence of CKD in Tobago men was lower compared to African American men, but, was comparable to Caucasian men. Hypertension and diabetes were significant predictors of CKD risk independent of age in Tobago men. We hypothesize that the disparate finding between African Americans and Tobago men was due to increased access to health care, and to socio-cultural and socioeconomic conditions among African Americans which improves coping and better survival. In Tobago men, we observed that the most important risk factor for CKD was hypertension. The risk of CKD was about two times higher with hypertension than with diabetes. Impaired glomerular function was more prevalent than albuminuria.

Excess body fat is a considerable cause of morbidity and mortality. The locations and distribution have also been shown to predict health outcomes including CVD, diabetes and hypertension, which are known risk factors for CKD. The most common correlates of body composition are BMI and WC. However, BMI and WC measures general-obesity and central

adiposity respectively and does not take into account lean mass. The use of DXA/PQCT discriminates between various body composition measures and therefore provides a reliable estimate of the role body composition play in relation to biomarkers of kidney function. However, it is widely known that serum creatinine and cystatin C are confounded by factors outside glomerular function. The influence on serum creatinine by age, gender, muscle mass, protein consumption and body composition make serum creatinine less sensitive as a biomarker of renal function. Also, the influence on cystatin C by adiposity, inflammatory biomarkers, and large doses of glucocorticoids use, hyperthyroid state and lean mass makes its use less than ideal. No study has been conducted in black population to evaluate the role of DXA/PQCT body composition on serum creatinine and cystatin C. To the best of our knowledge, this was the first project to evaluate this relationship among black subjects. In this project, we found that muscle area and to a lesser degree, muscle density were each associated with serum creatinine. Regional and general adiposities were each associated with cystatin C, but not with muscle area. Therefore, among Tobago blacks, when interpreting serum creatinine concentrations muscle and whole body lean mass should be considered. Also when interpreting cystatin C, body adiposity measures should also be considered. However, given high lean mass in Tobago men, cystatin C should be considered the biomarker of choice for estimating kidney function.

Bone and mineral disorder affects a substantial number of people in the U.S population. The incidence of bone disorders is on the increase, and it is paralleled by a rising number of CKD patients and a rising elderly population who also have a high risk of CKD. Bone loss is largely mediated by imbalances in serum minerals and biochemical compounds responsible for maintaining bone health. Imbalances begin from CKD stage 3 when the eGFR drops below 60ml/min/1.73/m2 and gradually worsens as eGFR falls below 15ml/min/1.73/m2. Bone disorder

is associated with several complications including fracture. The association of impaired kidney function on bone loss among black study participants has not been properly elucidated. In general, increasing quartiles of ACR, serum creatinine and cystatin C were each consistently associated with longitudinal bone loss. In addition, the rate of bone loss in Tobago black and U.S Caucasian men was similar among participants with mild to moderate CKD. Even though prior studies have associated higher peak bone mass to black race, it appears that higher peak bone mass did not confer protection against bone loss among our study participants. Neither was high yearly sunlight exposure, necessary for vitamin D synthesis in bone formation, protective. However, it may be that higher peak bone confers protection against fracture in older blacks compared to Caucasians.

14.2 STRENGTHS AND LIMITATIONS

This project was a population based study in which participants were a representative sample of Tobago black males 40 years and older. We benefited from having large sample size to test the hypothesis of our specific aims. The use of comparison groups for CKD prevalence estimation, enable us to underscore the relative difference in distribution of CKD among Tobago black with African American and Caucasian males.

PQCT and DXA scanning were used to measure various body compositions including general and regional adiposity distributions. PQCT procedures use modern technologies to assess three instead of two dimensional measurements of regional adiposities. The advantage of this is that volume, rather than area measurements were taken, which improves precision and accuracy in measurements of body composition.

In this project, important confounding factors were controlled based on extensive literature review and univariate analyses of important covariates. Serum creatinine and cystatin C, and ACR measures were used alongside each other to tackle limitations inherent in each individual biomarker.

Except for our specific aim 3, this was an observational cross-sectional study. As such, cause and effect cannot be established. Direct measurement of glomerular filtration rate was not possible. Instead, we relied on estimates of glomerular filtration by using MDRD equation. It is widely known that MDRD equation underestimates high eGFR and overestimates low eGFR. But we were able to partially overcome this limitation by including ACR measurements. Only one measurement of serum creatinine and cystatin C, and ACR were taken in 2004-2007. The use of one measurement to determine kidney function and albuminuria may be misleading. All self reported responses were not verifiable. Because all the participants in this study were blacks, our findings may not be generalized to other racial/ethnic groups.

14.3 FUTURE DIRECTION

Future direction should focus on environmental factors impacting CKD and their mechanisms of action and risk factors that are associated or contributing to risk of bone loss in Tobago men.

14.4 PUBLIC HEALTH SIGNIFICANCE

CKD is a major public health challenge of the 21st century. Epidemiological reports indicate that the incidence of CKD is rising globally and is fast becoming an epidemic, in particular among blacks. Hypertension and diabetes were the leading causes of CKD in Tobago men. We identified that hypertension in particular was the major cause of CKD and over half of the participants sampled had hypertension. Apart from CKD, hypertension is also a leading cause of CVD, which in turn is commonly found among CKD patients. Thus, diabetes, hypertension, CKD and CVD are all locked in a vicious cycle and interacting with one another to increase the risk of morbidity and mortality among Tobago blacks.

Bone loss is associated with fracture. Fracture in turn increases the risk of mortality. Although, the risk of fracture is high in the aging population, prior studies have also shown that the risk of fracture is seven times higher among patients in stage 5 of kidney disease and the risk of dying from fracture is two and half times higher among CKD patients. In addition, bone loss has been linked to vascular calcification. Recent reports show that vascular calcification was related to bone loss due to ESRD. The consequence is formation of atherosclerotic plaques, which in turn is associated with increased risk of CVD and CVD deaths.

Bone loss in CKD can be minimized by proper management of bone minerals, through medications and also by periodical DXA screening. The prevalence of CKD can be minimized by eliminating or modifying risk factors leading to hypertension and diabetes through proper dieting, lower salt and fat consumption, exercise and physical activities, weight control and early CKD screening. Concerted preventive strategies are needed to address the public health impact of CKD.

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