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Methods

Study design and participants

Data for the current analysis come from a cohort study that aimed to assess the effects of HIV, hepatitis C virus (HCV), substance use, and other factors on trajectories of kidney and cardiovascular disease markers. Participants were recruited from the Johns Hopkins Infectious Diseases clinic, other research studies, and through newspaper ads. We enrolled 292 participants (192 HIV-positive and 100 HIV-negative) in the original recruitment period (October 2010 to July 2012), and enrolled an additional 103 participants (45 HIV-positive and 58 HIV-negative) in a second recruitment period (December 2015 to October 2018). In the second recruitment period, we only enrolled HCV-positive participants with and without HIV. Inclusion criteria for the study included age 18 years or older, estimated GFR ≥ 60 mL/min/1.73m^2 by the Modification of Diet in Renal Disease equation (this cutoff was revised to ≥ 45 mL/min/1.73m2 in the second recruitment). Exclusion criteria included diabetes mellitus, history of radiocontrast allergy, insufficient venous access to place two peripheral intravenous catheters, pregnancy, uncontrolled blood pressure, collagen vascular disease, or severe or life threating comorbid conditions. Once enrolled, participants were followed with research visits approximately annually. Among all participants recruited, 207 and 120 were HIV-positive and HIV-negative AAs, respectively. In this analysis, we included only observations in which the participant had valid same day measures of GFR by iohexol disappearance from plasma (iGFR), serum creatinine, and serum cystatin C.

Participants and laboratory methods

At study visits, demographic, behavioral, and pharmacologic data were collected by interview and medical record review. To measure iGFR, we placed two peripheral intravenous catheters and infused a weighted dose of iohexol (5 mL: GE Healthcare, Amersham Division, Princeton, NJ) into one of the catheters. We drew blood samples from the second catheter at approximately 10, 30, 120, and 240 minutes, with actual values recorded. We measured plasma concentrations of iohexol from the timed samples by high performance liquid chromatography, and iGFR was calculated with a 2-compartment model described by Schwartz and colleagues. Iohexol GFR values prior to September 2014 were re-calibrated to account for a drift in iohexol measurements during this period that was described previously. Researchers reviewed graphical displays of iohexol plasma concentrations by time in each iGFR study for quality control and to identify findings that were inconsistent with the expected GFR-mediated disappearance of iohexol from plasma.

Additional measures included HIV serostatus, HCV serostatus, HIV RNA, HCV RNA, serum creatinine, cystatin C, glycosylated hemoglobin, and high sensitivity C-reactive protein (hsCRP). We measured serum creatinine with an enzymatic assay (Creatinine Plus, Roche Diagnostics, Basel, Switzerland) that was traceable to an isotope dilution mass spectrometry reference method. We measured cystatin C using a particle-enhanced turbidimetric immunoassay (Gentian, AS, Norway), with values standardized to certified reference material. We used flow cytometry to measure activated cluster of differentiation (CD)8+ lymphocytes, defined as CD8+ cells coexpressing CD38 and human leukocyte antigen DR isotope (HLA-DR) surface markers. HIV RNA was measured with the Amplicor HIV-1 MONITOR Test v1.5 (Roche Molecular Diagnostics, Pleasanton, CA) or Real Time HIV-1 (Abbott Laboratories, Abbott Park, IL). In a subset of study visits that occurred after January 2016, we measured total body mass, lean mass, and fat mass (all in kg) using DXA (Hologic, Horizon-W, Bedford, MA).

Definitions

We derived estimates of GFR (eGFRcr and eGFRcr-cys) from the CKD-EPI equations[1]. For each equation, we calculated eGFR both retaining the race calibration factor (standard equation) and omitting the race factor. We defined body mass index (BMI) as mass (kilograms) divided by height (meters) squared. We defined suppressed viral load as HIV RNA <400 copies/mL. HCV-infection was defined as positive HCV serology with a detectable HCV RNA at cohort enrollment. Albuminuria was defined as a urine albumin-creatinine ratio > 30mg/g. We defined bias as the difference between eGFR and iGFR, with units in mL/min/1.73m^2. Positive bias values correspond to overestimation of iGFR by eGFR and negative bias values correspond to underestimation of iGFR by eGFR. We defined accuracy as a binary indicator of whether eGFR was within ±30% of iGFR.

Statistics

We used Fisher's exact and Wilcoxon rank sum tests to compare categorical and continuous variables, respectively, between HIV-positive and HIV-negative participants. Analyses were stratified by HIV status. For each equation (eGFRcr and eGFRcr-cys), we compared accuracy and bias (relative to iGFR) of estimates in which the race calibration factor was retained or omitted. We used multilevel mixed models (logistic for accuracy and linear for bias), which allowed efficient use of all observations while accounting for the within-visit linked structure of the data (i.e., multiple estimates of GFR bias or accuracy in the same participant at the same visit) and repeated observations in the same individuals over multiple visits, assuming a random intercept for multiple measures in a visit nested within individuals over multiple

visits. Next, we explored whether participant demographic and clinical characteristics were differentially associated with the accuracy and bias of eGFRcr estimates in which the race calibration factor was retained or omitted. Time-invariant covariates included sex, race, smoking status, HCV infection, hypertension history, blood pressure, albuminuria, nadir CD4 count, and baseline CD4 count. Although some of these variables could vary over time, only baseline measurements were included in models. Time-varying covariates, which were updated at each visit, included age, iGFR, eGFRcr, eGFRcr-cys, hsCRP, percentage activated CD8+ lymphocytes, and HIV RNA. The time-varying dependent variables were analyzed either as a linear (bias) or a logistic (accuracy) model, adding a random effect (linear or log-odds intercepts, respectively) to account for non-independence of the data within participants. In HIV-positive participants, we compared the ability of the eGFRcr equation with race term retained or omitted to categorize participants at two clinically relevant {, 2013 #3061} iGFR breakpoints (60 and 90 mL/min/1.73m2) by calculating area under the receiver operating characteristic (ROC), in which sensitivity is plotted against 1 – specificity. The area under the ROC curve varies between 0.5 and 1.0, with higher values representing better classification.

In exploratory analyses that were restricted to a subset of participant observations in which DXA measurements were done, we assessed for relationships between eGFRcr bias and measures of body composition (BMI, total body mass, fat mass, and lean mass) using scatterplots, Spearman rank correlation coefficients (ρ), and linear regression. Next, we assessed whether HIV and HCV infection status were associated with lean mass in a linear regression model that controlled for sex, age, and fat mass.

The study was approved by the Johns Hopkins University School of Medicine Institutional Review Board, and participants provided written informed consent.

Supplement Table 1. Baseline characteristics of African American cohort participants enrolled in Baltimore, Maryland, 2010 to 2018.

Characteristic[1]	HIV-positive (n=207)	HIV-negative (n=120)	P value
Women	72 (35)	25 (21)	0.008
Age (years)	49 (45, 54)	50 (45, 55)	0.45
Body mass index, kg/m^2	25.1 (22.2, 30.7)	26.1 (23.3, 31.1)	0.079
Smoking status			0.72
Never smoker	48 (23)	27 (23)	0.98
Quit smoking >6 months prior	23 (11)	13 (11)	
Current smoker	136 (66)	80 (67)	
Hepatitis C infected	112 (54)	47 (39)	0.011
Hypertension history	79 (38)	35 (29)	0.12
Systolic blood pressure, mm Hg	122 (110, 132)	126 (114, 135)	0.037
Diastolic blood pressure, mm Hg	71 (65, 80)	73 (67, 82)	0.12
Glycosylated hemoglobin, %	5.4 (5.2, 5.7)	5.6 (5.3, 5.8)	0.024
Urine albumin-creatinine ratio > 30mg/g	38 (18)	12 (10)	0.055
Serum creatinine, mg/dL	0.9 (0.8, 1.1)	1.0 (0.8, 1.1)	0.57
Serum cystatin C, mg/L	0.96 (0.83, 1.15)	0.89 (0.77, 1.05)	0.002
Glomerular filtration rate (mL/min/1.73m^2)			
iGFR	88 (74, 100)	93 (82, 106)	0.012
eGFRcr [race term retained]	101 (83, 117)	103 (92, 114)	0.44
eGFRcr [race term omitted]	87 (71, 101)	89 (80, 99)	0.44
eGFRcr-cys [race term retained]	93 (74, 107)	97 (86, 111)	0.007
eGFRcr-cys [race term omitted]	86 (69, 99)	90 (79, 103)	0.007
High sensitivity C-reactive protein, mg/L	1.6 (0.6, 3.9)	1.6 (0.6, 5.2)	0.50
Activated CD8+ lymphocytes[2], %	31 (20, 48)	11 (8, 19)	<0.001

Supplement Table 1. Baseline characteristics of African American cohort participants enrolled in Baltimore, Maryland, 2010 to 2018.

Characteristic[1]	HIV-positive (n=207)	HIV-negative (n=120)	P value
Taking antiretroviral therapy	189 (91)	-	-
Tenofovir disoproxil fumarate	126 (61)	-	-
Tenofovir alafenamide	14 (7)	-	-
Protease inhibitor	120 (58)	-	-
Non-nucleoside reverse transcriptase inhibitor[3]	68 (33)	-	-
Integrase strand transfer inhibitor[3]	32 (15)	-	-
Pharmacologic booster[3,4]	132 (64)	-	-
Nadir CD4 lymphocyte count, cells/mm^3	158 (52, 316)	-	-
Baseline CD4 lymphocyte count, cells/mm^3	464 (256, 627)	-	-
HIV RNA ≤400 copies/mL	166 (80)	-	-
Contributed ≥ 2 observations	172 (83)	90 (75)	0.085
Number of observations[5]	4 (3.5, 5)	4 (3, 5)	0.004
Follow-up time (years)[5]	4.7 (3.0, 5.7)	4.1 (3.0, 5.2)	0.012

iGFR, iohexol-based glomerular filtration rate (GFR) estimate; eGFRcr, eGFRcys, and eGFRcr-cys are GFR estimates derived from the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations based on serum concentrations of creatinine, cystatin C, and both markers, respectively. [1] CD, cluster of differentiation

[1] Categorical variables shown as frequency (%); continuous variables shown as median (25th percentile, 75th percentile)

[2] CD8+ lymphocytes expressing human leukocyte antigen (HLA)-DR and CD38

[3] The association between antiretroviral drugs shown to inhibit tubular secretion of creatinine (dolutegravir, cobicistat, and rilpivirine) were recently assessed and no statistically significant associations with accuracy or bias of eGFRcr were found. [7]

[4] Ritonavir or cobicistat used increase exposure to another antiretroviral drug

[5] Among participants with ≥ 2 observations

Supplement Table 2. Factors associated with the accuracy and bias of the Chronic Kidney Disease Epidemiology Collaboration (CKD EPI) creatinine-based glomerular filtration estimating equation, with and without an adjustment factor among HIV-negative African Americans

Factor[3] (n observations)	Accuracy[1], % (P value)		Bias, mL/min/1.73m^2 (P value)	
	eGFRcr	eGFRcr [no race adjustment]	eGFRcr	eGFRcr [no race adjustment]
Sex				
Female (84)	80	91	14.1	0.4
Male (295)	88 (0.144)	87 (0.31)	2.5 (<0.001)	-10.7 (<0.001)
Age[4], years				
≤ 49.5 (151)	83	87	4.2	-9.6
> 49.5 (228)	89 (0.22)	89 (0.61)	5.7 (0.50)	-7.3 (0.27)
Body mass index, kg/m^2				
≤ 25.7 (151)	86	87	4.2	-9.2
> 25.7 (228)	87 (0.93)	89 (0.68)	5.8 (0.54)	-7.5 (0.48)
iGFR[4], mL/min/1.73m^2				
< 90 (178)	77	91	13.1	0.4
≥ 90 (201)	97 (<0.001)	85 (0.104)	-2.1 (<0.001)	-16.0 (<0.001)
Hepatitis C status				
Not infected (278)	90	88	2.2	-10.8
Infected (101)	78 (0.017)	88 (0.98)	10.9 (0.001)	-2.9 (0.002)
hsCRP[4], mg/L				
≤ 1.7 (174)	86	89	5.1	-8.5
> 1.7 (205)	87 (0.99)	87 (0.51)	5.2 (0.96)	-7.9 (0.75)

Supplement Table 2. Factors associated with the accuracy and bias of the Chronic Kidney Disease Epidemiology Collaboration (CKD EPI) creatinine-based glomerular filtration estimating equation, with and without an adjustment factor among HIV-<u>negative</u> African Americans

Factor[3] (n observations)	**Accuracy[1], % (P value)**		**Bias, mL/min/1.73m^2 (P value)**	
	eGFRcr	**eGFRcr [no race adjustment]**	**eGFRcr**	**eGFRcr [no race adjustment]**
Activated CD8+ cells[4], %				
≤ 20.3 (292)	87	87	5.0	-8.3
> 20.3 (87)	86 (0.85)	91 (0.38)	5.5 (0.82)	-7.9 (0.87)

eGFRcr and eGFRcys are glomerular filtration rate estimates derived from the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations[1] based on serum concentrations of creatinine and cystatin C, respectively, CD, cluster of differentiation

[1] Accuray defined as percentage of equation estimates that are within ± 30% of iohexol-based glomerular filtration rate measure.

[2] Bias defined as the difference between the biomarker-estimated and iohexol-based glomerular filtration rate.

[3] Continuous factors are dichotomized at the median or at an established cutpoint.

[4] Time-varying factors.

Supplement Table 3. Cross-sectional accuracy and bias of biomarker-based estimating equations compared with glomerular filtration rate measured by iohexol disappearance from plasma in HIV-positive and HIV-negative African American participants.

Performance measure	HIV-positive (781 observations in 207 individuals)	HIV-negative (379 observations in 120 individuals)	P value (comparison by HIV status)
Accuracy[1], % (95% CI)			
1. eGFRcr	78 (76, 81)	88 (85, 91)	<0.001
2 eGFRcr [no race adjustment]	86 (84, 89)	88 (85, 91)	0.39
3 eGFRcys	83 (80, 86)	85 (82, 89)	0.26
4 eGFRcr-cys	88 (86, 90)	90 (87, 93)	0.34
5 eGFRcr-cys [no race adjustment]	91 (89, 93)	94 (92, 97)	0.041
P value (within-group comparisons of estimating equations)			
1 vs. 2	<0.001	0.89	
1 vs. 3	0.008	0.178	
1 vs. 4	<0.001	0.25	
1 vs. 5	<0.001	<0.001	
2 vs. 3	0.011	0.137	
2 vs. 4	0.19	0.31	
2 vs. 5	0.001	<0.001	
3 vs. 4	<0.001	0.013	
3 vs. 5	<0.001	<0.001	
4 vs. 5	0.045	0.009	
Bias[2], difference in mL/min/1.73m^2 (95% CI)			
1. eGFRcr	9.1 (7.2, 11.0)	5.1 (2.5, 7.7)	0.016
2. eGFRcr [no race adjustment]	-3.9 (-5.8, -2.1)	-8.2 (-10.7, -5.7)	0.007
3. eGFRcys	-3.4 (-5.4, -1.3)	2.2 (-0.6, 5.0)	0.002
4. eGFRcr-cys	2.6 (1.0, 4.2)	4.6 (2.4, 6.8)	0.148
5. eGFRcr-cys [no race adjustment]	-4.0 (-5.5, -2.4)	-2.6 (-4.7, -0.5)	0.29
P value (within-group comparisons of estimating equations)			
1 vs 2	<0.001	<0.001	
1 vs 3	<0.001	0.36	
1 vs 4	<0.001	0.28	
1 vs. 5	<0.001	<0.001	
2 vs. 3	0.001	<0.001	
2 vs. 4	<0.001	<0.001	
2 vs. 5	0.33	<0.001	
3 vs. 4	<0.001	0.046	
3 vs. 5	0.016	<0.001	
4 vs. 5	<0.001	<0.001	

Supplement Table 3. Cross-sectional accuracy and bias of biomarker-based estimating equations compared with glomerular filtration rate measured by iohexol disappearance from plasma in HIV-positive and HIV-negative African American participants.

Performance measure	HIV-positive (781 observations in 207 individuals)	HIV-negative (379 observations in 120 individuals)	P value (comparison by HIV status)

CI, confidence interval; eGFRcr, eGFRcys, and eGFRcr-cys are glomerular filtration rate estimates derived from the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations[1] based on serum concentrations of creatinine, cystatin C, and both markers, respectively

[1] Accuray defined as percentage of equation estimates that are within ± 30% of iohexol-based glomerular filtration rate measure.

[2] Bias defined as the difference between the biomarker-estimated and iohexol-based glomerular filtration rate.

Supplement Table 4. Association of HIV and hepatitis C virus status on total lean mass in a cohort of African American participants enrolled in Baltimore, Maryland, 2010 to 2018

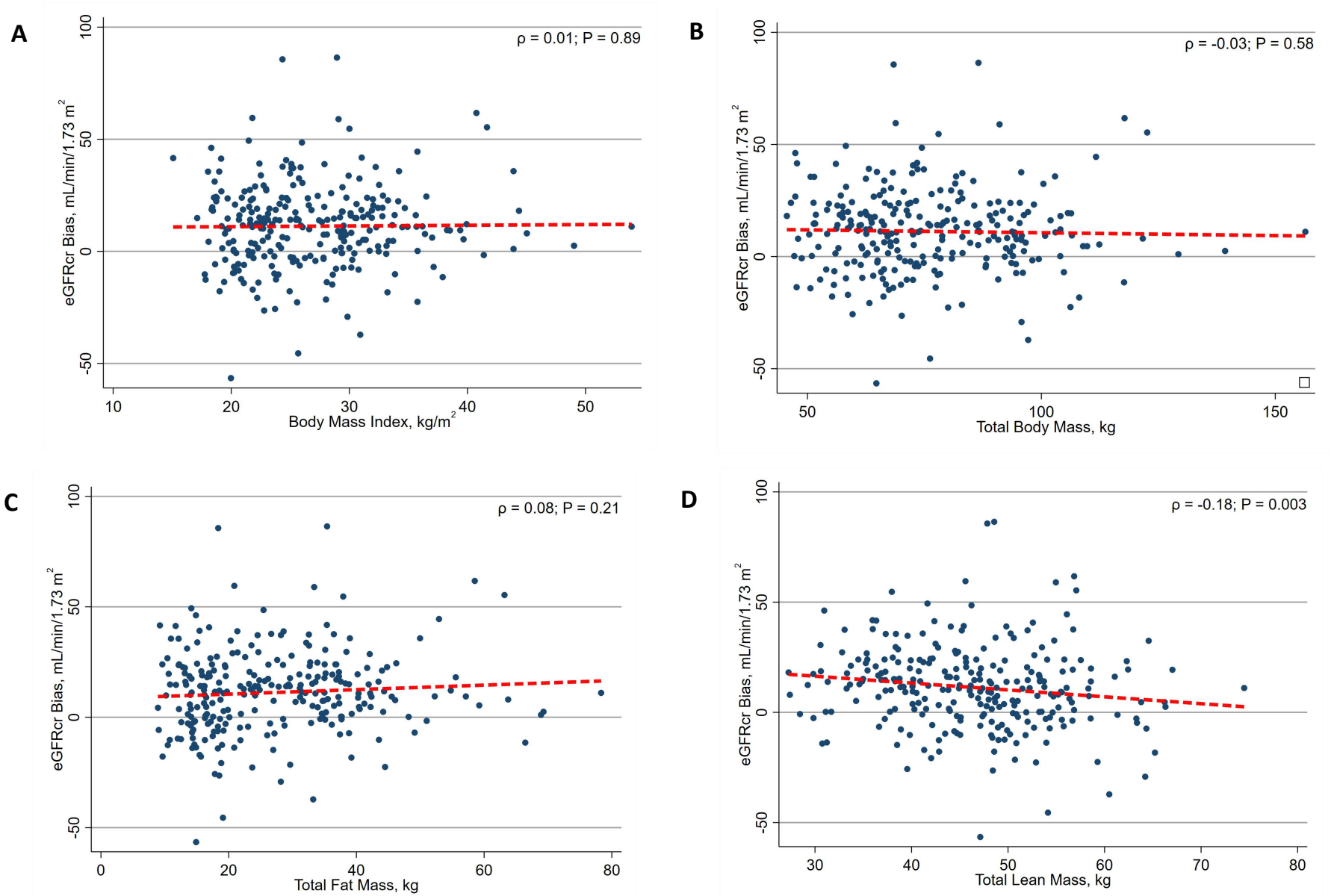
	Unadjusted model		Adjusted model[1]	
Variable	**Mass, kg (95% CI)**	**P value**	**Mass, kg (95% CI)**	**P value**
HIV/HCV status				
HIV (-) / HCV (-)	Reference		Reference	
HIV (-) / HCV (+)	-3.9 (-8.0, 0.2)	0.062	-1.2 (-3.8, 1.4)	0.35
HIV (+) / HCV (-)	-5.2 (-8.9, -1.5)	0.006	-2.9 (-5.2, -0.6)	0.015
HIV (+) / HCV (+)	-7.1 (-10.5, -3.6	<0.001	-2.5 (-4.7, -0.3)	0.026
Age, per 5-year increase			-0.7 (-1.2, -0.2)	0.003
Sex				
Female			Reference	
Male			14.5 (12.8, 16.2)	<0.001
Total body fat, per gg			0.4 (0.3, 0.4)	<0.001

HIV, human immunodeficiency virus; HCV, hepatitis C virus; (-) and (+) denote negative or positive infection status, respectively.

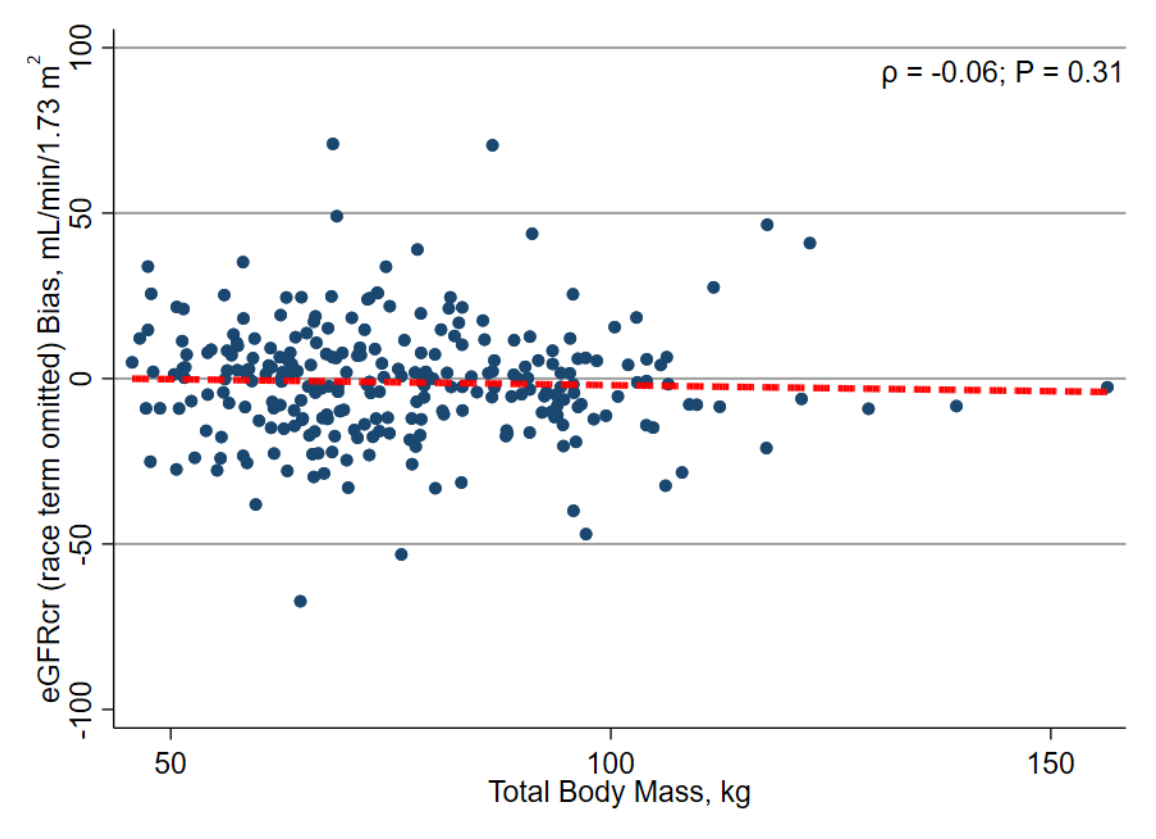
[1] Adjusted for factors shown in table

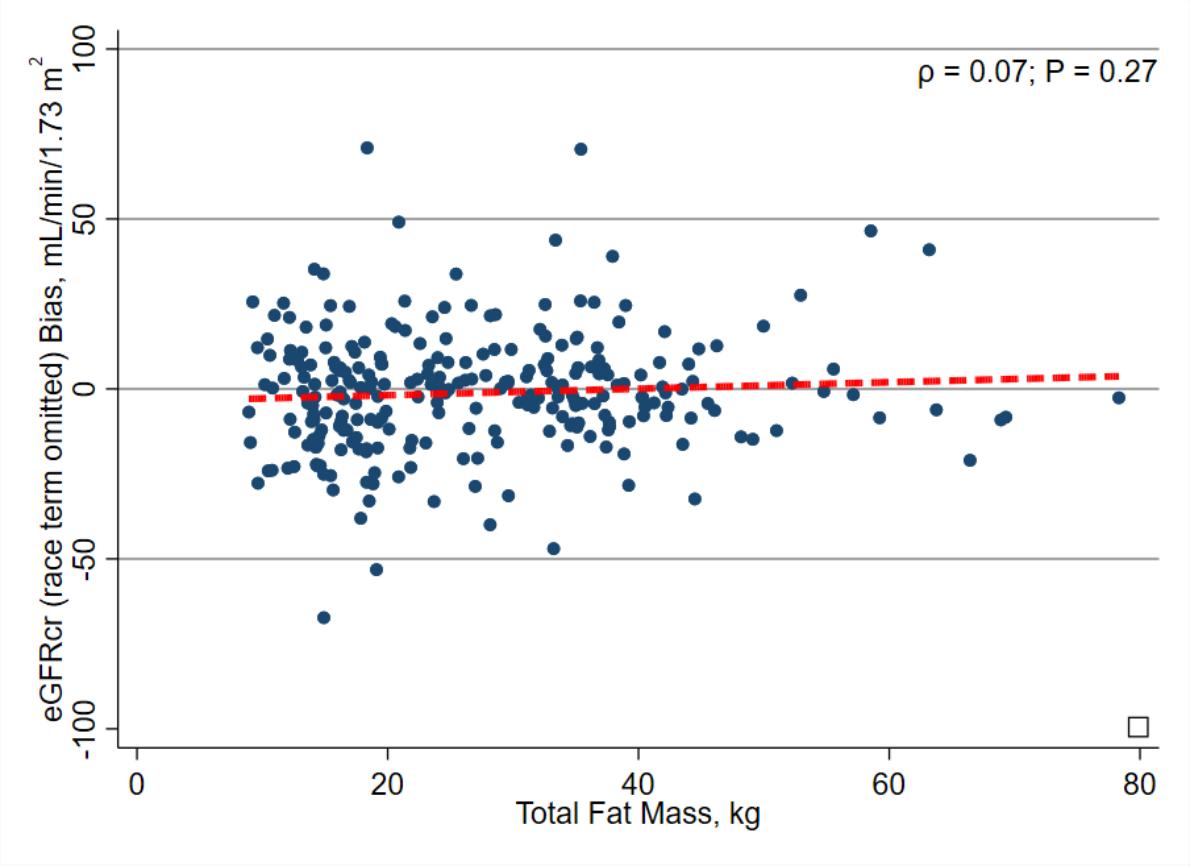
Supplement Figure 1. Association of eGFRcr bias (relative to measured iohexol GFR) with measures of body size and composition: total body mass index (A), total body mass (B), total fat mass (C), and total lean mass (D) in HIV-positive

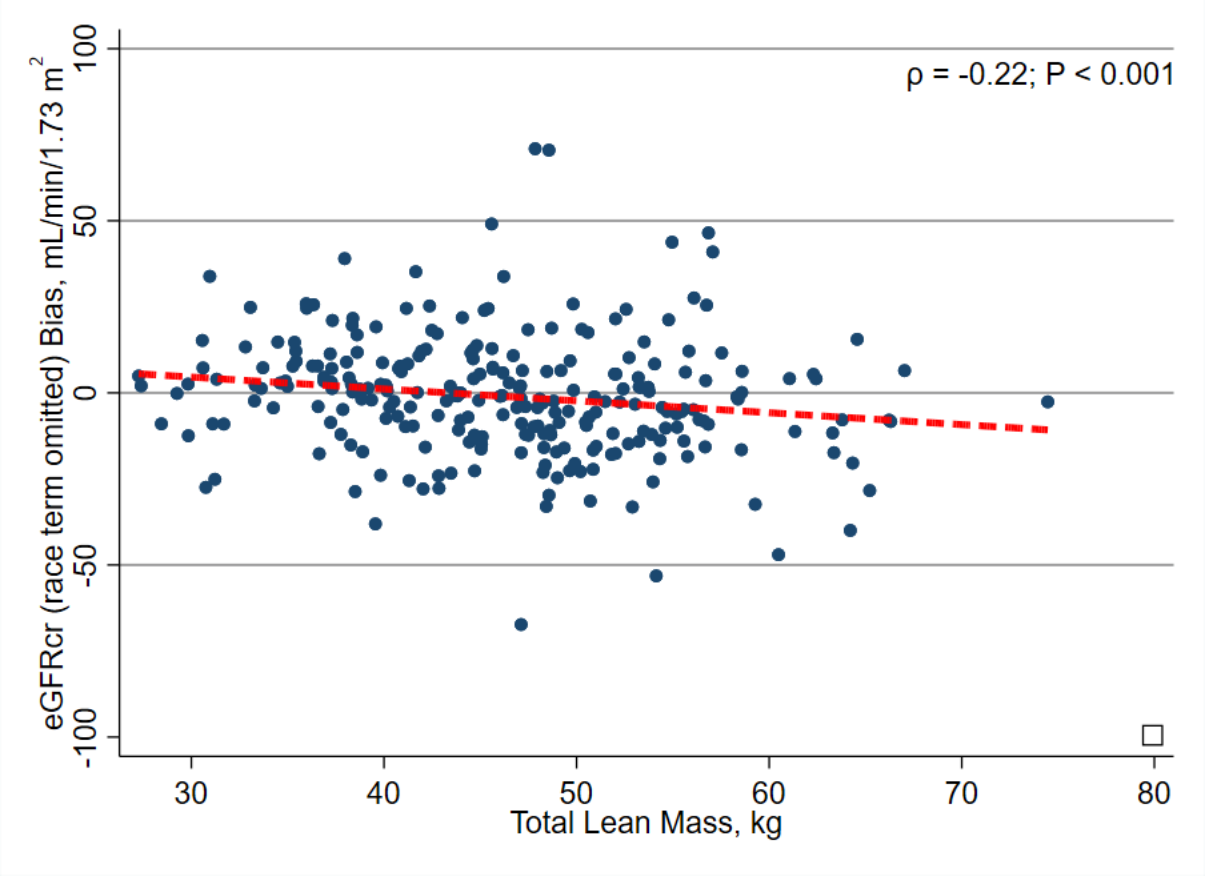
AAs. Circles represent individual participant observations. Spearman rank correlation coefficients (ρ) and P values are shown for each figure. The rd dashed line represents the least squares regression line.



Supplement Figure 2. Scatterplots and regression lines showing associations between body composition measures and bias of eGFRcr (race calibrating term omitted)




ρ = 0.07; P = 0.27
eGFRcr (race term omitted) Bias, mL/min/1.73 m²
100
50
0
-50
-100
0
20
40
60
80
Total Fat Mass, kg


ρ = -0.22; P < 0.001
eGFRcr (race term omitted) Bias, mL/min/1.73 m²
100
50
0
-50
-100
30
40
50
60
70
80
Total Lean Mass, kg