THE EPIDEMIOLOGY OF GLOMERULAR HYPERFILTRATION AMONG MEN WITH HIV IN THE ERA OF HIGHLY ACTIVE ANTIRETROVIRAL THERAPY

by

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II. Dissertation abstract

Background Men infected with HIV and receiving highly active antiretroviral therapy are at higher risk of metabolic and cardiovascular abnormalities as well as accelerated renal function decline and chronic kidney disease (CKD). Glomerular hyperfiltration, defined as elevated glomerular filtration rate (GFR) to pathologically high levels, is associated with diabetes and hypertension and is a treatable risk factor for CKD. The epidemiology of hyperfiltration has not been described in an HIV population. The purposes of this dissertation is to a) describe the prevalence of elevated GFR using directly measured iohexol GFR, a gold standard; b) describe the incidence of hyperfiltration using the serum creatinine-based CKD-EPI estimated GFR equation, a clinical standard; and c) investigate the effect of hyperfiltration on accelerated GFR decline in the Multicenter AIDS Cohort Study.

Methods Data consisted of a nested cross-sectional study within the MACS comprising 241 HIV-uninfected and 367 HIV-infected men with iohexol GFR, and all MACS data in the era of HAART comprising approximately 1373 HIV-uninfected and 1255 HIV-infected men. Hyperfiltration was classified using adapted definitions, including estimating the 90^{th} percentiles among HIV-uninfected men. Competing risks analyses, with age (after 30 years) as the time scale, were used to assess the effect of HIV-infection on incident hyperfiltration. To determine the effect of hyperfiltration on GFR decline, downward inflection points were identified.

Results There was a higher prevalence of elevated GFR among HIV-infected men compared to HIV-uninfected men (25% vs. 17%; adjusted odds ratio: 1.70, 95%CI: 1.11,

2.61) based on directly measured GFR. Using estimated CKD-EPI GFR, HIV infection was associated with increased risk of incident hyperfiltration among non-blacks at younger ages that diminished over time. A higher non-significant risk was observed among blacks. Hyperfiltration was not associated with accelerated 5-year GFR decline. Compared to uninfected men, men treated for HIV-infection had a faster 5-year GFR decline.

Conclusions Treated HIV infection was associated with an increased independent risk of prevalent and incident hyperfiltration, and varied by race. HIV infection, but not hyperfiltration, was associated with accelerated short term GFR decline. Therapies for metabolic, cardiovascular and renal abnormalities, including hyperfiltration, remain important considerations for HIV management.

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1. Introduction

1.1 HIV treatment in the era of HAART and comorbidities

With the advent of highly active antiretroviral therapy (HAART), life expectancy with HIV infection has increased and AIDS mortality has decreased [1]. However, accelerated aging and the frailty phenotype are more common among those with HIV and receiving therapy [2]. Indeed, in the HAART era, those with HIV infection are at higher risk of non-AIDS comorbidities such as cardiovascular, metabolic and renal disorders more typically associated with age [3–6]. For example, HAART-treated people with HIV are known to live longer but also exhibit accelerated kidney function decline and higher incidence of chronic kidney disease (CKD) [6,7]. Importantly, this population also has a higher incidence of disorders such as hypertension and diabetes that have renal, cardiovascular and metabolic etiologies. Therefore, identifying precursors and modifiable risk factors for cardiovascular and metabolic comorbidities are essential for current HIV treatment and preventive care.

1.2 Glomerular hyperfiltration

One such risk factor identified in HIV-uninfected populations is glomerular hyperfiltration, which refers to elevated glomerular filtration rate (GFR) to pathologically high levels. Hyperfiltration is considered an early marker of kidney damage in the context of pre-diabetes and pre-hypertension leading to subsequent accelerated GFR decline [8]. Hyperfiltration can occur naturally without lasting kidney damage (for example, during pregnancy [9]). However, it is generally accepted that hyperfiltration is also an initiator and accelerator of kidney disease [10] and a risk factor for proteinuria [8], in the presence

of other metabolic and cardiovascular comorbidities. Several studies have described increased prevalence of hyperfiltration among people with metabolic and cardiovascular abnormalities [11–15], as well as increased risk of accelerated GFR decline [10].

Hyperfiltration is hypothesized to be an adaptive response to a reduction in functional nephron number. Pressure within the glomerulus can increase to high levels due to decreased volume flow from the efferent arteriole relative to the afferent arteriole. Figure 1.1 presents a schematic of blood flow within a nephron depicting this process. This difference in flow may be due to substances or hormones (such as insulin), as well as increased systemic blood pressure (as measured by systolic and diastolic blood pressure). It is hypothesized that abnormal hormonal levels due to glucose intolerance and abnormal tubular resorption of glucose and sodium contribute to the development of hyperfiltration. This mechanism, largely derived from animal studies [9,16–18], is hypothesized to be responsible for the increased prevalence of hyperfiltration among diabetics, although this has not been directly confirmed in humans.

The chronic increased pressure that defines hyperfiltration is thought to directly cause pathologic damage to the glomerulus. Increased hemodynamic pressures of the filtering units are markers of kidney dysfunction and precede microalbuminuria (proteinuria), GFR decline, further insulin resistance and hypertension [10,13,19]. In particular, in the absence of treatment, there is a progressive decline in GFR with a commensurate increase in proteinuria.

Figure 1.2, described by Palatini [8] and reproduced here, presents the theoretical model of hyperfiltration proposed by Brenner and colleagues [20]. This model describes a period of increasing GFR ("Normal Filtration Phase 1") followed by a period of

hyperfiltration. The third period is characterized by a sharp decline in GFR ("Normal Filtration Phase 2"), accompanied by an increasing level of proteinuria. It should be noted that any single observation within a normal GFR period (i.e., Phase 1 or Phase 2) is not sufficient to categorize an individual, in the absence of proteinuria data. Additionally, it should be noted that normal renal function is expected to decrease over time. However, in this model, the decline described in Normal Filtration Phase 2 is faster relative to an expected age-related decrease.

Importantly, hyperfiltration is considered a treatable and modifiable risk factor. Mild intervention includes dietary changes such as lower protein and sodium intake [9]. More intensive treatment might involve aggressive antihypertensive therapy including a regimen of angiotensin-converting-enzyme inhibitors (ACEi) and/or angiotensin II receptor blockers (ARBs), which preferentially dilate the efferent arteriole of the glomerulus, reducing pressure as well as pathologically high GFR.

1.3 Metabolic, cardiovascular and renal health related to hyperfiltration in HIV-infected populations

Many of the identified risk factors of hyperfiltration are also more common among HIV-infected populations. Specifically, HIV-populations treated with antiretroviral therapy are at increased risk for diabetes [4,5] and hypertension [3]. Additionally, outcomes associated with hyperfiltration, such as accelerated GFR decline and incidence of CKD are also more common among those with HIV infection [6,21]. Several studies have also demonstrated much higher incidence of proteinuria (i.e., urine protein:creatinine ratio > 200 mg/g) among those with HIV infection compared to an uninfected population [22,23]. However, causes of higher proteinuria among those with

HIV are largely unknown [23] and investigation into early markers of kidney damage are warranted, particularly in a high risk HIV population.

There are several potential mechanisms by which HIV infection and HAART may lead to hyperfiltration and CKD. Firstly, HIV directly infects kidney epithelial cells and the kidney is a reservoir of HIV infection [24–26]. It is possible that HIV infection in the kidney may alter renal functioning. Indeed, HIV-associated nephropathy is a common clinical concern [27,28]. It is possible that early stages of HIV-associated nephropathy may be characterized by the presence of hyperfiltration. Secondly, certain HAART drugs (e.g., thymidine analogs) are metabolized and excreted renally, which can lead functional changes and damage to the kidney. Indeed, tenofovir, indinavir and atazanavir are known to be nephrotoxic and chronic use may lead to acute kidney injury, a precursor to CKD [29–31]. In addition to these direct effects of HIV infection and HAART on the kidney, there may also be indirect effects, such as the development of dyslipidemia and insulin resistance leading to diabetes and other metabolic and CVD risk factors [32,33].

1.4 **Measuring GFR**

As a brief summary of measuring renal function clinically and for research purposes, GFR can be measured directly or estimated based on biomarkers correlated with renal function. Plasma disappearance of iohexol is a direct measure of GFR and is considered the gold standard for renal function [34,35]. In short, a known amount of contrast medium, such as iohexol, is injected intravenously as a single bolus. Iohexol is a non-radioactive contrast medium that is not secreted, metabolized or reabsorbed by the kidney, with exclusive elimination by the kidneys and an established safety profile [34]. Plasma concentrations of iohexol obtained at specific times (up to 5 or 6 hours) after

injection are measured to describe a disappearance curve. Quantifying the diminishing concentrations of iohexol as a rate summarizes the filtration rate of the kidneys over this time period with units milliliters per minute cleared (ml/min). GFR is also standardized to body surface area of $1.73m^2$, which corresponds to an average adult male. The final units are ml/min per $1.7m^2$. While the plasma disappearance of iohexol offers a precise and reliable measurement of GFR, it is not practical in routine clinical care since the protocol is long and can be burdensome. Its use in research settings is more limited as well given the complexity of data collection and measurement, as well as study participant burden.

In contrast, estimated GFR (eGFR) is determined from easily measured biomarkers as a function of sex, race and age in a simple equation [36]. The most clinically used biomarker to estimate GFR is serum creatinine, although equations have been developed to include other markers such as serum cystatin C [37]. Measurement of serum biomarkers is common in clinical care and offers a fast assessment of renal function. Examples of these equations are the Modification of Diet in Renal Disease (MDRD) Study equation [38] and the CKD-EPI equation [36] whose units are also ml/min|$1.73m^2$. Since equations have been mostly developed in populations with or at high risk of CKD, they perform better at lower levels of GFR. At high levels of GFR, these equations may be biased or unstable, but the CKD-EPI equation is the least biased of available options [39]. Despite the overall good performance of the CKD-EPI equation at all levels of GFR, there is still significant error and misclassification compared to directly measured GFR. Indeed, 15.9% of CKD-EPI eGFR measurements were not within 30% of the measured GFR values in a validation analysis of the equation

[36], indicating substantial misclassification. Nonetheless, given the ease of use of eGFR and its pervasive clinical presence, it is a valuable tool for clinical decision making, and by extension, in applied research settings.

1.5 **Defining hyperfiltration**

Despite hyperfiltration being a putative antecedent of GFR decline, cardiovascular and metabolic comorbidities, there is no single functional clinical definition of the condition. One problematic aspect of defining hyperfiltration is due to age-related GFR decline and other health factors associated with GFR. The presence of declining GFR as a part of normal aging has been acknowledged, yet it is not clear what constitutes normal decline [40,41]. There are several examples of hyperfiltration definitions proposed in published research, which have used directly measured GFR and estimated GFR. While some studies do not use an age-varying threshold, we restrict our current discussion to studies that allow thresholds for hyperfiltration to vary by age.

Hyperfiltration definitions may be divided into two categories: conceptually derived definitions and population-based definitions. A conceptually derived definition uses a known level of high GFR and decreases that value commensurately by age, to account for normal age-related decreases in renal function. For example, Premaratne et al. [12] define hyperfiltration by a GFR threshold of 130 ml/min|1.73m^2 with a 1 ml/min decrease per year in this threshold after age 40 to account for age-related decline. This age-specific threshold is simple, clinically relevant and the initial level of high GFR has a physiological basis. However, this threshold approach is not based on data, and does not account for sub-groups that may have different thresholds.

Population-based definitions of GFR are based on data from a normal population. While there is a need for defining normal reference ranges by age [40], several studies have sought to define a normal population and derive a threshold for elevated GFR [15,42]. Examples of these include elevated GFR defined as being greater than the 90th percentile of eGFR adjusted for age, sex, weight, height and use of antihypertensive therapy [42]; or simply higher than the 95th percentile of 10-year age bins [15]. Okada et al [15] report age- and sex-specific 95th percentiles of 99,140 men and women between the ages of 20 and 89 from 4 health check sites in Japan, representing one region. The normal population in this case is the study population which is representative of the target population. The benefit to this approach is that the comparison group is well-defined, directly applicable to the study population and is free of physiological assumptions, in contrast to the conceptually derived definitions. However, this approach relies mainly on the statistical properties of the data and may not be physiologically tenable from a clinical perspective. Furthermore, defining a normal population can be problematic since some argue that the population should be completely free of comorbidities (similar to the definition presented by Melsom et al. [42]), while others may argue that the prevalence of comorbidities should be consistent between the source population and target population (similar to Okada et al. [15]).

1.6 **Overall goals of the dissertation**

Since hyperfiltration is a treatable and modifiable risk factor, it should warrant special clinical consideration in HIV management and monitoring. This importance is underscored by the renal excretion of some forms HAART: if GFR is too high, it is possible that medications may be excreted too quickly resulting in subtherapeutic

exposures. Additionally, preventing or treating hyperfiltration may reduce the risk of accelerated GFR decline and incidence of CKD, as well as potentially improve management of HIV and reduce adverse infection-related outcomes.

However, a full characterization of hyperfiltration has not yet been accomplished in an HIV-infected population. It is therefore unclear the extent to which hyperfiltration is associated with HIV infection and whether hyperfiltration leads to adverse outcomes in this population. The goal of this dissertation is to describe the epidemiology of hyperfiltration among men with and without HIV, using data from the Multicenter AIDS Cohort Study (MACS). The MACS includes directly measured GFR on a subset of participants as well as serum creatinine-based eGFR on the full cohort in the era of HAART. This is an ideal setting to investigate hyperfiltration, as the data include the gold standard (measured GFR) and clinical standard (eGFR) of renal function. The specific aims of this dissertation are the following:

1.6.1 Specific Aim 1

A. To define hyperfiltration from directly measured GFR and describe the prevalence of hyperfiltration in a cross-sectional MACS subsample of men with and without HIV.
B. To identify metabolic, cardiovascular and behavioral variables associated with hyperfiltration.
C. To identify HIV- and therapy-related variables associated with hyperfiltration.

1.6.2 Specific Aim 2

A. To derive a population-based definition of hyperfiltration using CKD-EPI estimated GFR among HIV-uninfected men.

B. To describe the incidence of hyperfiltration using this definition among MACS men in the HAART era, by HIV infection status.

C. To describe the risk of hyperfiltration associated with HIV infection in a competing risks setting.

1.6.3 Specific Aim 3

A. To describe trajectories of CKD-EPI eGFR decline after a hyperfiltration event and compare with the trajectories from an age- and HIV infection matched sample free of hyperfiltration.

B. To apply a generalized method to identify an inflection point in GFR trajectories, that is, a point at which eGFR declines rapidly.

C. To determine if treated HIV infection modifies the effect of hyperfiltration on eGFR decline.

1.7 **Study population and Data**

This dissertation uses data from the Multicenter AIDS Cohort Study (MACS), an ongoing prospective cohort study which has investigated the natural and treated history of HIV infection among high-risk homosexual and bisexual men in 4 sites: Baltimore MD/Washington DC, Chicago IL, Los Angeles CA, and Pittsburgh PA. The study was initiated in 1984 with 4 enrollment periods between 1984 and 2013 (and currently ongoing). Enrollment criteria were being at risk of HIV infection by sexual activity, infected with HIV, 18 years of age or older, no active malignancy or opportunistic infection that was AIDS-defining, and providing informed consent. AIDS and other clinical diagnoses were confirmed by medical record reviews in the recruitment process.

Datasets in these analyses were restricted to those subjects contributing data in the era of HAART (after June 1996).

The study design of the MACS is based on structured semi-annual visits with a standardized physical exam, subject-reported behavioral and clinical information and specimen collection (for immediate laboratory analysis as well as repository storage). Biomarkers were collected using structured protocols. CD4+ cell counts and HIV RNA (Roche) were measured at each local site, lipids were measured at Heinz Research Laboratory (Pittsburgh) and blood and urine chemistry markers (including serum creatinine) were measured at a local biochemistry laboratory (Quest) with results sent to the Center for Analysis and Management of Multicenter AIDS Cohort Study (CAMACS). A detailed description of the MACS study design has been previously presented [43]. This dissertation presents MACS data collected through September 30, 2013 (inclusive).

Within the MACS cohort, a nested representative sample comprising about 250 seronegative and 500 seropositive MACS subjects underwent a direct measure of GFR by a protocol of plasma clearance of iohexol between May 2008 and December 2010. All serum samples were measured at a central biochemistry laboratory (University of Rochester) with results sent to CAMACS.

1.8 **Structure and organization of dissertation**

The dissertation is organized into six chapters. The first presents introductory material describing hyperfiltration and the rationale for investigating this condition in an HIV-infected population, as well as the specific aims. The second chapter presents Specific Aim 1 in an analysis of a substudy nested within the MACS in which

approximately 500 HIV-infected men and 250 HIV-uninfected men underwent a directly measured GFR by an iohexol plasma disappearance protocol. This cross-sectional analysis of hyperfiltration was recently published in the journal *AIDS*, accepted September 2013 and appeared in print January 2014 [44]. The original publication is reproduced for Chapter 2 per copyright permissions granted by the publisher. The third chapter presents Specific Aim 2 utilizing serum creatinine-based CKD-EPI eGFR data in the MACS during the era of HAART, and is restricted to HIV-uninfected men and HIV-infected men receiving HAART. The fourth chapter presents Specific Aim 3 using a matched study design and the same data source as in the analyses presented in Chapter 3. Chapters 3 and 4 are written as stand-alone manuscripts and therefore these chapters present the same methods for defining elevated eGFR in an HIV-uninfected normal population, albeit in different contexts, in order to ensure clarity of methods. Chapter 5 presents a discussion and concluding remarks, including a summary of the findings, strengths, limitations and opportunities for further work. Chapter 6 presents figures and tables associated with the preceding chapters.

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2 HIV therapy, metabolic and cardiovascular health are associated with glomerular hyperfiltration among men with and without HIV infection

HIV therapy, metabolic and cardiovascular health are associated with glomerular hyperfiltration among men with and without HIV infection

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Objective: Diabetes and hypertension, common conditions in antiretroviral-treated HIV-infected individuals, are associated with glomerular hyperfiltration, which precedes the onset of proteinuria and accelerated kidney function decline. In the Multicenter AIDS Cohort Study, we examined the extent to which hyperfiltration is present and associated with metabolic, cardiovascular, HIV and treatment risk factors among HIV-infected men.

Design: Cross-sectional cohort using direct measurement of glomerular filtration rate by iohexol plasma clearance for 367 HIV-infected men and 241 HIV-uninfected men who were free of chronic kidney disease.

Methods: Hyperfiltration was defined as glomerular filtration rate above 140−1 ml/min per 1.73 m^2 per year over age 40. Multivariate logistic regression was used to estimate the odds ratios (ORs) of prevalent hyperfiltration for metabolic, cardiovascular, HIV and cumulative antiretroviral exposure factors.

Results: Among individuals without chronic kidney disease, the prevalence of hyperfiltration was higher for HIV-infected participants (25%) compared to uninfected participants (17%; $P = 0.01$). After adjustment, HIV infection remained associated with hyperfiltration [OR 1.70, 95% confidence interval (CI) 1.11–2.61] and modified the association between diabetes and hyperfiltration, such that the association among HIV-uninfected men (OR 2.56, 95% CI 1.33–5.54) was not observed among HIV-infected men (OR 1.19, 95% CI 0.69–2.05). These associations were independent of known risk factors for hyperfiltration. Indicators of hyperglycemia and hypertension were also associated with hyperfiltration as was cumulative zidovudine exposure.

Conclusion: Hyperfiltration, a potential modifiable predictor of kidney disease progression, is significantly higher among antiretroviral-treated HIV-infected men. Furthermore, HIV-infection nullifies the association of diabetes and hyperfiltration present in HIV-uninfected men.

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Introduction

The use of HAART has resulted in marked reductions in AIDS-related mortality and opportunistic disease among HIV-infected persons [1,2], yet these patients are now at increased risk of death as a result of chronic noninfectious age-related comorbid conditions, including chronic kidney disease (CKD) [3,4]. In the context of HAART-treated HIV infection and aging, CKD often results from metabolic abnormalities, such as diabetes mellitus and hypertension [5,6]. In the general population, proteinuria and impaired kidney function related to these conditions can be preceded by glomerular hyperfiltration, in which the glomerular filtration rate (GFR) increases to above normal levels [7–11], due to compensatory hemodynamic alterations within the kidney. Metabolic dysfunction characterized by impaired fasting glucose [12], diabetes mellitus [9,11,13], hypertension, and obesity [9] – conditions for which HIV-infected persons receiving HAART are at higher risk [14,15] – has also been linked to hyperfiltration.

Because hyperfiltration is an early indicator of kidney dysfunction and is potentially reversible by aggressive antihypertensive therapy or dietary changes [7,13], establishing its relevance for HIV clinical care is important. However, whether and to what extent glomerular hyperfiltration is present among HIV-infected persons receiving HAART, and whether metabolic and cardiovascular risk factors and HIV-related factors are associated with hyperfiltration in this population are unclear. To address these questions, we used directly measured GFR data from a representative subsample of the Multicenter AIDS Cohort Study (MACS), a cohort of men with or at risk for HIV infection. We aimed to: determine the prevalence of hyperfiltration; investigate the association of HIV infection and metabolic, cardiovascular and behavioral factors with hyperfiltration; and investigate HIV disease severity and HAART as potential risk factors for hyperfiltration.

Methods

Study population

The MACS is a prospective observational cohort study of the natural and treated histories of HIV infection among 6972 homosexual and bisexual men enrolled in four United States metropolitan areas from 1984 to 2003. Details of the study design have been previously described [16]. In brief, demographics, medical history, and clinical characteristics were collected semi-annually. Standard protocols included physical examinations, blood pressure measurements, blood and urine collection, and self-administered interviews.

The current study included men who underwent direct iohexol-based GFR measurement (iGFR) [17–19] between August 2008 and January 2011. These participants were a subsample of the full MACS cohort [19], chosen by random selection using a ratio of approximately two HIV-infected participants for each HIV-uninfected participant. Additionally, all participants co-infected with the hepatitis C virus (HCV), as determined by presence of serum anti-HCV antibody or plasma HCV RNA [20], were eligible for iGFR measurement. Participants who received renal replacement therapy, were diagnosed with cancer in the preceding 3 years, or were unable to complete data collection due to contrast allergy were excluded. A central laboratory measured blood glucose, insulin, lipid panels (Heinz, Pittsburgh, Pennsylvania, USA), and HCV antibodies (Tricore, Albuquerque, New Mexico, USA). HIV-1 infection was defined by positive serum ELISA with confirmatory Western blot. Plasma HIV RNA levels were ascertained by the Roche Amplicor assay (Hoffman-LaRoche, Nutley, New Jersey, USA) sensitive to 50 copies/ml, and $CD4^+$ lymphocyte counts/ml were measured using standardized flow cytometry [21]. This study was approved by Institutional Review Boards at all participating sites.

Dependent variables

The primary outcome was hyperfiltration, defined from GFR measured by the plasma disappearance of iohexol using a 2-compartment 4-point model standardized to a body surface area of 1.73 m^2 as previously described [19]. To address the expected decline in GFR associated with age, we defined hyperfiltration as iGFR at least 140 ml/min per 1.73 m^2 for men 40 years and younger and subtracted 1 ml/min per 1.73 m^2 for each year over age 40 [22]. Among men with GFR below this hyperfiltration threshold, we excluded men with GFR below 60 ml/min per 1.73 m^2 or with proteinuria [urine protein-to-creatinine ratio (UPCR) >200 mg/g] [7,23,24]. The remaining men without markedly impaired GFR (i.e. 'normofiltration') served as the comparison group. UPCR was measured at each site, and was based on the mean of three measurements for 1 year prior to iGFR measurement. Men with missing UPCR data were excluded ($n = 8$).

Independent variables

Metabolic variables included BMI (kg/m^2, obesity defined as $\geq 30\,kg/m^2$); serum high-density lipoprotein (HDL) and non-HDL cholesterol; dyslipidemia [fasting total cholesterol ≥200 mg/dl, low-density lipoprotein (LDL) ≥130 mg/dl, HDL <40 mg/dl, triglycerides ≥150 mg/dl, or use of lipid-lowering medications with self-reported/clinical diagnosis of dyslipidemia]; fasting glucose level; hemoglobin A1c (HbA1c); insulin resistance [Homeostatic Model Assessment – Insulin Resistance (HOMA-IR]; diabetes (HbA1c ≥6.5%, fasting glucose >126 mg/dl, or diagnosis of diabetes with use of

medications); and metabolic syndrome [25]. High fasting glucose (>100 mg/dl) and HbA1c (≥6.5%) were also used as indicators of hyperglycemia [12]. Cardiovascular variables were SBP and DBP, uncontrolled hypertension (SBP ≥140 or DBP ≥90 mmHg), and history of hypertension (uncontrolled hypertension or diagnosis of hypertension with use of antihypertensive medications). Behavioral variables included current smoking status at the time of iGFR measurement, stimulant use (cocaine, amphetamines, or methamphetamines), and other drug use (marijuana and inhalant nitrates). For all continuous variables, the mean of available data from the visits in 1 year prior to and including the iGFR visit was used as a summary level. For binary variables such as diabetes, the presence of these conditions was determined by at least two occurrences in the visits prior to and including the iGFR measurement in a 1-year period (i.e. two out of three measurements, about 6 months apart). Since variables within metabolic and cardiovascular domains were expected to be highly correlated, we did not simultaneously include them in multivariate analyses.

To investigate the association of HIV and HAART on hyperfiltration, we restricted the sample to men receiving HAART at the time of iGFR measurement, as few HIV-infected participants were HAART-naive ($n = 4$). Exposure to antiretroviral therapy (ART) was characterized by: years from any ART initiation to the time of iGFR measurement (i.e. time since ART initiation, either monotherapy, combination therapy, or potent therapy with three or more agents); years from HAART initiation (i.e. a combination regimen of three or more ART agents) to the time of iGFR measurement; and cumulative exposure [i.e. person-years of use, for each subclass of ARTs: non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors, and nucleoside reverse transcriptase inhibitors (NRTIs)]. Cumulative exposure was also calculated for specific antiretroviral drugs that have been associated with either metabolic or cardiovascular derangements (such as hyperglycemia or dyslipidemia, associated with thymidine-analog reverse transcriptase inhibitor medications used in earlier HAART regimens [15,26]) or for drugs that are considered nephrotoxic [27]. The specific medications evaluated were indinavir (IDV), ritonavir (RTV), atazanavir (ATV) for protease inhibitors; and zidovudine (ZDV), abacavir (ABC), tenofovir (TDF), and stavudine (d4T) for NRTIs. If the participant discontinued ART use between study visits, exposure time was calculated as half the time between study visits. For IDV, RTV, ATV, ABC, and d4T, in which more than 50% of participants were unexposed, these variables were classified by 'any exposure'. As a sensitivity analysis, we also categorized ART-exposed years as no exposure and by 4-year intervals.

Statistical analyses

The one-way Wilcoxon rank-sum test and Fisher's exact test were used to compare differences by filtration status within each HIV infection group with the hypothesis that comorbidities were more common among participants with hyperfiltration. Multivariate logistic regression was used to estimate odds ratios (ORs) of prevalent hyperfiltration. Confounders were identified *a priori* based on biological plausibility and published research, and included age, race, BMI, angiotensin-converting enzyme inhibitor (ACEi)/angiotensin receptor blocker (ARB) use, stimulant drug use, current smoking status [28,29], and HIV disease severity (as measured by $CD4^+$ cell counts) [30]. Although age after 40 years was part of the hyperfiltration definition, age was also included in multivariate models to control for potential residual confounding since it is strongly related to renal function. In investigating the effect of ART exposure on the odds of prevalent hyperfiltration, we additionally adjusted for time since any ART initiation and ART use in the pre-HAART era (i.e. prior to 1 July 1996 [31]). As weight gain may be in the causal pathway between ART exposure and hyperfiltration, we also performed a sensitivity analysis excluding BMI as a confounder, and instead used height as an indicator of body size.

Interactions between HIV status and categorical putative hyperfiltration risk factors (diabetes, hypertension, and metabolic syndrome) were assessed and included if significant. Multiple imputation (10 repetitions) was used to complete missing data for unbiased estimates of effects, and the corresponding 95% confidence intervals (95% CIs) are presented. A dataset comprising complete cases was analyzed as a sensitivity analysis and yielded similar inferences as the multiple imputation approach. Statistical significance was assessed at the 0.05 level. All statistical analyses were completed using SAS 9.2 (SAS Institute, Cary, North Carolina, USA).

Results

Cohort characteristics

Of the 741 men who underwent an iGFR study, 720 (97%) had a valid iGFR and were included in our analysis (260 HIV-uninfected and 460 HIV-infected men). Of the HIV-infected men, 456 (99%) had initiated ART prior to the iGFR study. The HIV-uninfected group was older, with a median age of 53 years, vs. 50 years in the infected group. The median iGFR was 105 and 109 ml/min per 1.73 m^2 for HIV-uninfected and infected men, respectively. About 4% ($n = 10$) of HIV-uninfected and 3% ($n = 16$) of HIV-infected men had iGFR below 60 ml/min per 1.73 m^2; these men were excluded from further analysis. We also excluded 6 HIV-uninfected and 69 HIV-infected men with other evidence of CKD: near-normal iGFR (i.e. 60 ml/min per 1.73 m^2 < iGFR < hyperfiltration threshold) and proteinuria [24], resulting in an analytic sample of 608 men free of CKD. Among HIV-uninfected men with hyperfiltration,

2.6% had proteinuria. In contrast, among HIV-infected men with hyperfiltration, 10% had proteinuria.

Metabolic, cardiovascular and behavioral characteristics by HIV status and filtration status

Table 1 compares participant demographic and clinical characteristics by HIV serostatus and filtration status, comprising 241 HIV-uninfected participants (17% with hyperfiltration) and 367 HIV-infected participants (25% with hyperfiltration), without CKD (17 vs. 25%; $P = 0.01$). Among HIV-uninfected participants, men with hyperfiltration had a higher proportion of diabetes (38 vs. 21%) compared to men with normofiltration; however, both groups had similar proportions of individuals with obesity, hypertension, and metabolic syndrome.

Among HIV-infected men, those with hyperfiltration were older (median 51 vs. 49 years) than men with normofiltration. HIV-infected participants with hyperfiltration also had higher fasting blood glucose (105 vs. 100 mg/dl) and HOMA-IR levels (3.9 vs. 3.2), and were more likely to be obese (26 vs. 15%). Men with hyperfiltration were more likely to have a history of hypertension (50 vs. 37%) and uncontrolled hypertension (26 vs. 15%), but the same proportion reported ACEi/ARB use as those with normofiltration (14%). Stimulant use was more common among men with hyperfiltration (22 vs. 14%), but current smoking and other drug use did not differ between groups.

Table 2 presents ORs of prevalent hyperfiltration associated with HIV infection adjusted for age, race, stimulant use, current smoking status, ACEi/ARB use, and BMI. HIV-infected participants were 1.70 times more likely to exhibit hyperfiltration than HIV-uninfected participants (95% CI 1.11–2.61). The effect of stimulant use was similar and borderline significant (OR 1.71, 95% CI 0.99–2.95). In contrast, race, age, and current ACEi/ARB use were not associated with odds of hyperfiltration.

Figure 1 presents results when extending the above model to include the main effects for metabolic and

Table 1. Description of study participants by HIV and filtration status [median (IQR), *n* (%)].

Characteristics	HIV-uninfected			HIV-infected		
	Normofiltration[a] ($n = 200$)	Hyperfiltration[b] ($n = 41$)	*P*	Normofiltration[a] ($n = 275$)	Hyperfiltration[b] ($n = 92$)	*P*
Age (years)	54 [48,61]	53 [47,61]	0.33	49 [44,55]	51 [47,57]	0.04
Black race	63 (32%)	15 (37%)	0.32	99 (36%)	22 (24%)	0.99
HCV infection	23 (12%)	4 (10%)	0.75	34 (13%)	4 (5%)	0.99
Metabolic and cardiovascular variables						
BMI (kg/m^2)	26.6 [24.2,29.6]	25.7 [23.7,29.3]	0.31	25.8 [23.6,28]	26.3 [23.9,29.7]	0.09
Obese (BMI >26 kg/m^2)	46 (23%)	10 (24%)	0.49	41 (15%)	24 (26%)	0.01
HDL cholesterol (mg/dl)	49 [41,58]	54 [45,59]	0.06	44 [38,53]	44 [37,52]	0.20
Non-HDL cholesterol (mg/dl)	138 [114,160]	131 [114,151]	0.14	143 [119,165]	143 [119,166]	0.40
Dyslipidemia	137 (73%)	24 (65%)	0.88	221 (84%)	73 (84%)	0.56
Hemoglobin A1c (%)	5.6 [5.4,5.8]	5.7 [5.4,5.9]	0.22	5.5 [5.2,5.7]	5.5 [5.2,5.8]	0.35
Insulin (μIU/Ml)	11.6 [8.8,17.3]	11.8 [8.4,15.7]	0.46	13.1 [9.8,18.7]	14 [9.8,22.7]	0.11
Fasting glucose (mg/dl)	98 [91,105]	100 [94,109]	0.08	100 [94,106]	105 [97,113]	<0.01
FG >100 mg/dl and ≤126 mg/dl	71 (39%)	14 (41%)	0.47	115 (47%)	41 (54%)	0.16
FG >126 mg/dl	14 (8%)	5 (15%)	0.16	15 (6%)	8 (11%)	0.14
HOMA-IR	3.0 [2.0,4.6]	3.1 [2.1,4.4]	0.39	3.2 [2.2,4.7]	3.9 [2.6,6.3]	0.04
Diabetes	41 (21%)	15 (38%)	0.02	72 (26%)	26 (28%)	0.40
Metabolic syndrome[c]	112 (57%)	24 (62%)	0.36	180 (65%)	66 (72%)	0.16
SBP (mmHg)	126 [118,135]	128 [115,136]	0.39	123 [115,132]	127 [118,136]	0.02
DBP (mmHg)	76 [72,83]	77 [71,84]	0.49	77 [71,82]	78 [72,83]	0.32
SBP >140 or DBP >90 mmHg	45 (23%)	10 (28%)	0.36	41 (15%)	22 (26%)	0.02
History of hypertension	87 (44%)	22 (56%)	0.11	101 (37%)	46 (50%)	0.02
ACEi/ARB use	38 (19%)	5 (12%)	0.90	38 (14%)	13 (14%)	0.53
Behavioral variables						
Current smoker	47 (24%)	11 (27%)	0.39	91 (33%)	29 (32%)	0.66
Stimulant use	30 (15%)	6 (16%)	0.55	38 (14%)	20 (22%)	0.05
Other drug use (marijuana/inhalant nitrates)	72 (37%)	16 (42%)	0.32	126 (46%)	47 (51%)	0.22

ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; FG, fasting glucose; HCV, hepatitis C virus; HOMA-IR, Homeostatic Model Assessment – Insulin Resistance; IQR, interquartile range. One-sided *P*-values based on Wilcoxon rank-sum test for continuous variables and Fisher's exact test for categorical variables, comparing normofiltration and hyperfiltration within HIV infection status group.

[a]Normofiltration defined as iGFR less than 140 ml/min per 1.73 m^2 − ml/min per year over age 40 and iGFR greater than 60 ml/min per 1.73 m^2 and urine protein: creatinine <200 mg/g.

[b]Hyperfiltration defined as iGFR above 140 ml/min per 1.73 m^2 − ml/min per year over age 40.

[c]Metabolic syndrome defined as having three or more of: waist at least 102 cm, fasting triglycerides at least 150 mg/dl, HDL-cholesterol below 40 mg/dl, fasting glucose at least 100 mg/dl, or diabetes diagnosis with medication use.

Table 2. Odds ratios of prevalent hyperfiltration from multivariate logistic regression.

Characteristics	OR (95% CI)
HIV-infected	1.70 (1.11–2.61)
Per 1 year increase in age	1.01 (0.98–1.03)
Black race	0.66 (0.41–1.05)
Stimulant use	1.71 (0.99–2.95)
ACEi/ARB use	0.77 (0.43–1.38)
Current smoker	1.06 (0.66–1.69)
Per 1 log increase in BMI	2.44 (0.71–8.34)

ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; CI, confidence interval; OR, odds ratio.

cardiovascular factors. The effect of HIV remained consistently strong across all models, with ORs ranging from 1.63 to 1.80. Indicators of hyperglycemia were associated with higher odds of prevalent hyperfiltration (HbA1c $\geq$6.5%, OR 3.03, 95% CI 1.25–7.38; fasting glucose $>$100 mg/dl, OR 1.58, 95% CI 1.03–2.42). The prevalence of hyperfiltration was similar by metabolic syndrome status. The association of diabetes with hyperfiltration differed by HIV status ($P=0.05$ for interaction). Specifically, diabetes was associated with hyperfiltration among HIV-uninfected individuals (OR 2.56, 95% CI 1.33–5.54), but not among HIV-infected individuals (OR 1.19, 95% CI 0.69–2.05; not shown). However, compared to HIV-uninfected individuals without diabetes (reference in this model represented by the vertical line at 1), HIV-infected individuals (regardless of diabetes status) were more than twice as likely to have hyperfiltration; this comparison was statistically significant. Uncontrolled hypertension was associated with higher odds of hyperfiltration (OR 1.61, 95% CI 0.99–2.60) as was history of hypertension (OR 2.10, 95% CI 1.33–3.33). The latter association was similar by HIV status ($P=0.91$ for interaction). The inclusion of hypertension in the model assessing the interaction of diabetes and HIV on hyperfiltration yielded similar results (model 7; Fig. 1).

HIV and antiretroviral therapy characteristics associated with hyperfiltration

Table 3 displays HIV and treatment-related factors among HIV-infected participants stratified by filtration status. Indices of HIV disease severity were similar by hyperfiltration status, whereas some ART factors differed between the two groups. The hyperfiltration and normofiltration groups had similar median times since any ART initiation, but the median cumulative HAART use was longer in the hyperfiltration group than the normofiltration group (median time was 7.3 vs. 6.3 years). Cumulative protease inhibitor and NNRTI years were

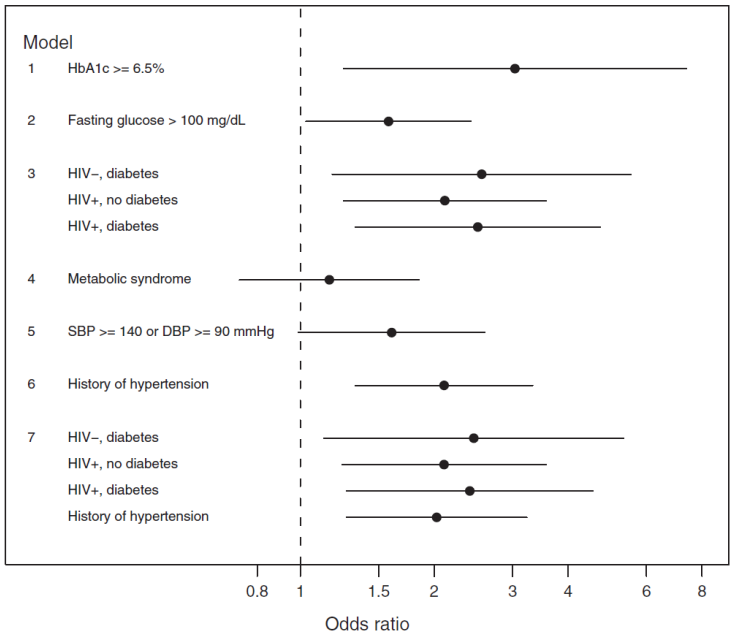

Fig. 1. The odds ratios (●) with 95% confidence intervals of prevalent hyperfiltration associated with metabolic and cardiovascular factors among men without CKD ($n=608$). The reference group (indicated by the vertical line at 1.0) is the one without the variable of interest in each model. Each model was adjusted for HIV infection, age, race, stimulant drug use, ACEi/ARB use, current smoking status and BMI, whose effects are shown in Table 2. The reference groups for model 3 and model 7 comprise HIV-uninfected men with no diabetes, and HIV-uninfected men with no diabetes and no history of hypertension, respectively. ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; CKD, chronic kidney disease.

Table 3. HIV and HIV treatment-related characteristics by filtration status and adjusted odds ratios of prevalent hyperfiltration [*n* (%), median (IQR) and OR (95% CI)].

Variable	Normofiltration ($n=275$)	Hyperfiltration ($n=92$)	Adjusted OR (95% CI)[a]
Nadir CD4$^+$ cell count <350/μl	225 (82%)	73 (79%)	0.69 (0.36–1.30)
Current CD4$^+$ cell count <350/μl	49 (18%)	21 (23%)	1.48 (0.79–2.78)
Current detectable HIV RNA	57 (21%)	16 (18%)	0.91 (0.45–1.86)
History of AIDS diagnosis	39 (14%)	10 (11%)	0.71 (0.33–1.54)
Time since ART initiation[b] (years)	12.3 [7.6, 19.8]	12.8 [8.4, 19.1]	1.48 (0.86–2.55)[d]
Cumulative HAART[c] (years)	6.3 [3.8, 9]	7.3 [4.4, 9.3]	1.31 (0.87–1.99)[d]
Cumulative NNRTI (years)	2.7 [0.3, 6.2]	3.8 [0.3, 6.9]	1.15 (0.81–1.64)[d]
Cumulative PI (years)	4.1 [0.1, 8]	4.3 [0.2, 8]	1.00 (0.75–1.33)[d]
Cumulative IDV (years)	0.0 [0.0, 0.3]	0.0 [0.0, 0.7]	
Any IDV exposure	76 (29%)	31 (35%)	1.41 (0.81–2.46)
Any RTV exposure	25 (9%)	9 (10%)	1.21 (0.58–2.55)
Cumulative ATV (years)	0.0 [0.0, 1.2]	0.0 [0.0, 0.2]	
Any ATV exposure	87 (33%)	22 (25%)	0.71 (0.41–1.24)
Cumulative NRTI (years)	17.4 [10.5, 23.3]	19.7 [11.9, 25.6]	1.12 (0.96–1.31)[d]
Cumulative ZDV (years)	2.0 [0.0, 5.9]	4.1 [0.2, 8.0]	1.53 (1.12–2.10)[d]
Cumulative TDF (years)	2.4 [0.0, 4.6]	2.0 [0.0, 3.9]	0.67 (0.40–1.14)
Cumulative ABC (years)	0.0 [0.0, 3.1]	0.0 [0.0, 3.1]	
Any ABC exposure	114 (43%)	36 (41%)	0.91 (0.55–1.50)
Cumulative d4T (years)	0.0 [0.0, 3.3]	0.0 [0.0, 3.5]	
Any d4T exposure	130 (47%)	46 (50%)	1.03 (0.60–1.76)

95% CI, 95% confidence interval; ABC, abacavir; ATV, atazanavir; d4T, stavudine; IDV, indinavir; IQR, interquartile range; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; OR, odds ratio; PI, protease inhibitor; RTV, ritonavir; TDF, tenofovir; ZDV, zidovudine.
[a]Odds ratios are calculated from multiple logistic regression models adjusting for age, race, BMI, ACEi/ARB use, current smoking status, stimulant drug use, current and nadir CD4$^+$ cell count <350, time since ART initiation and any ART use prior to 1 July 1996 (pre-HAART era).
[b]Time since ART initiation refers to first known date of any antiretroviral therapy (i.e. monotherapy, combination therapy, or potent therapy).
[c]HAART refers to potent combination therapy according to December 2009 guidelines (a regimen of three or more drugs).
[d]Odds ratios for a 5-year increase in cumulative medication exposure.

similar between the two groups, whereas men with hyperfiltration had higher median cumulative NRTI years (19.7 vs. 17.4). This difference in cumulative ART use was primarily accounted for by ZDV use (median 4.1 vs. 2.0 years).

The association of HIV disease severity indicators and therapy with hyperfiltration, adjusted for age, race, current and nadir CD4$^+$ cell count, time since first ART exposure and ART use in the pre-HAART era, BMI, ACEi/ARB use, current smoking status, and stimulant drug use, are presented by the adjusted odds of prevalent hyperfiltration ratios in the right-most column of Table 3. None of the indicators of HIV disease stage were associated with hyperfiltration, including nadir/current CD4$^+$ cell count below 350/μl, and detectable plasma HIV RNA level. Indicators of ART exposure were not associated with hyperfiltration, with the exception of ZDV. For a 5-year increase in ZDV exposure, the odds of hyperfiltration significantly increased by 53% (95% CI 1.12–2.09). A sensitivity analysis of cumulative ART exposure by 4-year categories yielded similar results. For ZDV specifically and with participants with no exposure to ZDV as the reference group, there was no association for less than 4 years of exposure (OR 0.85, 95% CI 0.42–1.70). However, the OR of prevalent hyperfiltration was 1.88 for 4–8 years of exposure (95% CI 0.93–3.81) and was 2.11 for more than 8 years of ZDV exposure (95% CI 0.99–4.49).

Discussion

In this MACS GFR substudy, the overall prevalence of measured glomerular hyperfiltration for men without CKD was significantly higher among those infected with HIV compared to uninfected men: 25 and 17%, respectively. From this group, men with HIV had 1.7 times higher odds of hyperfiltration than uninfected men, independent of demographic, metabolic, and cardiovascular factors. We further confirmed the findings of previous studies which identified hyperglycemia [12], diabetes mellitus [32], and hypertension [9], as independently associated with hyperfiltration in this unique population, although the effect of diabetes was mainly observed among HIV-uninfected men. Our findings suggest that hyperfiltration is common in this cohort and may be an important clinical consideration as an early indicator of kidney dysfunction among HIV-infected persons.

HIV infects renal epithelial cells [33] and disturbs podocyte structure and function [34]. The latter appears to involve up-regulation of the renin–angiotensin system [35], which is central to the pathophysiology of hyperfiltration. Alternatively, HIV itself, ART, and associated metabolic derangements may contribute to hyperfiltration via perturbations in insulin-like growth factor [36], leading to visceral adiposity and abnormal glucose metabolism [14,37]. In our cohort, since nearly

all HIV-infected men were also being treated with ART, we were unable to discern whether the elevated prevalence of hyperfiltration was due to HIV infection alone, ART use, or both.

In the general population, previous studies have found that elevated blood glucose and HbA1c are associated with higher odds of hyperfiltration, with ORs ranging from 1.3 [9] and 1.6–2.2 [12,38], as well as hypertension (OR 1.8) [9,39]. Studies also suggest hyperfiltration precedes the onset of albuminuria and kidney function decline, and is therefore an important marker of future CKD [23]. Indeed, among diabetic individuals, hyperfiltration has been associated with increased risk of diabetic nephropathy and CKD progression [32]. Among individuals with hypertension, the proportion of those with persistent hyperfiltration and those with hyperfiltration progressing to normofiltration who developed microalbuminuria was 16 and 36%, respectively, compared to only 5% of those who never experienced hyperfiltration, over a median follow-up of 8.5 years [40].

Our results show that HIV is also associated with higher odds of hyperfiltration, and that HIV infection modifies the diabetes–hyperfiltration relationship: among HIV-uninfected individuals, diabetes was strongly associated with hyperfiltration; however, among HIV-infected individuals, there was no further association between diabetes and hyperfiltration, but these individuals were at higher risk for hyperfiltration than their HIV-uninfected peers. This association was observed univariately (Table 1) and in adjusted models (Fig. 1). The effect of diabetes on hyperfiltration may be secondary to the dominant effect of HIV and its treatment.

Two recent publications documented the association of diabetes and HIV on decreased renal function [41] and end-stage renal disease (ESRD) [42]. In these studies, the presence of either HIV or diabetes was associated with a higher risk of diminished GFR [41] and ESRD among African Americans [42], a relationship which was similar to the increased odds of hyperfiltration in our study. In contrast to our findings, however, those who had both diabetes and HIV were at even greater risk of incidence of low eGFR below 45 ml/min compared to those with HIV or diabetes only [41]. Similar to the results for ESRD [42], we did not find an additive effect of HIV and diabetes on hyperfiltration. These results suggest that hyperfiltration may mediate some portion of the effects of diabetes and HIV on ESRD, but future research is needed to investigate this relationship.

Although not all associations were statistically significant, we consistently found increased odds of hyperfiltration in association with most ART factors, suggesting that prolonged ART exposure may increase the risk of hyperfiltration. Whereas this relationship was strongest for ZDV use (OR 1.53 per 5-year increase in ZDV exposure), increased ZDV exposure may be a marker for longer HIV infection duration, and longer HIV infection is the risk factor for hyperfiltration. Alternatively, ZDV use may be a proxy for historically poorer HIV suppression, since it was commonly used in the pre-HAART era; this was a possibility we could not explore. Despite the diminished use of ZDV, ZDV exposure may be clinically relevant for identifying patients with hyperfiltration since there are HIV-infected patients in care with prior or current exposure to ZDV. Indeed, there may also be a threshold effect of ZDV on hyperfiltration that is more common with chronic use, or the effect may manifest later among persons with a longer duration of HIV infection. The protective, but nonsignificant trend, associated with TDF exposure was also notable since TDF is nephrotoxic. Individuals at risk for kidney disease may have been less likely to be prescribed TDF than others (i.e. channeling bias). Alternatively, increased TDF exposure may have caused pathologic nephron loss, potentially counteracting any hyperfiltration effects due to other disease or therapy processes. Third, TDF renal toxicity is thought to be directed towards the proximal tubule rather than the glomerulus; therefore, TDF-treated individuals may not necessarily be at higher risk of glomerular hyperfiltration. Lastly, better virologic control and HIV management with TDF may reduce the risk of hyperfiltration.

Of note, in multivariate analysis, stimulant drug use was associated with increased odds of hyperfiltration (OR 1.71). The relationship between stimulant drug use and hyperfiltration has not been well characterized in the literature, but may be related to the effects of stimulant drug use on the sympathetic nervous system and on the regulation of beta cell function and dopamine (potentially affecting glomerular perfusion). Studies have suggested that cocaine and other stimulants may perturb insulin secretion and lipid/glucose homeostasis [43,44]. The observed association between stimulants and hyperfiltration may be related to a shared relationship with hormonal dysregulation, hyperglycemia, and renal function. Alternatively, stimulant use may be an indicator for socioeconomic or lifestyle factors that adversely affect disease management, adherence to medications, or overall general health, and thereby increase the risk of hyperfiltration.

Whereas ACEi/ARB use was not significantly associated with decreased odds of hyperfiltration, the directionality of effect was consistent with reduced GFR due to preferential dilation of the efferent arteriole (OR 0.77). In contrast, we were unable to explain the lower prevalence of hyperfiltration among black participants (OR 0.66), although this effect was also nonsignificant. This was surprising given the higher risk of diabetes, diabetic nephropathy, and ESRD in the black population,

and the documented higher risk of hyperfiltration among blacks with hypertension [45].

One limitation of this study is that we are unable to causally link identified metabolic, cardiovascular, and HIV infection factors with hyperfiltration. However, the physiologic framework of the HIV renal reservoir, the influence of ART on metabolic and cardiovascular health, as well as previous literature, suggest that metabolic and cardiovascular changes likely initiate hyperfiltration [10,32]. Furthermore, since previous direct measurements of GFR had not been obtained, we were unable to determine the duration of hyperfiltration, although a second iGFR measurement will be obtained in these men for future analyses. Whereas hyperfiltration is a known precursor to kidney damage, the duration of hyperfiltration necessary to precipitate kidney function decline is unclear. An early study among Pima Indians demonstrated GFR decline over a 4-year period after hyperfiltration was identified [46]. Future longitudinal data of iGFR in our cohort are needed to further understand risk factors for and consequences of hyperfiltration. An additional limitation is that the study sample was exclusively men, and whether these inferences extend to women is unknown.

In summary, we found that among men without CKD, HIV infection was associated with a higher prevalence of hyperfiltration compared to being uninfected. A major strength of this study was the use of directly measured GFR by iohexol to detect hyperfiltration, a better and more reliable method than estimating equations, particularly for high GFR levels [10,47]. Noninfectious metabolic conditions found commonly in aging HIV-infected individuals were associated with hyperfiltration. Additionally, among HIV-infected men, increased exposure to ART, particularly ZDV, was associated with higher odds of hyperfiltration. Since poor metabolic and cardiovascular health are known risk factors for glomerular hyperfiltration, which is an early clinical marker of kidney damage, evaluation and aggressive treatment of these risk factors [9,10] should be an important priority for health management in HIV-infected as well as non-HIV-infected populations.

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Conflicts of interest

The authors have no conflict of interests to declare.

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3 HIV infection and therapy are associated with higher incidence of hyperfiltration using creatinine-based CKD-EPI estimated glomerular filtration rate data in the Multicenter AIDS Cohort Study

3.1 Abstract

Objective Elevated glomerular filtration rate (GFR) has recently been shown to be associated with HIV infection using a directly measured iohexol protocol in a cross-sectional analysis using data from the Multicenter AIDS Cohort Study (MACS). As a putative risk factor for CKD and related to indicators of metabolic and cardiovascular abnormalities, it is important for clinical care since it is treatable with dietary interventions or aggressive antihypertensive therapy. The aims of this study were to a) define a threshold for elevated GFR appropriate for the MACS using the creatinine-based CKD-EPI estimated GFR (eGFR) equation; b) determine the incidence of chronic hyperfiltration; and c) estimate the risk of hyperfiltration associated with treated HIV infection in a competing risks setting.

Design Prospective study design comprising MACS participants free of low eGFR (> 90 ml/min|1.73m^2) and elevated eGFR. Elevated eGFR was defined by the CKD-EPI eGFR age- and race-specific 90th percentile among the HIV-uninfected men. At baseline, the study population at risk of incident hyperfiltration comprised 546 HIV-uninfected men and 574 HIV-infected men receiving HAART. Endpoints included chronic hyperfiltration (first of two eGFR > 90th percentile within 1 year), low eGFR (first of two eGFR< 90 ml/min|1.73m^2 within 1 year, or first eGFR < 70 ml/min|1.73m^2), or last observed visit as of September 30, 2013.

Methods Quantile regression for all HIV-uninfected men was used to determine age- and race-specific 90th percentiles of eGFR, and was bootstrapped to obtain 95% confidence intervals for the estimates. For incidence and survival analyses, the time scale was age, specifically years from age 30. Age-specific incidence rates and incidence rate ratios were based on Poisson regression, and non-parametric Kaplan-Meier estimates incorporating late entries were used to account for study entry after age 30. Competing risks proportional hazards regression was used to estimate the relative subhazard associated with treated HIV infection, accommodating the competing risk event of low eGFR. Sensitivity analyses investigated the impact of differing thresholds to define eGFR on the incidence of hyperfiltration and inferences.

Results The equation for the estimated 90th percentile of eGFR was 118.951 ml/min – 0.726 × years after age 30 for non-blacks and 130.39 ml/min – 0.653 × years after age 30 for blacks, based on HIV-uninfected men. The median ages for each race and HIV infection category was between 41 and 46, with a median follow-up time between 2.5 and 3.5 years. The cumulative incidence rates of hyperfiltration for non-blacks were 5.3 and 4.8 per 100 person-years for HIV-uninfected men and HIV-infected men, respectively. Among blacks, the cumulative incidence rates were 3.5 for HIV-uninfected and 5.7 per 100 person years for HIV-infected men. However, in age-adjusted analyses, non-black men with HIV were more likely to develop hyperfiltration prior to age 35 than uninfected non-black men (IRR= 3.41, 95%CI: 1.22, 9.56); black men with HIV were more likely to develop hyperfiltration after age 45 than uninfected black men (IRR for ages 45 to 50= 2.79, 95%CI: 1.12, 6.95). This was confirmed graphically with Kaplan-Meier survival plots and in a competing risk analyses accounting for late entries and low eGFR as a

competing event. The subhazard ratio (SHR) for HIV infection at age 30 among non-blacks was 3.7 (95%CI: 1.6, 8.4) and this SHR declined 7% each year (SHR= 0.93, 95%CI: 0.89, 0.96). For blacks, the subhazard ratio comparing HIV-infected to HIV-uninfected did not vary by age and showed an increased, but non-significant estimated risk of hyperfiltration (SHR= 1.6, 95%CI: 0.96, 2.7), regardless of age. Sensitivity analyses suggested that the estimates of incidence rates were highly sensitive, yet overall inferences about risk of hyperfiltration associated with HIV infection did not change.

Conclusions Men treated with HAART for HIV infection are at higher risk for incident hyperfiltration after age 30, particularly at ages younger than 45 among non-blacks; there was a higher but non-significant risk of hyperfiltration among blacks. Clinical monitoring of eGFR should include consideration of persistently elevated levels, as well as low levels, in this high risk population.

3.2 Introduction

Glomerular hyperfiltration is defined as elevated glomerular filtration rate (GFR) to pathologically high levels, potentially leading to accelerated renal function decline and chronic kidney disease (CKD) [1]. Elevated GFR has recently been shown to have a higher prevalence among HIV-infected men compared to uninfected men in the Multicenter AIDS Cohort Study (MACS) in a cross-sectional analysis using directly measured GFR by plasma disappearance of iohexol [2]. Metabolic and cardiovascular abnormalities, including elevated fasting blood glucose [3,4], and the presence of Type 2 diabetes [5–7] and hypertension [8,9] have been shown to be associated with elevated GFR primarily in non-HIV populations, but also in the MACS [2]. Indeed, many of these metabolic and cardiovascular risk factors associated with hyperfiltration are also related to HIV infection and highly active antiretroviral therapy (HAART) [10,11]. Furthermore, HIV infection and HAART are considered causes of CKD through numerous pathways [12–18].

Hyperfiltration is considered a modifiable risk factor of CKD, treatable by a low-protein diet or antihypertensive therapy, using ACEi or ARBs, which preferentially dilate the efferent arterioles [19]. Since hyperfiltration is a precursor of CKD and its risk may be mitigated or eliminated with intervention, its early detection may be an important clinical tool to improve outcomes, particularly in HIV populations receiving HAART that are at high risk of CKD and poor outcomes.

Initial evidence of increased risk of hyperfiltration associated with HIV was a higher prevalence of elevated GFR in the MACS, with 17% among HIV-uninfected men compared to 25% among HIV-infected men receiving HAART [2]. However, there are

few studies investigating the incidence of hyperfiltration in general, and none that characterize incidence in an HIV population. Few studies also describe hyperfiltration from a widely-used clinical measure of GFR: the CKD-EPI serum creatinine-based estimated GFR (eGFR) equation [20]. Therefore, the purposes of this study using MACS data were to a) present a threshold of elevated GFR using the CKD-EPI eGFR equation, based on HIV-uninfected men who are comparable to the HIV-infected men receiving HAART, and b) describe the incidence of chronic hyperfiltration in both groups, and c) determine the risk of hyperfiltration associated with HIV infection treated with HAART.

3.3 **Methods**

3.3.1 Study population

The Multicenter AIDS Cohort Study is a longitudinal observational study of the natural and treated history of HIV among homosexual and bisexual men with infected with HIV or at risk of acquiring HIV. Initiated in 1984, the study comprises 7087 men from 1984 through 2012 in four recruitment waves (1984; 1987 to 1991; 2001 to 2003; 2012 to September 30, 2013), at the time of the current analyses. Details of the MACS have been previously described [21]. Since untreated HIV infection is strongly associated with CKD [22] and GFR decline [14], and the administration of HAART is the standard of HIV care, these analyses restrict to HIV-infected men who have initiated HAART. Collection of serum creatinine data from HIV-uninfected men began in 2002, well into the era of widespread use of HAART (available starting in 1996) and was therefore comparable to data from HIV-infected men of the same era.

Standardized protocols collected height, weight, fasting blood samples, blood pressure and behavioral variables, as previously described [2]. Metabolic variables in this

analysis included body mass index (BMI); obesity (defined as BMI ≥ 30 kg/m^2); fasting glucose level; serum high-density lipoprotein (HDL); low-density lipoprotein (LDL); diabetes (defined as fasting glucose > 126 mg/dl, or diagnosis of diabetes with use of medications); dyslipidemia (defined as fasting total cholesterol ≥ 200 mg/dl, LDL ≥ 130 mg/dl, HDL < 40 mg/dl, triglycerides ≥ 150 mg/dl, or use of lipid lowering medications with self-reported/clinical diagnosis of dyslipidemia); and metabolic syndrome [23]. Cardiovascular variables included systolic blood pressure (SBP; mmHg); diastolic blood pressure (DBP; mmHg); uncontrolled hypertension (SBP ≥ 140 mmHg or DBP ≥ 90 mmHg); and use of antihypertensive medications. Behavioral variables included smoking status (current or non-current) and stimulant use (defined as cocaine, amphetamine or methamphetamine use in the preceding 6 months). Serum creatinine was measured from blood samples at laboratories local to the site as part of standardized blood work up to calculate eGFR using the CKD-EPI eGFR equation [20].

3.3.2 Estimating 90th percentile threshold for defining elevated eGFR

To establish a threshold of elevated eGFR, HIV-uninfected men older than 30 years were used as a reference population for HIV-infected men. Our conceptual framework assumes that had the HIV-infected men never been infected, their eGFR distribution would be identical to the HIV-uninfected men. Since this uninfected population shares many of the same health and risk behaviors as the HIV-infected men in the MACS, they serve as an appropriate counterfactual group.

Quantile regression was used to estimate the 90th percentile of eGFR (dependent variable) as a function of age after 30 years (independent variable), since there is a normal age-related renal function decline [24–26]. Black race is associated with an

overestimation bias using the CKD-EPI equation [27]; therefore, models were stratified by race (non-black and black). From these models, a 90th percentile level of eGFR could be determined that is dependent on the age and race of a given subject, and this level served as a threshold of elevated eGFR. This approach was adapted from previous studies that used a normal population to derive a threshold of elevated eGFR [3,9].

Since subjects contributed multiple eGFR observations, 95% confidence intervals were estimated by bootstrapping to account for correlated within-subject observations. A total of 2000 datasets were created for each race group with subjects randomly selected (with replacement) comprising the same number of observed individuals (1083 non-black and 290 for black men) in the original dataset. All eGFR observations from each selected subject were used in each bootstrapped set. The 95% confidence intervals were defined as the 2.5th and 97.5th percentiles from the distribution of intercepts and slopes from the 2000 quantile regression models.

The empirical 90th percentile for each 5-year age bin was used to assess model fit. As a sensitivity analysis, log-transformed eGFR was assessed as an alternative to a linear model, but this transformation did not improve the fit to the data.

3.3.3 Data structure and outcomes for time to event analyses

In order to determine the incidence of chronic hyperfiltration, the analytic dataset was restricted to subjects with normal renal function at baseline, as defined as first observed visit where age ≥ 30 years. Specifically, the data comprise men who were free of elevated eGFR (i.e., eGFR ≥ age- and race-specific 90th percentile) and were free of evidence of CKD (i.e., eGFR < 90 ml/min|1.73m^2). For the purposes of these analyses,

we assumed men were free of hyperfiltration prior to study entry. The time scale was age after 30 years; therefore, all inferences are conditional on being event-free up to age 30.

Chronic hyperfiltration, the outcome of interest, was defined as having at least two occurrences of elevated eGFR (i.e., ≥ age and race-specific 90^{th} percentiles) within one year (i.e., out of 3 consecutive semi-annual visits). Requiring persistently high eGFR levels to defined chronic hyperfiltration minimized the potential misclassification due to transient elevated eGFR. Subjects could exit the study at the occurrence of low eGFR, defined as the first of two eGFR observations less than 90 ml/min|1.73m^2 within 1 year, or a single observation where eGFR < 70 ml/min|1.73m^2), or at the last observed visit.

3.3.4 Statistical analysis

There were four components to the analyses describing the incidence of hyperfiltration among HIV-infected and uninfected men in the MACS. First, we present incidence rates and incidence rate ratios to characterize the occurrence of hyperfiltration, by infection status and race. Second, we present non-parametric Kaplan-Meier estimates of survival to describe the time to hyperfiltration with age as the time scale. In these first two analyses, subjects were censored at the occurrence of low GFR or last observed visit. However, low GFR is a competing risk event for hyperfiltration, such that once a person declines to low renal functioning, they are no longer at risk of hyperfiltration. Censoring these events can be appropriate in order to describe the unconditional risk of hyperfiltration [28] and is reasonable to use for describing general incidence rates and Kaplan-Meier estimates. Nonetheless, since low eGFR is a true competing risk event, the third component assessed hyperfiltration in a competing risk setting, using the subhazard ratio to describe the effect of HIV infection on hyperfiltration. Lastly, the fourth

component of the analyses explored the sensitivity of these findings using different thresholds to define elevated eGFR, and by extension, chronic hyperfiltration. Data management was conducted in SAS 9.2 (SAS Institute, Cary, North Carolina, USA), survival analyses were performed in STATA version 11 (STATACorp, College Station, Texas), and graphics were produced in SPLUS 8.2 (TIBCO Software).

3.3.5 Incidence rates and incidence rate ratios

Incidence rates (IRs) and incidence rate ratios (IRRs) were calculated by a Poisson linear model for count data by five-year age bins and HIV infection status, with separate models for each race group (non-black and black). Subjects were censored at low eGFR or the last observed visit.

3.3.6 Kaplan-Meier estimation of incidence by survival step functions

Non-parametric Kaplan-Meier estimates accounting for late entries provided graphical depictions of the incidence of hyperfiltration and estimated times, with 95% confidence intervals, for the *p*th percentiles free of hyperfiltration. Censoring likewise was based on low eGFR or the last observed visit. The survival function was summarized by the estimated times of events for the 10^{th}, 25^{th}, 50^{th} and 75^{th} percentiles by HIV infection status and race groups. The log-rank test compared the equality of survival function.

3.3.7 Competing risks regression

To account for the competing risk event of low eGFR, we used the approach proposed by Fine and Grey to estimate the subhazard ratio (SHR), in the context of a proportional hazard model using the subdistribution of a competing event [29]. In short,

this method appropriately differentiates between individuals who remain at risk of having the event after they exit the study because they were no longer followed (i.e., are truly censored) and those who exit the study because they are no longer at risk of the event occurring (i.e., have low eGFR), by estimating the cumulative incidence function using two cause-specific hazards (the event of interest and the competing risk event). This is in contrast to the Kaplan-Meier estimator which estimates the cumulative incidence function from the hazard function of the event of interest only. The primary independent variable was HIV infection. In the presence of non-proportional hazards, we also explored allowing the SHR for HIV infection to vary as a linear function of time. Since we were interested in the population-level risk, the primary model did not adjust for confounders. However, as a secondary analysis, we included known risk factors at baseline (obesity, fasting blood glucose > 100 g/dL and uncontrolled hypertension) as covariates in the model as a comparison, especially since these variables are putative mediators of incident hyperfiltration. Death was not included as a competing event since only one subject death was observed.

3.3.8 Sensitivity analyses

To investigate the effect of threshold changes on the inferences from the main analyses, a sensitivity analysis was conducted using different age- and race-specific thresholds to define elevated eGFR (and subsequently chronic hyperfiltration). The Kaplan-Meier analyses were replicated using three different thresholds based on a) previously published findings of the normal age-related decline in GFR, and b) the confidence limits of the bootstrapped quantile regression estimates. Race-specific intercepts from the equation were rounded to the nearest 1 ml/min|1.73m^2 and the slopes

were varied to obtain three different race-specific thresholds. From previously published literature, Delayne et al. [25] report the best study estimate of age-related decline in healthy men is –8 ml/min|1.73m^2 per 10 years [30], which we express as –0.8 ml/min|1.73m^2 per year. The race-specific lower 95% confidence limit and upper 95% confidence limits were used for the other two analyses.

3.4 **Results**

3.4.1 Determination of elevated eGFR

To establish a threshold of elevated eGFR, estimates of age- and race-specific 90th percentiles were derived from 1373 men free of HIV infection in the MACS. Since there is a known overestimation bias among blacks using the CKD-EPI eGFR equation, the models were stratified by race. A total of 1083 uninfected non-black subjects contributed 15456 eGFR observations; 290 uninfected black subjects contributed 3482 observations. The equation for the estimated 90th percentile eGFR level as a function of age was (95% confidence intervals in subscript):

(1) Non-black 90th percentile eGFR=

$$_{118.563}118.951_{119.531} + (_{-0.747}-0.726\ _{-0.712})\times \text{ years after age 30}$$

The equation for blacks was:

(2) Black 90th percentile eGFR=

$$_{129.184}130.390_{134.914} + (_{-0.833}-0.653\ _{-0.608}) \times \text{ years after age 30}$$

Figure 1 graphically depicts these equations along with the empirical 90th percentiles by five year age bins, indicating good model fit.

3.4.2 Baseline clinical and demographic characteristics

Figure 2 presents the study flow, by HIV infection status for inclusion in this analysis. At first visit after age 30, 39.7% (546/1373) of HIV-uninfected men were free of low or elevated eGFR (i.e., eGFR $\geq$ 90 ml/min|1.73m^2 and less than the age- and race-specific 90th percentiles) while 45.7% (574/1255) of HIV-infected men receiving HAART were in the same category. About 14.5% (182/1255) of the HIV-infected group had prevalent elevated eGFR compared to 8.6% (118/1373) of the HIV-uninfected men. The HIV-infected men also had a lower proportion with eGFR < 90 ml/min|1.73m^2 compared to the HIV-uninfected men (39.8% vs. 51.6%, respectively). These prevalent differences by eGFR category were significant (chi-square p< 0.001). For the analytic sample used to describe the incidence of hyperfiltration, the HIV-uninfected group comprised 546 men (386 non-blacks and 160 blacks) and the HIV-infected group comprised 574 men (377 non-black subjects and 197 black subjects).

Table 1 presents the demographic and clinical characteristics of the analytic sample, specifically subjects with normal eGFR at baseline. Among non-blacks, the median age at entry into this sample was 47 years [IQR= 41, 53] for HIV-uninfected men (n= 386) and 43 years [IQR= 38, 48] for HIV-infected men (n= 377) and this difference was significant (p< 0.001). CKD-EPI eGFR did not differ by infection status at study entry: the median level for HIV-uninfected men was 100 ml/min|1.73m^2 [IQR= 96, 104] and was 101 [IQR= 96, 106] among HIV-infected men. HIV infection was associated with lower weight and body mass index and a lower prevalence of obesity (7% vs. 19%,

p< 0.001), but a poorer lipid profile: 84% of HIV-infected men had dyslipidemia, compared to 72% of HIV-uninfected men (p< 0.001). There was no difference in hypertension status (prevalence of uncontrolled hypertension was 16% for both groups), but HIV-infected men were slightly less likely to receive antihypertensive therapy (10% vs. 14%, p= 0.072). HIV-infected men were more likely to be current smokers (32% vs. 25%, p= 0.03) and have used illegal stimulants, specifically cocaine or uppers, in the previous year (21% vs. 12%, p< 0.001).

Among black subjects, the HIV-uninfected men (n= 160) and HIV-infected men (n= 197) did not differ by age at study entry (42 and 41 years, respectively; p= 0.796). Black HIV-infected men had higher eGFR at entry than the HIV-uninfected men (108 vs. 103 ml/min|1.73m^2, p < 0.01); these levels were also higher than the non-black group. HIV infection was also associated with lower body mass and prevalence of obesity. Although not statistically significant, the HIV-infected men had a higher prevalence of dyslipidemia (69% vs. 60%, p= 0.156). There were no differences by hypertensive status, antihypertensive therapy, current smoking status or use of stimulants in the past year.

Table 1 also presents a description of the longitudinal data and observed follow-up time. For non-black men, the overall median follow-up time was about 3 years for HIV-uninfected men (1496.2 total person-years) and 2.5 years for HIV-infected men (1367.8 total person years). In this group of 386 HIV-uninfected men, 21% had a hyperfiltration event; 42% had a low eGFR event and exited the study; and 38% had neither event and exited the study at their last observed visit with a median follow-up time of 5.2 years. Among the non-black HIV-infected group, 12 men became infected and initiated treatment while under study observation for a total of 389 men. Of these,

17% had a hyperfiltration event; 51% exited with low eGFR; and 32% exited with no event and a median follow-up time of 3.9 years. The distribution of these three events was significant by HIV infection status (p= 0.026) and was largely driven by a high incidence of low eGFR events among the HIV-infected men (51% vs. 42%). Among black men, the overall median follow-up time was similar between HIV-uninfected and HIV-infected men: 3.4 years and 3.5 years, respectively; and the total observed follow-up time was 664.2 person years and 791.7 person years, respectively. A total of 5 black men became infected and initiated therapy contributing to both groups until their infection status changed; thus, the denominator for HIV-infected black group comprised 202 men. The distributions of events were borderline significant by HIV infection status: 14% of HIV-uninfected men had incident hyperfiltration, 31% had low eGFR and 55% for neither hyperfiltration nor low eGFR events at study exit, and in this group with no events, the median follow-up time was 5.3 years. For HIV-infected black men, 22% had a hyperfiltration event; 33% had a low eGFR event and 45% exited the study with neither event, and in this group with no event, the median follow-up time was 4.0 years.

Table 2 describes disease and therapy-related characteristics among those with HIV infection and receiving therapy, stratified by race to provide context for this HIV-infected population. At study entry, the median CD4+ cell counts were 477 [IQR: 301, 667] for non-blacks and was 415 [IQR: 266, 554] for blacks. About 33% of non-blacks and 38% of blacks had CD4+ cell counts less than 350 at baseline; and 44% of non-blacks and 58% of blacks had a detectable HIV RNA load, despite having initiated HAART. For non-black men and black men, 56% and 43% received any ART prior to HAART initiation, respectively. The time since HAART initiation was 6.2 years for non-

blacks and 7.1 years for blacks. Overall, the characteristics of HIV infection and therapy use were similar by race.

3.4.3 Incidence rates

Tables 3a and 3b presents the age-specific incidence rates and incidence rate ratios associated with HIV infection, by race. It is important to note that men who progressed to low eGFR (at least two occurrences of eGFR less than 90 ml/min|1.73m^2 within 1 year, or one occurrence of eGFR < 70 ml/min|1.73m^2) were censored in these analyses. Among non-black men, the cumulative incidence among HIV-uninfected men was 5.28 per 100 person-years (95%CI: 4.24, 6.58) and among HIV-infected men was 4.83 per 100 person-years (95%CI: 3.79, 6.14). The IRR, comparing the IR among HIV-infected men to HIV-uninfected men, was 0.91 (95%CI: 0.66, 1.27), a non-significant difference but this effect was heavily confounded and modified by age. Indeed, there was substantial variability by age group: hyperfiltration was more common among younger HIV-infected men, while incident hyperfiltration was more frequent among older HIV-uninfected men. The IR in the youngest age group (30 to 35 years) was 13.65 per 100 person years (95%CI: 7.92, 23.5) but was only 4.00 per 100 person years (95%CI: 1.67, 9.62) among HIV-uninfected men; the IRR was 3.41 (95%CI: 1.22, 9.56), a significant difference. This effect diminished with increasing age, when hyperfiltration was more common among HIV-uninfected men at older age groups. In the age group 60 to 65 years, the HIV-uninfected incidence was 22.21 per 100 person years (95%CI: 14.89, 33.13), in contrast to the HIV-infected group in which the IR was 13.13 (95%CI: 5.9, 29.23), although this effect was not statistically significant (IRR: 0.59; 95%CI: 0.24, 1.45).

The cumulative incidence of hyperfiltration among black men was 3.46 per 100 person years among HIV-uninfected men (95%CI: 2.30, 5.21) and 5.68 per 100 person-years among HIV-infected men (95%CI: 4.24, 7.61). The IRR was borderline significant (IRR: 1.64, 95%CI: 0.99, 2.71; p= 0.053), although this estimate does not take into account age. The HIV-uninfected black men had the lowest cumulative incidence out of the four groups, while the other three groups had similar rates. A comparison of the age-based IRs among black men revealed an opposite trend compared to the non-black: at younger ages (i.e., 30 to 45 years), there was no difference in incidence of hyperfiltration by HIV infection status (IRRs between 0.87 and 1.20, all non-significant). However, there was a higher incidence between ages 45 and 60 years for those with HIV infection: the IRR was 2.79 (95%CI: 1.12, 6.95) for the ages between 45 and 50, but was not statistically significant for ages 50 to 55, and 55 to 60 (IRRs were 2.26 and 2.18, respectively). This heterogeneity in IRs by age between non-black and black subjects justified race stratification for the subsequent analyses.

3.4.4 Kaplan-Meier estimates with late entry

Figure 3 presents the non-parametric Kaplan-Meier step functions of the incidence of hyperfiltration accounting for late entry after age 30. The results reflect the inferences derived from the IR and IRR analyses: among non-blacks, HIV-infected men had a higher incidence at younger ages (prior to age 40), but the HIV-uninfected men had the same or higher incidence at older ages (after age 50). There were no differences by infection status for non-blacks (log-rank p= 0.495), but the difference was significant among black subjects (p= 0.045). The age at which 25% of HIV-infected men had hyperfiltration was 32.5 (95%CI: 30.6, 34.1); for HIV-uninfected men, that age was 37.8

(95%CI: 30.8, 44.0). The median age at hyperfiltration among HIV-infected men was 35.2 (95%CI: 31.5, 39.2), but was 49.5 among HIV-uninfected men (95%CI: 37.8, 56.5). These results should be interpreted cautiously since there were several age-years in which no events occurred (about 41 to 48 years for HIV-infected men, and 39 to 43 years for HIV-uninfected men). This stability was likely an artifact of the data and may not be clinically meaningful. Among black subjects, the incidence was similar until about age 47 at which point the incidence of hyperfiltration among HIV-infected subjects increased. The ages at which 25% of black men had hyperfiltration for the HIV-infected and uninfected groups were 41.4 years (95%CI: 30.9, 46.8) and 37.3 years (95%CI: 33.0, 48.6), respectively; the median ages were 48.2 years (95%CI: 0.86, 54.3) and 51.8 year (95%CI: 33.0, 64.2), respectively. These large confidence intervals indicate relatively few events and shorter duration of follow-up time.

3.4.5 Competing risks

In the previous analyses of incidence rates and survival step function, those who had low eGFR were censored. However, low eGFR was also a competing risk event for hyperfiltration. A substantial proportion of men had low eGFR relative to the hyperfiltration event. Among non-black men, 49.1% exited the study with low eGFR (170 HIV-uninfected and 209 HIV-infected men); among black men, 35.2% had this competing risk (52 HIV-uninfected men and 75 HIV-infected men). Since a very high proportion experienced low eGFR, methods were applied to take into account this competing risk in determining the effect of HIV on the incidence of hyperfiltration. The subhazard ratio [29], similar to the hazard ratio presented in a Cox proportional hazard model, describes the subdistribution of hyperfiltration in the presence of competing risks

and semiparametrically estimates the proportional hazard of the effect of HIV on hyperfiltration. However, it is clear from the Kaplan-Meier estimates that the hazards were non-proportional between HIV-infected and HIV-uninfected (i.e., the survival step functions cross at age 55) among non-blacks. Therefore, the analysis included exploring a time and HIV infection interaction, which can be interpreted as how age modifies the effect of HIV infection on hyperfiltration as a linear function. This interaction was significant among non-blacks, but was not significant among blacks.

Figure 4 presents the proportional subhazard ratios of the univariate effect of HIV infection on hyperfiltration, by race. Among non-blacks, there was a strong HIV effect at younger ages, but this effect diminished over time. At age 30, the subhazard ratio of hyperfiltration was 3.69 (95%CI: 1.63, 8.36), yet this subhazard ratio decreased by 7% for each year after age 30 (SHR= 0.93, 95%CI: 0.89, 0.96). The confidence intervals indicate that shortly after age 40, the SHR estimate was no longer significant; by age 47, the SHR point estimate was very close to null (reference line at 1). Among blacks, there was no significant effect of time and, as depicted in Figure 4, the constant SHR showed that black men with HIV infection had a 1.6 times higher subhazard of hyperfiltration compared to HIV-uninfected black men (SHR= 1.60, 95%CI: 0.96, 2.67) and this was borderline significant (p= 0.07).

To investigate the potential role of mediation, a secondary competing risks analysis adjusted for variables associated with HIV infection at baseline (described in Table 1) and potentially on the causal pathway between HIV infection and hyperfiltration. Specifically, these variables included obesity, dyslipidemia, uncontrolled hypertension and use of antihypertension medications, and were included as covariates in

the regression model. There were no inferential differences between the unadjusted and adjusted models: the effect modification related to age among non-blacks remained significant and was of similar magnitude (SHR at age 30= 3.56, 95%CI: 1.51, 8.28; SHR of HIV infection with a 1-year increase in age= 0.93, 95%CI: 0.89, 0.96); and the borderline significant subhazard ratio that did not vary by age among black men was essentially the same (SHR= 1.51 95%CI: 0.89, 2.58).

3.4.6 Sensitivity analyses results

To investigate how different thresholds affect the estimates of incident hyperfiltration, different slopes were used to define elevated eGFR. Specifically, the estimated 90th percentile at age 30 (i.e., the intercept) was rounded to 119 and 130 ml/min|1.73m^2 for non-black and black populations, respectively, and the change per each year after age 30 was varied according to previously published literature and the 95% confidence limits based on the bootstrapped quantile regression models from the HIV-uninfected men in the MACS. The expected decline among normal, healthy individuals reported by Poggio et al. was -0.8 ml/min per year, and this did not differ by race [30]. The empirical lower 95% confidence interval was -0.747 ml/min per year for non-black men and was -0.833 ml/min per year for black men. The upper 95% confidence interval was -0.712 ml/min per year for non-black men, and was -0.608 ml/min per year for black men. The lowest threshold for elevated eGFR is based on the published source of renal function decline, while the highest threshold is based on the upper 95% confidence interval. Figure 5 presents the Kaplan-Meier survival curves using the three cutoffs for each race category, stratifying by HIV infection (in the same format as Figure 3). Table 4 summarizes the cumulative incidence rates and the results from the

competing risks analyses for the main analyses and when using the three different thresholds for elevated eGFR cutoffs. For non-black individuals, varying the threshold for elevated eGFR to define hyperfiltration did not alter the inferences derived from the main analyses: HIV infection was associated with increased incidence of hyperfiltration at younger ages, but not at older ages. The lack of events between ages 40 and 45 presented in Figure 3 of the main analysis did not persist in the sensitivity analyses using lower thresholds (Figure 5a and 5b), indicating a higher sensitivity in detecting events in this age range.

Among black men, when the two lower thresholds were used, more hyperfiltration events were captured and the survival functions more closely resembled those of the non-blacks, however, there was no substantial change in the inferences from the competing risks analyses. There was a higher incidence of hyperfiltration among HIV-infected compared to uninfected men, and the difference occurred earlier than that observed in the main analysis, although the effect of HIV infection was non-significant and not as large as among non-blacks. When the upper 95th confidence interval bound was used, the results were essentially identical to the main analysis in which there was no difference in survival functions by infection status until about age 47, at which point men with HIV infection had an accelerated incidence rate. This difference was not statistically significant, as described in the competing risks analyses. Overall, the determination of elevated eGFR for defining a hyperfiltration event had a larger impact on the characteristics of the survival curves and cumulative incidence rates, but varying this definition did not change the overall inferences from the main analyses.

3.5 **Discussion**

The primary purpose of this study was to investigate and describe the incidence of chronic glomerular hyperfiltration in the MACS, and characterize the effect of HIV infection as a putative risk factor for increased incidence. Since hyperfiltration is considered a modifiable risk factor of accelerated GFR decline leading to CKD, its incidence in the HIV-infected population is clinically important, since this population is at high risk for CKD [22]. These analyses suggest that treated HIV infection is associated with increased incidence of hyperfiltration, particularly at younger ages (ages 30-45) among non-black men, in this population of homosexual and bisexual men ranging in age from 30 to 65 years. The cumulative incidence rates were similar regardless of HIV infection status among non-black men, yet there were significant age-based differences. Among black men, there was an increased, but non-significant, risk of incident hyperfiltration in HIV-infected men. The directionality and effect size in this group was similar to that of non-blacks, and the non-significance may have been due to a smaller sample size: the number of black men in this study was less than half that of the non-black men (357 black men compared to 763 non-black men).

The estimated incidence rate among non-black men aged 30 to 35 was about 14 per 100 person years, although this rate declined dramatically with age, largely due to the increased incidence of low eGFR. We considered low eGFR a competing risk event to hyperfiltration: for example, it was unlikely a 40 year old non-black subject with eGFR equal to 67 ml/min|1.73m^2 would be at risk of exceeding the elevated eGFR threshold of 111.7 ml/min|1.73m^2 (a difference of about 45 ml/min|1.73m^2). Since HIV infection and HAART treatment are known risk factors for accelerated eGFR decline [14], the excess

of HIV-infected men relative to uninfected men exiting the study due to low eGFR, particularly at older ages was expected. This was borne out when accounting for low eGFR as a competing risk. The subhazard ratio among HIV-infected men was high at younger ages (at 30 years, the estimated SHR was 3.69) but declined thereafter, and was null by age 47 which was close to the median age at entry of the study population. This decreasing SHR was likely due to men with HIV declining to low GFR and exiting the study and who were no longer at risk for hyperfiltration. The corresponding median years since any antiretroviral therapy initiation and HAART initiation were 9.2 years and 6.2 years, respectively indicating a substantial portion of life with HIV infection and treatment. It is therefore not unexpected that a high proportion of non-black men experienced low eGFR (51% in HIV-infected men compared to 41% among HIV-uninfected non-black men) prior to any observed hyperfiltration in this time period.

The incidence rate was higher at later ages among black men, between 7 and 9 per 100 person years among those between the ages of 45 and 60, than at younger ages (between 3 and 4 per 100 person-years for ages 30 to 45). The incidence of low eGFR was not different between HIV-infected and HIV-uninfected men (33% vs. 31%), in contrast to the non-black sample, indicating that there are likely important clinical differences between non-black and black men in the present analysis. One possible explanation is due to the higher 90^{th} percentile threshold for elevated eGFR among blacks, which potentially allowed more black men with higher eGFR levels to enter the study, relative to the non-black men. Given the documented systematic bias in the CKD-EPI eGFR equation among blacks [27], and the consistently high eGFR levels observed

in Figure 1, there is a strong basis for stratifying by race and presenting race-specific results.

Overall, these results were congruent with the cross-sectional analysis of directly measured GFR in which 25% of HIV-infected men and 17% of HIV-uninfected men had prevalent hyperfiltration [2]. In that analysis, the adjusted prevalence odds ratio was 1.70 (95%CI: 1.11, 2.61). While prevalence odds ratios and subhazard ratios are not directly comparable, both underscore an association between treated HIV infection and hyperfiltration. A major strength of the current study was maximizing the availability of longitudinal data to determine incident hyperfiltration by restricting study entry to those with eGFR levels in the normal range (eGFR between 90 ml/min|1.73m^2 and less than the age- and race-specific 90th percentile).

Another important contribution of this study included describing the 90th percentiles by age, stratified by race, since it has been reported that the CKD-EPI eGFR equation overestimates GFR at high levels among blacks [27]. Quantile regression was used to estimate the 90th percentiles starting at age 30, and the model was bootstrapped to estimate the 95% confidence intervals to account for the within-person repeated measurements. A simple linear model fit the data well and the intercept (at age 30) was about 119 ml/min|1.73m^2 for non-blacks and 130 ml/min|1.73m^2 for blacks, which is consistent with normal-high levels. Additionally, the estimated decline after age 30 for the 90th percentile was -0.726 ml/min per year (95%CI: -0.747, -0.712) and -0.653 ml/min per year (95%CI: -0.833, -0.608) for the non-black and black men, respectively. These results compare favorably with previously published estimates of elevated eGFR levels. In a Japanese population, Okada et al. reported that the 95th percentile of serum

creatinine-based eGFR for men aged 20 to 29 was 117 ml/min|1.73m^2 (n= 2189) and for men aged 30 to 39, the 95th percentile was 108 ml/min|1.73m^2 (n= 3866) [9]. The authors used the 95th percentiles within 10-year age bins to define elevated eGFR, in contrast to our study which used a parametric model to allow the threshold to be dependent on age as a continuous variable. In another methodologically strong study, Poggio et al. [30] presented an age-related decline among healthy individuals as -0.8 ml/min|1.73m^2 per year, which is slightly less than the KDOQI general guideline describing a loss of -1 ml/min per year [31]. The strength of the Poggio et al. [30] study was the use of directly measured GFR and a population of confirmed healthy individuals. The estimates of GFR decline by age at the 90th percentile using quantile regression were very close to the -0.8 ml/min per year; indeed, among non-blacks, the 95% confidence interval contained this value. Furthermore, Lindeman et al. [32] presented the mean creatinine clearance decline among healthy men as 0.75 ml/min per year, which is also very close to our estimates. A summary of age-related GFR decline in the most recent KDIGO guidelines [33] present 6 studies of healthy men, with estimates ranging from -1.2 ml/min per year to -0.36 ml/min per year (with only one study estimating a steeper decline of less than -1 ml/min per year).

In the sensitivity analyses, we explored how different thresholds to define elevated eGFR (and subsequently hyperfiltration) affected the incidence characteristics and risks associated with HIV infection. The different thresholds were based on published research on expected decline among healthy individuals [30], and the bounds of the 95% confidence intervals of the slope, based on bootstrapped quantile regression. It should be noted that the differences in slope were clinically insignificant: for example,

among non-blacks the slopes used were -0.726 (main), -0.8 (published [30]), -0.747 (lower 95% confidence interval bound) and -0.712 (upper 95% confidence interval bound). However, these small differences had important differences in defining hyperfiltration: for HIV-uninfected non-blacks, the cumulative incidence rates ranged from 4.48 to 9.67 per 100 person-years; for HIV-infected non-blacks, these rates ranged from 3.8 to 8.2 per 100 person-years. This heterogeneity was reflected in the Kaplan-Meier estimates in which lower thresholds for elevated eGFR were related to smoother step-functions. Nonetheless, in the competing risks analyses, the effect sizes were comparable and the inferences remained unchanged, indicating robustness in the estimates of risk associated with HIV. These analyses highlight a need to improve eGFR estimation at high levels, particularly to understand clinically meaningful differences at elevated renal function as well as the development of an acceptable and valid clinical definition of hyperfiltration using estimating equations is needed.

There were several strengths to this analysis. The use of serum creatinine-based eGFR as the primary measurement of renal function to defined hyperfiltration provides a clinically meaningful context to these findings since this tool is widely used and easily calculated in a clinical setting. Furthermore, the consistency with the previous findings of the association between treated HIV infection and hyperfiltration which used directly measured iohexol GFR (which is less common in clinical use), is encouraging. This study also used three different methods to describe the epidemiology of incident hyperfiltration, including basic age-adjusted incidence rates, non-parametric Kaplan-Meier estimates incorporating late entries and a competing risk analysis, all of which provided similar inferences. A methodologic strength to this study was using age as the time scale and

accounting for late entries. This method accommodates the survivorship for subjects that are observed after age 30 (i.e., for an individual entering after age 30, they were not at risk for hyperfiltration prior to entry into the study). This survivorship induces a deficit of fast progressors (i.e., men who developed hyperfiltration prior to observation), who were not observed. This study design assumes the subjects observed were free of hyperfiltration prior to study entry, and therefore all interpretations are based on conditional survivorship at age 30 years. Another methodologic strength was the use of competing risk proportional hazards analysis, which described an age-dependent effect of HIV infection on the risk of hyperfiltration among non-blacks. Indeed, in the original paper describing this method [29], the authors specifically commented on including time and covariate interactions, which was directly applicable in our dataset, quantifying an important aspect of the association between incident hyperfiltration and HIV infection. When excluding the time by HIV infection interaction among non-blacks, the SHR was non-significant and in the opposite direction (SHR: 0.81; 95%CI: 0.57, 1.13), underscoring the perils of assuming proportional hazards in the presence of non-proportionality by time in a competing risks setting.

There were several limitations in this analysis. Firstly, although eGFR is widely used clinically, it is not a direct measure of renal function, such as plasma disappearance of iohexol, a gold standard. It is known that eGFR does not perform as well at high levels of renal function (compared to low levels) since the equations were developed in mainly CKD populations. However, despite this, the CKD-EPI equation has been shown to perform better at high levels than alternative equations [27]. Another limitation was the high incidence of low GFR, particularly among HIV-infected individuals. It is unclear

how an overwhelming presence of this competing risk event influenced the estimates of risk; however, the inferences are consistent with the incidence rates and Kaplan-Meier step functions. These findings are only applicable to men, and not women, and in this age range, in particular. Studies have shown differences by sex in terms of normal GFR levels [25,33], as well as high levels [9]. Another major limitation was a lack of proteinuria or albuminuria data, which are indicators of kidney damage and strong predictors of future CKD [33]. GFR is still the most important predictor of CKD staging and is widely used clinically [34], however, our ability to correctly classify men as being completely free of CKD was limited without albuminuria data. Lastly, the assumption of being hyperfiltration free prior to study entry may be too strong. It has been reported that GFR can fluctuate widely within individuals, with periods of increasing, stable and declining renal function [35]. In our analyses, subjects entered the study at the first available eGFR observation after age 30 that was at least 90 ml/min|1.73m^2 and less than the age- and race-specific 90th percentile; data regarding previous hyperfiltration or low eGFR were not available.

In conclusion, based on data from healthy, HIV-uninfected men, age- and race-specific 90th percentile levels were estimated using simple equations. These equations provided a threshold to define elevated eGFR among both HIV-uninfected men and HIV-infected men, who were receiving HAART. This analysis presented data demonstrating an increased risk of incident chronic hyperfiltration associated with HAART-treated HIV infection, using the CKD-EPI eGFR equation. Since hyperfiltration is a putative accelerator of GFR decline and a precursor to CKD, which are more common among those with HIV infection, patients presenting with chronically high eGFR along with

indicators of metabolic and cardiovascular derangements, should receive clinical consideration for dietary modifications and/or ACE inhibitor or ARB use to lower GFR levels.

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4 Estimated GFR decline after chronic hyperfiltration among men with and without HIV in the Multicenter AIDS Cohort Study

4.1 Abstract

Objective Glomerular hyperfiltration by direct measures of glomerular filtration rate (GFR) is more common among men infected with HIV, likely due to metabolic and cardiovascular effects of infection and antiretroviral therapy. Hyperfiltration is also a putative risk factor of accelerated GFR decline, yet its effect in the context of treated HIV infection is unknown. The purpose of this study was to describe 5 year trajectories of eGFR after the occurrence of hyperfiltration.

Design Prospective longitudinal cohort study of creatinine-based CKD-EPI eGFR with up to 5 years of follow-up after hyperfiltration event in the Multicenter AIDS Cohort Study comprising men over 30 years of age. Control men (i.e., no history of CKD or hyperfiltration) were matched to the men with hyperfiltration (ratio of 4:1) by age within 0.5 years, race, HIV infection status and hypertensive status. Data were restricted to HIV-uninfected men and HIV-infected men who had initiated highly active antiretroviral therapy (HAART).

Methods Quantile regression of data comprising multiple observations from 1373 HIV-uninfected men was used to determine age- and race-specific 90^{th} percentile thresholds to define elevated eGFR. Hyperfiltration was defined as at least two occurrences of elevated eGFR within 1 year. Longitudinal data up to 5 years after index visit (hyperfiltration or match) using a previously validated general method to identify downward inflection points (IP). This method used a segmented regression model with eGFR as the outcome

and time as the independent variable to describe individual eGFR trajectories before and after an IP. In cases in which an IP was not identified, a slope-intercept model was used. The distributions of eGFR decline after the IP or from the slope-intercept model were compared by filtration status (normofiltration vs. hyperfiltration), stratified by HIV infection status.

Results The age- and race-specific 90th percentiles were described by the equations 119.0 – 0.726 × years after age 30 for non-black men and 130.4 – 0.653 × years after age 30 for black men. A total of 90 HIV-infected and 87 HIV-uninfected men were classified as having hyperfiltration with at least 6 subsequent eGFR observations to describe trajectories; 321 and 337 normofiltering controls were matched to these groups, respectively. Among HIV-infected men, those with hyperfiltration were more likely to have started any antiretroviral therapy (ART), and HAART earlier. About 68% of those with hyperfiltration had a downward IP compared to only 35% of the normofiltering controls ($p < 0.001$). The median eGFR change per year among HIV-infected men was -2.3 and -3.8 ml/min|1.73m^2 per year for hyperfilterers and normofilterers, respectively; and for HIV-uninfected men the median change was -1.8 and -2.0 ml/min|1.73m^2 per year, respectively. There were no differences by filtration status in adjusted analyses. While there was no significant effect of hyperfiltration on the rate of eGFR decline, those with treated HIV infection were more likely to have a faster decline than HIV-uninfected men.

Conclusions Hyperfiltration was not associated with accelerated GFR decline in the subsequent five years in this population, although this may be due to measurement error issues related to eGFR data. Identifying inflection points may be a meaningful way to

model longitudinal GFR data in epidemiologic studies of hyperfiltration. This study replicates the effect of accelerated GFR decline associated with treated HIV infection among men with prevalent normal and high eGFR, but hyperfiltration does not appear to be a significant contributor to this effect.

4.2 **Introduction**

Elevated glomerular filtration rate (GFR) is associated with metabolic and cardiovascular derangements [1–4], such as diabetes, high fasting blood glucose and hypertension. In a recent cross-sectional study using directly measured iohexol-based GFR, HIV-infected men had a higher prevalence of elevated iGFR than HIV-uninfected men [5]. The increased prevalence of elevated GFR was associated with increased exposure to antiretroviral therapy (ART), notably zidovudine (ZDV), a thymidine analog. Elevated GFR has been associated with accelerated renal function decline over four years using a directly measured GFR among Type 2 diabetics [1,6]. A model for hyperfiltration posits that after a period of increasing GFR leading to a phase of chronic hyperfiltration, there is a rapid decline in GFR [2]. However, the duration of chronic hyperfiltration prior to declining GFR is not well defined or understood. Additionally, it is unclear how hyperfiltration is related to GFR decline among patients with HIV infection, who are also at higher risk of chronic kidney disease (CKD) [7].

Published research is limited in describing the effect of hyperfiltration on renal function decline using the CKD-EPI estimated GFR (eGFR) equation, a widely used clinical and epidemiologic tool [8]. One reason for this deficiency may be the demonstrated lack of precision of eGFR at high levels of renal function [9] and an absence of clinical criteria for elevated eGFR. As an example of this lack of precision, Melsom [10] reported an association of impaired fasting blood glucose with increased measured GFR, but not estimated GFR. Nonetheless, physicians must use eGFR for assessments and decision making, in the absence of directly measured GFR, for routine clinical care.

An additional challenge in identifying elevated eGFR and accelerated GFR decline is the presence of age-related decline, which is considered a normal process [11,12]. The physiologic age-related GFR decline has been identified as early as 1950 [13] and has two implications for identifying elevated GFR and describing GFR decline. First, a threshold for classifying elevated GFR must respect that normal GFR declines over time. This has been accomplished by a theory-based method that sets a threshold and subtracts 1 ml/min per year after age 40 [14] to account for an age-related decline. An alternative method empirically derived the threshold as a percentile based on age- and sex-specific distributions of a normal population [3]. Both approaches account for the known age-related decline in eGFR. The second implication is that normal age-related decline must be considered when making inferences about accelerated GFR decline, for which hyperfiltration is hypothesized to be a risk factor. Given that GFR is expected to decline with age, even among normal, healthy individuals, it is necessary to present accelerated GFR decline in this context. Specifically, to investigate the effect of hyperfiltration, it is important to compare those with the condition to those with normofiltration of similar ages.

The purposes of this study were to a) describe a definition of elevated eGFR and chronic hyperfiltration using empirically-based percentile thresholds among HIV-uninfected men; b) describe the trajectories of eGFR after chronic hyperfiltration using a matched study design; and, c) determine the effect of hyperfiltration on eGFR decline in HIV-infected men using data from the Multicenter AIDS Cohort Study (MACS). The ideal comparison group to determine the effect of hyperfiltration as a putative accelerator

of GFR decline comprise men with the same age-distribution and HIV infection status, and free of chronic kidney disease (CKD).

4.3 **Methods**

4.3.1 Study population

The MACS is a longitudinal epidemiologic study of the natural and treated history of HIV infection. The recruitment and follow-up study design has been previously described [15]. There were a total of 7087 men recruited at the time of analysis (data collected through September 30, 2013), and these data were restricted to subjects contributing serum creatinine data in the era of highly active antiretroviral therapy (HAART), as defined by time since June 15, 1996 (n= 2628), and, if infected with HIV, receiving HAART . Semi-annual study visits included structured interviews, physical examinations and collections of biological specimens. Serum creatinine measurements from a local site laboratory (Quest Laboratories) were obtained as part of standard renal work-up beginning in 2006; retrospective measurement of stored serum samples were obtained for all treated HIV-infected subjects prior to this time point who had available serum before and after the initiation of antiretroviral therapy. Of the 2628 men, 12 contributed as both HIV-uninfected and HIV-infected subjects as they acquired infection while under study observation. In total, there were 1373 HIV-uninfected subjects and 1255 HIV-infected subjects. Baseline was defined as the first visit with measured serum creatinine in the HAART era.

4.3.2 Defining elevated eGFR and chronic hyperfiltration

Estimated GFR was determined from the serum creatinine-based CKD-EPI equation for men [8]. To establish a working definition of hyperfiltration based on longitudinal eGFR data, we characterized the eGFR distributions by age and race among HIV-uninfected participants. The HIV-uninfected participants comprise an appropriate comparison group to the HIV-infected men, since they share many of the same behavioral and lifestyle characteristics, based on study entry criteria. As the nearest counterfactual, the HIV-uninfected men provide a reference for the HIV-infected participants' eGFR levels, had they not been infected.

Defining elevated eGFR as a single threshold is not appropriate since GFR decreases with age [11,12]. Also, an overestimation bias among black people for high levels of GFR using the CKD-EPI equation has been presented [9]. Therefore, defining pathologically high eGFR should take into account age and race. The age- and race-adjusted 90^{th} percentile serve as the threshold to determine a high eGFR level ("elevated eGFR") in an approach modified from Okada et al [3] and Melsom et al [10]. Quantile regression, stratifying by race (non-black and black), was used to determine the 90^{th} percentiles of eGFR for a given age. Model fit was assessed by the empirical median and 90^{th} percentile levels by 5-year age bins. The 50^{th} percentiles were also derived from the same quantile regression model to investigate a potential bias by race at lower (normal) eGFR levels.

Hyperfiltration was defined as the occurrence of at least two observations above this threshold (with elevated eGFR) within 1.1 years. Since chronic hyperfiltration causes pathologic changes to the kidneys, this definition sought to identify those with

consistently elevated eGFR, minimizing misclassification from transiently high eGFR and regression to the mean after a single occurrence. The analytic dataset was restricted to subjects free of CKD (eGFR > 90 ml/min|1.73m^2) and below the age- and race-specific 90th percentile at baseline in order to determine incident hyperfiltration.

4.3.3 Longitudinal assessment of eGFR and matched study design

In order to determine the effect of hyperfiltration on eGFR decline, we used a matched study design in which control, or comparison, subjects were matched to each case of chronic hyperfiltration. The controls comprised men with no history of observed hyperfiltration or CKD (since CKD is a predictor of accelerated GFR decline).

Cases were defined at the age of the first occurrence of elevated eGFR. Potential controls were matched to each case by the following criteria: within 1 year of age (i.e., +/- 0.5 years), same race, HIV status and recruitment wave (pre- or post-2001 recruitment; the later recruitment targeted minorities and men of lower socioeconomic status). After a first round of matches, there was an excess of uncontrolled hypertension among controls relative to cases. Since hypertension among cases is a putative confounder (related to unexposed and the outcome of GFR decline), we additionally matched on this variable. Subjects could serve as controls for multiple cases at different ages, but not multiple times for the same case. Additionally, controls matched multiple times at the same age were excluded such that there were no identical repeated controls. Cases did not serve as controls prior to their hyperfiltration event occurring since trajectories of controls should not include potential hyperfiltration. Up to 4 controls for each case were randomly selected, or all controls were selected when there were less than 4 controls available.

Descriptive statistics by demographic and clinical characteristics of cases and controls are presented at the time of hyperfiltration or match, hereafter referred to as the index visits, as well as up to 5 years prior to and after the index date for select variables. Clinical characteristics were based on four domains: metabolic, cardiovascular and behavioral domains for all subjects and HIV health indicators for HIV-infected men. Metabolic variables included body mass index (kg/m^2), treated as continuous and categorized (obese), high density lipoprotein (HDL) and low density lipoprotein (LDL), dyslipidemia (defined as fasting total cholesterol ≥ 200 mg/dl, LDL ≥ 130 mg/dl, HDL < 40 mg/dl, triglycerides ≥ 150 mg/dl, or use of lipid lowering medications with self-reported/clinical diagnosis of dyslipidemia), fasting glucose, treated as continuous and categorized (impaired is equal to fasting glucose > 100 mg/dL), and a diagnosis of diabetes (defined as fasting glucose > 126 mg/dl, or diagnosis of diabetes with use of medications). Cardiovascular variables included systolic blood pressure (SBP), diastolic BP (DBP), uncontrolled hypertension (SBP > 140 mmHg or DBP > 90 mmHg). Behavioral variables included current and ever cigarette use and current use of stimulants (cocaine, amphetamine or methamphetamine in the past year). Indicators of HIV management and disease severity included current and nadir CD4+ cell count (per μl), as continuous and categorized (< 350 per μl), detectable viral load, history of an AIDS-defining illness, initiated ART, years since ART initiation, initiated HAART, and years since HAART initiation, including parameters for medication adherence.

For the 5 years preceding and after the index date, the mean of continuous variables for each time period was calculated for each individual. The medians and interquartile ranges of these subject-specific means are presented. The presence of any

detectable viral load in these two periods summarized this variable among HIV-infected men.

Conditional logistic regression accounting for matched risk sets was used to determine univariate differences at baseline, and prior to and after the index visit, as presented in Table 1. The dependent variable was hyperfiltration status and risk sets were based on the ratio of hyperfilterers to controls (1:*m*, where *m* is between 1 and 4). Similar results were obtained when using Fisher's exact test and Wilcoxon rank sum test to detect differences without accounting for risk sets (results not shown). Statistical significance was assessed at the $p< 0.05$ level.

4.3.4 Characterizing eGFR decline by downward inflection points

Since an accelerated decline is expected after a period of chronic hyperfiltration, occurring at an inflection point (IP) [2], we sought a more flexible model than individual linear trajectories or a random effects mixed model which estimates a mean overall change. A regression model with polynomial terms was not used since it is unclear whether all people with hyperfiltration have an accelerated decline that can be adequately summarized in a three- or four-term model. In an approach previously presented for describing changes in T-cell counts among HIV-infected individuals [16], an IP was identified for each subject to characterize eGFR changes before and after this time point. In this analysis, for subjects with hyperfiltration, we included data from the year preceding the second occurrence of elevated eGFR (to denote a period of increasing or stable elevated eGFR levels) and data for up to 5 years following this second elevated eGFR. Study entry for this analysis among normofiltration subjects was the first preceding eGFR within one year of the index visit. In order to appropriately characterize

eGFR trajectories before and after the IP with several data points, this analysis was restricted to subjects with a minimum of 6 eGFR measurements (at least 3 prior to and including the second elevated eGFR and at least 3 after the second elevated eGFR).

Using the same notation presented by Gange et al. [16], identification of the IP was defined by the following equation:

$$(1) \qquad eGFR_{ij} = B_{i0}^{(k)} + B_{i1}^{(k)}(t_{ij} - t_i^{(k)})^- + B_{i2}^{(k)}(t_{ij} - t_i^{(k)})^+ + e_{ij}^{(k)}$$

where $eGFR_{ij}$ is the jth eGFR measurement for the ith subject, at time t_{ij}. $t_i^{(k)}$ is the time of the kth eligible time, that is, the midpoint between t_{ij} and t_{ij+1}, in order to identify the IP. The kth eligible time was further restricted to having at least 3 data points prior to and 3 data points after the identified IP. The model parameters may be interpreted as follows: the estimated eGFR level at the IP is B_{i0}, B_{i1} is the slope prior to the IP, and B_{i2} is the slope after the IP. Models were fit for each kth eligible time for each subject (i.e., three parameters were obtained for each kth time). The model which minimized the residual variance of the data (i.e., the sum of the $[e_{ij}^{(k)}]^2$) was selected as the final IP model for the subject. Figure 1a presents an example of this approach, displaying eligible IP times, the final selected IP, and the corresponding parameters from the model described above.

Since the conceptual model of hyperfiltration conforms to a downward inflection point (i.e., $B_{i1}^{(k)} > B_{i2}^{(k)}$ indicating an accelerated decline), for subjects in which $B_{i1}^{(k)} \leq B_{i2}^{(k)}$, a slope-intercept linear model was fit to the data of the form:

$$(2) \qquad eGFR_{ij} = \alpha_{i0} + \alpha_{i1} \times t_{ij} + e_{ij}$$

where α_{i0} is the estimated eGFR level for the *i*th individual at time ($t_{ij} = 0$; i.e., the first observation) and α_{i1} is the slope for the $_i$th individual over the observed study time in ml/min per year (i.e., the change in eGFR per year). The error term e_{ij} is assumed to be normally distributed with mean equal to 0 and variance σ as in a standard linear regression.

Figure 1b presents the case in which there is no downward IP as indicated by the discontinuous line (i.e., $B_{i1}^{(k)} \leq B_{i2}^{(k)}$) and the two parameter model presented in equation (2) is fit to the data as the final model for that individual (solid line).

The distributions of $B_{i2}^{(k)}$ among subjects with an identified IP, and α_{i1} among subjects without an identified IP were compared by filtration and infection status by box-percentile plots [17] and non-parametrically compared using the Wilcoxon rank sum test.

In adjusted analyses, separate linear regression models by HIV infection status were conducted with the best slope for each subject as the dependent variable, defined as either $B_{i2}^{(k)}$ (subjects with an identified IP) or α_{i1} (subjects without an identified IP). Covariates included for adjustment were glucose, SBP and DBP (in the log scale) since these have been consistently reported in the literature as risk factors for hyperfiltration. All continuous variables were centered at the population medians. For HIV-infected men, time since ART initiation was also included as a covariate. The estimated adjusted mean GFR change per year was reported for a reference subject within each group of interest (normofiltration and hyperfiltration, by HIV infection status) whose covariates corresponded to the group mean. Analyses were conducted with incremental additions of covariates to present how estimates change with their inclusions in the model. Among the control groups, there were instances in which multiple slopes were contributed by the

same subject, but at different ages. To account for the potential correlation of slopes among repeated subjects and obtain valid standard error estimates, generalized estimating equations (GEE) were used.

As a sensitivity analysis, data from the 4:1 matching design were restricted to 1:1 matching with unique subjects to create equally balanced number of hyperfilterers and matched normofilterers. Hyperfilterers without matched normofilterers were excluded from the analyses. The distribution of slopes were described and adjusted analyses used GEE to account for potential correlation within each matched pair.

Matching, identification of inflection points and graphics were performed in RStudio (0.96.316). All other analyses were performed in SAS 9.2 (SAS Institute, Cary, North Carolina, USA).

4.4 **Results**

4.4.1 Estimation of 50th and 90th percentile level of eGFR based on HIV-uninfected men, by race

In order to determine a threshold for elevated eGFR, the 90th percentile by age was estimated by quantile regression separately for black and non-black races, since there is a known overestimation bias among blacks at high levels of GFR, using all data from HIV-uninfected men older than 30 years. A total of 3482 observations were contributed by 290 black men and 15457 observations from 1083 non-black men. We also investigated whether lower levels of eGFR differed by race, by estimating the median eGFR levels in the same stratified approach. The estimated median and 90th percentiles of eGFR as a function of age among HIV-uninfected black and non-black men from these regression models are depicted as lines in Figure 2 (solid lines for non-black and

discontinuous lines for black sub-groups). The points represent these percentiles from 5 year age bins from age 30 and indicate appropriate fit of the model to the data (solid dots for non-black and open dots for black sub-groups). The median eGFR levels by age were very similar between races, but the 90th percentiles were systematically and consistently higher among black men. Indeed, the estimate of the 90th percentiles for black men was 130.4 – 0.65 × years after age 30; for non-black men, the equation was 119 – 0.73 × years after age 30. The GFR decline associated with age was about the same by race. Previous literature has documented an overestimation bias of eGFR among black subjects relative to measured GFR that is not present among non-black subjects [9], which likely accounts for this difference. Indeed, at median (i.e., normal) levels the difference by race was not nearly as extreme. Given the previous report of eGFR overestimation among black people, and the large difference at high levels of eGFR by race in our study population (despite sharing similar health and risk profiles and very similar median levels), there was justification to use race-specific age-based threshold to identify elevated eGFR and hyperfiltration.

4.4.2 Identification of subjects with hyperfiltration and matching for comparison group with normofiltration

Of the 574 HIV-infected men with available eGFR data and who did not have low or elevated eGFR at study entry, a total of 111 were identified with incident hyperfiltration, based on eGFR exceeding age- and race-specific thresholds at least twice in a calendar year. Among HIV-uninfected men (n= 546) with the same prevalent eGFR restrictions, a total of 102 were identified with chronic hyperfiltration. Subjects were excluded if they had less than 6 observations from the study time spanning 1 year prior to

the second elevated eGFR and 5 years after the second elevated eGFR. For this analysis investigating the effect of chronic hyperfiltration on eGFR decline, a total of 90 (81.1%) HIV-infected men and 87 (85.3%) HIV-uninfected men were included who met the criteria for hyperfiltration and with sufficient data to characterize GFR decline.

To provide an appropriate comparison group for the sets of HIV-uninfected and HIV-infected men with hyperfiltration, we selected controls who were defined as men free of CKD (i.e., eGFR > 90 ml/min|1.73m^2) and elevated eGFR (i.e., eGFR < 90th age- and race-specific percentile) in a matching approach. For each subject with hyperfiltration, we randomly selected controls who were within 1 year of age at the time of the second occurrence of elevated eGFR, of the same race (black or non-black), recruitment wave (pre- or post-2001 cohort), hypertensive status (uncontrolled or controlled at time of hyperfiltration or match), and HIV infection status. Subjects with hyperfiltration were eligible for selection prior to the event of hyperfiltration occurring and a maximum of 4 control men were selected per each case. A total of 230 HIV-uninfected men were selected as controls for the 87 HIV-uninfected men with hyperfiltration, and 274 HIV-infected men were matched as controls for the 90 HIV-infected men with hyperfiltration.

4.4.3 Characteristics of men with hyperfiltration and matched controls

Table 1 presents the characteristics of normofiltration controls and hyperfiltration cases. Per the matching protocol, the distributions of age, race, cohort enrollment and uncontrolled hypertension status were the same between normofilterers and hyperfilterers within HIV infection status. HIV-infected men were younger than HIV-uninfected men, more likely to be black and recruited after 2001. The median eGFR at the time of second

elevated eGFR was 107.6 for HIV-uninfected men and 116.7 ml/min|1.73m^2 for HIV-infected men; for the matched normofiltration controls, the median levels were 95.0 and 98.0 ml/min|1.73m^2, respectively. Regardless of HIV infection status, those with hyperfiltration had a longer observed time prior to hyperfiltration and slightly shorter observed time after the hyperfiltration event. HIV-uninfected subjects with normofiltration had a shorter time prior to matching since prospective measurement of serum creatinine was initiated in 2005. In contrast, HIV-infected subjects who had started HAART had serum creatinine measured retrospectively from available repository-stored blood samples and therefore contributed more data.

Among the HIV-uninfected group, the hyperfilterers were similar to the normofilterers based on characteristics at the time of identified hyperfiltration, with the exception of stimulant use. Stimulant use was higher among the normofilterers compared to the hyperfilterers (18% vs. 7%; p= 0.037). For the observed time prior to index visit, the hyperfilterers had a higher subject-specific mean eGFR (median mean level was 102.7 vs 96.3 ml/min|1.73m^2; p< 0.001), but similar mean fasting glucose and mean DBP levels. After the index visit in this group of HIV-uninfected men, the hyperfiltering group still had a higher 5-year mean eGFR (102.2 vs 97.1 ml/min|1.73m^2; p< 0.001) and a slightly shorter follow-up time (median years were 4.5 vs. 4.6; p< 0.001). There was no statistical difference between hyperfilterers and normofilterers for fasting glucose, SBP or DBP in the years after the index visit.

Among the HIV-infected group, at the time of index visit, men with hyperfiltration were more likely to have lower DBP (medians= 74 mmHg vs. 78 mmHg, p= 0.004). Additionally, they were slightly less likely to have used stimulants in the

preceding year compared to the matched normofiltering controls (18% vs. 27%, p= 0.208), but this difference was not significant. Both prior to and after the hyperfiltration event, GFR was higher and SBP and DBP lower among the hyperfilterers compared to the normofilterers. Similar differences in follow-up time prior to and after the event that were observed among HIV-uninfected men were also observed among the HIV-infected men.

Previously identified comorbidities and clinical indicators associated with hyperfiltration, such as diabetes, high fasting glucose and elevated BP [5], were not associated in this selected population based on a matched study design.

Table 2 presents the clinical characteristics of HIV-infected men and statistical differences (i.e., p-values) based on conditional logistic regression, by hyperfiltration status. At the time of index visit, the proportion of men with CD4+ cell count < 350 was higher among hyperfilterers compared to normofilterers (30.2% vs. 21.4%, respectively; p= 0.028). However, detectable viral load was similar by hyperfiltration status (37.9% vs. 33.3%; p= 0.376), as was having a previous AIDS diagnosis (10.0% vs. 11.3%, p= 0.776). Hyperfilterers had a longer time since ART initiation (median years= 9.4 vs. 7.1; p= 0.014), as well as longer time since HAART initiation (median years= 6.5 vs. 5.6; p= 0.011). HIV disease severity, as measured within 5 years preceding the index visit, was similar between the two groups: the proportion with mean CD4+ cell count < 350 was 22.2% for hyperfilterers and 21.7% for normofilterers (p= 0.456); the proportion with any detectable viral load occurrences was 61.1% for hyperfilterers and 55.2% for normofilterers (p= 0.056). Within 5 years of follow-up after the index visit, 21.8% of hyperfilterers had a mean CD4+ cell count < 350, while 17.1% of normofilterers did (p=

0.130). There was also no difference in having any detectable viral load in this time frame between hyperfilterers and normofilterers (47.1% vs. 52.8%, respectively; p= 0.518). There were no differences by mean CD4+ cell count or having a first AIDS diagnosis in this time frame after hyperfiltration.

4.4.4 Effect of hyperfiltration on longitudinal GFR decline

From the 681 total subjects (hyperfilterers and matched normofilterers controls), individual changes were described by identifying an IP or using a slope-intercept model. For hyperfilterers, 71.3% of HIV-uninfected men (n= 62 of 87) and 64.4% HIV-infected men (n= 58 of 90) had an identified downward IP. In contrast, among normofilterers, 33.5% of HIV-uninfected men (n= 77 of 230) and 42.0% of HIV-infected men (n= 115 of 274) had a downward IP. Regardless of HIV infection status, hyperfilterers were much more likely to have a downward IP compared to normofilterers (67.8% compared to 38.1%; χ^2 p< 0.001). Among hyperfilterers with a downward inflection point, the median time of inflection point (starting from 1 year prior to second elevated eGFR) was 1.33 years [IQR: 1.21, 1.81 years] for HIV-uninfected men, and 1.56 years [IQR: 1.26, 2.52 years] for HIV-infected men.

Figure 3 presents the distributions of slopes of the downward IPs or, in the absence of a downward IP, overall changes in GFR. The median GFR change among HIV-uninfected normofilterers was -2.0 ml/min|1.73m^2 per year and was -1.7 ml/min|1.73m^2 per year for hyperfilterers, and this difference was not significant (p = 0.901). For HIV-infected subjects, the median change among normofilterers was -3.8 ml/min|1.73m^2 per year, and was -2.3 ml/min|1.73m^2 for hyperfilterers, and this was borderline significant (p= 0.054). When pooling HIV-infected subjects (i.e.,

normofiltration and hyperfiltration groups combined), the HIV-infected subjects had a faster decline than the pooled uninfected subjects ($p< 0.001$).

To control for potential confounding, previously published literature indicate that fasting glucose, SBP and DBP are particularly important risk factors for hyperfiltration [2,4,10] and the levels in the 5 years preceding the index visit were included in adjusted analyses. Table 3 presents the unadjusted and adjusted mean GFR changes based on the IP analysis using linear regression models. The unadjusted analyses present means that are lower than the median levels described in Figure 3 due to the left-skewing of the distributions, but overall the inferences remain consistent. In all adjusted analyses, there was no significant difference in decline between normofilterers and hyperfilterers, regardless of HIV infection status. The estimated GFR decline was faster for those with HIV infection regardless of filtration status, compared to HIV-uninfected men. In adjusted analyses for mean glucose, SBP and DBP prior to index visit, the estimated GFR decline for a reference subject was -2.82 ml/min per year (95%CI: -3.72, -1.91) among HIV-uninfected normofilterers and was -2.16 ml/min per year (95%CI: -3.59, -0.74) for HIV-uninfected hyperfilterers ($p= 0.396$); in contrast, among HIV-infected men, the estimated decline for the same reference individual was -6.07 ml/min per year (95%CI: -7.46, -4.69) for men with normofiltration and -4.69 ml/min per year (95%CI: -6.52, -3.29) for those with hyperfiltration ($p= 0.215$). When adjusting for ART initiation in addition to glucose and BP variables, the inferences remained unchanged: among normofilterers, the mean decline was -5.89 ml/min per year (95%CI: -7.13, -4.63); for hyperfilterers, the mean decline was -4.60 ml/min per year (95%CI: -6.19, -3.01).

4.4.5 Sensitivity analysis restricting to 1:1 matching

As a sensitivity analysis, data from the results described above were restricted to men with normofiltration individually paired to those with hyperfiltration. Those with hyperfiltration and no matched controls (i.e., non-repeated matched normofilterers) were excluded. There were 61 pairs (hyperfilterers and normofilterers) of HIV-uninfected men and 59 pairs of HIV-infected men. Figure 4.4 presents the distributions of slopes which quantitatively were very similar to the main analyses presented in Figure 4.3, and identical inferentially: there were no differences between normofilterers and hyperfilterers, by HIV infection status. The proportions of hyperfilterers with an identified IP were 72% (44/61) and 61% (36/59) for HIV-uninfected and infected men, respectively. This was much higher than those with normofiltration: 31% (19/61) and 35% (21/59) had an identified IP among HIV-uninfected and infected men, respectively. Table 4.4 presents the adjusted estimated means and confidence intervals accounting for the possible correlation within pairs using GEE. These results were very close to the estimated means using the 4:1 matching presented in Table 4.3 and indicate robustness of these results using different matching methods.

4.5 **Discussion**

This study describes the eGFR decline after a period of chronic hyperfiltration among men with and without HIV compared to matched men with similar characteristics who were free of hyperfiltration and markedly low eGFR. Men with hyperfiltration did not have an accelerated eGFR decline compared to their matched controls. The proportion with a downward inflection point which indicates persistently high eGFR, followed by a relatively faster decline, was much more common among those with

hyperfiltration (68% vs. 38%). For hyperfilterers with an IP, an accelerated decline was observed after about 1.3 to 1.6 years of follow-up, shortly after the second occurrence of elevated eGFR. Nonetheless, in terms of identifying an individual's fastest rate of decline using a reasonable amount of data (at least 3 eGFR measurements), there was no difference by filtration status. These results persisted in the sensitivity analyses restricted to single matches of unique normofilterers paired with each hyperfiltration case (i.e., 1:1 matching).

There was, however, a significantly faster eGFR decline among HIV-infected men receiving treatment compared to HIV-uninfected men in the MACS. Despite the HIV-uninfected men being older than the HIV-infected men in this cohort (median age difference was about 4 years), the HIV-infected men still had a faster eGFR decline. This effect of HIV is consistent with published findings of persons with HIV infection. Choi et al. [18] reported an adjusted mean GFR decline of -4.7 to -1.9 ml/min|1.73m^2 for the periods prior to and after ART use, respectively. A large portion of all men in this study population were within this range, yet HIV-infected men had a higher proportion of eGFR decline less than -5 ml/min|1.73m^2 per year compared to HIV-uninfected men (37.9% vs. 19.9%, χ^2 $p<0.001$), which would be classified as fast progressors to CKD according to current practice guidelines [19]. Importantly, this study included men with normal to high-normal levels of eGFR (i.e., free of CKD) at baseline and provides a further characterization of a faster rate of GFR decline among HIV-infected men.

There were few differences by indicators of HIV severity among the HIV-infected subjects. Notably, hyperfilterers had a longer time since ART initiation (an indicator of pre-HAART therapy initiation) at the time of their hyperfiltration event (approximately

9.5 years compared to 7 years among HIV-infected normofilterers). This is consistent with our previous findings using directly measured iohexol GFR: those with elevated GFR had a longer cumulative time since ART initiation, and more specifically longer AZT use, a first-generation thymidine analog antiretroviral medication [5].

Indicators of HIV severity within 5 years prior to the index visit did not differ by filtration status. Curiously, the proportion with low CD4 at the index visit was higher among hyperfilterers compared to normofilterers (30% vs. 21%, p= 0.028), but this did not correspond to a substantially higher proportion with detectable viral load (38% vs. 33%, p= 0.376). It should be noted that these differences were minimal but a similar, and also non-significant, pattern was observed in our cross-sectional study using directly measured GFR [5]: those with elevated eGFR had a 48% higher odds of having CD4+ cell count < 350 (OR: 1.48; 95%CI: 0.79, 2.78), but a 9% lower odds of having a detectable viral load (OR: 0.91; 95%CI: 0.45, 1.86). A biological explanation of why lower CD4+ might be related elevated GFR in the absence of increased HIV replication is unclear, but it is worth noting the directionality is similar in these two different studies, albeit with non-significant effects. It is also possible that this observed phenomenon is simply a statistical artifact.

This analysis also characterized eGFR decline among relatively healthy (i.e., HIV-uninfected) men. The results indicated a substantial heterogeneity in renal function change among this group with normal ranges at study entry. These data are informative since there is a paucity of data describing normal age-related GFR decline. Delanaye and colleagues [11] have shown a dearth of research investigating GFR decline due to kidney senescence, in what would be considered normal aging and present a range of GFR

decline of 6 to 12 ml/min per 10 years. This is problematic since normal reference ranges for elderly individuals do not take this known phenomenon into account: CKD stages are based on absolute threshold that are not age dependent. For example, the authors argue that populations older than 70 years with a GFR less than 60 ml/min|1.73m^2 could still be considered normal, given other clinical characteristics. This has important implications in the epidemiology of HIV and renal function in which persons infected with HIV are living longer due to effect therapy and disease management: the disease burden of CKD may be overestimated, if the threshold for CKD is not constant across all age ranges. In these analyses, classifications of CKD staging adhered to the KDIGO definitions, in the absence of proteinuria data (i.e., GFR < 90 ml/min|1.73m^2 was classified as CKD Stage II).

This analysis used a previously described generalized method to identify an inflection point [16] in the context of GFR decline. This method was attractive for this particular research question since our conceptual model of hyperfiltration was based on an increasing or stable GFR at an elevated level followed by a rapid decline. We allowed the matched controls (i.e., those with normofiltration) to also follow this model (i.e., identify a possible inflection point) since many events could cause a rapid renal function decline, such as hypertension, diabetes, ART use or a nonspecific acute kidney injury. Such cases were still frequent among normofilterers (33% and 42% for HIV-uninfected and HIV-infected men, respectively) indicating that a non-trivial portion of people without hyperfiltration have GFR trajectories that are well-described by this inflection point method. To our knowledge, applying this method to longitudinal GFR data is novel, yet previous literature has documented the heterogeneity of GFR trajectories within-

individuals [20]. Indeed, Li et al. [20] have noted that individuals may have mixed periods of rapid decline, increasing levels, or even stable trajectories. While there was evidence of this in exploratory data analysis scrutinizing each individual trajectory, our research question was focused on characterizing GFR changes up to 5 years after identifying chronic hyperfiltration. It is possible to extend this method to identify multiple inflection points, but this would require more data over a longer period of time. Exploring new methods, perhaps extending the technique used in this analysis, would be helpful to empirically describe GFR changes over long periods of time that are not simply summarized by a linear decline. The main benefits of using a linear GFR decline are simplicity and utility when few data points are used. However, it is not an ideal tool for long-term GFR trajectories, particularly in the presence of CKD and incident end stage renal disease, in which non-linear patterns predominate [20–22].

There were several limitations to this study. Firstly, measurement of GFR by serum creatinine is an estimate of renal function, and has identified biases and potential limitations at high levels, largely due to the development of the CKD-EPI equation among subjects with lower renal function [8]. Indeed, current guidelines recommend censoring such GFR measurements at > 60 ml/min|1.73m^2. Stevens et al. [9] reported an overestimation bias at high levels of eGFR among blacks. Creating race-specific thresholds for elevated eGFR in this analysis attempted to account for this bias. However, it remains a significant limitation in describing GFR trajectories: as a normal age-related decline occurs, the eGFR may decline to normal-low levels in which the measurement is unbiased. Since the initial starting level (at identified hyperfiltration) had an overestimation bias, the decline may appear steeper among blacks than non-blacks as

their values decline to unbiased levels. This information bias would potentially lead to a higher proportion of black men with hyperfiltration having steeper eGFR decline, but this was not observed. Nonetheless, the CKD-EPI eGFR equation has a lower bias among blacks (and other subgroups) at high renal function (GFR > 90 ml/min|1.73m^2) than other estimating equations and is recommended for clinical use. Therefore, the data presented in this analysis do reflect patterns of clinical information, albeit with these limitations. Directly measured longitudinal GFR data would more directly address this problem, but the data were not available in this study.

The analyses were also limited by the lack of association between hyperfiltration and traditional risk factors such as blood pressure, hypertension, high fasting blood glucose, and diabetes. Previous studies have reported the presence of these associations using directly measured GFR that is not observed in the same dataset using corresponding eGFR [1]. This may be a significant limitation of the CKD-EPI eGFR instrument. For example, blood pressures were better among hyperfilterers compared to normofilterers, a directionality we did not expect. This may indicate that most hyperfilterers are healthier, which would explain the lack of association with GFR decline. Describing this limitation of estimated GFR in measuring pathologically high renal function may compel further work improving these equations.

Another limitation is the lack of proteinuria data in these analyses. High proteinuria is a strong predictor of CKD-related complications [19,23,24]. While these data were not available since the MACS only began collecting proteinuria in 2010, these data would have allowed for further stratification of CKD stage based on KDIGO guidelines [19]. Since HIV-infected persons are at high risk for pathologic proteinuria

[25] adjusting for baseline renal function with proteinuria or eliminating men with evidence of CKD based on proteinuria alone would bolster the quality of the analyses.

An additional limitation of this study is that the trajectories by exposure status are presented as a continuous distribution, yet it is likely that each group is made up of a mixture of people with different trajectories. For example, each group may contain people who have no progression (stable), slow decline (slow progressors), fast decline (fast progressors) or increasing renal function (increasers). Since about 68% of those with hyperfiltration had an identified inflection point (in contrast to 38% of those without hyperfiltration), there is some support to this hypothesis [23,25]. Future directions in this research may make use of latent class models, such as growth mixture modeling, to identify different trajectories and the probabilities of membership in each latent class.

In summary, we found that among HIV-infected and HIV-uninfected middle-aged men, those with glomerular hyperfiltration based on chronically elevated eGFR, were more likely to have a downward inflection point of accelerated eGFR decline within 5 years of follow-up. However, the eGFR decline after this inflection point was not significantly different than the decline associated among those without hyperfiltration in an age- and race-matched control group, when stratifying by HIV infection status. Those with HIV infection were more likely to have accelerated eGFR decline compared to the older HIV-uninfected group. Given the limitations of eGFR at high levels of renal function, these results should be interpreted cautiously. Patients suspected of chronic hyperfiltration may not exhibit accelerated eGFR decline within 5 years, but should be monitored for other risk factors of incident CKD such as proteinuria, hypertension and diabetes. Refinement of estimating GFR equations to include high levels of renal function

would be helpful for clinical use, investigating the epidemiology of hyperfiltration and to impart confidence in describing longitudinal trajectories of eGFR in elevated ranges.

4.6 **References**

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5 Discussion and conclusion

The purpose of this dissertation was to describe the epidemiology of hyperfiltration among men with HIV infection using data from the MACS. The three analyses present results describing the prevalence of hyperfiltration using a gold standard measurement of GFR (Specific Aim 1); the incidence of hyperfiltration based on estimated GFR, the clinical standard for renal function (Specific Aim 2); and the associated estimated GFR decline after hyperfiltration over a 5 year period (Specific Aim 3).

5.1 Summary of results

5.1.1 Summary of Chapter 2: Prevalence of hyperfiltration in nested iohexol GFR substudy of the MACS

Chapter 2 presented results from a cross-sectional nested subsample of the MACS comprising 608 subjects with no evidence chronic kidney disease (CKD) who underwent a directly measured iohexol GFR protocol. Hyperfiltration was conceptually defined as a GFR greater than 140 ml/min|1.73m^2 – 1 ml/min|1.73m^2 per year over age 40. Using this definition, men with treated HIV infection had an increased prevalence of hyperfiltration compared to HIV-uninfected men (unadjusted prevalence was 25% vs. 17%, p= 0.01). In adjusted analyses, HIV infection was associated with 70% higher odds of hyperfiltration compared to men who were HIV-uninfected (prevalence odds ratio= 1.70, 95%CI: 1.11, 2.61). Previously identified risk factors for hyperfiltration, such as elevated blood glucose [1,2], diabetes [3–5], and hypertension [6] were also associated with hyperfiltration in this population. Importantly, this analysis presented an association of stimulant drug use (such as cocaine, amphetamines or methamphetamines) with hyperfiltration, a

relationship that had not been previously described in the literature. This is perhaps not surprising since stimulant drug use has been linked to metabolic abnormalities [7,8] and also modifies sympathetic nervous system functioning. This analysis also described a negative interaction associated with diabetes and HIV infection, such that among HIV-uninfected men, diabetes was associated with an increased prevalence of hyperfiltration (which is consistent with previously published literature [4,5]); however, a novel contribution of this study showed that there was no additive effect of diabetes among HIV-infected men, who overall had an increased prevalence of hyperfiltration compared to HIV-uninfected men. Lastly, this analysis described an association between hyperfiltration and increased ART exposure, particularly first-generation thymidine analogs (zidovudine). It is unclear whether this association was due to a longer duration of HIV infection or increased exposure to zidovudine, it is nonetheless clinically meaningful as a potential surrogate for CKD risk.

5.1.2 Summary of Chapter 3: Incidence of chronic hyperfiltration and risk associated with HIV infection in the MACS during the era of HAART using estimated GFR

Chapter 3 presented the incidence of hyperfiltration among men free of low eGFR and elevated eGFR at baseline in the era of HAART using data from the MACS. The chapter also presented the results from quantile regression models deriving estimated age- and race-specific 90^{th} percentile equations for use as a threshold for elevated eGFR, and provided 95% confidence intervals for the estimated parameters by bootstrapping. Using this population-based threshold, chronic hyperfiltration was defined as at least two eGFR observations above the age- and race-specific 90^{th} percentiles within 1 year. Incidence rates, non-parametric Kaplan-Meier survival functions and subhazard ratios

(accounting for competing risk events) were used to describe the relationship between treated HIV infection and hyperfiltration, stratified by race. Importantly, study entry was conditional on being event-free up to age 30 (a strong assumption), and the time scale was age after 30 years, which provided a meaningful context for interpretation, in contrast to simply using the first observed study visit. Late entry methods were used in these three analyses. The results from these analyses indicated an increased hyperfiltration incidence rate among younger non-black, HIV-infected men, compared to non-black, HIV-uninfected men. Among black men, HIV infection was associated with increased hyperfiltration incidence rates after age 45. The median ages of hyperfiltration among HIV-infected men was 35.2 years for non-blacks and 48.2 years for blacks; for HIV-uninfected men, the median ages at hyperfiltration were 49.5 years for non-blacks and 52.8 years for blacks. However, it should be noted that the sensitivity analyses showed a wide variability of median ages, depending on minor variations in the threshold used to define hyperfiltration.

Since men who experienced low eGFR were no longer at risk of hyperfiltration in our conceptual framework, low eGFR was considered a competing risk. Using competing risk proportional hazards regression, and allowing the effect of HIV infection to vary by age, among non-blacks, HIV infection was associated with increased hazard of hyperfiltration at age 30 (SHR: 3.69, 95%CI: 1.63, 8.36), and this subhazard ratio declined by 7% per year (SHR: 0.97, 95%CI: 0.89, 0.96). The estimated subhazard ratio was null at about 47 years. Among blacks, the effect of HIV on the subhazard of hyperfiltration did not vary by age, and HIV infection was associated with an increased risk of hyperfiltration (SHR: 1.60), and this effect was borderline non-significant

(95%CI: 0.96, 2.67). In a sensitivity analyses investigating the impact of modifying the threshold for elevated eGFR according to a) previous literature [9] and b) the 95% confidence intervals from the bootstrapped quantile regression, we noted that estimates of incidence were fairly sensitive, and this was most clear in the Kaplan-Meier survival functions. However, varying the threshold did not have any impact on the overall inferences, that HIV infection is associated with increased risk of incident eGFR hyperfiltration at younger ages among non-black men (i.e., between 30 and about 45 years of age), but at older ages in a population of black men (i.e., after 45 years of age).

5.1.3 Summary of Chapter 4: GFR decline after hyperfiltration

Chapter 4 presented results describing 5-year eGFR decline after hyperfiltration and in a matched comparison group, stratified by HIV infection. This chapter presented the derivation of the 90th percentile threshold described in Chapter 3, as well as the 50th percentile in the same population stratified by race, and showed the overestimation bias associated with eGFR at high levels among blacks, compared to non-blacks [10]. Using a matching algorithm, men free of hyperfiltration and CKD (i.e., normofiltration) were matched to each hyperfiltration case by age, race, HIV infection and hypertensive status at a ratio of up to 4:1. To identify and characterize accelerated GFR decline after a period of hyperfiltration, as described by Palatini [11] (Chapter 1, Figure 1), we applied a previously described generalized method to identify an inflection point [12], or a point at which GFR descends more rapidly. Subject-specific slopes after an inflection point, or over the observed 5-year time period in the absence of an inflection point, were calculated. In unadjusted and adjusted analyses, those with hyperfiltration did not have a significantly faster eGFR decline compared to the matched dataset without

hyperfiltration. Among HIV-infected men, there was a higher proportion with large GFR decline (more than -20 ml/min per year) among the hyperfilterers, but overall, there was no difference in GFR decline. Despite the null finding of the effect of hyperfiltration on GFR decline, this study did show that treated HIV infection, in a population with normal and elevated eGFR, was associated with a faster eGFR decline compared to those without HIV infection.

5.2 **Strengths**

5.2.1 Quality of data and two important instruments for measuring GFR

One of the strengths of the data in this study was the use of two important instruments to measure GFR. Chapter 2 used iohexol GFR in a small, nested subsample of the MACS, and this directly measured GFR is the gold standard for quantifying renal function. The results from these data give confidence in estimates of the prevalence of elevated eGFR, since the quality of measurement was high. However, iohexol GFR is not widely used clinically, and therefore it is unclear how the results from this study may be applied in real-world setting. To that end, Chapters 3 and 4 used the serum creatinine-based CKD-EPI eGFR, an instrument that is widely used clinically and is considered the standard in clinical care. Importantly, the increased incidence of eGFR-defined hyperfiltration associated with HIV infection was entirely consistent with the increased prevalence of elevated iohexol GFR. Of note, the higher prevalence of elevated eGFR among HIV-infected men presented in the subject flow figure of Chapter 3 (Figure 2) was also fairly congruent with the cross-sectional iohexol GFR analysis: 14.5% of HIV-infected men had prevalent elevated eGFR (182/1255) compared to 8.6% of HIV-uninfected men at baseline (118/1373).

5.2.2 Application of biostatistical methods

There were also several methodological strengths of these studies. In describing the incidence of hyperfiltration (Chapter 3), the time scale was age after 30 years. This provides a relevant and meaningful context for the research question, in contrast to observed study time as the time scale. The cumulative incidence rates for hyperfiltration indicated no difference by HIV infection status among non-black men, yet there were large differences by age, which is important epidemiologically. However, most men did not enter the study at age 30. We therefore used late entry methods to account for the deficit of men who developed hyperfiltration closer to age 30 but were not observed (i.e., survivorship bias).

Competing risks were also applied in this setting (Chapter 3) given the importance of differentiating subjects who were truly censored (i.e., assumed to have the event at an unobserved time that is after the last observed visit) and those who were no longer at risk for hyperfiltration (i.e., persons whose GFR is too low to reasonably increase to hyperfiltration levels). Using methods described by Fine and Gray [13], the subhazard ratio for HIV infection was estimated accounting for the competing risk event (i.e., low eGFR).

Lastly, the analyses in Chapter 4 applied a generalized method for identifying an inflection point using longitudinal GFR data. This method was originally designed for longitudinal T-cell trajectories in an HIV [12], but had not been used in describing changes in renal function. This methodologic approach precisely fit our conceptual framework of hyperfiltration and GFR decline [11] and was an appropriate tool for this analysis. Several studies have highlighted the epidemiologic and clinical importance of

non-linear GFR trajectories in the context of CKD [14,15], and this simple and easily accessible method should be a consideration for these types of research questions.

5.2.3 Describing age-related decline in renal function

Another strength of the study was a description of age-related GFR decline among healthy HIV-uninfected men, which was estimated to be between -0.8 and -0.6 ml/min per year at 90th percentile levels of GFR (presented in 3.1), and was similar at median levels of GFR (presented Figure 4.2). These estimates compare favorably with previously published research [9,16,17] in non-CKD populations. This consistency was encouraging and is an important contribution to the literature since our data comprised creatinine-based CKD-EPI eGFR, in contrast to others studies that have used directly measured GFR data to characterize GFR decline. It should be noted that these were population-based estimates of eGFR decline, and did not explicitly model within-person trajectories, as in a random effects linear mixed model. In Chapter 3, bootstrapping was used to account for within-person correlation and estimate the variability in the dataset. The point estimate of intercept and slope was derived from treating each eGFR observation as independent. This is one methodologic difference between describing decline while estimating the age- and race-specific 90th percentile (as in Chapter 3) and characterizing decline after hyperfiltration as empirical subject-specific changes in GFR (as in Chapter 4).

5.3 **Limitations and future directions**

5.3.1 Establishing a threshold for hyperfiltration

There were several limitations in these analyses. As briefly mentioned in Chapter 1, there are several assumptions and limitations associated with different methods to define elevated eGFR. For Chapter 2, elevated eGFR was defined as a threshold of 140 ml/min|1.7m^2 minus 1 ml/min per year over age 40. While this level would be considered extremely high, it is somewhat arbitrary given the continuous distribution of GFR. For example, it is not clear that a 45 year old man who has a GFR of 132ml/min|1.73m^2 is pathologically different than if he had a GFR of 136: the former would be classified as having normal GFR, while the latter would exceed the threshold of 135 ml/min|1.73m^2. This inherent limitation in dichotomizing a continuous outcome also applies to the population-based threshold described in Chapters 3 and 4. This is potentially problematic since the sensitivity analysis in Chapter 3 (Figure 3.5) revealed remarkable differences in characterizing incidence when the threshold changed very slightly (less than 0.1 ml/min per year in some case, which is clinically insignificant). Additionally, it is possible that a single threshold for defining elevated GFR across all ages (i.e., one threshold level that does not address potential age related decline) is more appropriate for this condition. This approach would be similar to establishing a single threshold of GFR for defining CKD (i.e., < 60 ml/min regardless of age). Given the sensitivity of results using different thresholds incorporating an age-related decline (Chapter 3), the inferences using a single threshold for elevated GFR would indeed be different as fewer hyperfiltration events would occur.

Future work might investigate hyperfiltration as a continuous variable, and use different criteria for defining hyperfiltration. Such an approach may include prediction models for hyperfiltration-related outcomes, such as proteinuria, or even physiological outcomes based on kidney biopsies.

5.3.2 Improving equations at high levels of renal function

Another limitation in Chapters 3 and 4 is the use of the CKD-EPI estimating equation at high levels of GFR. It is clear that this equation performs better at lower levels of renal function primarily because it was mainly developed in populations at risk for or with CKD [18]. While the CKD-EPI equation was not designed for use at high GFR levels, an evaluation of performance at elevated renal functioning indicated it was the least biased option in general. While this equation was biased among blacks at high levels, this bias has been identified and defined [10]. Interestingly, this bias was identified and presented in Chapter 4 by comparing black HIV-uninfected men with non-black HIV-uninfected men, with both groups sharing similar health and risk profiles, using quantile regression in the absence of a gold standard GFR measurement. Since there is relatively poorer performance of eGFR at high levels, future work should be dedicated towards refining and improving the equations in measuring elevated GFR. This would be helpful for clinicians in decision making and profiling risk, but also for research purposes, especially given the pervasiveness of serum creatinine measurements, which is now commonly measured in routine blood tests.

5.3.3 Quantifying error associated with GFR

Yet another limitation in measurement of GFR from estimating equations is a lack of quantified error associated with each measurement. The data that are used to define an

eGFR for a given person is age, sex, race and serum creatinine level. Since there is no information bias associated with sex and race, and arguably age as well, these variables are not sources of error. The remaining sources of error are serum creatinine and the directly measured GFR by which the estimated GFR is derived. The within-individual variability of serum creatinine and directly measured GFR (within days or weeks of first measurement) has not been adequately described. Lab-based variability is another source of error that is also not well characterized, but recent standardization of serum creatinine measurements have allowed for comparisons across labs. This is another limitation applicable to Chapters 3 and 4, yet it should be noted that for the purposes of these research questions clinical eGFR was most relevant, and the data for these specific aims were reflective of real-world settings. Formal studies investigating different sources of error in estimating GFR equations would be of great benefit in order to quantify the expected variability for a given eGFR value.

5.3.4 Error associated with individual slopes

The inflection point analysis presented in Chapter 4 ascribed individual empirical slopes to each subject. This method was limited since these empirical slopes were subject-specific estimates that summarized the overall trend of longitudinal eGFR, and did not define or account for error associated with this estimate. While these slopes were valid estimates of eGFR changes over time, future methods might refine this approach by incorporating an estimate of variability associated with the data. Similar approaches have been used previously to describe longitudinal changes in GFR [19,20], and have shown to be especially effective at characterizing heterogeneity of GFR decline in subpopulations [21]. There was a slight indication of this among HIV-infected men with hyperfiltration

(Figure 4.3) who had a higher proportion with extremely fast GFR decline, however, this interpretation is cautiously made since this effect was represented by very few subjects.

5.3.5 Defining normal age-related decline

A common issue related to all the analyses is that of normal age-related decline. There was a consistently strong association between age and declining GFR (Figure 4.2) and this relationship was an assumption embedded in many aspects of the analyses. In particular, age-related decline was assumed in the two methods of defining the threshold of elevated eGFR. This assumption was well-founded and based on evidence both in previously published literature and in the MACS data. However, the question of what constitutes normal age-related decline remains open and contentious. Levey and colleagues have noted that even within older age groups, lower GFR was predictive of adverse outcomes and mortality, thus justifying a single threshold for classifying CKD [22]. However, others believe age-related decline is non-pathological and better attempts can be made to incorporate what is known about normal age-related decline into clinical definitions [17]. Future work in defining hyperfiltration should take this into account as well. For the purposes of this dissertation, the HIV-uninfected men served as the counterfactual for the HIV-infected men. The underlying assumption of this comparison is that had the HIV-infected men never acquired the virus, their levels would be identical to the observed HIV-uninfected men. This assumption freed us from external criteria for defining hyperfiltration, but it was encouraging that the results were consistent with previous findings. The attempts in this dissertation to define hyperfiltration based on adapting previously published criteria, as well as strong theoretical underpinnings (i.e.,

the counterfactual), in the absence of an established clinical definition, may provide a framework for future studies to investigate the epidemiology of hyperfiltration.

5.4 **Concluding remarks**

These results provide evidence that treated HIV infection is indeed a risk factor for hyperfiltration, at least among men between the ages of 30 and 60 years. However, the results from Chapter 4 indicate that accelerated eGFR decline may not be an inevitable outcome of hyperfiltration. Indeed, changes in GFR after hyperfiltration were not significantly different from changes in the matched comparison group. A major limitation of this study is that those in the matched comparison group (i.e., those with normofiltration), may have had hyperfiltration prior to study entry that was never observed. Alternatively, these men may already have decreasing GFR at the time of match due to other pathologies, and this may explain the comparable distributions of GFR decline by filtration status. Nonetheless, the distributions of slopes also reveal a modest excess of fast decliners among HIV-infected hyperfilterers. As described in the discussion of Chapter 4, it is possible that those with identified hyperfiltration are a mixture of people with varying pathologies of GFR function (including no pathology). Identifying and classifying people with high GFR into hyperfiltering groups of differing risks should be a goal for epidemiologic and clinical research going forward. Such a definition might include criteria based on glucose levels, blood pressure, previous metabolic history, or simply body mass index.

Since the analyses presented in Chapter 2 [23] and previous research indicate a strong association of metabolic and cardiovascular abnormalities with hyperfiltration [1,2,5,6], increased clinical attention to these factors might mitigate any adverse effects

of hyperfiltration. For example, a patient presenting with hyperglycemia and hypertension is already indicated for therapies to manage blood sugar levels and blood pressure. In this scenario, identification of hyperfiltration may provide further justification for urgency of therapy and goals to improve adherence, but would likely not change the treatment course for this patient. On the other hand, monitoring GFR in conjunction with hyperglycemia and blood pressure may improve indicators of therapy effectiveness.

Given the lack of evidence for accelerated GFR decline associated with hyperfiltration (Chapter 4), CKD should remain the primary focus of clinicians treating an HIV-infected population [24], until evidence is presented that hyperfiltration is a reliable predictor of CKD and/or adverse outcomes. Studies have described numerous causes of CKD, especially in the context of HIV infection [25–27], and it is likely that hyperfiltration is one of these causes [5,11,28]. In light of this, further research should investigate the proportion of CKD risk attributable to hyperfiltration among persons infected with HIV in the context of carefully conducted, standardized cohort studies.

In conclusion, this dissertation provides evidence that HIV infection is a risk factor of incident hyperfiltration and that traditional metabolic and cardiovascular risk factors associated with HAART appear to play a role in the prevalence of hyperfiltration. This is clinically important since hyperfiltration is a modifiable and treatable condition. While hyperfiltration was not significantly associated with accelerated GFR decline, treated HIV infection was a risk factor for faster GFR decline in a population with normal and elevated eGFR. Indicators of metabolic, cardiovascular and renal health, including hyperfiltration, as well as duration of infection and therapy should remain important

clinical considerations in the management of HIV infection and overall health in this high risk population.

5.5 References

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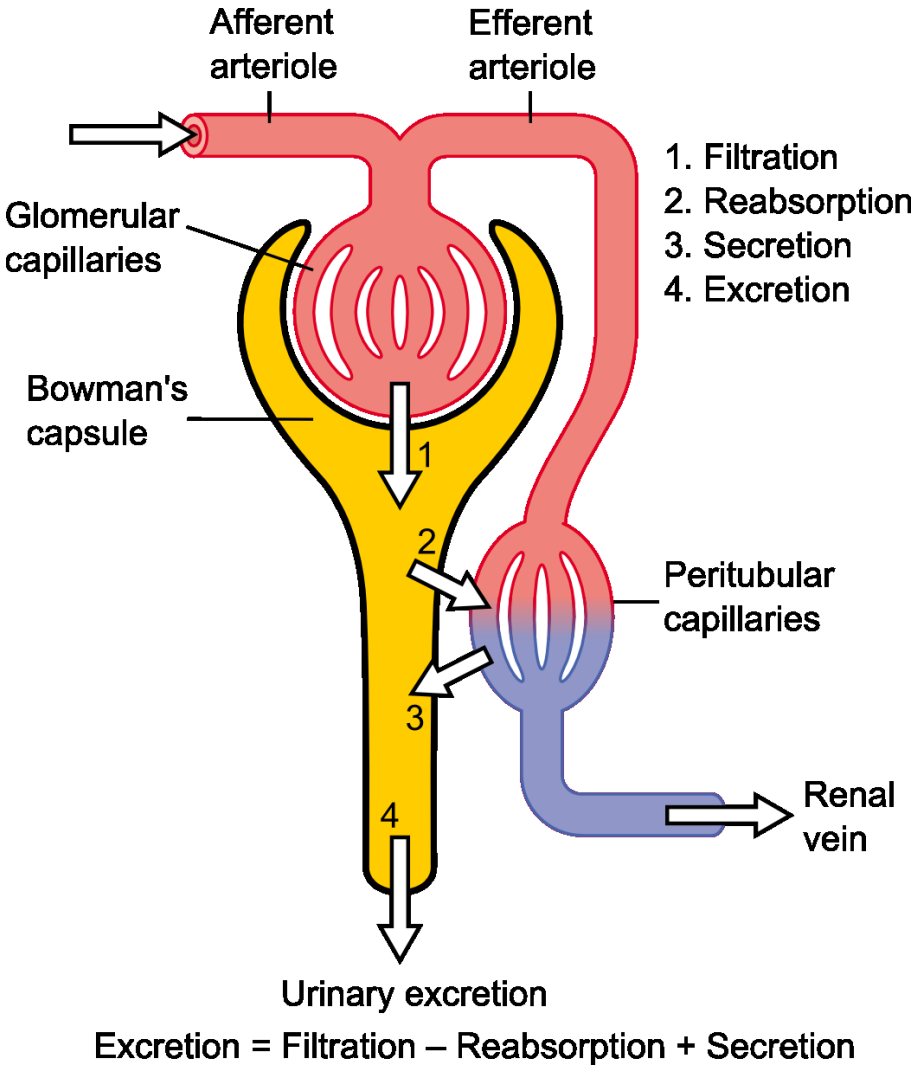
6 Figures and Tables

6.1 Figures for Chapter 1

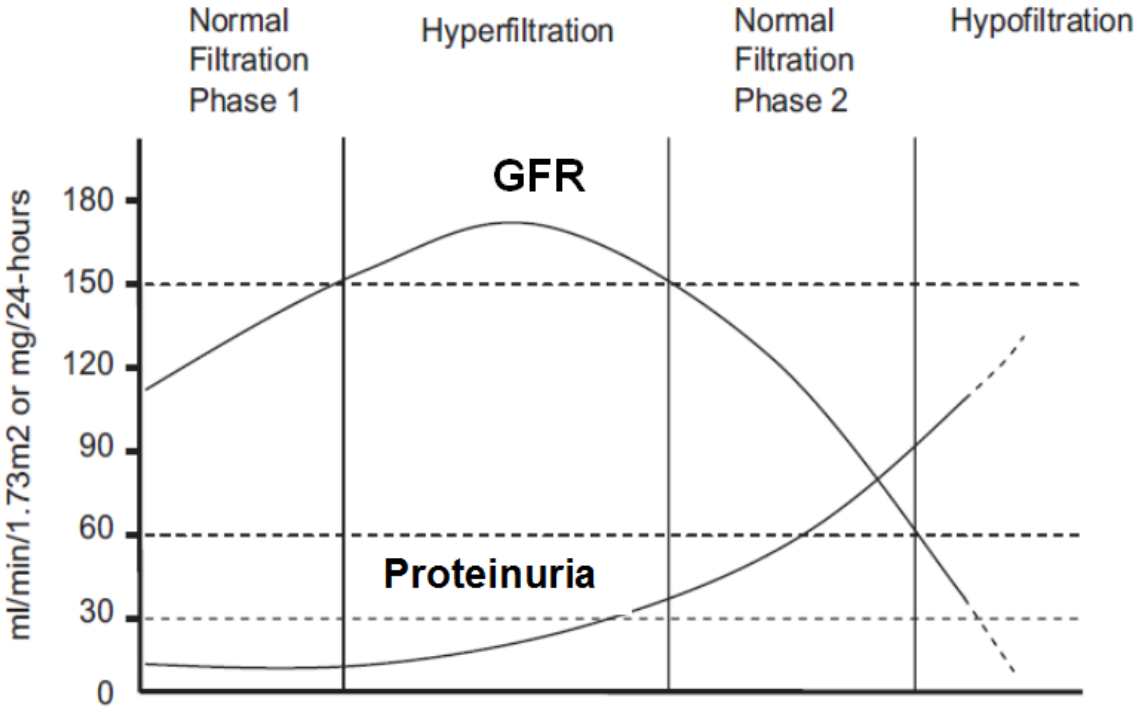
6.1.1 Figure 1.1. Schematic of blood flow and filtration mechanisms in a nephron.



Source: "Physiology of Nephron" by Madhero88. Licensed under Creative Commons Attribution 3.0 via Wikimedia Commons - https://commons.wikimedia.org/wiki/File:Physiology_of_Nephron.png#mediaviewer/File:Physiology_of_Nephron.png Retrieved 8/26/2014.

6.1.2 Figure 1.2 Conceptual framework of time course of GFR and albumin excretion rate (AER, mg/24 hrs) based on the theory of hyperfiltration proposed by Brenner et al. (1996).

Conceptual framework of time course of GFR and albumin excretion rate (AER, mg/24 hrs) based on the theory of hyperfiltration proposed by Brenner et al. (1996). The threshold of 150 ml/min/1.73m^2 is arbitrary in this model.

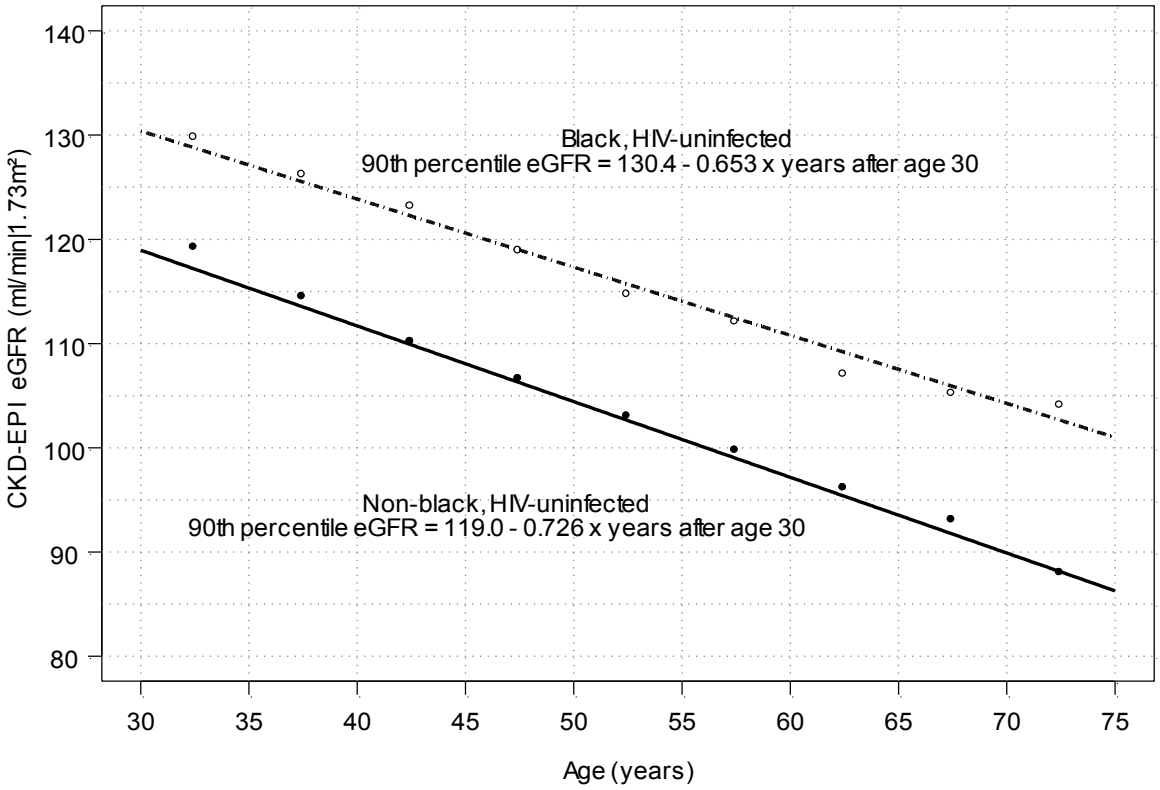


Source: Palatini P. Glomerular hyperfiltration: a marker of early renal damage in pre-diabetes and pre-hypertension. *Nephrol Dial Transplant*, 2012, 0:1-7.

6.3 **Figures and Tables for Chapter 3**

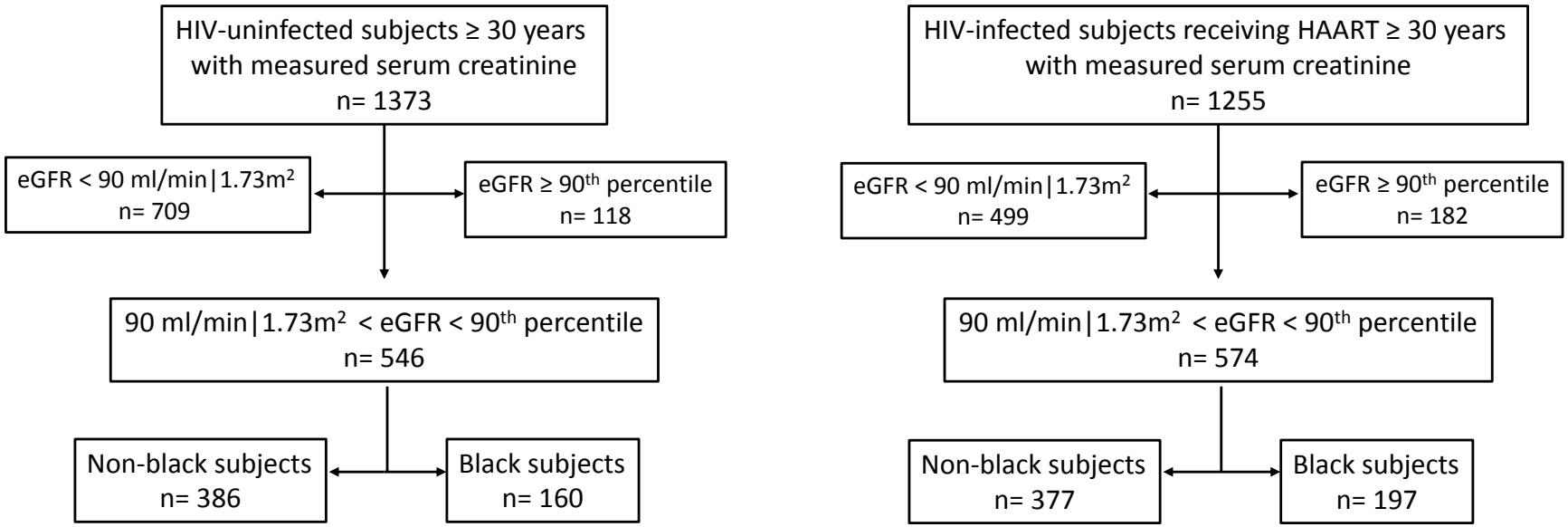
6.3.1 Figure 3.1. Estimated 90th percentiles based on quantile regression models, stratified by race (non-black and black).

Data points are 90th percentile values by 5 year age bins to assess model fit.



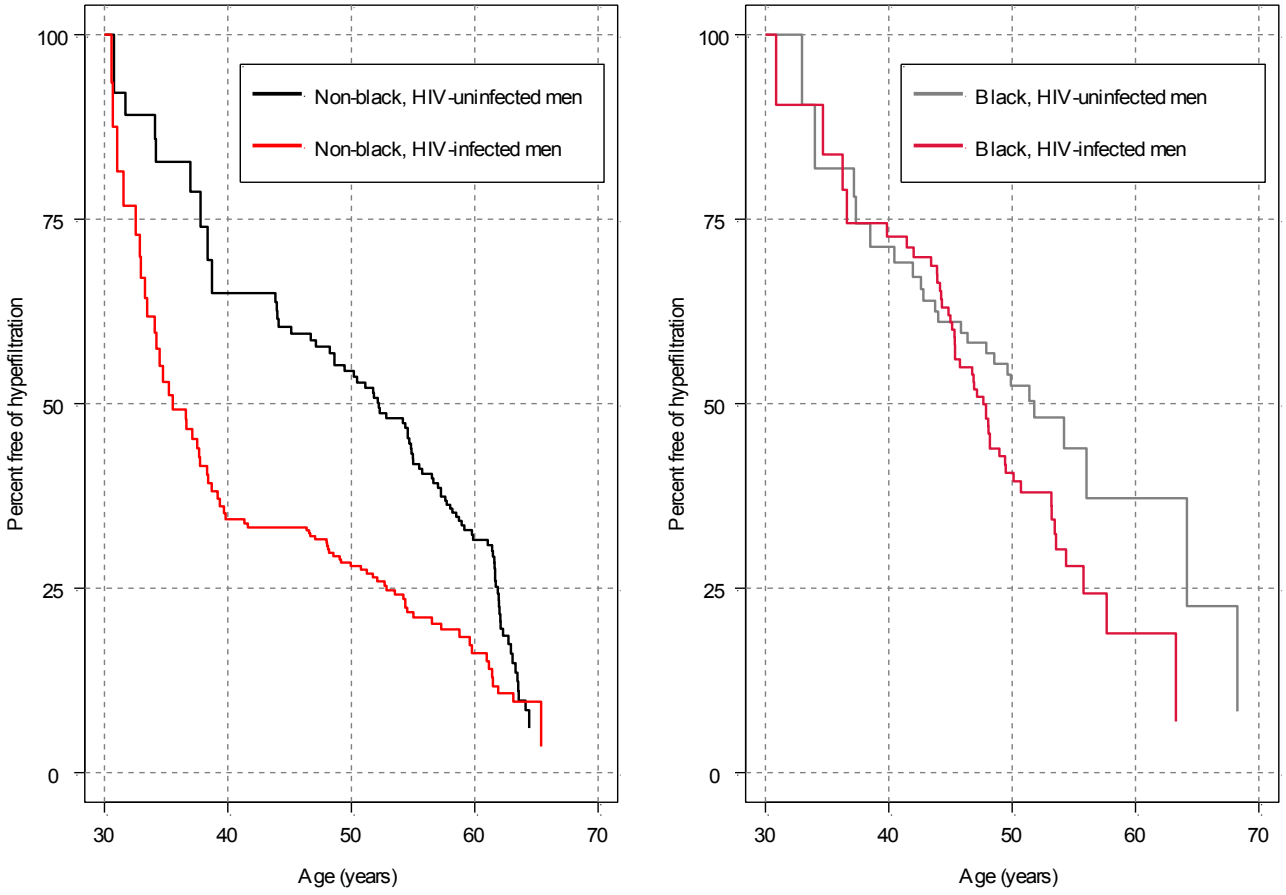
6.3.2 Figure 3.2. Diagram of subject flow for study selection of study population.

The study population comprised men free of evidence of CKD (CKD-EPI eGFR < 90 ml/min|1.72m^2) or elevated eGFR (CKD-EPI eGFR > 90th percentiles specific to age and race), stratified by HIV infection status.

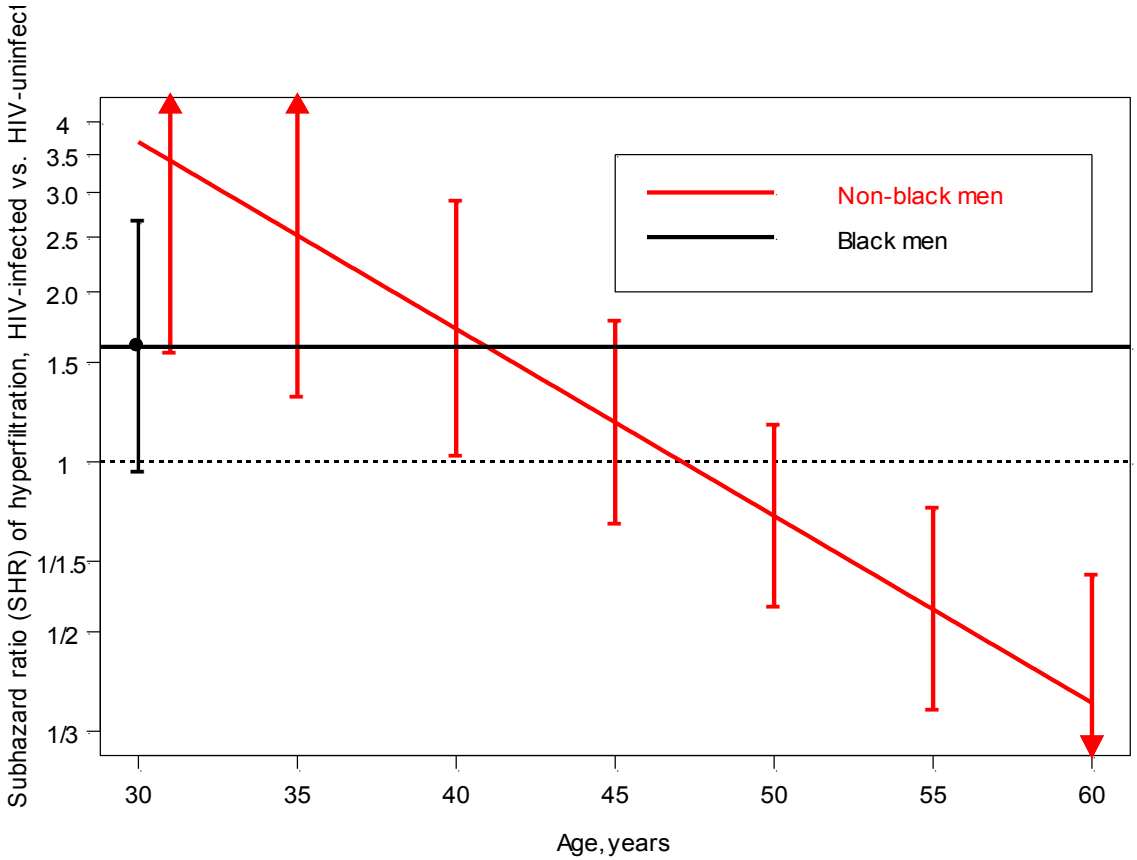


6.3.3 *Figures 3.3a and 3.3b. Kaplan-Meier incidence of chronic hyperfiltration by race and infection status using age (after 30 years) as the time scale.*

Panel A presents non-black subjects (n= 763; HIV-uninfected n= 386; HIV-infected men n= 377) and Panel B describes black subjects (n= 357; HIV-uninfected n= 160; HIV-infected n= 197).



6.3.4 Figure 3.4. Subhazard ratios of the effect of HIV infection on hyperfiltration, by non-black race and black race.



6.3.5 Figures 3.5a-3.5c. Sensitivity analyses presenting Kaplan-Meier survival step functions based on different thresholds to define hyperfiltration, stratified by race.

These thresholds correspond to previously published expected decline of -0.8 ml/min|1.73m^2 among healthy individuals (Figure 3.5a; [30]); the lower (Figure 3.5b) and upper (Figure 3.5c) 95% confidence limit of the slope of 90th percentiles based on bootstrapped quantile regression.

Figure 3.5a. Elevated eGFR threshold based on an expected decline of -0.8 ml/min per year.

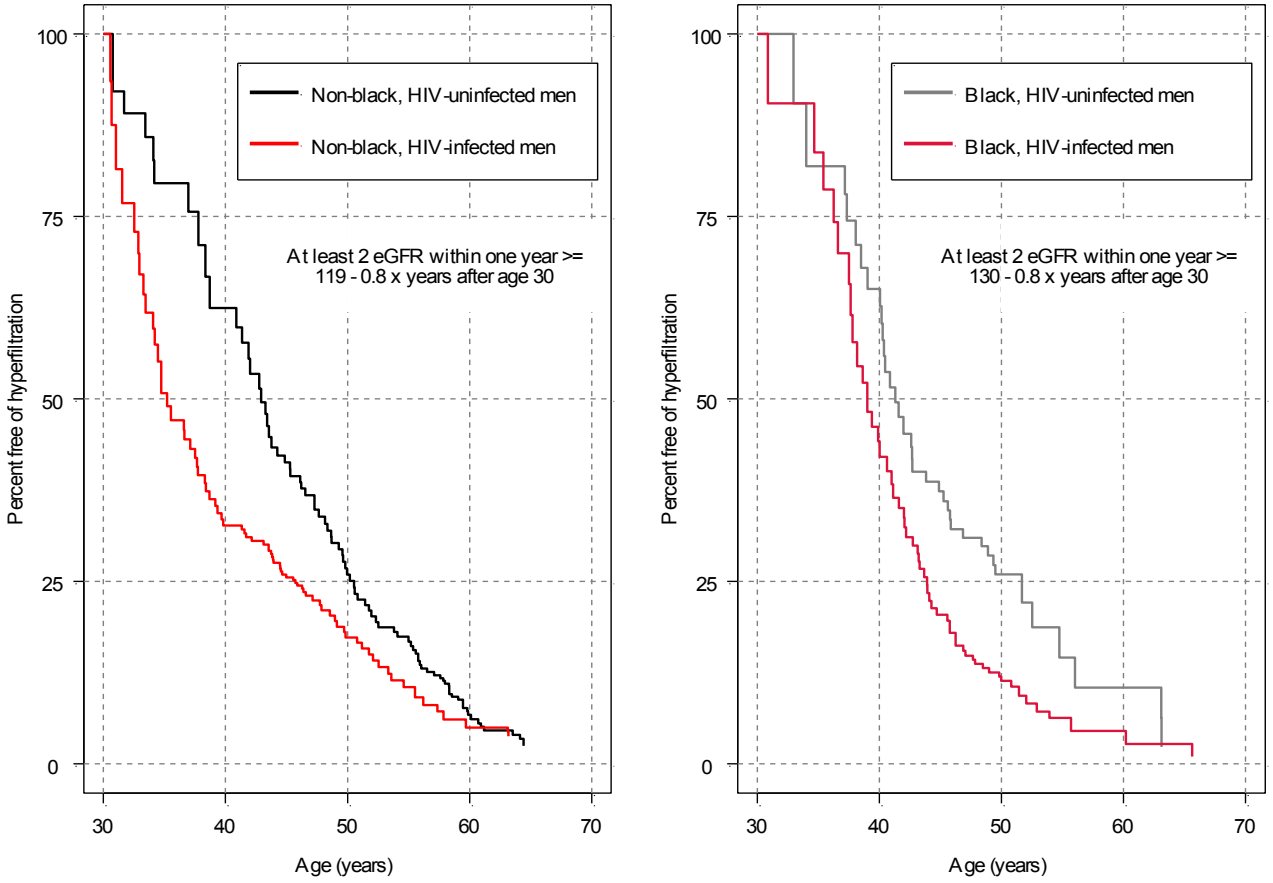


Figure 3.5b. Elevated eGFR threshold based on a decline of -0.747 ml/min per year for non-black men, and -0.833 for black men, which corresponds to the lower 95% confidence intervals from the bootstrapped quantile regression.

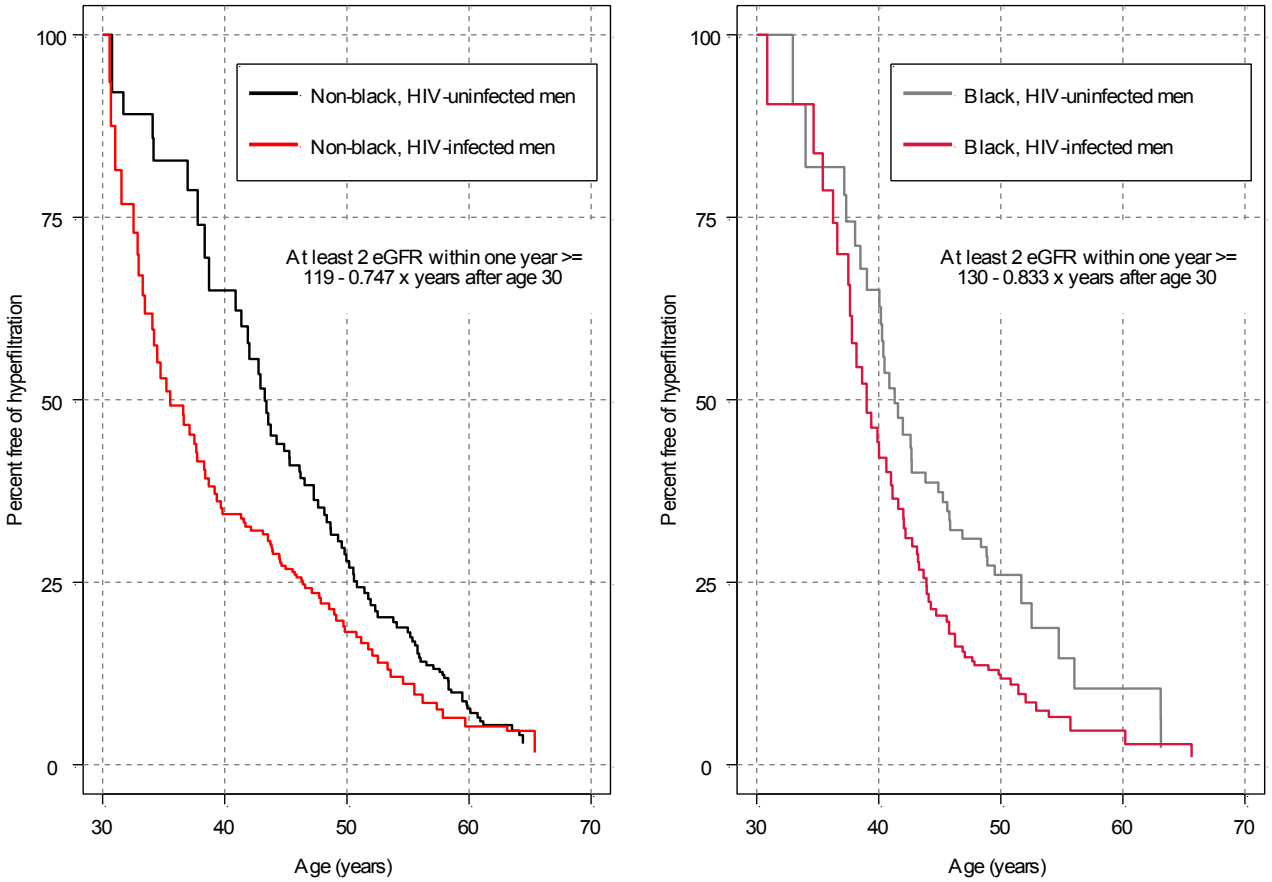
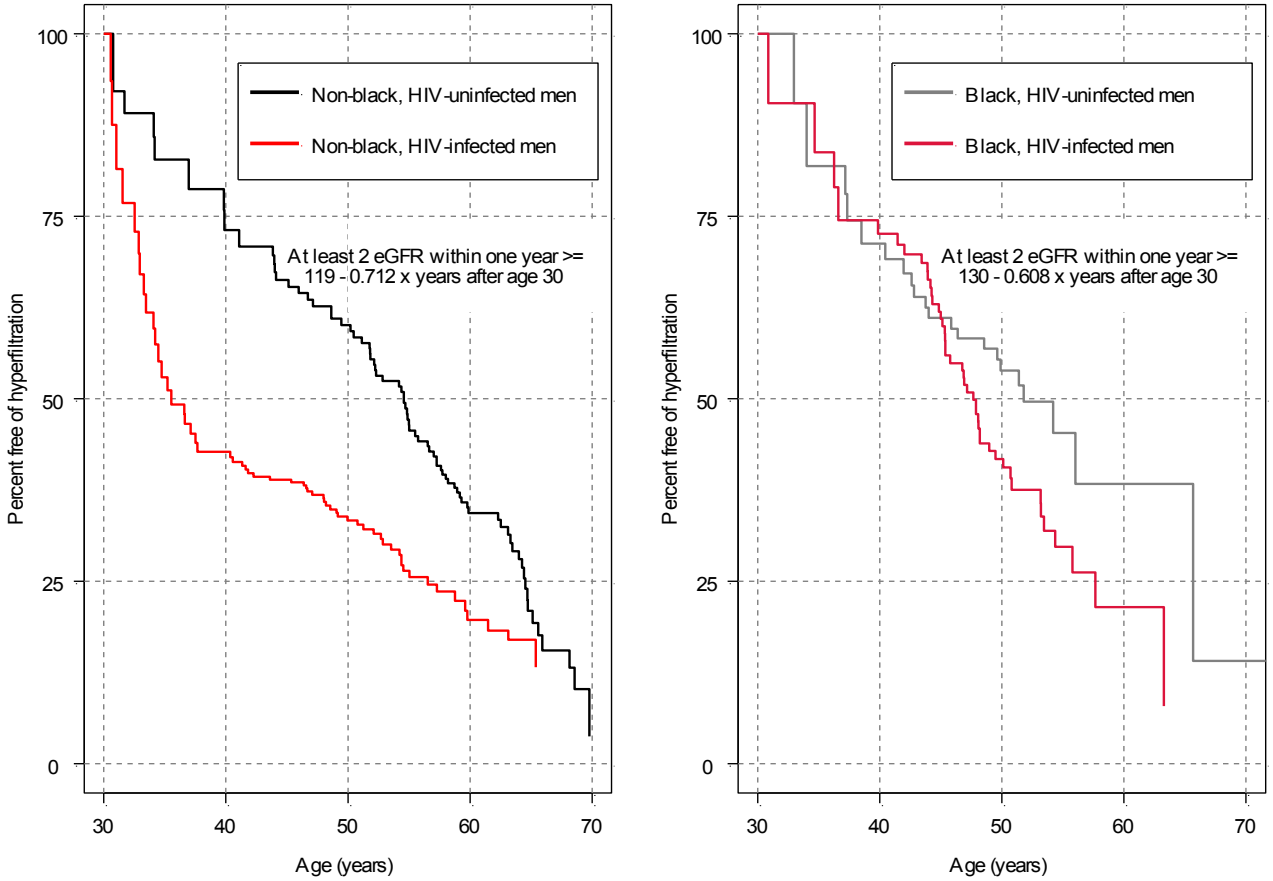


Figure 3.5c. Elevated eGFR threshold based on a decline of -0.712 ml/min per year for non-black men, and -0.608 for black men, which corresponds to the upper 95% confidence intervals from the bootstrapped quantile regression.



6.3.6 Table 3.1. Clinical characteristics and descriptive statistics of subjects at study entry, by race and HIV infection status.

	Non-black men			*Black men*		
Variable	*HIV-uninfected n= 386*	*HIV-infected n= 377*	*P-value*	*HIV-uninfected n= 160*	*HIV-infected men n= 197*	*P-value*
Year of study entry	2003.97 [2003.74, 2004.53]	2003.87 [1999.56, 2004.79]	0.001	2004.07 [2003.87, 2004.53]	2004.33 [2003.88, 2005.70]	0.006
Age, years	46.6 [41.3, 52.5]	43.4 [38.4, 48.4]	<.001	42.3 [37.7, 45.7]	41.4 [38.1, 46.4]	0.796
Post-2001 recruitment	29.8% (115)	38.2% (144)	0.015	82.5% (132)	78.7% (155)	0.422
CKD-EPI eGFR, ml/min|1.73m^2	100 [95.8, 104.3]	101.4 [95.8, 106.1]	0.100	103.2 [96.5, 109.4]	107.6 [97.8, 115.3]	0.003
Height, m	1.8 [1.7, 1.8]	1.8 [1.7, 1.8]	0.621	1.8 [1.7, 1.8]	1.8 [1.7, 1.8]	0.380
Weight, kg	78.9 [70.7, 91.6]	77.1 [69.5, 84.2]	0.002	81.2 [73.8, 95.7]	76.9 [69.9, 86.4]	0.002
Body mass index, kg/m^2	25.9 [23.1, 30.7]	24.8 [23, 27.9]	0.003	26.7 [24.1, 32.5]	24.9 [22.7, 28]	<.001
Obese (BMI > 30 kg/m^2)	18.6% (65)	7.1% (24)	<.001	25.2% (37)	12.1% (22)	0.002
Fasting blood glucose, mg/dL	94 [88, 102]	95 [87, 102]	0.905	94 [88, 104]	95 [88, 106]	0.758
Fasting glucose > 100 mg/dL	27.1% (82)	28.6% (60)	0.763	35.1% (39)	37.7% (49)	0.690
Diabetes	4.6% (15)	5.3% (12)	0.842	9.9% (12)	9.1% (13)	0.836
High density lipoproteins, mg/dL	48.6 [41, 57]	41.8 [35.5, 49.2]	<.001	51 [43.8, 60.9]	46.4 [38.7, 54.5]	<.001
Low density lipoproteins, mg/dL	122 [100, 144.5]	110 [88, 139]	0.001	114 [88, 138]	101 [84, 131]	0.060
Dyslipidemia	72.8% (243)	83.9% (256)	<.001	59.8% (70)	68.7% (103)	0.156
Metabolic syndrome	19.5% (70)	23% (60)	0.318	16.1% (24)	12.6% (22)	0.425
Systolic blood pressure, mmHg	120 [112, 130]	120 [112, 130]	0.877	123 [115, 132]	120 [112, 130]	0.088
Diastolic blood pressure, mmHg	78 [70, 82]	79 [70, 84]	0.850	79 [70, 86]	76 [69, 84]	0.049
Uncontrolled hypertension	16% (56)	16.3% (56)	1.000	23.8% (35)	20% (37)	0.423

	Non-black men			*Black men*		
Variable	*HIV-uninfected n= 386*	*HIV-infected n= 377*	*P-value*	*HIV-uninfected n= 160*	*HIV-infected men n= 197*	*P-value*
Taking antihypertensive medications	13.9% (53)	9.7% (36)	0.072	12.5% (20)	10.2% (20)	0.505
Current smoker	25.2% (95)	32.4% (120)	0.029	60.9% (95)	55.2% (107)	0.327
Current stimulant use	11.7% (44)	20.9% (77)	<.001	26.9% (42)	30.2% (58)	0.552
Longitudinal data						
Observed follow-up time	2.95 [1.05, 6.36]	2.51 [0.96, 5.12]	0.101	3.43 [1.00, 6.63]	3.45 [1.00, 6.30]	0.577
Total observed follow-up time	1496.2	1367.8		664.2	791.7	

[a] There were 12 non-black men and 5 black men who became infected while under study observation. They contributed person-time to the HIV-uninfected group and the HIV-infected with antiretroviral therapy group.

6.3.7 Table 3.2. Descriptive statistics of indicators of HIV-disease severity and antiretroviral therapy at baseline and study exit, by race.

	HIV-infected men receiving antiretroviral therapy	
Variable	*Non-black n= 377*	*Black n= 197*
Baseline CD4+ cell count	477 [301, 667]	415 [266, 554]
Baseline CD4+ cell count < 350	32.7% (118)	37.6% (71)
Nadir CD4+ cell count	272 [169, 394]	260 [144, 372]
Nadir CD4+ cell count < 350	67.0% (244)	69.7% (129)
Baseline detectable HIV RNA	43.7% (157)	58.0% (109)
Previous AIDS diagnosis	15.4% (58)	11.2% (22)
Received any ART prior to HAART	56.2% (212)	43.2% (85)
Years since ART initiation at study exit	9.23 [5.00, 15.21]	9.75 [5.29, 17.79]
Years since HAART initiation at study exit	6.22 [2.88, 9.74]	7.11 [3.99, 10.43]

6.3.8 Table 3.3a. Incidence rates per 100 person years and incidence rate ratios comparing HIV-infected non-black men with HIV-uninfected non-black men (reference).

	Non-black race						
	HIV-uninfected men			HIV-infected men			
Age category	Events	Person years	Incidence rate per 100 person years	Events	Person years	Incidence rate per 100 person years rate	Incidence rate ratio
[30 to 35)	5	124.91	4.00 (1.67, 9.62)	13	95.27	13.65 (7.92, 23.5)	**3.41 (1.22, 9.56)**
[35 to 40)	4	93.47	4.28 (1.61, 11.4)	15	169.36	8.86 (5.34, 14.69)	2.07 (0.69, 6.24)
[40 to 45)	4	193.92	2.06 (0.77, 5.50)	2	341.81	0.59 (0.15, 2.34)	0.28 (0.05, 1.55)
[45 to 50)	7	328.44	2.13 (1.02, 4.47)	12	377.65	3.18 (1.80, 5.60)	1.49 (0.59, 3.79)
[50 to 55)	17	348.69	4.88 (3.03, 7.84)	11	228.90	4.81 (2.66, 8.68)	0.99 (0.46, 2.1)
[55 to 60)	18	298.76	6.02 (3.80, 9.56)	6	108.69	5.52 (2.48, 12.29)	0.92 (0.36, 2.31)
[60 to 65)	24	108.06	22.21 (14.89, 33.14)	6	45.69	13.13 (5.9, 29.23)	0.59 (0.24, 1.45)
Total	79	1496.24	5.28 (4.24, 6.58)	66	1367.75	4.83 (3.79, 6.14)	0.91 (0.66, 1.27)

6.3.9 Table 3.3b. Incidence rates per 100 person years and incidence rate ratios comparing HIV-infected black men with HIV-uninfected black men (reference).

	Black race						
	HIV-uninfected men			HIV-infected men			
Age category	Events	Person years	Incidence rate per 100 person years	Events	Person years	Incidence rate per 100 person years	Incidence rate ratio
[30 to 35)	2	46.63	4.29 (1.07, 17.15)	2	53.46	3.74 (0.94, 14.96)	0.87 (0.12, 6.19)
[35 to 40)	3	103.85	2.89 (0.93, 8.96)	3	109.34	2.74 (0.88, 8.51)	0.95 (0.19, 4.71)
[40 to 45)	6	187.30	3.20 (1.44, 7.13)	10	260.63	3.84 (2.06, 7.13)	1.20 (0.44, 3.30)
[45 to 50)	6	198.59	3.02 (1.36, 6.72)	20	237.19	8.43 (5.44, 13.07)	**2.79 (1.12, 6.95)**
[50 to 55)	3	92.52	3.24 (1.05, 10.05)	7	95.38	7.34 (3.5, 15.39)	2.26 (0.59, 8.75)
[55 to 60)	1	24.46	4.09 (0.58, 29.03)	2	22.42	8.92 (2.23, 35.66)	2.18 (0.20, 24.06)
[60 to 65)	1	7.72	12.95 (1.82, 91.96)	1	7.23	13.83 (1.95, 98.15)	1.07 (0.07, 17.06)
Total	23	664.17	3.46 (2.30, 5.21)	45	791.74	5.68 (4.24, 7.61)	1.64 (0.99, 2.71)

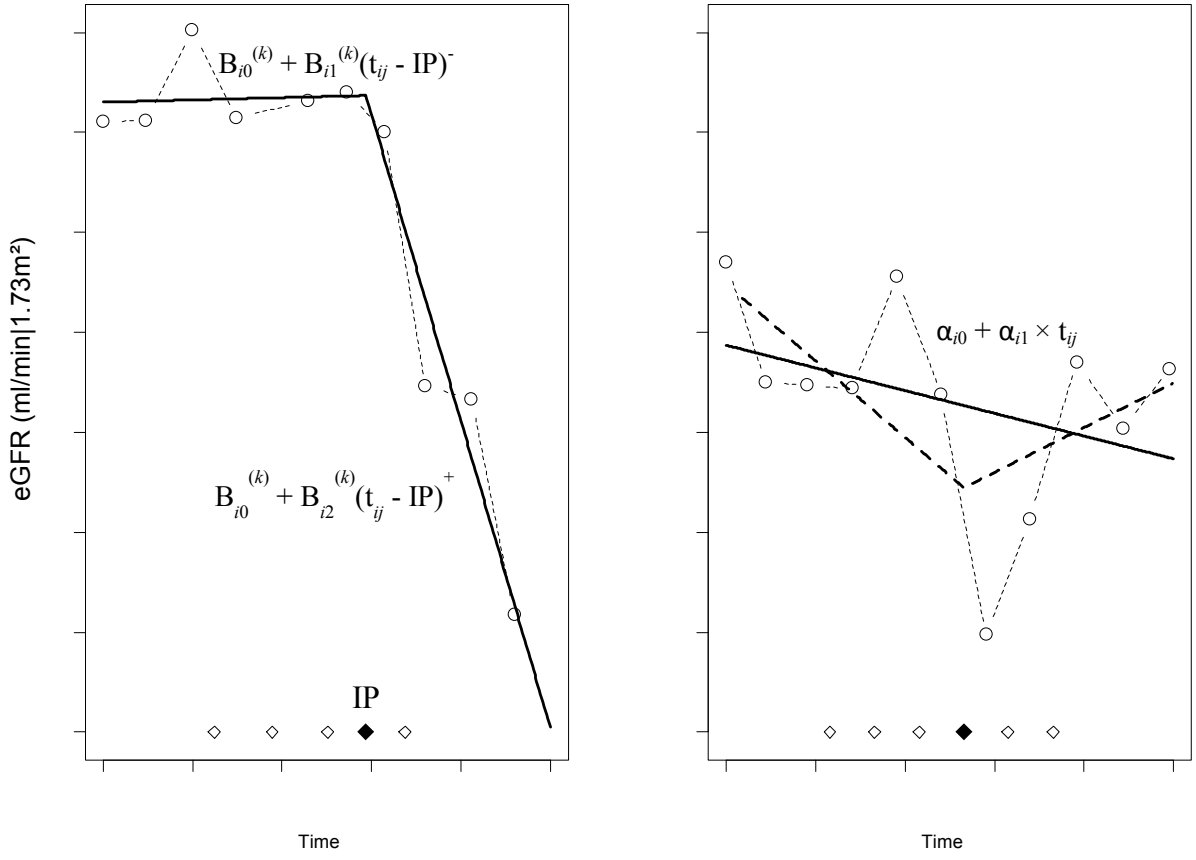
6.3.10 Table 3.4. Sensitivity analyses based on different thresholds to define elevated eGFR describing number of events, cumulative incidence rate and subhazard ratios from competing risks proportional hazards models.

	HIV-uninfected		HIV-infected			
Elevated eGFR equation	Hyperfiltration events	Cumulative incidence rate per 100 py (95%CI)	Hyperfiltration events	Cumulative incidence rate per 100 py (95%CI)	HIV-infected vs. HIV-uninfected subhazard ratio at age 30 (95%CI)	HIV-infected vs. HIV-uninfected subhazard ratio per 1 year increase (95%CI)
Non-black men						
Main model	79	5.28	66	4.83	**3.69**	**0.93**
118.95 – 0.726 × years after age 30		(4.24, 6.58)		(3.79, 6.14)	**(1.63, 8.36)**	**(0.89, 0.96)**
Adapted from Poggio et al. (2009)	81	9.67	74	8.22	**3.10**	**0.92**
119 – 0.8 × years after age 30		(7.78, 12.03)		(6.55, 10.33)	**(1.45, 6.65)**	**(0.88, 0.96)**
Main model lower 95%CI slope	78	9.25	73	8.05	**3.18**	**0.92**
119 – 0.747 × years after age 30		(7.41, 11.55)		(6.40, 10.13)	**(1.44, 6.98)**	**(0.88, 0.96)**
Main model upper 95%CI slope	73	4.48	58	3.80	**3.66**	**0.92**
119 – 0.712 × years after age 30		(3.56, 5.63)		(2.94, 4.92)	**(1.59, 8.40)**	**(0.89, 0.96)**
Black men						
Main model	23	3.46	45	5.68	1.60	1
130.39 – 0.726 × years after age 30		(2.30, 5.21)		(4.24, 7.61)	(0.96, 2.67)	
Adapted from Poggio et al. (2009)	37	8.28	53	12.44	1.44	1
130 – 0.8 × years after age 30		(6.00, 11.43)		(9.50, 16.30)	(0.90, 2.29)	
Main model lower 95%CI slope	37	8.29	52	12.26	1.41	1
130 – 0.833 × years after age 30		(6.01, 11.45)		(9.34, 16.09)	(0.88, 2.26)	
Main model upper 95%CI slope	22	3.25	44	5.54	1.64	1
130 – 0.608 × years after age 30		(2.14, 4.93)		(4.12, 7.44)	(0.97, 2.76)	

6.4 **Figures and Tables for Chapter 4**

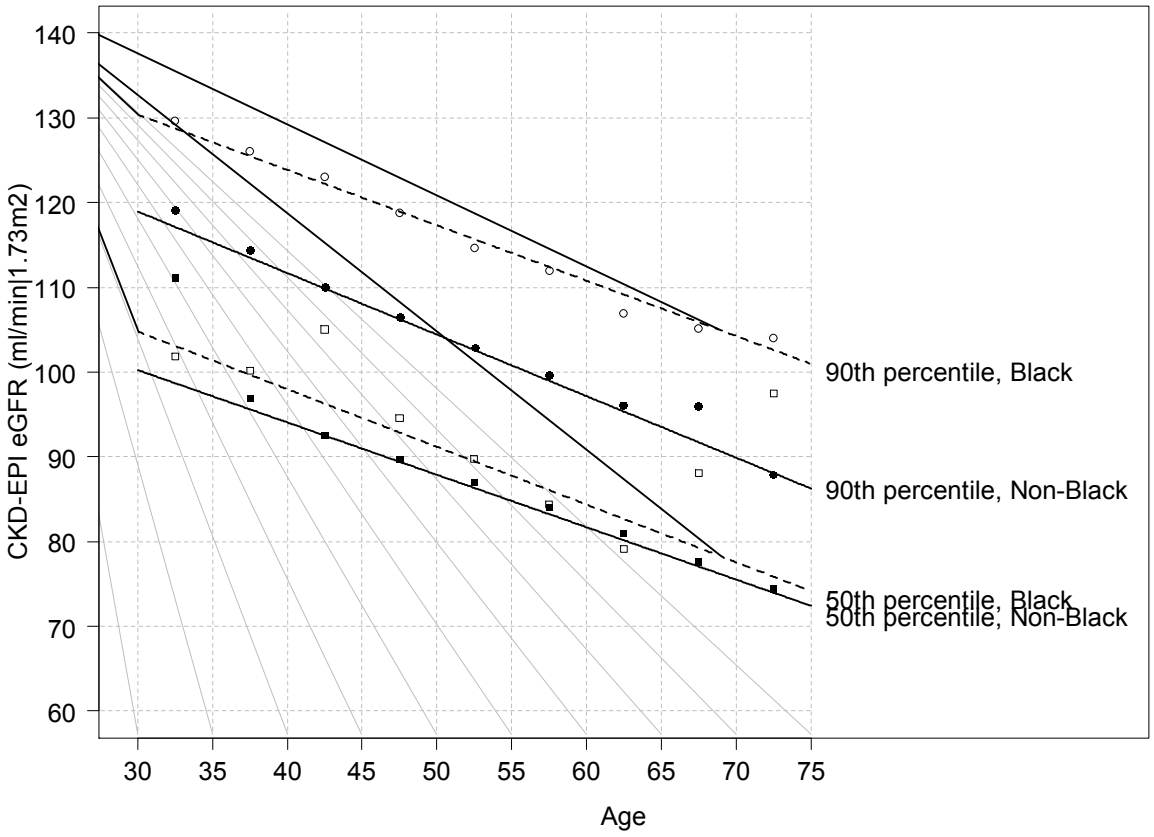
6.4.1 Figure 4.1. Graphical depiction of method to define individual slope in the presence of a downward inflection point (Figure 4.1a) and in the absence of a downward inflection point (Figure 4.1b).

Figure 4.1a. Graphical depiction of approach to identify downward inflection points with corresponding parameters from model. Figure 1b. Graphical depiction of simple linear (slope-intercept) decline since the identified inflection point was not downward, as indicated by thick discontinuous line. Open circles (○) represent observed eGFR, open diamonds (◊) represent eligible IP, solid diamonds (♦) represent identified IP from the model minimizing residual variance, solid lines represent final model.

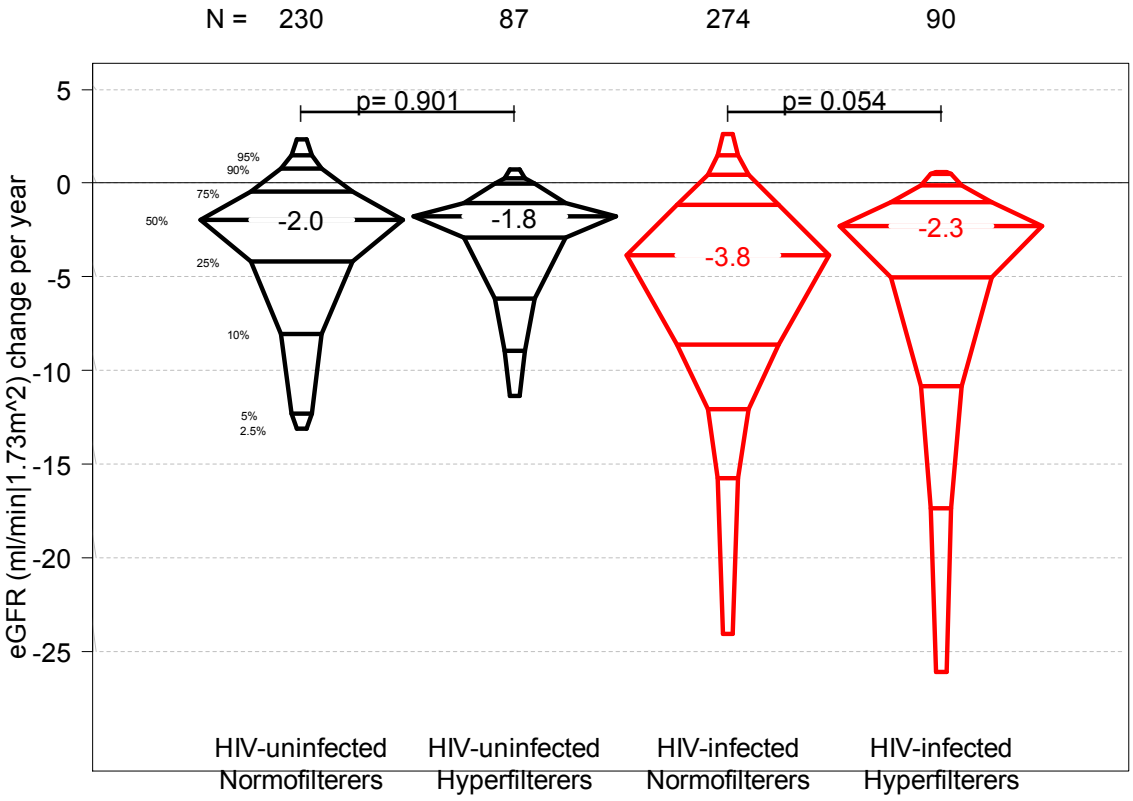


6.4.2 *Figure 4.2. Estimation of 50th and 90th percentiles of eGFR among HIV-uninfected men, stratified by race.*

Distributions (50th and 90th percentiles) of eGFR among HIV-uninfected black (n= 3482 observations contributed by 290 men) and non-black men (n= 15457 observations contributed by 1083 men). Lines are based on quantile regression estimating the 50th and 90th percentiles of eGFR on age after 30 years; dots depict medians and 90th percentiles for 5 year age bins.

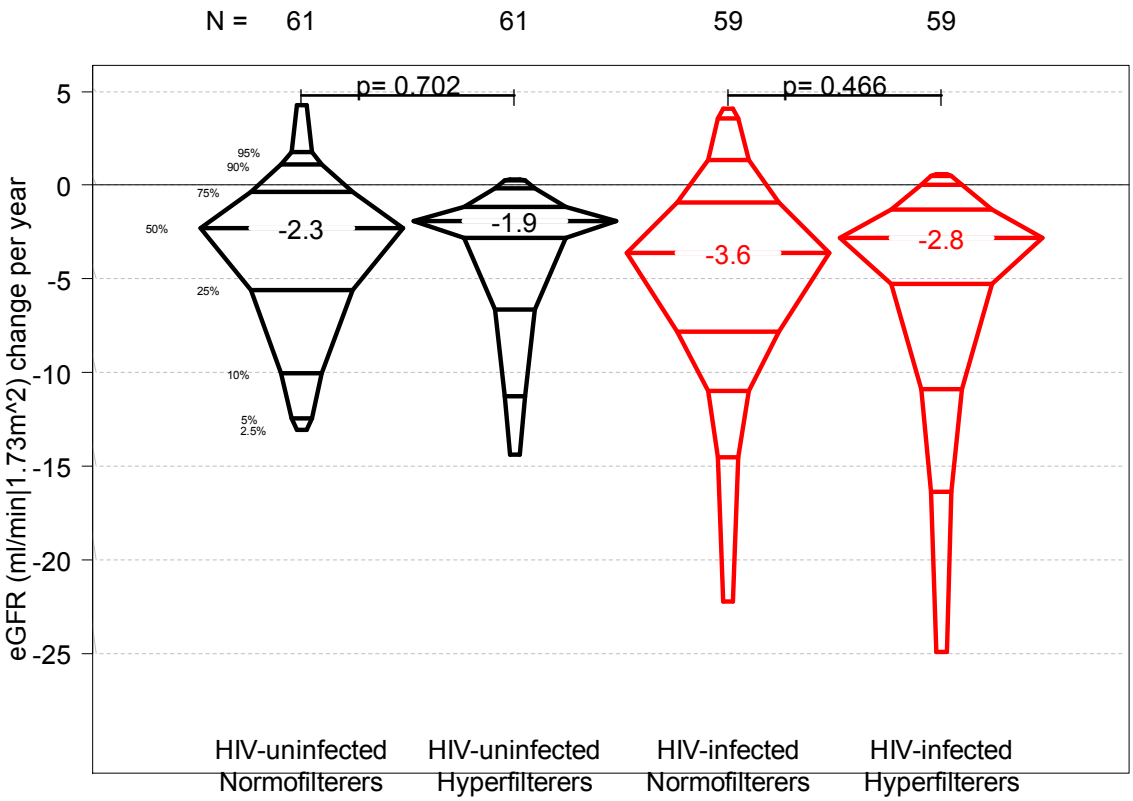


6.4.3 Figure 4.3. Distributions of changes in eGFR based on identified IP or single slope models, by HIV-infection and filtration status.



6.4.4 *Figure 4.4. Distributions of changes in eGFR based on identified IP or single slope models, by HIV-infection and filtration status based on a one-to-one matching design as a sensitivity analysis.*

The normofilterers comprise unique subjects, individually matched to the hyperfilterers by age, race, infection status, cohort enrollment and hypertensive status.



6.4.5 Table 4.1. Descriptive statistics of demographic, clinical and longitudinal data of MACS men, stratified by HIV and filtration status, based on matching study design. Median [IQR] and % (n).

Variable	*HIV-uninfected, normofilterers n= 230*	*HIV-uninfected, hyperfilterers n= 87*	*P-value*	*HIV-infected, normofilterers n= 274*	*HIV-infected, hyperfilterers n= 90*	*P-value*
At time of index (hyperfiltration or match)						
Age, years	52 [44.6, 57.9]	54.8 [44.6, 61.9]	0.377	46.7 [40, 50.9]	46.8 [38.8, 51.1]	0.709
Black race	21.3% (49)	24.1% (21)	NA	39.1% (107)	42.2% (38)	NA
Post-2001 cohort	34.3% (79)	29.9% (26)	NA	60.6% (166)	60% (54)	NA
eGFR, ml/min|1.73m^2	95.0 [91.0, 99.8]	107.6 [100.9, 118.7]	0.101	98.0 [91.2, 104.6]	116.7 [111.6, 123]	**0.002**
Body mass index, kg/m^2	25.1 [22.9, 29]	26.8 [23.9, 30.4]	0.313	24.8 [23, 27.2]	24.3 [21.9, 27]	0.902
Obese	19.1% (41)	26.3% (21)	0.197	9.1% (23)	8% (6)	0.828
HDL, mg/dL	48.8 [40, 57.2]	50.6 [40.5, 58.7]	0.353	43.7 [38, 50]	47.3 [39.7, 53.5]	0.157
LDL, mg/dL	117.5 [97, 139]	112.5 [96, 135]	0.913	109 [86, 132]	100 [83, 122.5]	0.180
Dyslipidemia	72% (144)	74.4% (58)	0.468	79.3% (180)	75% (54)	0.645
Fasting glucose, mg/dL	95 [89, 106]	98 [92, 104]	0.835	96 [89, 105]	97 [87, 104]	0.728
Fasting glucose > 100 mg/dL	35.7% (65)	39.2% (29)	0.642	38.4% (71)	39.4% (26)	0.326
Diagnosed diabetes	12.1% (24)	10.1% (8)	0.816	11.6% (24)	11.8% (8)	0.591
Metabolic syndrome	23.6% (50)	27.2% (22)	0.626	24.1% (55)	22.9% (16)	0.648
SBP, mmHg	123 [115, 131]	126 [116, 132]	0.552	124 [114, 132]	120 [113, 130.5]	0.477
DBP, mmHg	77 [72, 84]	76 [71, 80]	0.062	78 [71, 84]	74 [68, 80]	**0.004**
Uncontrolled hypertension	14.9% (32)	13.9% (11)	NA	14.6% (38)	11.8% (9)	NA

Variable	*HIV-uninfected, normofilterers n= 230*	*HIV-uninfected, hyperfilterers n= 87*	*P-value*	*HIV-infected, normofilterers n= 274*	*HIV-infected, hyperfilterers n= 90*	*P-value*
Current smoker	22.6% (51)	27.6% (24)	0.299	37% (101)	43.2% (38)	0.333
Stimulant use (cocaine, methamphetamines)	17.9% (40)	7.3% (6)	**0.037**	26.9% (73)	18.4% (16)	0.208
Up to 5 years prior to index (hyperfiltration or match)						
Number of observations	3 [2, 7]	7 [4, 9]	**<.001**	4 [2, 7]	6 [3, 8]	**0.013**
Time observed, years	2 [1, 3.8]	4 [2.6, 4.6]	**<.001**	2.5 [1.3, 4.3]	3.4 [1.5, 4.5]	**0.004**
Mean GFR	96.3 [93.7, 99.6]	102.7 [96.8, 109.6]	**<.001**	101.4 [96.7, 105.6]	110.1 [105.6, 116.1]	**<.001**
Mean fasting glucose	96 [90, 104]	95 [90, 102]	0.734	95 [88, 103]	96 [91, 104]	0.796
Mean SBP	123 [118, 132]	126 [117, 131]	0.619	125 [117, 132]	120 [113, 129]	0.057
Mean DBP	80 [74, 84]	77 [71, 82]	0.190	80 [75, 83]	75 [69, 80]	**0.002**
Up to 5 years after index (hyperfiltration or match)						
Number of observations	9 [7, 9]	8 [5, 9]	**0.001**	9 [8, 10]	8 [6, 9]	**<.001**
Time observed, years	4.6 [4.4, 4.9]	4.5 [3, 4.8]	**<.001**	4.6 [4.4, 4.8]	4.5 [3.9, 4.8]	**0.001**
Mean GFR	91.1 [86.9, 93.7]	102.2 [96.2, 109.5]	**<.001**	91.0 [85.2, 96.1]	108.4 [102.1, 114.7]	**<.001**
Mean fasting glucose	97 [89, 107]	95 [89, 100]	0.644	97 [91, 104]	97 [91, 105]	0.957
Mean SBP	126 [119, 134]	127 [118, 134]	0.292	127 [119, 134]	124 [115, 131]	**0.019**
Mean DBP	78 [72, 83]	76 [72, 82]	0.342	79 [74, 84]	76 [70, 80]	**<.001**

6.4.6 *Table 4.2. HIV- and therapy related characteristics among HIV-infected subjects with normofiltration and hyperfiltration.*

Variable	*HIV-infected, Normofilterers n= 274*	*HIV-infected, Hyperfilterers n= 90*	*P-value*
At time of index (hyperfiltration or match)			
CD4+ cell count	532 [383, 753]	504 [324, 685]	0.184
CD4+ cell count < 350	21.4% (57)	30.2% (26)	**0.028**
Nadir CD4+ cell count	292 [219, 392]	294 [170, 410]	0.140
Detectable viral load	33.3% (90)	37.9% (33)	0.376
Previous AIDS diagnosis	11.3% (31)	10.0% (9)	0.776
Any ART prior to HAART	47.8% (131)	51.1% (46)	0.602
Time since ART initiation	7.1 [3.9, 13.9]	9.4 [5.8, 18.1]	**0.014**
Time since HAART initiation	5.6 [2.1, 8.0]	6.5 [3.9, 9.3]	**0.011**
Up to 5 years prior to index			
Mean CD4+ cell count	533 [384, 731]	485 [370, 703]	0.158
Mean CD4+ cell count < 350	21.7% (48)	22.2% (20)	0.456
Any detectable viral load	55.2% (122)	61.1% (55)	0.056
Up to 5 years after index			
Mean CD4+ cell count	564 [431, 738]	598 [360, 749]	0.526
Mean CD4+ cell count < 350	17.1% (46)	21.8% (19)	0.130
Any detectable viral load	52.8% (142)	47.1% (41)	0.518
First AIDS diagnosis	6.2% (17)	2.2% (2)	0.409

6.4.7 Table 4.3. Unadjusted and adjusted mean GFR change (ml/min|1.73m^2 per year) by HIV-infection and filtration status from linear regression using subject-specific slopes as the outcome.

Covariates included for adjustment were based on the mean levels 5 years prior to the hyperfiltration event or match (fasting glucose, systolic blood pressure, and diastolic blood pressure); and time since antiretroviral therapy initiation was at the time of index visit (hyperfiltration event or match). Estimated adjusted means from linear regression models accounting for repeated subjects using generalized estimating equations (GEE) are presented for a reference subject described in footnote.

Mean GFR (ml/min|1.73m^2) change per year	*HIV-uninfected, normofiltration n= 230*	*HIV-uninfected, hyperfiltration n= 87*	*HIV-infected, normofiltration n= 274*	*HIV-infected, hyperfiltration n= 90*
Unadjusted	-3.01	-2.81	-5.33	-4.60
(95%CI)	(-3.82, -2.19)	(-3.69, -1.93)	(-6.33, -4.33)	(-6.01, -3.20)
p-value	Reference	0.749	Reference	0.410
Adjusted for glucose levels 5 years prior to index[a]	-3.26	-2.84	-5.89	-4.84
(95%CI)	(-4.23, -2.28)	(-3.70, -1.97)	(-7.08, -4.70)	(-6.24, -3.43)
p-value	Reference	0.526	Reference	0.243
Adjusted for glucose levels, SBP, DBP 5 years prior to index[b]	-2.82	-2.16	-6.07	-4.91
(95%CI)	(-3.72, -1.91)	(-3.59, -0.74)	(-7.46, -4.69)	(-6.52, -3.29)
p-value	Reference	0.396	Reference	0.215
Adjusted for glucose levels, SBP, DBP and time since ART initiation [c]	NA	NA	-5.89	-4.60
(95%CI)			(-7.13, -4.65)	(-6.19, -3.01)
p-value			Reference	0.178

[a] For reference individual with mean fasting blood glucose equal to 100 mg/dL over 5 years prior to index.

[b] For reference individual with mean fasting blood glucose equal to 100 mg/dL, SBP equal to 120 mmHg and DBP equal to 80 mmHg over 5 years prior to index.

[c] For reference individual with mean fasting blood glucose equal to 100 mg/dL, SBP equal to 120 mmHg, DBP equal to 80 mmHg over 5 years prior to index, and 7 years since ART initiation at index.

6.4.8 Table 4.4. Unadjusted and adjusted mean GFR change (ml/min|1.73m^2 per year) by HIV-infection and filtration status from linear regression using subject-specific slopes as the outcome based on a one-to-one matched design as a sensitivity analysis.

Covariates included for adjustment were based on the mean levels 5 years prior to the hyperfiltration event or match (fasting glucose, systolic blood pressure, and diastolic blood pressure); and time since antiretroviral therapy initiation was at the time of index visit (hyperfiltration event or match). Estimated adjusted means from linear regression models using generalized estimating equations (GEE) are presented for a reference subject described in footnote. GEE was used to account for within-pair correlations.

Mean GFR (ml/min|1.73m^2) change per year	*HIV-uninfected, normofiltration n= 61*	*HIV-uninfected, hyperfiltration n= 61*	*HIV-infected, normofiltration n= 59*	*HIV-infected, hyperfiltration n= 59*
Unadjusted	-3.25	-3.11	-5.02	-4.58
(95%CI)	(-4.38, -2.12)	(-4.29, -1.93)	(-6.86, -3.17)	(-6.13, -3.04)
p-value	Reference	0.863	Reference	0.668
Adjusted for glucose levels 5 years prior to index[a]	-3.62	-3.11	-6.02	-4.70
(95%CI)	(-5.17, -2.06)	(-4.27, -1.94)	(-8.40, -3.65)	(-6.26, -3.15)
p-value	Reference	0.593	Reference	0.234
Adjusted for glucose levels, SBP, DBP 5 years prior to index[b]	-3.35	-2.76	-5.85	-4.32
(95%CI)	(-4.90, -1.80)	(-4.07, -1.45)	(-8.47, -3.23)	(-6.05, -2.58)
p-value	Reference	0.552	Reference	0.299
Adjusted for glucose levels, SBP, DBP and time since ART initiation [c]	NA	NA	-5.91	-4.40
(95%CI)			(-8.65, -3.18)	(-5.96, -2.83)
p-value			Reference	0.233

[a] For reference individual with mean fasting blood glucose equal to 100 mg/dL over 5 years prior to index.

[b] For reference individual with mean fasting blood glucose equal to 100 mg/dL, SBP equal to 120 mmHg and DBP equal to 80 mmHg over 5 years prior to index.

[c] For reference individual with mean fasting blood glucose equal to 100 mg/dL, SBP equal to 120 mmHg, DBP equal to 80 mmHg over 5 years prior to index, and 7 years since ART initiation at index.

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Updated October 21, 2014

8 CURRICULUM VITAE

Derek Kai Sing Ng, ScM

PERSONAL DATA

Department of Epidemiology

Johns Hopkins Bloomberg School of Public Health

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Baltimore, MD 21205

Phone: 410-614-5112

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Dual American/Canadian Citizenship

EDUCATION AND TRAINING

2014 Candidate for Doctor of Philosophy, Epidemiology

Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

Advisor: Lisa P. Jacobson, ScD

Thesis: The epidemiology of glomerular hyperfiltration among men with HIV in the era of highly active antiretroviral therapy

Pre-doctoral trainee: Epidemiology and Biostatistics of Aging T32AG000247

2009 Master of Science, Epidemiology

Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

2005 Bachelor of Arts with Distinction, Psychology

Yale University, New Haven, CT

PROFESSIONAL EXPERIENCE

2011 - present	**Sr. Biostatistician**, Part-time Johns Hopkins Bloomberg School of Public Health Department of Epidemiology, STATEPI group Baltimore, MD
2009 - 2011	**Sr. Biostatistician**, Full-time Johns Hopkins Bloomberg School of Public Health Department of Epidemiology, STATEPI group
2008 - 2009	**Student research associate** Johns Hopkins Bloomberg School of Public Health Welch Center for Epidemiology and Clinical Research
2007 - 2008	**Research assistant** Johns Hopkins Bloomberg School of Public Health Cochrane Eyes and Vision Group US Project
2005 - 2007	**Junior associate** PinneyAssociates, Inc. Health consulting and risk management Pittsburgh, PA
2004 - 2005	**Research assistant** Department of Molecular Psychiatry and Neuroscience Yale School of Medicine, New Haven, CT
2004 - 2005	**Research assistant** Yale Center for the Advancement of Perioperative Health Yale-New Haven Hospital
2004	**Intern, program coordinator** Bridgehaven Mental Health Services Louisville, KY

2003	**Research Assistant** Developmental Neuroimaging Lab Yale School of Medicine

PROFESSIONAL ACTIVITIES

2007 - 2008	**Society of Epidemiologic Research** Program co-chair of Student Caucus Annual meeting, San Diego, CA
2008 - 2009	**Epidemiology Student Organization** Masters Student Representative Johns Hopkins Bloomberg School of Public Health

EDITORIAL ACTIVITIES

Peer Review

2013	*American Journal of Kidney Disease*
2013	*Journal of Hospital Medicine*

AWARDS

2008	Johns Hopkins Bloomberg School of Public Health Department of Epidemiology Master's Tuition Scholarship
2005	Yale Silliman Cup for college service

PUBLICATIONS

Journal Articles (Peer-reviewed)

1. Caldwell-Andrews AA, Kain ZN, Mayes LC, Kerns RD, **Ng D**. Motivation and maternal presence during induction of anesthesia. *Anesthesiology*. 2005 Sep;103(3):478-83. PMID: 16129970

2. Egner PA, Chen JG, Wang JB, Wu Y, Sun Y, Lu JH, Zhu J, Zhang YH, Chen YS, Friesen MD, Jacobson LP, Muñoz A, **Ng D**, Qian GS, Zhu YR, Chen TY, Botting NP, Zhang Q, Fahey JW, Talalay P, Groopman JD, Kensler TW. Bioavailability of Sulforaphane from two broccoli sprout beverages: results of a short-term, cross-over clinical trial in Qidong, China. *Cancer Prevention Research*. 2011 Mar;4(3):384-95. PMID: 21372038 PMCID: PMC3076202

3. **Ng DK**, Schwartz GJ, Jacobson LP, Palella FJ, Margolick JB, Warady BA, Furth SL, Muñoz A. Universal GFR determination based on two time points during plasma iohexol disappearance. *Kidney International*. 2011 Aug;80(4):423-30. PMID: 21654718 PMCID: PMC3146568

4. Dodson JL, Jerry-Fluker J, **Ng DK**, Moxey-Mims M, Schwartz GJ, Dharnidharka VR, Warady BA, Furth SL. Urological disorders in chronic kidney disease in children cohort: clinical characteristics and estimation of glomerular filtration rate. *Journal of Urology.* 2011 Oct;186(4):1460-6. PMID: 21855938

5. Ladha KS, Young JH, **Ng DK**, Efron DT, Haider AH. Factors affecting the likelihood of presentation to the emergency department of trauma patients after discharge. *Annals of Emergency Medicine*. 2011 Nov;58(5):431-7. PMID: 21689864

6. Kensler TW, **Ng DK**, Carmella SG, Chen M, Jacobson LP, Muñoz A, Egner PA, Chen JG, Qian GS, Chen TY, Fahey JW, Talalay P, Groopman JD, Yuan JM, Hecht SS. Modulation of the metabolism of airborne pollutants by glucoraphanin-rich and sulforaphane-rich broccoli sprout beverages in Qidong, China. *Carcinogenesis*. 2012 Jan;33(1):101-7. PMID: 22045030

7. Samuels J, **Ng D**, Flynn JT, Mitsnefes M, Poffenbarger T, Warady BA, Furth S. Ambulatory blood pressure patterns in children with chronic kidney disease. *Hypertension.* 2012 Jul;60(1):43-50. PMID: 22585950

8. **Ng DK**, Brotman D, Lau B, Young JH. Insurance status, not race, is associated with mortality after an acute cardiovascular event in Maryland. *Journal of General Internal Medicine* 2012 Oct; 27(10):1368-76. PMID: 22821570

9. Omoloja A, Jerry-Fluker J, **Ng DK**, Abraham AG, Furth S, Warady BA, Mitsnefes M. Secondhand smoke exposure is associated with proteinuria in children with chronic kidney disease. *Pediatric Nephrology*, 2013 Aug;28(8):1243-51. PMID: 23584848.

10. Hidalgo G, **Ng DK**, Moxey-Mims M, Minnick ML, Blydt-Hansen T, Warady BA, Furth S. Association of income level with kidney disease severity and progression among children with CKD: A report from the Chronic Kidney Disease in Children study. *American Journal of Kidney Disease*, 2013 Dec;62(6):1087-94. PMID: 23932090

11. Chen JG, Egner PA, **Ng D**, Jacobson LP, Muñoz A, Zhu YR, Qian GS, Wu F, Yuan JM, Groopman JD, Kensler TW. Reduced aflatoxin exposure presages decline in liver cancer mortality in an endemic region of China. *Cancer Prevention Research*, 2013 Oct;6(10):1038-45. PMID: 23963804

12. **Ng DK**, Jacobson LP, Brown TT, Palella FJ, Martinson JJ, Bolan R, Miller ER 3rd, Schwartz GJ, Abraham AG, Estrella MM. HIV therapy, metabolic and cardiovascular health are associated with glomerular hyperfiltration in the Multicenter AIDS Cohort Study. *AIDS*, 2014 Jan 28;28(3):377-86. PMID: 24670523 PMCID: PMC3972628

13. Flynn JT, **Ng DK**, Chan GJ, Samuels J, Furth S, Warady B, Greenbaum LA. The effect of abnormal birth history on ambulatory blood pressure and disease progression in children with chronic kidney disease. *The Journal of Pediatrics*, 2014 Mar 31. pii: S0022-3476(14)00191-7. PMID: 24698454

14. Barletta GM, Flynn J, Mitsnefes M, Samuels J, Friedman LA, **Ng DK,** Cox C, Poffenbarger T, Warady B, Furth S. Heart rate and blood pressure variability in children with chronic kidney disease: A report from the CKiD Study. *Pediatric Nephrology,* 2014 Jun;29(6):1059-65. PMID: 24488505

15. Egner PA, Chen JG, Zarth AT, **Ng DK** [co-first author], Kensler KH, Jacobson LP, Muñoz A, Johnson JL, Groopman JD, Fahey JW, Talalay P, Zhu J, Chen TY, Quan GS, Carmella SG, Hecht SS, Kensler TW. Rapid and sustainable detoxication of airborne pollutants by broccoli sprout beverage: results of a randomized clinical trial in China. *Cancer Prevention Research.* OnlineFirst, June 9, 2014. PMID: 24913818

CURRICULUM VITAE

Derek Kai Sing Ng

PART II

TEACHING

2014-15	**Lecturer**
1st term	Advanced Methods in the Design and Analysis of Cohort Studies (340.728)
	Lecture: Graphical Methods in Epidemiology - Enhanced Bland-Altman Graph: Assessing Agreement of Two Measures
	Primary instructors: Alvaro Munoz, Christopher Cox
2011-12	**Teaching Assistant**
4th term	Methodologic Challenges in Epidemiologic Research (340.754)
	Co-TA: Kara Rudolph
	Primary instructors: Tom Glass, Danielle Fallin, Bryan Lau

RESEARCH GRANT PARTICIPATION

2009 - present	**Sr. Biostatistician**, Chronic Kidney Disease in Children Cohort Study Data Management and Analysis Center
	PI: Alvaro Muñoz, PhD
2009 - present	**Sr. Biostatistician**, Chemo/Dietary Prevention in Dept. of Environmental Health Sciences
	PI: Thomas Kensler, PhD
2012 - present	**Student researcher**, Center for the Analysis and Management of the Multicenter Aids Cohort Study
	PI: Lisa P. Jacobson, ScD
2009 - 2011	**Sr. Biostatistician**, Cardiac Clinical Imaging at the Johns Hopkins School of Medicine
	PIs: Joao Lima, MD; Christopher Cox, PhD

PRESENTATIONS

Scientific meetings

1. **Ng DK**, Young JH. Insurance as a mediator of ethnic mortality differences in a cohort of patients with CVD, 1993-2007. Oral presentation for the Society for Epidemiologic Research Annual Meeting, Anaheim, CA, June 24, 2009.

2. Hidalgo G, Furth SL, **Ng DK**, White CT. Family Socioeconomic Status (SES) and Disease Severity in Children with CKD: Results from the Chronic Kidney Disease in Children (CKiD) Cohort Study. Poster presented for American Society of Nephrology Annual Meeting, Denver, CO, November 20, 2010.

3. Omoloja A, Jerry-Fluker J, Abraham AG, **Ng DK**, Warady BA, Furth SL, Mitsnefes M. Urine Cotinine in Adolescents with Chronic Kidney Disease: A Report from the Chronic Kidney Disease in Children (CKiD) Cohort Study. Poster presented for American Society of Nephrology Annual Meeting, Denver, CO, November 20, 2010.

4. Kupferman JC, **Ng DK**, Flynn JT, Furth SL, Warady BA, Mitsnefes M. Longitudinal Relationship between Blood Pressure and Left Ventricular Mass in Children with Chronic Kidney Disease. Poster presented for American Society of Nephrology Annual Meeting, Philadelphia, PA, November 10, 2011.

5. Barletta GM, Flynn JT, Mitsnefes M, **Ng DK**, Warady BA, Samuels JA, Poffenbarger T, Furth SL. Heart Rate and Blood Pressure Variability in Children with Chronic Kidney Disease: Report from CKiD Study. Poster presented for American Society of Nephrology Annual Meeting, Philadelphia, PA, November 10, 2011.

6. Hidalgo G, **Ng DK**, Warady BA, Moxey-Mims MM, Furth SL. Family Income and Type of Renal Replacement Therapy (RRT) in Children with Chronic Kidney Disease (CKD). Results from the CKiD Study. Poster presented for American Society of Nephrology Annual Meeting, Philadelphia, PA, November 10, 2011.

7. Egner PA, Chen JG, **Ng DK**, Jacobson LP, Muñoz A, Lu JH, Zhu YR, Qian GS, Chen TY, Wu F, Wang JM, Groopman JD, Kensler TW. Reduction in aflatoxin exposure drives decrease in primary liver cancer in Qidong, China. Poster presented for American Association for Cancer Research Annual Meeting, Washington, DC, April 6, 2013.

Invited seminars and lectures

1. *Ratio of fast to slow areas of iohexol disappearance curves as a function of slow GFR*, CKiD Steering Committee Meeting, Baltimore MD, October 2, 2009.

2. *Congruence of CKiD and MACS data for estimation of 2-compartment GFR from 1-compartment GFR*. Statistics in Epidemiology Research Meeting, Baltimore, MD, October 27, 2009.

3. *Update on local and central serum creatinine measurements.* CKiD Steering Committee Meeting, Atlanta, GA, April 22, 2010.

4. *MACS equations for estimation of GFR*. Multicenter AIDS Cohort Study annual meeting, Rockville, MD, May 5, 2010.

5. *Bioavailability of sulforaphane from two forms of broccoli sprout beverages: results of a short-term, cross-over clinical trial in Qidong, People's Republic of China.* Statistics in Epidemiology Research Meeting, Baltimore, MD, August 10, 2010.

6. *Uncovering the utility of the point at 240 minutes in a 3-point iohexol GFR protocol.* CKiD Steering Committee Meeting, Baltimore, MD, January 14, 2011.

7. *Family socioeconomic status and disease severity in children with CKD.* CKiD Steering Committee Meeting, Baltimore, MD, January 14, 2011.

8. *Description of CKiD 2-point protocol studies.* CKiD Steering Committee Meeting, Dallas, TX, April 14, 2011.

9. *Measurement of kidney function and methods to characterize its decline: Results from CKiD and MACS.* Co-presented with Alvaro Muñoz, PhD and Christopher B. Pierce, MHS. Department of Epidemiology Seminar Series, October 17, 2011.

10. *Monitoring of CKiD 2-point protocol studies.* CKiD Steering Committee Meeting, Arlington, VA, January 23, 2012.

11. *Bland-Altman to the "Not-so-bland" Bland-Altman: Understanding agreement through novel refinements of the Bland-Altman in clinical use and cohort studies.*

General Epidemiology and Methods Journal Club, Baltimore, MD, February 14, 2012.

12. *"Matching methods for causal inference: a review and a look forward"; Applications in cohort studies*. General Epidemiology and Methods Journal Club, Baltimore, MD, October 16, 2012.

13. *The epidemiology of renal hyperfiltration in men with HIV*. Statistics in Epidemiology Research Meeting, Baltimore, MD, November 13, 2012.

14. *The epidemiology of renal hyperfiltration in men with HIV*. Department of Epidemiology Seminar Series, Baltimore, MD, November 28, 2012.

15. *Preliminary results of renal hyperfiltration in the MACS*. Multicenter AIDS Cohort Study semi-annual meeting: Renal Working Group, Baltimore, MD, December 6, 2012.

16. *Risk factors for glomerular hyperfiltration in the MACS.* Statistics in Epidemiology Research Meeting, February 12, 2013.

17. *Renal hyperfiltration in men with HIV*. Renal Disease Interest Group, Baltimore, MD, February 13, 2013.

18. *Propensity scores and inverse probability weighting methods in CKiD*. CKiD Steering Committee Meeting, Phoenix, AZ, April 25, 2013.

19. *Incidence of glomerular hyperfiltration in the MACS*. Multicenter AIDS Cohort Study semi-annual meeting: Renal Working Group, Pittsburgh, PA, November 6, 2013.

20. *Elevated eGFR and chronic glomerular hyperfiltration in the Multicenter AIDS Cohort Study.* Epidemiology and Biostatistics of Aging, Research in progress seminar series, January 27, 2014.

21. *Natural history of glomerular hyperfiltration*. Multicenter AIDS Cohort Study annual meeting: Renal Working Group, Bethesda, MD, May 21, 2014.

22. *Birth history and blood pressure in CKiD: application of propensity score matching methods.* Chronic Kidney Disease in Children Observational Study Monitoring Board (OSMB) meeting, Bethesda, MD, June 18, 2014.