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Low Thigh Muscle Mass is Associated with Coronary Artery Stenosis Among HIV-Infected and -Uninfected Men: The Multicenter AIDS Cohort Study (MACS)

Short title: Muscle Mass and Obstructive Coronary Disease

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Abstract

Background. HIV-infected individuals are at increased risk for both sarcopenia and cardiovascular disease. Whether an association between low muscle mass and subclinical coronary artery disease (CAD) exists and is modified by HIV-serostatus is unknown.

Methods: We performed cross-sectional analysis of 513 male MACS participants (72% HIV-infected) who underwent mid-thigh computed tomography (CT) and non-contrast cardiac CT for coronary artery calcium (CAC) during 2010-2013. Of these, 379 also underwent coronary CT angiography for non-calcified coronary plaque (NCP) and obstructive coronary stenosis ≥50%. Multivariable-adjusted Poisson regression was used to estimate prevalence risk ratios of associations between low muscle mass (<20th percentile of the HIV-uninfected individuals in the sample) and CAC, NCP and obstructive stenosis.

Results: The prevalence of low thigh muscle mass was similar by HIV-serostatus (20%). There was no association of low muscle mass with CAC or NCP. However, low thigh muscle mass was significantly associated with a greater than 2-fold higher prevalence of obstructive coronary stenosis, after adjustment for demographics and traditional CAD risk factors [PR 2.46 (95% CI 1.51, 4.01)]. This association remained statistically significant after adjustment for adiposity, systemic inflammation, or physical activity. There was no significant interaction by HIV-serostatus (p-interaction=0.90).

Conclusions: In this exploratory analysis, low thigh muscle mass was significantly associated with subclinical obstructive coronary stenosis ≥50%. Additional studies involving larger sample sizes and prospective analyses are needed to confirm the utility of measuring mid-thigh muscle mass for identifying individuals at increased risk for advanced subclinical CAD who might benefit from more aggressive risk factor management.

KEY WORDS: Muscle mass, sarcopenia, HIV-infection, coronary atherosclerosis, coronary artery stenosis

1. INTRODUCTION:

With advances in treatment for HIV infection and subsequent declines in AIDS-related mortality,[1] cardiovascular disease (CVD) has emerged as a leading cause of death among HIV-infected individuals. Specifically, HIV-infected individuals have higher rates of myocardial infarction[2, 3] and increased prevalence of subclinical coronary atherosclerosis compared to HIV-uninfected individuals with similar risk factor profiles.[4, 5] The pathogenesis of coronary artery disease (CAD) in HIV-infected individuals is complex and may be related to both traditional and non-traditional CVD risk factors.

One potential risk factor for CAD among HIV-infected individuals that has not been well-studied is muscle wasting, a condition that could be modifiable with strength training.[6] HIV-infection, particularly in persons with a history of more advanced HIV disease, is associated with lean muscle mass loss[7] and sarcopenia.[8] Since skeletal muscle constitutes about 60% of the total body mass, any changes in this large and metabolically active system can have profound adverse health outcomes, including frailty and onset of acute and chronic disease states.[9]

In the general population, sarcopenia has been associated with insulin resistance and systemic inflammation, known risk factors for atherogenesis.[10, 11] Sarcopenia also has linked to measures of subclinical atherosclerosis including carotid artery intimal medial thickness,[12] arterial stiffness,[13] and coronary artery calcium (CAC).[14] Whether low muscle mass is also associated with non-calcified coronary plaque, which is more prevalent in HIV-infected compared to non-infected individuals,[4, 5] as well as with the severity of obstructive coronary artery stenosis, remains unknown. Furthermore,

whether the association of low muscle mass with subclinical coronary atherosclerosis is modified by HIV-serostatus is yet to be explored.

Currently there is no consensus clinical definition of sarcopenia because of a lack of standardized methods of ascertainment of functional skeletal muscle mass.[15] Prior studies have used either measures of muscle mass, muscle strength or a combination of both as markers for sarcopenia.[16] However, the mid-thigh muscle mass cross-sectional area ascertained by computed tomography (CT) has been proven to be a useful index of sarcopenia as well as a useful tool for monitoring treatment response in older patients.[17] Prior work from the Multicenter AIDS Cohort Study (MACS) found that thigh muscle cross-sectional area was correlated with grip strength, and that HIV-infected men had lower muscle quality and more pronounced decline in thigh muscle density with increasing age compared to similar HIV-uninfected men.[18] In sum, a single slice mid-thigh CT is noninvasive and easily obtained among individuals undergoing CT imaging for other indications and may offer additional prognostic information.

Therefore, our study sought to investigate three questions: (1) whether low thigh muscle mass is associated with subclinical coronary atherosclerosis as measured by both CAC and non-calcified coronary plaque; (2) whether low thigh muscle mass is associated with the severity of coronary stenosis; and (3) whether such associations, if present, differ by HIV-serostatus. We hypothesized that low thigh muscle mass area will be independently associated with CAC, non-calcified plaque, and severity of stenosis, independent of traditional CVD risk factors. In exploratory analyses, we evaluated whether such associations are stronger among HIV-infected compared to HIV-uninfected individuals, and whether associations are additionally independent of

adiposity, systemic inflammation, or physical activity levels – factors which may mediate any relationships found.

2. METHODS:

2.1 Population:

The Multicenter AIDS Cohort Study (MACS) is an ongoing multi-center prospective cohort study of the natural and treated histories of HIV-1 infection in men who have sex with men. Almost 7,000 HIV-infected and HIV-uninfected participants have been enrolled in three time periods (1984-85, 1987-91, 2001-03) in 4 sites: Baltimore, Maryland/ Washington, DC; Chicago, Illinois; Pittsburgh, Pennsylvania; and Los Angeles, California.[19] Research visits occur every 6 months and include questionnaires, physical examination, blood testing (including long-term serum storage), and urine analysis. Detailed description of the MACS has been previously published.[19]

Between 2010-2013, 1006 MACS men aged 40 to 70 years, weighing less than 136 kg, and having no history of cardiac surgery or percutaneous coronary intervention were recruited into a CVD sub-study (MACS-CVD2).[4] Eligible men underwent non-contrast cardiac CT scanning to determine CAC score. Among MACS-CVD2 participants who completed the non-contrast cardiac CT, a subset (n=759) also underwent contrast coronary CTA to measure total plaque burden, plaque composition and coronary stenosis. Exclusion criteria for CTA were contrast allergy, atrial fibrillation, or chronic kidney disease (estimated GFR <60 mL/min/1.73m^2) within 30 days of imaging. Also, 607 MACS-CVD2 participants who also participated in a previous (2004-2006) CVD substudy[20] were recruited to undergo a mid-thigh CT scan at the time of their MACS-CVD2 cardiac CT as part of another ancillary study evaluating measures of

body composition.[18] Ninety-four participants had missing or invalid mid-thigh CT scans for the measurement of muscle mass area. The current analysis was conducted on 513 men with both thigh muscle mass area and non-contrast chest CT data for CAC analysis, and 379 men with thigh muscle mass area and CTA data for analyses of non-calcified plaque and severity of coronary stenosis (**Figure 1**).

The Institutional Review Boards of all participating sites approved the MACS and MACS-CVC2 studies, and all participants signed informed consent.

2.2 Measurement of Muscle Mass:

For this analysis, low muscle mass was the independent variable of interest. At the time of the cardiac CT scanning, a mid-thigh CT scan was obtained with a 3-10 mm thick (cross-sectional) single image for each subject at the midpoint between the anterior superior iliac crest and the patella. Images were sent to a core CT reading center (Los Angeles Biomedical Research Institute at Harbor-UCLA). Manual tracings were performed to delineate subcutaneous fat and muscle compartments; tissue attenuation was measured in Hounsfield Units (HU). Areas of thigh muscle mass, expressed in square centimeters, were calculated by summing the area of pixels in the slice with CT values from 0-100 HU, defined by the attenuation values described by Goodpaster et al,[21] to exclude subcutaneous fat and bone.

Previously in the MACS cohort, “muscle weakness” (as part of the frailty definition) was defined as present if grip strength measured using a dynamometer was less than 20th percentile of the HIV-uninfected men in the sample.[22] To mirror this definition, participants with total areas of thigh muscle mass <20th percentile of the HIV-uninfected men in this sample were also classified as having “low muscle mass”. This

corresponded to <124.6 cm^2. We also examined thigh muscle mass as a continuous variable per 1 standard deviation (SD) lower value.

2.3 Cardiac CT and assessment of subclinical coronary atherosclerosis:

The dependent outcomes of interest included: (1) presence any CAC (Agatston score >0), (2) presence of significant CAC (Agatston score ≥100) (3) presence of any coronary plaque on CT angiography (total plaque score (TPS) >0), (4) presence of any non-calcified plaque (non-calcified plaque score (NCPS) >0), and (6) moderate to severe coronary artery stenosis (≥50%).[23]

Cardiac CT scans were obtained following procedures as previously described.[24] Briefly, participants received a beta-blocker or calcium channel blocker if needed for heart rate control just before the time of scanning, followed by sublingual nitroglycerin before administration of IV contrast unless contraindicated. Coronary CTA was performed with electrocardiogram-triggered protocols and median radiation dose of 1.9 mSv (interquartile range, 1.7-2.7 mSv). CT images were analyzed at the core CT reading center (Los Angeles Biomedical Research Institute at Harbor-UCLA) by trained, experienced readers blinded to participant clinical information.

CAC scores obtained from non-contrast CTs were calculated using the Agatston method.[25] Coronary CTA images were examined to characterize coronary plaque burden (presence, size, and composition of plaque) and degree of coronary stenosis in all segments following the modified 15-segment model of the American Heart Association.[26] Coronary stenosis ≥50% were considered significant.[27]

Total plaque score was calculated as the sum of individual plaque size scores across all coronary segments that showed any plaque, with a maximum score of 45.[28]

Composition of plaque for each coronary segment was categorized as noncalcified or calcified. Noncalcified plaque was defined as any discernable structure clearly assignable to the vessel wall with a CT density less than the contrast-enhanced coronary lumen but greater than the surrounding connective in at least 2 independent planes. Calcified plaque was defined as any structure with CT attenuation >130 HU visualized as distinct from the intravascular lumen in at least 2 independent planes. The noncalcified plaque score (NCPS) was calculated as the sum of the individual NCPS across all coronary segments.

2.4 Covariates:

Demographic data including race (self-identified) were recorded at enrollment. We categorized race as White non-Hispanic, Black non-Hispanic, and other (which included Hispanics, American Indian or Alaskan Native, Asian or Pacific Islander, and other). As part of routine MACS visits, study participants were seen every 6 months, and data were collected regarding medication use, CVD risk factors, and HIV clinical variables, by questionnaires, physical examination, and blood tests. For this analysis, we used data collected at the MACS visit closest to the CT date.

Blood pressure was measured using standardized procedures, and hypertension defined as systolic blood pressure over 140 mmHg, diastolic blood pressure over 90 mmHg, or self-reported use of medication for hypertension. Body mass index (BMI) was categorized as <18, 18-<25, 25-<30, ≥30 kg/m^2. Smoking status was self-reported at each visit as never, former, or current smoker. Physical activity was self-reported using the International Physical Activity Questionnaire[29] which captures three levels of activity (low, medium, or high activity).

Glucose, total cholesterol, and high-density lipoprotein cholesterol (HDL-C), levels were measured from fasting blood samples. Serum creatinine level was measured at each MACS visit and within 30 days prior to CT scanning for participants who had contrast injection; eGFR was determined by the Modification of Diet in Renal Disease equation.[30]

Serum levels of inflammatory biomarkers were measured from blood samples drawn at the time of the CT visit and assayed at the University of Vermont Laboratory for Clinical Biochemical Research in Burlington, Vermont as previously described.[23, 31] For our analyses, we chose *a priori* to evaluate levels of high-sensitivity C-reactive protein (hsCRP) and interleukin 6 (IL-6) because in the general population levels of these markers are associated with subclinical coronary atherosclerosis.[32] We also chose to evaluate levels of monocyte activation markers soluble CD14 (sCD14), soluble CD163 (sCD163), and chemokine (C-C motif) ligand 2 (CCL2) because they have previously been shown to be elevated in antiretroviral therapy (ART) treated HIV-infected men and to be associated with atherosclerosis.[23]

Hepatitis C virus (HCV) infection was defined as either a positive enzyme immunosorbent assay (EIA) for HCV antibody in plasma or serum, or detection of HCV RNA in plasma using a quantitative polymerase chain reaction assay (Roche Molecular Systems ultrasensitive assay with a limit detection of 50 copies/mL). Men who were EIA-positive with undetectable HCV RNA for at least 3 years were considered HCV-uninfected. HIV disease characteristics evaluated included plasma HIV RNA levels, CD4+ T-lymphocyte cell counts (CD4) measured by flow cytometry, history of AIDS-defining illness, and use and duration of ART.

2.5 Statistical Analysis:

For participants with missing key covariates, multiple imputation was used to impute for missing data: systolic blood pressure (n=2 persons); use of antihypertensive medications (n=6); use of diabetes medications (n=9); fasting glucose (n=8); use of lipid-lowering medications (n=15); total and HDL cholesterol levels (n=2); body mass index (n=4); hsCRP levels (n=10); IL-6 (n=10); HCV status (n=8); sCD163 levels (n=31); sCD14 levels (n=42); CCL2 levels (n=8); and physical activity (n=101). The distributions of demographic and clinical variables by muscle mass status (i.e. $<20^{th}$ percentile or $\geq 20^{th}$ percentile) by HIV-serostatus were reported as counts (proportions), means (standard deviations) or median (interquartile interval). Comparisons were made using Student's t-test, Wilcoxon rank sum test or chi-square test as appropriate.

Since the prevalence of measures of subclinical coronary atherosclerosis were greater than 10%, a modified Poisson regression analysis with robust variance was used to estimate the prevalence risk ratios (PR) with 95% confidence intervals (CI) of associations between low muscle mass area and each subclinical coronary atherosclerosis measure in separate models.[33]

Multivariable-adjusted models were performed as follows. **Model 1** adjusted for demographic factors including age, race, CT scanning center, study cohort (pre- versus post-2001), and HIV-serostatus. **Model 2**, which was our primary model, additionally adjusted for established CVD risk factors including systolic blood pressure, smoking status, fasting glucose level, total and HDL cholesterol, use of lipid lowering, antihypertensive, and diabetes medications, and HCV-infection status. Three additional models were performed to explore potential mechanisms potentially underlying

associations seen in our primary model. **Model 3** additionally adjusted for categories of BMI. Since adiposity and low muscle mass can co-exist (i.e., sarcopenic obesity) and confer even greater CVD risk when present together,[34] we wanted to see if BMI attenuated any associations found between low muscle mass and subclinical coronary atherosclerosis. **Model 4** adjusted for Model 2 covariates plus levels inflammatory markers (hsCRP, log-transformed IL-6, sCD163, sCD14 and log-transformed CCL2). Inflammation may mediate associations between sarcopenia and CAD;[10] therefore, we wanted to determine if effects were attenuated after accounting for levels of inflammatory markers. Finally, to evaluate whether low physical activity mediates associations between sarcopenia and coronary plaque, **Model 5** adjusted for Model 2 covariates plus physical activity categories (low, medium high). Finally, among HIV-infected men only, we evaluated a supplementary model (**Model 6**) that adjusted for Model 2 covariates plus HIV-related factors including CD4 cell count, presence of detectable HIV RNA >200 copies, history of AIDS, use of ART, and duration of ART.

For our main Model (Model 2), Wald tests were used to formally test for two-way multiplicative interactions of thigh muscle mass status with HIV-serostatus in relation to subclinical coronary atherosclerosis measures. All statistical analyses were performed using Stata 12 (StataCorp Lp, College Station, TX). A *p*-value of <0.05 was considered to be statistically significant.

3. RESULTS:

The characteristics of study participants (n=513) by thigh muscle mass area, stratified by HIV-serostatus are displayed in **Table 1**. The study population included 368 (72%) HIV-infected men and 145 (28%) HIV-uninfected men. The prevalence of low

thigh muscle mass area (<20th percentile of HIV-uninfected men) was the same, 20%, regardless of HIV-serostatus. Both HIV-infected and uninfected men with low thigh muscle mass were more likely have lower BMI compared to their respective counterparts with higher muscle mass. Among HIV-infected men, those with low thigh muscle mass were significantly more likely to have a history of AIDS and a lower mean CD4+T cell count at the time of their scan.

HIV-infected men with low thigh muscle mass had significantly higher blood levels of IL-6, sCD163 and sCD14 compared with HIV-infected men with greater thigh muscle mass (**Table 2**). In unadjusted analyses, the distribution of the presence of CAC and non-calcified plaque on CTA did not significantly differ by muscle mass status regardless of HIV-serostatus. However, HIV-infected men with low thigh muscle mass tended to have more coronary artery stenosis ≥50% on CTA (**Table 3**).

Of the 513 men who underwent thigh muscle mass assessment and non-contrast cardiac CT, there were no significant associations between low muscle mass and prevalent CAC (CAC>0) or prevalent significant CAC (CAC≥100) in any of the multivariable-adjusted models (**Table 4**). Among the 379 men with muscle mass and coronary CTA data, low muscle mass was not significantly associated with the presence of any plaque (TPS >0) and non-calcified plaque (NCPS >0) (**Table 4**).

However, low muscle mass was significantly associated with a 2.5-fold increased prevalence of obstructive coronary stenosis ≥50% seen on CTA, after adjustment for demographic factors and traditional CVD risk factors [2.46 (95% CI: 1.51, 4.01)] **(Table 4, Model 2)**. This association remained significant even in models that further adjusted for potential mediators of this association, including BMI, inflammatory markers, and

physical activity. However, there was no significant interaction by HIV-serostatus (p for interaction=0.90). Given *a priori* interest in differences by HIV-serostatus, the results of the associations between muscle mass and obstructive coronary stenosis stratified by HIV-serostatus are shown in **Supplemental Table A**, but given the absence of any significant interaction by HIV-serostatus, these findings should be considered exploratory. The association of low muscle mass with obstructive CAD was still statistically significant in HIV-infected men even after adjusting for ART and HIV-disease related factors (**Supplemental Table A, Model 6).** Findings were also generally similar when muscle mass was considered as a continuous variable. Lower muscle mass (per 1 standard deviation lower) was associated with 30% increased risk of obstructive coronary stenosis [1.30 (1.01, 1.68), **Table 4, Model 2**].

4. DISCUSSION

In this well-characterized predominately HIV-infected population, we found that the prevalence of low thigh muscle mass area ascertained through a single cross-sectional mid-thigh CT was similar among HIV-infected and HIV-uninfected men. We found no association between low thigh muscle mass and the presence of CAC and other types of coronary plaque on CTA. However, low thigh muscle mass area (<20th percentile) was associated with a 2.5-fold prevalence of moderate to severe subclinical coronary stenosis (≥50%) after adjustment for demographic and traditional CVD risk factors. This association remained significant after further adjustments for BMI, levels of inflammatory markers, and physical activity.

The present study is the first to our knowledge to evaluate associations between CT-assessed low muscle mass and subclinical coronary atherosclerosis assessed by both non-contrast cardiac CT and CTA, and the first to comparatively evaluate such associations by HIV-serostatus. Although some studies have evaluated the association of low skeletal muscle mass with subclinical atherosclerosis in other vascular beds (i.e. carotid intimal medial thickness, ankle brachial index, etc),[12, 13, 35] fewer have included subclinical coronary atherosclerosis data ascertained through cardiac CT,[14] which is a more powerful prognostic indicator of future CAD and CVD risk.[36, 37] Our finding of an association of low muscle mass with higher prevalence of obstructive coronary artery stenosis ≥50% is novel. It is unclear whether low muscle mass leads to obstructive coronary stenosis in a causal fashion, though potential biologic mechanisms include insulin resistance and inflammation,[10] or through reduced physical activity level.[38] However, our results remained significant even after further adjustment for physical activity and levels of inflammatory biomarkers. These findings are clinically relevant since coronary stenosis ≥50% is associated with a 10-fold higher risk for cardiovascular events as well as a 6-fold higher risk for death, myocardial infarction, and unstable angina, independent of CAC.[39] So obstructive CAD, even when asymptomatic, is a marker of high CVD risk, implying potential benefit from more intensive preventive efforts (i.e. targeted lifestyle intervention and preventive pharmacotherapies). Additional prospective studies are needed to investigate associations between changes in skeletal muscle mass with progression of coronary artery stenosis over time.

It is unclear why low thigh muscle mass was not associated with the prevalence of CAC and other coronary plaques types apparent on CTA. Ko et al. reported that low

skeletal muscle mass index was inversely associated with CAC prevalence in the general population.[14] It is possible that since both sarcopenia and obstructive CAD take time to develop, low muscle mass may be more strongly associated with advanced subclinical CAD rather than just the presence of any subclinical coronary atherosclerosis. Perhaps, other traditional and non-traditional factors associated with CAD in this population might have a greater contribution to early forms of subclinical CAD and thus overshadow the relative contribution from low muscle mass.

Surprisingly, HIV-infected men had the same prevalence of low thigh muscle mass area compared to HIV-uninfected men. However, Natsag et al. found that muscle quality was lower among HIV-infected men enrolled in MACS due to greater fatty infiltration despite similar cross-sectional areas.[18] Contrary to our hypotheses of an effect modification by HIV-serostatus, we actually found that low muscle mass was similarly associated with subclinical obstructive CAD among both HIV-infected and uninfected men. However given our small sample size and low prevalence of obstructive CAD, we are unable to conclusively exclude the possibility of a difference.

If the results of the present study are confirmed in other populations, this might have some potential clinical implications for preventive cardiology, especially in the aftermath of recent recommendations by the US Preventive Services Task Force for lung cancer screening using chest CT imaging.[40] Many older adults might now be undergoing CT imaging for other indications, and potentially, the addition of a single non-contrast mid-thigh CT slice to assess for low muscle mass could be useful in stratifying risk for advanced subclinical coronary atherosclerosis who may benefit from

more aggressive risk factor management and preventative therapies (i.e. aspirin, statins, etc).

4.1 Strengths and Limitations

A major strength of this study is the detailed assessment of subclinical coronary atherosclerosis and plaque composition using CTA in addition to non-contrast coronary CT scans in a well-characterized cohort. Nonetheless, this analysis has some limitations: First, we studied only men and our findings may not necessarily be generalizable to women who are known to have different distributions of body fat compared to men.[41] Second, the cross-sectional design of this study limits our ability to determine temporal relationships and causality. Residual confounding and reverse causation may explain the associations seen, although we adjusted for numerous demographic and clinical factors, and the men were free of clinical CAD (defined by no prior revascularizations) at the time of CT scanning. Again, additional studies are needed to investigate whether muscle mass predicts change in coronary artery stenosis over time. Third, we performed testing of thigh muscle mass with multiple coronary atherosclerosis markers of interest. It is possible that the association we found may be due to chance, although the association with obstructive coronary stenosis was robust across all models tested and was present among individuals with and without HIV-infection. Fourth, there is no consensus of the definition of low muscle mass by thigh CT and we used a cut-point of $<20^{th}$ percentile (of our HIV-uninfected sample) to be similar to the muscle weakness definition used in the same cohort.[22] Thus, this cut-point is somewhat arbitrary and should be considered exploratory, but the associations with obstructive CAD were similar when low muscle mass was treated as a continuous

measure (per 1 SD lower). Finally, sarcopenia is more traditionally defined by low appendicular lean skeletal muscle mass obtained by dual energy x-ray absorptiometry, which was not performed in our study. However, the usefulness of a single slice CT-assessment of thigh muscle mass has been noted in other studies,[17] and makes it an attractive potential prognostic marker for CAD risk that can be easily obtained in individuals who are already undergoing non-cardiac CT screening for other indications.

5. CONCLUSION:

In summary, low muscle mass was found to be associated with subclinical obstructive coronary stenosis ≥50% but not with presence or extent of specific coronary plaque types, independent of demographics and multiple traditional CVD risk factors. Low mid-thigh muscle mass ascertained through a single non-contrast CT slice may be a marker of more advanced subclinical CAD. Further studies involving larger sample sizes and longitudinal follow up are needed to evaluate the prognostic value of mid-thigh muscle CT assessment in identifying individuals with advanced subclinical CAD who could benefit from more aggressive risk factor management.

Figure legends

Figure 1. Inclusion and exclusion of study participants

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Author Contributions:

Drs. Michos, Tibuakuu, and Saxena designed the study. Dr. Tibuakuu wrote the initial draft of the manuscript. Dr. Zhao performed the statistical analyses and provided critical revisions to the paper. Dr. Post obtained the NIH-funding for the MACS-CVD ancillary

study, was involved in the CT data collection, and provided critical revisions to the paper. Dr. Budoff interpreted the cardiac CT scans for the study (CT Core Lab) and provided critical revisions to the paper. Drs. Brown, Jacobson, Guallar, Palella, Witt, Koletar, Margolick, and Mr. Korada provided critical revisions to the paper. Dr. Michos, Dr. Tibuakuu, and Dr. Zhao take fully responsibility for its content. All authors reviewed the final draft and approve of its submission. The manuscript was also approved by the MACS Executive Committee.

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