Official title: Inflammatory pathogenesis of coronary atherosclerosis in HIV.

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JHM IRB - eForm A – Protocol

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1. Abstract

a. Provide no more than a one page research abstract briefly stating the problem, the research hypothesis, and the importance of the research.

Survival in people with HIV has significantly improved with the use of antiretroviral therapy (ART) but HIV+ people now experience an increasing burden of chronic diseases, including coronary artery disease (CAD). HIV patients manifest an increased risk of CAD and its consequences possibly due to interplay of inflammation with traditional risk factors, some of the latter accentuated by ART. In addition, epicardial adipose tissue (EAT), a metabolically-active paracrine source of local inflammatory mediators, accumulates in HIV+ people and may also contribute to local CAD development. Mechanistic studies of the role of inflammation in the pathogenesis of CAD in HIV+ people taking ART are desperately needed to define the importance of inflammation per se and to guide the testing of new practical treatment approaches.

Although current imaging methods can document anatomic CAD that has developed over years, there has not been a noninvasive means to identify and quantify the central, early mechanisms contributing to the pathogenesis of CAD such as coronary endothelial dysfunction. Two very recent advances now make it possible to overcome those hurdles and test the role of inflammation in HIV-associated CAD.

First, colchicine is an anti-inflammatory agent used for over 20 years to treat gout and recurrent pericarditis. Low dose colchicine (LDC) reduces inflammatory biomarkers and was shown recently to reduce cardiovascular events by more than 65% ($p<0.001$) in stable CAD patients(1). In addition, we have recent evidence that colchicine does not suppress markers of immune function in HIV+ people. Thus LDC offers a practical, clinically relevant, untested means to probe the impact of inflammation on CAD pathogenesis in HIV+ people.

Second, inflammation undoubtedly enhances the development and progression of coronary atherosclerosis via several mechanisms, but endothelial dysfunction is believed to be one common result of these mechanisms. Coronary endothelial function (CEF) plays a pivotal role in the development, progression, and clinical manifestations of CAD and endothelial dysfunction is a marker for sub-clinical disease, an independent predictor of cardiovascular events, and a target for successful medical interventions(2-9). Endothelial-dependent function was historically only assessed invasively in the catheterization laboratory by changes in coronary arterial diameter and flow in response to endothelial-dependent vasomotor interventions. We recently developed and validated the first noninvasive methods to study CEF: these MRI-based methods allow safe, non-invasive reproducible studies in the same individuals over time and in low risk populations (10, 11). Thus the impact of anti-inflammatory interventions on CAD pathogenesis in HIV+ people can now be assessed.

We propose here a randomized, double-blind, placebo-controlled, phase 2, proof-of-concept trial exploiting our new non-invasive CEF measures to determine whether LDC improves local CEF in HIV+ people with no history of clinical CAD.

2. Objectives (include all primary and secondary objectives)

To evaluate the role of inflammation on the pathogenesis of coronary atherosclerosis in HIV we will test the hypothesis that an anti-inflammatory intervention, low dose colchicine (LDC), improves impaired local coronary endothelial function in HIV+ people with no clinical CAD.

To test the hypotheses that 1) EAT in HIV+ people is associated with impaired local CEF, and 2) LDC improves CEF more in coronary segments with increased EAT, as a source of local inflammatory mediators, as compared to segments with less EAT.

Primary Endpoint: Coronary endothelial function at 8 weeks; specifically, change in coronary blood flow (CBF) from rest to that during IHE stress (% rest and as ml/min) at 8 weeks.

Secondary Endpoints:

1. Change in coronary artery CBF from rest to IHE stress (as ml/min and as % rest) at 24 weeks;
2. Change in coronary artery CSA from rest to IHE stress (as mm^2 and as % rest) at 8 and 24 weeks;
3. hsCRP and IL-6 at 8 and 24 weeks and change in hs-CRP& IL-6 between baseline and 8 and 24 weeks;
4. Brachial flow mediated dilatation (FMD) at 8 and 24 weeks: change in FMD between baseline and 8 and 24 weeks;

To make full use of the data, changes in CEF from baseline to 8- and 24-weeks will be analyzed, along with changes in FMD and changes in inflammatory biomarkers (hsCRP, IL-6, VCAM-1, TNFa) from baseline to 8- and 24-weeks. Also, the relationship between change in inflammatory markers from baseline to 8 weeks (and 24 weeks) and change in CEF between baseline and 8 and 24 weeks will be quantified. CD4+ cell counts and HIV RNA (viral load) will be measured in subjects at baseline and 24 weeks. Additional variables for evaluation are coronary plaque burden (CWT, NWI, and percent luminal stenosis).

Safety endpoints: metabolic panel and complete blood count, withdrawal due to side-effects

3. Background (briefly describe pre-clinical and clinical data, current experience with procedures, drug or device, and any other relevant information to justify the research)

Since the advent of effective ART in the 1990's, HIV+ people are living longer and developing chronic diseases including cardiovascular disease that is currently the cause of death in 8%-15% of HIV+ people(15-18). HIV+ people have accelerated atherosclerosis and an approximate 50%-70% increase in the risk of myocardial infarction (MI) as compared to a comparable, age-matched population(19-21). Several factors are thought to contribute to this increased CAD risk including over-representation of traditional risk factors, chronic inflammation, and vascular activation in HIV+ people. However, the importance and interaction of these factors are very poorly understood and so in 2012 the NHLBI AIDS Working Group convened and published "Advancing HIV/AIDS Research in Heart, Lung, and Blood Disease" to outline critical gaps in HIV research.

Three main knowledge gaps regarding the pathogenesis of HIV heart disease were identified and this protocol proposes to address two of them: 1) mechanisms of the interplay of HIV/inflammation, anti-retroviral therapy, and traditional risk factors in the development of CAD, and 2) characterization of atherosclerosis in HIV+ people through cardiovascular imaging. We propose to conduct a mechanistic, phase 2 trial that elucidates the role of inflammation in the pathogenesis of CAD in HIV+ people.

Coronary atherosclerosis is an inflammatory disease (22). Endothelial cell injury and activation occur at the earliest stages of atherosclerosis with expression of endothelial surface molecules (e.g. vascular cell adhesion molecule-1, (VCAM-1)), that attract leukocytes, mostly monocytes, which in turn invade the vessel wall, drawn by chemo-attractant molecules and then contribute to the development of fatty streaks (22, 23). HIV infection, as well as HIV tat and Nef proteins in the absence of infection, activate endothelial and circulating immune cells (24).

Although ART reduces circulating inflammatory biomarkers, they are often not reduced to normal levels and circulating virus and the extent of immune and inflammatory activation correlate with the presence and extent of carotid and coronary atherosclerosis. Despite these associations the effect of inflammation per se on the processes which result in coronary atherosclerosis and whether an anti-inflammatory strategy alters these processes in HIV+ people is not known.

Abnormal coronary endothelial function (CEF) is likely an important contributor to atherosclerosis and, therefore, a potential target for medical interventions (25-32). Inflammation and endothelial dysfunction are closely related at all stages of CAD. Exposure of the endothelium to proinflammatory cytokines impairs endothelium-dependent vascular relaxation and induces prothrombotic activity (33) while inflammatory markers like hsCRP correlate with abnormal brachial artery endothelial vasoreactivity(34). The effects of inflammation on endothelial function may be mediated through reactive oxygen species (ROS) that decrease the bioavailability of NO (35), and separately by events that lead to programmed cell death (36-38). ART improves brachial artery endothelial function in ART naïve HIV+ people(39). Although inflammatory biomarkers are associated with increased cardiac risk in HIV+ people, it is not known whether reducing inflammation will alter the fundamental biologic processes linked to CAD, such as abnormal CEF. Thus measures of CEF offer a fundamental pathophysiologic window into the processes responsible for CAD in HIV+ people and may provide a means to assess the relative importance, if any, of inflammatory markers and the impact of anti-inflammatory interventions on this important contributor to CAD.

Endothelial-dependent function is assessed by the direction and magnitude of changes in arterial area and flow in response to endothelial-dependent vasomotor interventions (26). The invasive nature of coronary angiography with Doppler flow measures used in traditional measures of CEF limited clinical and research investigations, particularly those benefiting from repeated studies and/or studies in clinically stable subjects. We describe here**,** a novel validated approach using 3T magnetic resonance imaging (MRI) to assess NO-mediated endothelial-dependent coronary vasomotor function noninvasively. We combined 3T coronary MRI with isometric handgrip exercise (IHE), a previously proposed endothelial-dependent stressor (40-45), as a new approach to quantify CEF noninvasively. Our published findings demonstrate that 3T MRI combined with IHE can detect vasodilatation and increased blood flow in healthy subjects and paradoxical vasoconstriction and reduced coronary blood flow in patients with CAD, hallmarks of CEF. In addition, the heterogeneous response in CAD patients to the endothelial-dependent stressor, with more abnormal endothelial responses in coronary arteries with a more severe stenosis, calls into question the ability of peripheral endothelial function measures to accurately reflect the spectrum of CEF present within a given patient with CAD. The new capacity to noninvasively quantify CEF is a significant innovation that also provides new biologic insights.

Role of Inflammation: Following the pathologic recognition of the role of inflammatory cells in acute coronary syndromes, corticosteroids were initially investigated as anti-inflammatory agents in CAD patients. Enthusiasm for corticosteroids was tempered by equivocal benefit (46) and concerns about wound healing, myocardial rupture, and adverse metabolic effects (47). Nonsteroidal anti-inflammatory drugs (NSAIDs) were later associated with higher MI and event rates in CAD patients (48). Thus, initial anti-inflammatory strategies were not successful.

However there is renewed interest in anti-inflammatory strategies because patients with elevated inflammatory biomarkers (e.g., hsCRP) are at increased risk of primary and secondary cardiovascular events (49-52) and because statins have anti-inflammatory properties (53-55) and lower cardiovascular mortality (56-59). Nevertheless cardiovascular event rates remain high in statin treated HIV+ people (54, 60) and statins alone do not fully suppress inflammation in many patients (54, 61). Thus it is not known whether an anti-inflammatory approach would be effective in improving CEF in HIV, which systemic inflammatory biomarkers best predict the local coronary response (if any), and whether the local CEF response is heterogeneous and related to the extent

of underlying atherosclerosis (Aim 1) and/or surrounding epicardial adipose tissue (Aim 2) in a given HIV+ patient. To answer the question of whether inhibiting inflammation per se improves coronary endothelial dysfunction and impacts atherosclerosis in HIV+ people we need well-tolerated anti-inflammatory agents that do not have profound effects on lipids and do not significantly suppress the immune system in HIV+ people receiving ART.

Low dose colchicine (LDC) is an appealing choice to suppress inflammation in HIV+ people because it has been used for decades to treat inflammatory diseases and, in some populations, reduces inflammatory biomarkers, improves systemic endothelial function and is associated with fewer cardiac events.

Colchicine and cardiac disease: Colchicine is an anti-inflammatory agent used to treat gout, recurrent pericarditis, post-pericardiotomy syndrome, Bechet's disease and sometimes life-long (<2mg/day) to treat Familial Mediterranean Fever (FMF) (62-65). Colchicine is believed to act through inhibition of microtubule assembly in immune cells, including neutrophils and monocytes, which leads to reduced cytokine production, modulation of chemokine and prostanoid production and inhibition and down-regulation of lymphocyte and endothelial cell adhesion molecule surface expression (66, 67). Colchicine is concentrated in leukocytes due to a prolonged half-life in those cells (~60 hrs) as compared to that in plasma (~20 min) (68-70).

Because LDC manifests many potent anti-inflammatory effects, has been in clinical use for decades, and does not have adverse effects on lipids, it is an appealing choice for the treatment of inflammatory heart diseases. Chronic LDC was studied for the prevention of postoperative atrial fibrillation (70). Following cardiac surgery, 336 patients were randomized to placebo or colchicine (0.25-0.5mg twice daily) for 30 days. The incidence of atrial fibrillation was reduced in those receiving LDC (12.0% versus 22.0%, P<0.021; relative risk reduction, 45%; number needed to treat, 11) with shorter in-hospital and rehabilitation stays. No severe side effects were noted and the rates of withdrawal and side effects were similar in placebo and LDC groups. In another trial, patients with paroxysmal atrial fibrillation undergoing ablation were randomized to placebo or colchicine (0.5mg twice daily) for three months. LDC reduced recurrent atrial fibrillation by about 60% (odds ratio 0.38, p=0.01; number needed to treat 5.6)(71). Notably, the inflammatory biomarkers, hsCRP and IL-6, were reduced by 35% and 19%, respectively (both p<0.01) after only 3 days of LDC in these cardiac patients. In a recent randomized 240 patient trial of colchicine (0.5mg BID) for acute pericarditis, LDC reduced symptoms, hospitalizations and relapse rates, with discontinuation rates similar to those in patients taking placebo (72).

Several observational studies in the last 3 years suggest a positive effect of LDC on cardiovascular outcomes. In a 2012 retrospective study, gout users taking colchicine had a lower incidence of MI (1.2% vs 2.6%, p<0.03) and exhibited trends toward reduced all-cause mortality and lower CRP level versus those who did not take colchicine, despite similar baseline risk factors (73). In stable CAD patients with hsCRP>2mg L^{-1}, LDC (0.5mg twice daily) reduced hsCRP levels in four weeks by about 60%(74). More recently, the Low Dose Colchicine for Secondary Prevention of Coronary Artery Disease (LoDoCo) Trial results were reported(1). In this prospective, randomized, observer-blinded endpoint (PROBE) study, 532 patients with stable CAD received either placebo or colchicine (0.5mg/day) in addition to usual care. Patients were followed for a median of 3 years. LDC significantly reduced the primary composite endpoint of acute coronary syndrome, out-of-hospital cardiac arrest, and stroke by 67% (hazard ratio: 0.33; 95% confidence interval [CI] 0.18 to 0.59; p < 0.001; number needed to treat: 11). This occurred in a CAD population on aggressive background cardiovascular therapy. The LDC benefit was observed early, continued to accrue over time, and was largely driven by a reduction in coronary events (1). 11% of colchicine patients withdrew during the first 30 days and another 11% over the ensuing 3 years, mostly due to the well-known gastrointestinal side effects. An accompanying editorial commented that the 67% reduction in coronary events is about twice that seen with statins (47). Taken together, these studies demonstrate that LDC reduces inflammatory biomarkers rapidly in

patients with CAD (possibly as early as 3 days), is safely tolerated in the vast majority of CAD patients for several years, and appears to reduce cardiac events (pending confirmation) by an unidentified mechanism. This protocol proposes to use LDC to mechanistically probe for the first time the role of inflammation in the pathogenesis of CAD in HIV+ people.

Epicardial adipose tissue: a paracrine source of inflammatory cytokines contributing to CAD in HIV.

Visceral adipose tissue (VAT) is metabolically active and its extent correlates more closely with increased cardiovascular risk than does subcutaneous adipose tissue in the general population (75-77). VAT releases several inflammatory cytokines associated with atherosclerosis, including TNF-α and IL-6, which in turn activate production of pro-atherosclerotic mediators such as ICAM, VCAM, PAI-I and endothelin-1 (78, 79). VAT is increased in the lipodystrophy syndrome associated with HIV (80, 81). Epicardial adipose tissue (EAT), shares a similar embryologic origin to VAT, releases inflammatory cytokines and is metabolically active and associated with increased cardiovascular risk (82-85). Importantly, EAT is increased in HIV+ people taking ART and associated with atherosclerosis (86-91). Studies relating EAT to cardiovascular risk factors and atherosclerosis in HIV populations are relatively recent and have relied on noninvasive imaging measures of EAT thickness, typically by echocardiography, DEXA, and/or EAT volumes by CT and MRI. Volumetric CT and MRI measures of EAT are more reproducible than thickness measures and in some recent studies, MRI is considered the gold-standard, in part due to its ability to chemically clearly distinguish lipid and water resonances (92-94).

The hypothesis that EAT releases inflammatory cytokines that contribute through paracrine mechanisms to local coronary atherosclerosis is supported indirectly by observations that epicardial coronary artery segments coursing through cardiac muscle (“bridges” that do not contact EAT) do not develop atherosclerosis (95-97). Although a local, inflammatory-paracrine contribution of EAT to accelerated coronary atherosclerosis in HIV is appealing and directly in line with the aims of a recent NIH request for application (RFA HL14-023), *there is no direct evidence linking EAT with the local pathogenesis of CAD via inflammatory or other mechanisms in HIV+ people*.

We propose to evaluate this for the first time by combining EAT and CEF imaging techniques with the LDC intervention to probe the role of inflammation in HIV+ people. Because endothelial dysfunction is a critical step in the pathogenesis of atherosclerosis and because we can detect spatially heterogeneous CEF with MRI, we will use MRI to study CEF and relate CEF to local atherosclerosis and local EAT, in the same examination. We will study for the first time, in observational fashion, the baseline relationships among EAT, CEF and early atherosclerosis in HIV+ people as well as probe, in interventional fashion, the effects of an anti-inflammatory agent on those relationships in HIV+ people.

We searched the Johns Hopkins HIV Clinic database and observed that mean circulating neutrophil and CD4 counts were measured in 48 HIV+ people before, during and after clinical treatment with colchicine. There was no significant suppression of neutrophil or CD4 counts by colchicine. Most patients received $\geq$0.6mg daily, the same or more than proposed here. Mean duration of colchicine use was 104 days (quartiles: 54:181 days; range 10-316 days). Thus 75% of HIV+ people treated clinically with colchicine in our database are treated at least ~8 weeks, the primary endpoint of our proposed trial, and this duration of colchicine administration is not associated with a decrease in mean cell counts.

Although the FDA would not provide us safety information on colchicine in HIV apart from that publicly available, due to proprietary concerns, the one supplier of colchicine in the US did. Dr. Morehead of Takeda Pharmaceuticals wrote in an email that their Medical Director of Pharmacovigilance (PV) reviewed the last 12 months in the PV database with a search that involved HIV-positive patients who may have taken colchicine and wrote to me that “No reports of safety concerns were found.” They also conducted literature searches and did not find information

suggesting safety concerns (adverse events or drug interactions) in HIV+ people other than those in prescribing information. Our proposed trial will provide critical LDC safety data in HIV+ people.

4. Study Procedures

a. Study design, including the sequence and timing of study procedures (distinguish research procedures from those that are part of routine care).

The following are research procedures:

Randomized, double-blind, placebo-controlled phase 2 trial of the effect of LDC on coronary and systemic endothelial function and inflammatory biomarkers in 102 HIV+ people with no clinical CAD. More specifically, HIV+ people on stable clinically-guided ART will undergo baseline MRI quantification of CEF and EAT. Those with abnormal CEF (an increase in coronary CBF during IHE of ≤7ml/min from the resting value) will then have assessment of brachial FMD by ultrasound, measurement of inflammatory markers and lipids, and then be randomized to 24 weeks of either (1) colchicine (0.6mg daily) or (2) matching placebo. The Johns Hopkins Research Pharmacy will conduct the randomization procedure, prepare study medications in similar appearing formulations, and maintain group assignment documentation, keeping investigators blinded. Stratified randomization will be performed for smoking (yes/no). In addition the Research Pharmacy will review the two groups after 50% of the subjects have been randomized for gender and prevalence of diabetes, hypertension and statin use and adjust subsequent group assignment if they are not balanced for potential confounding factors. CEF, FMD, and biomarker endpoints will be re-assessed at 8 and 24 (unless subjects experience a clinical event that increases inflammation, in which case evaluation will occur after the inflammatory clinical event resolves). Subjects will be evaluated every 6 weeks for safety.

Initial evaluation: A careful history and physical examination will be performed. Blood samples will be acquired for complete blood cell count, CD4+ count and HIV RNA level, routine chemistry panel including hepatic transaminases and creatinine, LDL and HDL cholesterol and triglycerides, as well as serum hsCRP, IL-6, IL-1β, TNFα, IFN-γ, ICAM-1, VCAM-1, sE-selectin, sP-selectin, thombomodulin, vWF, PAI-1, endothelin-1, adiponectin and other biomarkers. Patients will also be tested for hepatitis B and C.

Systemic Endothelial Function: Brachial Flow Mediated Dilatation (FMD) and Velocity: These studies will be conducted at the ICTR on participants in the fasting state (>10 hours) asked to refrain from drinking alcohol or beverages containing caffeine in the prior 24 h using a standardized protocol (98, 99). Supine subjects will have the brachial artery visualized with a high-resolution ultrasound probe proximal to the antecubital fossa. After baseline images and flow measurements, a pressure cuff on the upper arm will be inflated to 200–250 mmHg for 5 min. Blood flow will be measured during the 15 s following cuff release and vessel diameter between 60 and 90 s after cuff deflation. Flow-mediated dilation (FMD) and velocity time interval (VTI) will be calculated (98, 99). Images will be coded and analyzed in blinded fashion.

MRI methods for Coronary Vasoreactivity: an index of CEF: Patients will undergo a detailed baseline MRI study of CEF in the fasting state using MRI methodology at rest and during continuous IHE as previously described (10). The MRI will be used to measure cross-sectional area (CSA), coronary flow velocity (CFV), and coronary blood flow (CBF) changes in response to IHE stress (continuous isometric handgrip for 4 min at 30% of each subject's maximum, determined prior to entering the MRI), as previously reported(11),(10). Coronary black blood imaging will also be performed in the same coronary slices for vessel wall measurements: CWT, vessel wall area and normalized wall index (NWI), an index of arterial remodeling calculated as: vessel wall area divided by the total vessel area (11). In addition, fat-excited and Dixon EAT acquisitions will be obtained in the same coronary segments as measures of CEF and CWT, so

that the volume of EAT can be assessed in the same coronary segment. In the event of technical or other difficulties in obtaining adequate images, the MRI may be repeated with the participant's permission.

Fasting: Subjects will be asked to fast after midnight and delay eating breakfast and taking morning cardiovascular medicines until after the study procedure, which will be completed by noon. In insulin-dependent diabetics, we will ask the subjects to delay their morning insulin dose until after the study procedure and will complete their studies by 10am. In addition, we will have a snack available and access to a finger-stick glucometer for any insulin-dependent diabetics who become symptomatic.

MRI analysis: MR images will be analyzed in blinded fashion without operator knowledge of time or treatment group for CEF (eg changes in CBF, CBV, and CSA) as previously validated and described (10). In addition, black blood coronary images will be analyzed for coronary wall thickness (CWT), an early marker of coronary atherosclerosis, and NWI, as described (11). Fat images (from fat selective excitation and/or mDixon method) will be analyzed for EAT for maximum thickness, CSA, and segment volume in each coronary segment evaluated for CEF. Baseline, 8-week and 24-week images will be analyzed at the same time for each subject (again blinded to time and treatment group with equal numbers of placebo and LDC) to reduce variability and assure identical segments are analyzed at each time. If the participant withdraws from the study before week 24, we will request participant permission to perform an MRI and FMD at termination of study.

Blood Draw and Biomarker Analysis: Blood samples will be obtained from a peripheral vein using standard venipuncture techniques. Biomarkers will be analyzed in replicate by the ICTR CRU Core Laboratory directed by Dr. Neal Fedarko from blood specimens collected into collection tubes without anticoagulant and centrifuged 30 min after collection (time allowed for clotting) using a centrifuge with an integrated refrigeration system (at 4°C/1000 g for 15 min) and kept at -80°C. Each aliquot will be thawed and analyzed once, and no aliquots will be refrozen(71). Analysis will be performed with ELISA or multiplexed ELISA for the following biomarkers of inflammation/activation/clotting (coefficient of variation listed in parentheses): hsCRP (6%), IL-6 (5%), IL-1β (8%), TNFα (8%), IFN-γ, ICAM-1 (6%), VCAM-1 (4%), sE-selectin (6%), sP-selectin (4%), thombomodulin, vWF (3%), PAI-1 (3%), endothelin-1, adiponectin (5%), and serum monocyte activation markers (sCD163) (100), PCSK9 and other inflammatory markers. All of these can be acquired from <900ul of serum. To minimize variability of assays of longitudinal markers, the baseline, 8- and 24-week specimens of each individual will be analyzed on the same plate, with equal numbers of subjects who received placebo and LDC.

Safety surveillance: Patients will undergo surveillance safety monitoring every 6 weeks that will include complete blood counts, and liver and renal function tests. Dr. Lisa Christopher-Stine, Co-Director of the Johns Hopkins Myositis Center will serve as the Chair of the DSMB and will review laboratory results from a safety standpoint. She will not be involved in primary or secondary data analysis or interpretation. Patients will also return 4 weeks after the last dose of study drug for a safety evaluation.

Follow up End-point Evaluation: After 8 and 24 weeks of study-drug administration, subjects will undergo repeat evaluation, lipid and inflammatory biomarker analysis, CD4+ count and HIV RNA levels, as well as brachial FMD and coronary MRI with the same protocols used at baseline. In particular, coronary MRI will be repeated with an identical protocol and special attention taken to interrogate the same coronary segments as those studied at baseline, using anatomic landmarks

of coronary ostia and branch vessels- as we have done in the past (10). Study drug compliance will be assessed by questionnaire and pill count at 6, 12, 18 and 24 weeks.

b. Study duration and number of study visits required of research participants.

Each participant will be followed for a 7-8 month period with the possibility of 8 – 9 study visits.

c. Blinding, including justification for blinding or not blinding the trial, if applicable.

Participants will be randomly assigned to one of two study groups (placebo or LDC) after providing informed consent and meeting all inclusion criteria. To keep the investigators blinded, the Johns Hopkins research pharmacy will conduct the randomization procedure, prepare medications (colchicine and placebo) in similar appearing formulations, and maintain group assignment documentation. The Johns Hopkins research pharmacy has many decades of experience in this process including randomization for study group assignment, blinding of medications, and maintenance of appropriate records for group assignment. It is important to blind the investigators and the subjects so that there is no bias in reporting or recording of potential side effects.

d. Justification of why participants will not receive routine care or will have current therapy stopped.

All participants will receive routine care and will not have current therapy stopped because of participating in the study.

e. Justification for inclusion of a placebo or non-treatment group.

The placebo group is included as a separate control group and will be used as a comparator group. Anti-inflammatory strategies like colchicine are not a standard part of contemporary therapy for patients with coronary artery disease thus there is no ethical problem with withholding them in a placebo group. Because it is not known whether these anti-inflammatory agents improve coronary endothelial function and/or limit atherosclerosis in CAD patients it is critical to have a placebo group to determine the efficacy of the anti-inflammatory drugs and to evaluate their safety.

f. Definition of treatment failure or participant removal criteria.

Participants who experience significant symptoms will be evaluated and those or others who develop new laboratory abnormalities will be reviewed and labs possibly repeated. If any of the following criteria are met (CBC<2000, platelets<50,000, Hct<27%, GFR <30ml/min or a >50% reduction in GFR compared to baseline values, and LFTs >3x upper limit of normal), the test would be repeated. If confirmed on repeat, there would be a temporary stop of study drugs. If the abnormality resolves, study drugs would be resumed at the same or lower dose. If the abnormality does not resolve or the temporary stop occurs on three consecutive measures, then the case will be reviewed for discontinuation from the study. Based on the clinical status, severity of the problem, and reversal of symptoms/laboratory findings, a decision will be made as to whether the study drug can be restarted under close observation (weekly surveillance) or the participant withdrawn from the study. Participants who wish to end participation in the trial at any time may do so upon request without penalty.

g. Description of what happens to participants receiving therapy when study ends or if a participant's participation in the study ends prematurely.

Participants withdrawn from receiving additional study drug due to an adverse experience will be followed by the Investigator until the outcome is determined. Every effort will be made to follow the subject for the full study period as per the schedule of study visits.

5. Inclusion/Exclusion Criteria

Inclusion Criteria:

A. Patients of either gender who are ≥21 years of age (no upper age limit), HIV positive and taking stable ART (no change in ART regimen in last 3 months),
B. HIV viral load <100 copies/mL (plasma HIV RNA concentration),
C. Abnormal CEF at baseline (≤7ml/min change in CBF during IHE as compared to resting value).

Exclusion criteria:

A. Patients unable to understand the risks, benefits, and alternatives of participation and give meaningful consent,
B. Patients with contraindications to MRI such as implanted metallic objects (pre-existing cardiac pacemakers, cerebral clips) or indwelling metallic projectiles,
C. History of clinical CAD, including acute coronary syndrome, myocardial infarction or revascularization,
D. Resting ECG with evidence of Q wave myocardial infarction,
E. Pregnant women,
F. Recent history, within the past 3 months, of cocaine or heroin use,
G. Moderate or greater renal impairment (estimated glomerular filtration rate <45ml/min),
H. Moderate-severe hepatic disease (elevation in hepatic transaminases >3x upper limit of normal),
I. Leukopenia (<3000/mm^3) or thrombocytopenia (<100,000/mm^3),
J. CD4<200 cell/mm^3,
K. Chronic inflammatory condition such as lupus or rheumatoid arthritis, ulcerative colitis or Crohn's disease,
L. Requirement for, or intolerance to, colchicine,
M. Women of childbearing potential (even if using oral contraceptive agents) or intention to breastfeed,
N. Chronic, continuous use of oral or IV steroid therapy or other immunosuppressive or biologic response modifiers or anti-inflammatory agents (chronic NSAIDs or ASA>81mg daily),
O. History of chronic pericardial effusion, pleural effusion, ascites or peripheral neuropathy manifested by both signs and symptoms,
P. Taking protease inhibitors (PI), cobicistat, or CYP3A4 inhibitors.

6. Drugs/ Substances/ Devices

a. The rationale for choosing the drug and dose or for choosing the device to be used.

The chosen low dose of colchicine is based on those used in clinical practice and recent studies showing that it reduces inflammation and improves endothelial function in patients with rheumatoid arthritis and other conditions. Colchicine is an anti-inflammatory agent used for over 20 years to treat gout, recurrent pericarditis, and post-pericardiotomy syndrome. Colchicine (1-2mg/day) is used chronically, sometimes life-long, to treat Familial Mediterranean Fever (FMF), where it is well tolerated (62-64). It is also used to treat Bechet's disease (65). The dose we have chosen, 0.6mg daily, is a dose that is available in the United States and most similar to the dose that reduced recurrent cardiac events in CAD patients in the Low Dose Colchicine for Secondary Prevention of Coronary Artery Disease (LoDoCo, 0.5mg daily) trial (1) where the drug was reasonably well tolerated. The 0.5 mg dose is not available in the United States. In addition, we provide evidence in the Preliminary Studies section that colchicine does not suppress immune

function in that it is does not lower CD4 or neutrophil counts in HIV+ people in the Johns Hopkins HIV clinic.

b. Justification and safety information if FDA approved drugs will be administered for non-FDA approved indications or if doses or routes of administration or participant populations are changed.

Low dose colchicine is an FDA approved medication that is, however, not approved for this indication. We received an exemption from the FDA that an IND is not needed. Safety information on the use of these drugs is detailed above.

c. Justification and safety information if non-FDA approved drugs without an IND will be administered. **NA**

7. Study Statistics

a. Primary outcome variable.
Coronary endothelial function at 8 weeks; specifically, change in coronary blood flow (CBF) from rest to that during IHE stress (% rest and as ml/min) at 8 weeks

b. Secondary outcome variables.
   (i) Change in coronary artery CBF from rest to IHE stress (as ml/min and as % rest) at 24 weeks
   (ii) Change in coronary artery CSA from rest to IHE stress (as mm^2 and as % rest) at 8 and 24 weeks
   (iii) hsCRP and IL-6 at 8 and 24 weeks and change in hs-CRP& IL-6 between baseline and 8 and 24 weeks
   (iv) Brachial FMD at 8 and 24 weeks: change in FMD between baseline and 8 and 24 weeks

To make full use of the data, changes in CEF from baseline to 8- and 24-weeks will be analyzed, along with changes in FMD and changes in inflammatory biomarkers (hsCRP, IL-6, VCAM-1, TNFa) from baseline to 8- and 24-weeks. Also, the relationship between change in inflammatory markers from baseline to 8 weeks (and 24 weeks) and change in CEF between baseline and 8 and 24 weeks will be quantified. Additional variables for evaluation are coronary plaque burden (CWT, NWI, and percent luminal stenosis).

Safety endpoints: metabolic panel and complete blood count, withdrawal due to side-effects

c. Statistical plan including sample size justification and interim data analysis.

The principal outcome in this double blind randomized trial of low dose colchicine versus placebo in HIV+ people without clinical CAD and abnormal CEF is the change in coronary blood flow (CBF) from rest to IHE at 8 weeks in the LDC and placebo groups. We chose to study the change in CBF because it 1.) reflects both macrovascular and microvascular changes related to the endothelial-dependent IHE stressor, 2.) offers a large dynamic range in responses between healthy subjects and HIV+ people, 3.) is reproducible over this period, and 4.) because endothelial function is an independent predictor of atherosclerotic progression and clinical events (25-27, 98, 99). The 8 week time was chosen to minimize confounding events occurring over longer times and because improvements in endothelial function have been observed in HIV+ people in as little as 4 weeks following the initiation of ART (39) and as little as 8 weeks of treatment with statins (101) or salsalate (102). We will also collect CEF measures at 24 weeks to evaluate safety and avoid missing a longer-term CEF effect, if present. Drugs that improve cardiovascular outcomes improve

endothelial function in fewer than 24 weeks, so there is little need to re-study beyond 24 weeks based on the literature (8, 103, 104). An increase in the IHE-CBF response in HIV subjects to 50% of that of healthy subjects does not assume complete normalization of CEF in HIV/CAD subjects with LDC but, instead, an increase that would be biologically significant and consistent with the changes in endothelial function observed with medications shown to reduce cardiovascular outcomes. For example, a landmark study demonstrated that statins improve CEF in CAD patients to approximately half of normal/maximal responses (8). Methotrexate, an anti-inflammatory agent, improved abnormal endothelial function to that of healthy subjects in patients with rheumatoid arthritis (105, 106). In studies without healthy subjects, comparisons are typically made with baseline or placebo measures and such studies consistently show that statins and ACE inhibitors improve abnormal endothelial function by ~50% or more in CAD patients (107-110). A similar 50% increase in endothelial function was also observed in HIV+ people treated for 8 weeks with statins (101). Thus based on the existing literature of the impact of established cardiovascular medications (statins, ACE-I) on endothelial function and based on our observations of coronary endothelial function (CBF increases during IHE in healthy subjects +43%±30% (mean±SD)) and does not increase in HIV/CAD patients, change of -2%±17% (mean±SD), we propose that there will be no increase in the 8-week CBF response to IHE in placebo-administered HIV+ people and that there will be +20% increase (i.e. approximately 50% of the 43% increase we observe in healthy individuals) in the LDC-administered HIV+ people. We assume that at 8 weeks:

(1) Mean CBF will be unchanged from rest to IHE in HIV+ people on placebo (ie -2% change during IHE)
(2) Mean CBF will increase by 20% from rest to IHE in HIV+ people on LDC (ie +20% increase during IHE)
(3) Standard deviation (variability) of 24% for both groups

The variability is conservatively based on the mean standard deviation in CBF response observed in this population and in healthy subjects. With a sample size of 35 in each group, we will have power of 92% (alpha=0.05, two-sided test) to detect such a difference in CBF-IHE response between the placebo and LDC groups (111). We will conservatively assume a 20% dropout rate over 8 weeks due to unwillingness to repeat MRI and to withdrawal and/or side effects from LDC (with 10% 30-day discontinuation rate in the LoDoCo Trial in CAD patients most without HIV (1)). Thus we plan to randomize 43 in each group for a total of 86 HIV+ people with subclinical CAD. Based on our preliminary studies, we expect that 15% of subjects undergoing the initial screening MRI will not meet the CEF inclusion criterion of an IHE-induced change in CBF $\leq$7ml/min of baseline. Therefore we plan to screen 102 HIV+ people with CAD with MRI-IHE in order to identify 86 who meet entry criteria for randomization so as to ultimately have 35 in each of the 2 groups complete the 8 week follow-up for the primary endpoint. For secondary endpoints occurring at the 24-week time, we anticipate that as many an additional 10% may dropout or decline follow-up MRI. However if as many as 20% dropout or decline to return for the 24 week MRI, that would leave ~25 in each group and there would be an 85% power to detect the same difference in the reduced sample size at 24 weeks (for secondary endpoints).

d. Early stopping rules.

Participants who experience significant symptoms will be evaluated and those or others who develop new laboratory abnormalities will be reviewed and labs possibly repeated. If any of the following criteria are met (CBC<2000, platelets<50,000, Hct<27%, GFR <30ml/min or a >50% reduction in GFR compared to baseline values, and LFTs >3x upper limit of normal), the test would be repeated. If confirmed on repeat, there would be a temporary stop of study drugs. If the abnormality resolves, study drugs would be resumed at the same or lower dose. If the abnormality does not resolve or the temporary stop occurs on three consecutive measures, then the case will be reviewed for discontinuation from the study. Based on the clinical status, severity of the problem, and reversal of symptoms/laboratory findings, a decision will be made as to whether the study drug can be restarted under close observation (weekly surveillance) or the patient withdrawn

from the study. Participants who wish to end participation in the trial at any time may do so upon request without penalty.

8. Risks

a. Medical risks, listing all procedures, their major and minor risks and expected frequency.

The MRI procedures do not expose patients to ionizing radiation and are not considered a significant risk (112). No long-term side effects have been noted at these magnetic field strengths. The potential risks of the MRI study are few and primarily relate to injury from metallic objects attracted to the MR scanner or the isometric handgrip exercise (see below). A blood pressure cuff is inflated and maintained for a few minutes during brachial endothelial function testing and this is associated with transient discomfort but no known major risks or long-term problems. The likelihood of MRI-related risks is minimized by thoroughly screening participants for metal prior to entering the scanner, and limiting access to the scanner area. A minority of individuals develop a claustrophobic sensation inside the magnet, which ceases when the subject exits the scanner. Patients with a prior history of being a machinist, welder, metal worker, or a similar activity that poses the potential risk of metal exposure to the eyes, will be asked to undergo screening orbit x-rays to rule out the presence of metal fragments. The amount of radiation exposure from the required series of skull x-rays is approximately 0.03 rems.

Isometric handgrip exercise performed in the MRI scanner results in modest increases blood pressure and heart rate and can result in paradoxical vasoconstriction in atherosclerotic coronary vessels, and is relatively safe (41). The cited side effect in patients with coronary disease of handgrip exercise is non-sustained ventricular ectopy, which occurred in one percent of patients and which spontaneously resolved after termination of the exercise (113). Voice contact between subject and MR system operator will be available at all times, during both coronary endothelial function studies (isometric handgrip exercise), as well as direct observation via a video camera in the scan room. In addition, a Cardiologist will be available during the MRI examinations. Isometric hand-grip exercise is terminated prematurely if participants develop symptoms that would typically stop their activities or if abnormal heart rate or blood pressure findings develop. We have performed isometric hand-grip exercise testing in conjunction with cardiac spectroscopy studies since 1988 (114) and have not had any significant complications.

Low dose colchicine is fairly well tolerated but has a relatively narrow therapeutic window. There were no serious adverse events reported in the LoDoCo trial (using a similar colchicine dose 0.5 mg QD to the dose proposed here 0.6mg QD) over three years although 11% of individuals within 30 days and another 11% over the next 3 years withdrew from the study. The most common reasons for withdrawal were unrelated intercurrent illnesses (3.9%), patient choice (1.8%), intestinal upset (2.5%), myalgia (0.9%), myositis (<0.5%), rash (<0.5%), alopecia (<0.5%), itch (<0.5%) and peripheral neuritis (<0.5%) (1). In a study of cardiac patients with atrial fibrillation, no serious adverse events were noted but diarrhea was reported in 8.6% (vs 1.3% on placebo) and nausea in 4.9% (vs 3.8% in placebo) and one case of elevated LFTs which reversed after stopping drug (115).

Flow mediated dilatation of the brachial artery: A blood pressure cuff is inflated and maintained for a few minutes with the testing of brachial endothelial function and this is associated with transient discomfort but no known major risks or long term problems.

Having blood drawn may produce discomfort or minor bleeding and the possibility of bruising at the site of the needle puncture. There is also a slight risk of infection at the site of the needle puncture. Some people may experience nausea, light-headedness, and fainting in association with a blood draw.

b. Steps taken to minimize the risks.

MRI will be performed with exposure to static and time-dependent magnetic fields within FDA guidelines (112). Special precautions will be taken to exclude patients with implanted metallic objects, including pacemakers, cerebral clips, or prior occupational exposure to small metallic projectiles (eg, lathe operators). Those subjects with a history suggesting possible risk will undergo orbital x-rays and those at risk will be excluded. All participants will be carefully screened by the investigators for the exclusions stated above. A Cardiologist will be present for each MRI study. Heart rate will be monitored continuously during MR studies via ECG, and blood pressure monitored with a remotely activated sphygmomanometer during isometric handgrip stress. Participants will be in direct verbal contact with the MR system operator at all times. Participants who experience a worsening condition or who wish to end the exam at any time may do so upon request without penalty. The scanner area is equipped with standard medical emergency equipment (including a crash cart) for a hospital-based clinical MR center with appropriately trained personnel in attendance.

To protect against and minimize the potential risks of low dose colchicine, patients with pre-existing liver or renal disease, blood dyscrasia, protease inhibitor use and other conditions that might increase risk will be excluded from participation (please see Exclusion Criteria above). In addition, patients will undergo a careful history and physical and screening laboratory examination at baseline to identify the presence of any of conditions which might increase their risk from the medications but not have been known to the patients or documented in their medical records. In addition, patients will be evaluated closely at 6-week intervals while on study drug for side-effects and surveillance metabolic and hematologic screening studies. The patients will also return 4 weeks after completing the study (28 weeks after randomization and initial dosing) for a follow-up safety surveillance. That evaluation will not affect the study drug dosing of that participant but if safety concerns are identified, it could be used to minimize risk in future participants. Patients who experience significant symptoms will be evaluated and those and any others who develop laboratory abnormalities will have the study drug withheld. Based on the clinical status, severity of the problem, and reversal of symptoms/laboratory findings, a decision will be made as to whether the study drug can be restarted under close observation (weekly surveillance) or the patient withdrawn from the study. Subjects who wish to end participation in the trial at any time may do so upon request without penalty.

Trained personnel will perform the blood collection procedure and will make every effort to minimize any risks or discomfort.

c. Plan for reporting unanticipated problems or study deviations.

All unanticipated problems or study deviations will be reported to the Data Safety and Monitoring Committee and according to The Johns Hopkins Medicine Institutional Review Boards published guidelines.

d. Legal risks such as the risks that would be associated with breach of confidentiality.

Once the data are acquired, they will be assigned a code for each subject with identification secured and accessible only to the PI, senior investigators, and the research coordinator. Patient clinical information such as copies of clinical studies and imaging reports shall be filed in a locked filing cabinet with access under control of Dr. Weiss and Research Coordinator.. Patient information summarized and/or converted into electronic form (tables, images, etc) shall be identified by the assigned code. It is a Johns Hopkins Institutional policy that all identifiers in images and data acquired under IRB-approved research protocols must be removed if they leave the institution, for example in presentations.

e. Financial risks to the participants.

All study related costs will be paid from research sources, as stated in the consent form.

9. Benefits

a. Description of the probable benefits for the participant and for society.

Results from this study may benefit patients with HIV and coronary heart disease in the future by providing information on the role of anti-inflammatory medications and inflammatory biomarkers in coronary atherosclerosis of HIV+ people. This would support the use of anti-inflammatory medications to complement existing cardiovascular prevention strategies. However, we do not currently know whether subjects will have an immediate benefit. It could be that this agent (colchicine) reduces inflammation and improves coronary endothelial function and this would, at least in the short term, benefit the patients receiving those drugs. If the patients' physicians felt the subjects benefited, they could make the decision, apart from this study, to continue the colchicine if they are inclined to do so. Although there are small risks associated with low dose colchicine, the risks would be relatively short term since this is only a 24-week study (with at least 6 week surveillance). In addition, low dose colchicine is associated with reduced heart disease risk in patients with arthritis and in HIV- people with clinical CAD.

Contraindications to MRI studies as listed above will be strictly observed and should not constitute added risks for patients enrolled in this study. The low risk is reasonable in relation to the potential knowledge gained that may guide future therapeutic studies.

10. Payment and Remuneration

a. Detail compensation for participants including possible total compensation, proposed bonus, and any proposed reductions or penalties for not completing the protocol.

Participants will receive parking at each visit and will be offered a $10 meal voucher. Compensation of $75 will be given for each completed MRI (3 total) and $50 for each completed safety monitoring visit (4 total) for a possible total compensation of $425.

11. Costs

a. Detail costs of study procedure(s) or drug (s) or substance(s) to participants and identify who will pay for them.

There is no cost to the participants related to the study procedures or drugs.

Bibliography:

1. Nidorf SM, Eikelboom JW, Budgeon CA, Thompson PL. Low-Dose Colchicine for Secondary Prevention of Cardiovascular Disease. Journal of the American College of Cardiology. 2013;61(4):404-10.
2. Anderson TJ, Meredith IT, Yeung AC, Frei B, Selwyn AP, Ganz P. The Effect of Cholesterol-Lowering and Antioxidant Therapy on Endothelium-Dependent Coronary Vasomotion. New Engl J Med. 1995;332(8):488-93. doi: 10.1056/nejm199502233320802.
3. Halcox JPJ, Schenke WH, Zalos G, Mincemoyer R, Prasad A, Waclawiw MA, Nour KRA, Quyyumi AA. Prognostic Value of Coronary Vascular Endothelial Dysfunction. Circulation. 2002;106(6):653-8.
4. Hambrecht R, Wolf A, Gielen S, Linke A, Hofer J, Erbs S, Schoene N, Schuler G. Effect of Exercise on Coronary Endothelial Function in Patients with Coronary Artery Disease. New Engl J Med. 2000;342(7):454-60. doi: 10.1056/nejm200002173420702.
5. Nitenberg A, Chemla D, Antony I. Epicardial coronary artery constriction to cold pressor test is predictive of cardiovascular events in hypertensive patients with angiographically normal

coronary arteries and without other major coronary risk factor. Atherosclerosis. 2004;173(1):115-23. doi: 10.1016/j.atherosclerosis.2003.12.030.
6. Schächinger V, Britten MB, Zeiher AM. Prognostic Impact of Coronary Vasodilator Dysfunction on Adverse Long-Term Outcome of Coronary Heart Disease. Circulation. 2000;101(16):1899-906.
7. Suwaidi JA, Hamasaki S, Higano ST, Nishimura RA, Holmes DR, Lerman A. Long-Term Follow-Up of Patients With Mild Coronary Artery Disease and Endothelial Dysfunction. Circulation. 2000;101(9):948-54.
8. Treasure CB, Klein JL, Weintraub WS, Talley JD, Stillabower ME, Kosinski AS, Zhang J, Boccuzzi SJ, Cedarholm JC, Alexander RW. Beneficial effects of cholesterol-lowering therapy on the coronary endothelium in patients with coronary artery disease. New England Journal of Medicine. 1995;332(8):481-7.
9. Ludmer PL, Selwyn AP, Shook TL, Wayne RR, Mudge GH, Alexander RW, Ganz P. Paradoxical Vasoconstriction Induced by Acetylcholine in Atherosclerotic Coronary Arteries. New Engl J Med. 1986;315(17):1046-51. doi: 10.1056/nejm198610233151702.
10. Hays AG, Hirsch GA, Kelle S, Gerstenblith G, Weiss RG, Stuber M. Noninvasive Visualization of Coronary Artery Endothelial Function in Healthy Subjects and in Patients With Coronary Artery Disease. J Am Coll Cardiol. 2010;56(20):1657-65.
11. Hays AG, Kelle S, Hirsch GA, Soleimanifard S, Yu J, Agarwal HK, Gerstenblith G, Schär M, Stuber M, Weiss RG. Regional coronary endothelial function is closely related to local early coronary atherosclerosis in patients with mild coronary artery disease: pilot study. Circulation Cardiovascular imaging. 2012;5(3):341-8.
12. Lai H, Detrick B, Fishman EK, Hollis BW, Gerstenblith G, Brinker JA, Bartlett J, Cofrancesco Jr J, Tong W, Tai H. Vitamin D deficiency is associated with the development of subclinical coronary artery disease in African Americans with HIV infection: a preliminary study. J Investig Med. 2012;60(5):801.
13. Lai H, Fishman EK, Gerstenblith G, Moore R, Brinker JA, Keruly JC, Chen S, Detrick B, Lai S. Vitamin D deficiency is associated with development of subclinical coronary artery disease in HIV-infected African American cocaine users with low Framingham-defined cardiovascular risk. Vascular health and risk management. 2013;9:729.
14. Lai H, Gerstenblith G, Fishman EK, Brinker J, Kickler T, Tong W, Bhatia S, Hong T, Chen S, Li J. Vitamin D deficiency is associated with silent coronary artery disease in cardiovascularly asymptomatic African Americans with HIV infection. Clin Infect Dis. 2012;54(12):1747-55.
15. Boccara F, Lang S, Meuleman C, Ederhy S, Mary-Krause M, Costagliola D, Capeau J, Cohen A. HIV and Coronary Heart Disease: Time for a Better Understanding. J Am Coll Cardiol. 2013;61(5):511-23.
16. Collaboration ATC, Gill J, May M, Lewden C, Saag M, Mugavero M, Reiss P, Ledergerber B, Mocroft A, Harris R. Causes of death in HIV-1-infected patients treated with antiretroviral therapy, 1996-2006: collaborative analysis of 13 HIV cohort studies. Clin Infect Dis. 2010;50(10):1387-96.
17. Lewden C, May T, Rosenthal E, Burty C, Bonnet F, Costagliola D, Jougla E, Semaille C, Morlat P, Salmon D. Changes in causes of death among adults infected by HIV between 2000 and 2005: The" Mortalite 2000 and 2005" surveys (ANRS EN19 and Mortavic). JAIDS J Acquired Immune Defic Syndromes. 2008;48(5):590-8.
18. Palella Jr FJ, Baker RK, Moorman AC, Chmiel JS, Wood KC, Brooks JT, Holmberg SD. Mortality in the highly active antiretroviral therapy era: changing causes of death and disease in the HIV outpatient study. JAIDS J Acquired Immune Defic Syndromes. 2006;43(1):27-34.

19. Lang S, Mary-Krause M, Cotte L, Gilquin J, Partisani M, Simon A, Boccara F, Bingham A, Costagliola D. Increased risk of myocardial infarction in HIV-infected patients in France, relative to the general population. AIDS. 2010;24(8):1228-30.
20. Triant VA, Lee H, Hadigan C, Grinspoon SK. Increased acute myocardial infarction rates and cardiovascular risk factors among patients with human immunodeficiency virus disease. J Clin Endocrinol Metab. 2007;92(7):2506-12.
21. Althoff KN, McGinnis KA, Wyatt CM, Freiberg MS, Gilbert C, Oursler KK, Rimland D, Rodriguez-Barradas MC, Dubrow R, Park LS. Comparison of risk and age at diagnosis of myocardial infarction, end-stage renal disease, and non-AIDS-defining cancer in HIV-infected vs uninfected adults. Clin Infect Dis. 2014:ciu869.
22. Ross R. Atherosclerosis — An Inflammatory Disease. New Engl J Med. 1999;340(2):115-26. doi: 10.1056/nejm199901143400207.
23. Libby P, Ridker PM, Maseri A. Inflammation and Atherosclerosis. Circulation. 2002;105(9):1135-43.
24. Zanni MV, Grinspoon SK. HIV-specific immune dysregulation and atherosclerosis. Current HIV/AIDS Reports. 2012;9(3):200-5.
25. Anderson TJ, Meredith IT, Yeung AC, Frei B, Selwyn AP, Ganz P. The effect of cholesterol-lowering and antioxidant therapy on endothelium-dependent coronary vasomotion. N Engl J Med 1995;332(8):488-93. PubMed PMID: 389.
26. Ludmer PL, Selwyn AP, Shook TL, Wayne RR, Mudge GH, Alexander RW, Ganz P. Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. N Engl J Med. 1986;315(17):1046-51. PubMed PMID: 3093861.
27. Halcox JP, Schenke WH, Zalos G, Mincemoyer R, Prasad A, Waclawiw MA, Nour KR, Quyyumi AA. Prognostic value of coronary vascular endothelial dysfunction. Circulation. 2002;106(6):653-8. PubMed PMID: 12163423.
28. Hambrecht R, Wolf A, Gielen S, Linke A, Hofer J, Erbs S, Schoene N, Schuler G. Effect of exercise on coronary endothelial function in patients with coronary artery disease. N Engl J Med. 2000;342(7):454-60. PubMed PMID: 10675425.
29. Nitenberg A, Chemla D, Antony I. Epicardial coronary artery constriction to cold pressor test is predictive of cardiovascular events in hypertensive patients with angiographically normal coronary arteries and without other major coronary risk factor. Atherosclerosis. 2004;173(1):115-23. PubMed PMID: 15177131.
30. Schachinger V, Britten MB, Zeiher AM. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. Circulation. 2000;101(16):1899-906. PubMed PMID: 10779454.
31. Suwaidi JA, Hamasaki S, Higano ST, Nishimura RA, Holmes DR, Jr., Lerman A. Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. Circulation. 2000;101(9):948-54. PubMed PMID: 10704159.
32. Treasure CB, Klein JL, Weintraub WS, Talley JD, Stillabower ME, Kosinski AS, Zhang J, Boccuzzi SJ, Cedarholm JC, Alexander RW. Beneficial effects of cholesterol-lowering therapy on the coronary endothelium in patients with coronary artery disease. N Engl J Med. 1995;332(8):481-7. PubMed PMID: 7830728.
33. Vane JR, Anggard EE, Botting RM. Regulatory functions of the vascular endothelium. N Engl J Med. 1990;323(1):27-36. PubMed PMID: 2113184.
34. Fichtlscherer S, Rosenberger G, Walter DH, Breuer S, Dimmeler S, Zeiher AM. Elevated C-reactive protein levels and impaired endothelial vasoreactivity in patients with coronary artery disease. Circulation. 2000;102(9):1000-6. PubMed PMID: 10961964.
35. Katakam PV, Tulbert CD, Snipes JA, Erdos B, Miller AW, Busija DW. Impaired insulin-induced vasodilation in small coronary arteries of Zucker obese rats is mediated by reactive oxygen species. Am J Physiol Heart Circ Physiol. 2005;288(2):H854-60. doi: 288/2/H854 [pii] 10.1152/ajpheart.00715.2004. PubMed PMID: 15650157.

36. Inoguchi T, Li P, Umeda F, Yu HY, Kakimoto M, Imamura M, Aoki T, Etoh T, Hashimoto T, Naruse M, Sano H, Utsumi H, Nawata H. High glucose level and free fatty acid stimulate reactive oxygen species production through protein kinase C--dependent activation of NAD(P)H oxidase in cultured vascular cells. Diabetes. 2000;49(11):1939-45. PubMed PMID: 11078463.
37. Sparagna GC, Hickson-Bick DL, Buja LM, McMillin JB. A metabolic role for mitochondria in palmitate-induced cardiac myocyte apoptosis. Am J Physiol Heart Circ Physiol. 2000;279(5):H2124-32. PubMed PMID: 11045945.
38. Zhou YT, Grayburn P, Karim A, Shimabukuro M, Higa M, Baetens D, Orci L, Unger RH. Lipotoxic heart disease in obese rats: implications for human obesity. Proc Natl Acad Sci U S A. 2000;97(4):1784-9. doi: 97/4/1784 [pii]. PubMed PMID: 10677535.
39. Torriani FJ, Komarow L, Parker RA, Cotter BR, Currier JS, Dubé MP, Fichtenbaum CJ, Gerschenson M, Mitchell CKC, Murphy RL. Endothelial Function in Human Immunodeficiency Virus-Infected Antiretroviral-Naive Subjects Before and After Starting Potent Antiretroviral TherapyThe ACTG (AIDS Clinical Trials Group) Study 5152s. J Am Coll Cardiol. 2008;52(7):569-76.
40. Brown BG, Josephson MA, Petersen RB, Pierce CD, Wong M, Hecht HS, Bolson E, Dodge HT. Intravenous dipyridamole combined with isometric handgrip for near maximal acute increase in coronary flow in patients with coronary artery disease. The American Journal of Cardiology. 1981;48(6):1077-85.
41. Brown BG, Lee AB, Bolson EL, Dodge HT. Reflex constriction of significant coronary stenosis as a mechanism contributing to ischemic left ventricular dysfunction during isometric exercise. Circulation. 1984;70(1):18-24.
42. Wray DW, Witman MAH, Ives SJ, McDaniel J, Fjeldstad AS, Trinity JD, Conklin JD, Supiano MA, Richardson RS. Progressive handgrip exercise: evidence of nitric oxide-dependent vasodilation and blood flow regulation in humans. American Journal of Physiology-Heart and Circulatory Physiology. 2011;300(3):H1101-H7.
43. Gilligan DM, Panza JA, Kilcoyne CM, Waclawiw MA, Casino PR, Quyyumi AA. Contribution of endothelium-derived nitric oxide to exercise-induced vasodilation. Circulation. 1994;90(6):2853-8.
44. Duffy SJ, New G, Harper RW, Meredith IT. Metabolic vasodilation in the human forearm is preserved in hypercholesterolemia despite impairment of endothelium-dependent and independent vasodilation. Cardiovasc Res. 1999;43(3):721-30.
45. Rubenfire M, Cao N, Smith DE, Mosca L. Usefulness of brachial artery reactivity to isometric handgrip exercise in identifying patients at risk and with coronary artery disease. The American Journal of Cardiology. 2000;86(11):1161-5.
46. Giugliano GR, Giugliano RP, Gibson CM, Kuntz RE. Meta-analysis of corticosteroid treatment in acute myocardial infarction. The American Journal of Cardiology. 2003;91(9):1055-9.
47. Vogel RA, Forrester JS. Cooling Off Hot Hearts: A Specific Therapy for Vulnerable Plaque? J Am Coll Cardiol. 2013;61(4):411-2.
48. Olsen A-MS, Fosbøl EL, Lindhardsen J, Folke F, Charlot M, Selmer C, Bjerring Olesen J, Lamberts M, Ruwald MH, Køber L, Hansen PR, Torp-Pedersen C, Gislason GH. Long-Term Cardiovascular Risk of Nonsteroidal Anti-Inflammatory Drug Use According to Time Passed After First-Time Myocardial Infarction: A Nationwide Cohort Study. Circulation. 2012;126(16):1955-63.
49. Koenig W, Lowel H, Baumert J, Meisinger C. C-reactive protein modulates risk prediction based on the Framingham Score: implications for future risk assessment: results from a large cohort study in southern Germany. Circulation. 2004;109(11):1349-53. Epub 2004/03/17. doi: 10.1161/01.CIR.0000120707.98922.E301.CIR.0000120707.98922.E3 [pii]. PubMed PMID: 15023871.

50. Pai JK, Pischon T, Ma J, Manson JE, Hankinson SE, Joshipura K, Curhan GC, Rifai N, Cannuscio CC, Stampfer MJ, Rimm EB. Inflammatory markers and the risk of coronary heart disease in men and women. N Engl J Med. 2004;351(25):2599-610. Epub 2004/12/17. doi: 351/25/2599 [pii]10.1056/NEJMoa040967. PubMed PMID: 15602020.
51. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med. 2000;342(12):836-43. Epub 2000/03/25. doi: 10.1056/NEJM200003233421202. PubMed PMID: 10733371.
52. Ridker PM, Rifai N, Rose L, Buring JE, Cook NR. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. N Engl J Med. 2002;347(20):1557-65. Epub 2002/11/15. doi: 10.1056/NEJMoa021993 347/20/1557 [pii]. PubMed PMID: 12432042.
53. Albert MA, Danielson E, Rifai N, Ridker PM. Effect of statin therapy on C-reactive protein levels: the pravastatin inflammation/CRP evaluation (PRINCE): a randomized trial and cohort study. JAMA. 2001;286(1):64-70. Epub 2001/07/04. doi: joc10590 [pii]. PubMed PMID: 11434828.
54. Ridker PM, Cannon CP, Morrow D, Rifai N, Rose LM, McCabe CH, Pfeffer MA, Braunwald E. C-reactive protein levels and outcomes after statin therapy. N Engl J Med. 2005;352(1):20-8. Epub 2005/01/07. doi: 352/1/20 [pii]10.1056/NEJMoa042378. PubMed PMID: 15635109.
55. Ridker PM, Danielson E, Fonseca FA, Genest J, Gotto AM, Jr., Kastelein JJ, Koenig W, Libby P, Lorenzatti AJ, MacFadyen JG, Nordestgaard BG, Shepherd J, Willerson JT, Glynn RJ. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. N Engl J Med. 2008;359(21):2195-207. Epub 2008/11/11. doi: NEJMoa0807646 [pii]10.1056/NEJMoa0807646. PubMed PMID: 18997196.
56. Ginsberg HN, Elam MB, Lovato LC, Crouse JR, 3rd, Leiter LA, Linz P, Friedewald WT, Buse JB, Gerstein HC, Probstfield J, Grimm RH, Ismail-Beigi F, Bigger JT, Goff DC, Jr., Cushman WC, Simons-Morton DG, Byington RP. Effects of combination lipid therapy in type 2 diabetes mellitus. N Engl J Med. 2010;362(17):1563-74. Epub 2010/03/17. doi: NEJMoa1001282 [pii]10.1056/NEJMoa1001282. PubMed PMID: 20228404; PMCID: 2879499.
57. Ford I, Murray H, Packard CJ, Shepherd J, Macfarlane PW, Cobbe SM. Long-term follow-up of the West of Scotland Coronary Prevention Study. N Engl J Med. 2007;357(15):1477-86. Epub 2007/10/12. doi: 357/15/1477 [pii]10.1056/NEJMoa065994. PubMed PMID: 17928595.
58. Topol EJ. Intensive statin therapy--a sea change in cardiovascular prevention. N Engl J Med. 2004;350(15):1562-4. Epub 2004/03/10. doi: 10.1056/NEJMe048061NEJMe048061 [pii]. PubMed PMID: 15007111.
59. Cannon CP, Braunwald E, McCabe CH, Rader DJ, Rouleau JL, Belder R, Joyal SV, Hill KA, Pfeffer MA, Skene AM. Intensive versus moderate lipid lowering with statins after acute coronary syndromes. N Engl J Med. 2004;350(15):1495-504. Epub 2004/03/10. doi: 10.1056/NEJMoa040583NEJMoa040583 [pii]. PubMed PMID: 15007110.
60. Baigent C, Keech A, Kearney PM, Blackwell L, Buck G, Pollicino C, Kirby A, Sourjina T, Peto R, Collins R, Simes R. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. Lancet. 2005;366(9493):1267-78. Epub 2005/10/11. doi: S0140-6736(05)67394-1 [pii]10.1016/S0140-6736(05)67394-1. PubMed PMID: 16214597.
61. Ridker PM, Morrow DA, Rose LM, Rifai N, Cannon CP, Braunwald E. Relative efficacy of atorvastatin 80 mg and pravastatin 40 mg in achieving the dual goals of low-density lipoprotein cholesterol <70 mg/dl and C-reactive protein <2 mg/l: an analysis of the PROVE-

IT TIMI-22 trial. J Am Coll Cardiol. 2005;45(10):1644-8. Epub 2005/05/17. doi: S0735-1097(05)00742-4 [pii]10.1016/j.jacc.2005.02.080. PubMed PMID: 15893181.

62. Cerquaglia C, Diaco M, Nucera G, Regina M, Montalto M, Manna R. Pharmacological and clinical basis of treatment of familial Mediterranean fever (FMF) with colchicine or analogues: an update. Current Drug Targets-Inflammation and Allergy. 2005;4(1):117-24.
63. Kiraz S, Ertenli I, Arici M, Calgüneri M, Haznedaroglu I, Celik I, Pay S, Kirazli S. Effects of colchicine on inflammatory cytokines and selectins in familial Mediterranean fever. Clin Exp Rheumatol. 1998;16(6):721-4.
64. Zemer D, Pras M, Sohar E, Modan M, Cabili S, Gafni J. Colchicine in the Prevention and Treatment of the Amyloidosis of Familial Mediterranean Fever. New Engl J Med. 1986;314(16):1001-5. doi: doi:10.1056/NEJM198604173141601.
65. Yazici H. Behçet's syndrome: An update. Curr Rheumatol Rep. 2003;5(3):195-9. doi: 10.1007/s11926-003-0066-9.
66. Molad Y. Update on colchicine and its mechanism of action. Curr Rheumatol Rep. 2002;4(3):252-6.
67. Perico N, Ostermann D, Bontempeill M, Morigi M, Amuchastegui CS, Zoja C, Akalin E, Sayegh MH, Remuzzi G. Colchicine interferes with L-selectin and leukocyte function-associated antigen-1 expression on human T lymphocytes and inhibits T cell activation. J Am Soc Nephrol. 1996;7(4):594-601.
68. Adler Y, Finkelstein Y, Guindo J, Rodriguez de la Serna A, Shoenfeld Y, Bayes-Genis A, Sagie A, Bayes de Luna A, Spodick DH. Colchicine treatment for recurrent pericarditis: a decade of experience. Circulation. 1998;97(21):2183-5.
69. Imazio M, Brucato A, Trinchero R, Spodick D, Adler Y. Colchicine for pericarditis: hype or hope? Eur Heart J. 2009;30(5):532-9.
70. Imazio M, Brucato A, Ferrazzi P, Rovere ME, Gandino A, Cemin R, Ferrua S, Belli R, Maestroni S, Simon C. Colchicine Reduces Postoperative Atrial Fibrillation Results of the Colchicine for the Prevention of the Postpericardiotomy Syndrome (COPPS) Atrial Fibrillation Substudy. Circulation. 2011;124(21):2290-5.
71. Deftereos S, Giannopoulos G, Kossyvakis C, Efremidis M, Panagopoulou V, Kaoukis A, Raisakis K, Bouras G, Angelidis C, Theodorakis A. Colchicine for Prevention of Early Atrial Fibrillation Recurrence After Pulmonary Vein IsolationA Randomized Controlled Study. J Am Coll Cardiol. 2012;60(18):1790-6.
72. Imazio M, Brucato A, Cemin R, Ferrua S, Maggiolini S, Beqaraj F, Demarie D, Forno D, Ferro S, Maestroni S. A randomized trial of colchicine for acute pericarditis. New Engl J Med. 2013;369(16):1522-8.
73. Crittenden DB, Lehmann RA, Schneck L, Keenan RT, Shah B, Greenberg JD, Cronstein BN, Sedlis SP, Pillinger MH. Colchicine Use Is Associated with Decreased Prevalence of Myocardial Infarction in Patients with Gout. The Journal of Rheumatology. 2012;39(7):1458-64.
74. Nidorf M, Thompson PL. Effect of colchicine (0.5 mg twice daily) on high-sensitivity C-reactive protein independent of aspirin and atorvastatin in patients with stable coronary artery disease. The American Journal of Cardiology. 2007;99(6):805.
75. Britton KA, Massaro JM, Murabito JM, Kreger BE, Hoffmann U, Fox CS. Body fat distribution, incident cardiovascular disease, cancer, and all-cause mortality. J Am Coll Cardiol. 2013;62(10):921-5.
76. Tchernof A, Després J-P. Pathophysiology of human visceral obesity: an update. Physiol Rev. 2013;93(1):359-404.
77. Mottillo S, Filion KB, Genest J, Joseph L, Pilote L, Poirier P, Rinfret S, Schiffrin EL, Eisenberg MJ. The Metabolic Syndrome and Cardiovascular RiskA Systematic Review and Meta-Analysis. J Am Coll Cardiol. 2010;56(14):1113-32.

78. Rondinone CM. Adipocyte-derived hormones, cytokines, and mediators. Endocrine. 2006;29(1):81-90.
79. Margaritis M, Antonopoulos AS, Digby J, Lee R, Reilly S, Coutinho P, Shirodaria C, Sayeed R, Petrou M, De Silva R. Interactions Between Vascular Wall and Perivascular Adipose Tissue Reveal Novel Roles for Adiponectin in the Regulation of Endothelial Nitric Oxide Synthase Function in Human Vessels. Circulation. 2013;127(22):2209-21.
80. Tungsiripat M, McComsey G. Pathogenesis and management of lipoatrophy. Current HIV/AIDS Reports. 2008;5(2):55-63.
81. Lake JE, Wohl D, Scherzer R, Grunfeld C, Tien PC, Sidney S, Currier JS. Regional fat deposition and cardiovascular risk in HIV infection: the FRAM study. AIDS Care. 2011;23(8):929-38.
82. Rabkin SW. Epicardial fat: properties, function and relationship to obesity. Obesity reviews. 2007;8(3):253-61.
83. Doesch C, Haghi D, Suselbeck T, Schoenberg SO, Borggrefe M, Papavassiliu T. Impact of Functional, Morphological and Clinical Parameters on Epicardial Adipose Tissue in Patients With Coronary Artery Disease. Circulation journal: official journal of the Japanese Circulation Society. 2012.
84. Mazurek T, Zhang L, Zalewski A, Mannion JD, Diehl JT, Arafat H, Sarov-Blat L, O'Brien S, Keiper EA, Johnson AG. Human epicardial adipose tissue is a source of inflammatory mediators. Circulation. 2003;108(20):2460-6.
85. Baker AR, Harte AL, Howell N, Pritlove DC, Ranasinghe AM, da Silva NF, Youssef EM, Khunti K, Davies MJ, Bonser RS, Kumar S, Pagano D, McTernan PG. Epicardial Adipose Tissue as a Source of Nuclear Factor-κB and c-Jun N-Terminal Kinase Mediated Inflammation in Patients with Coronary Artery Disease. The Journal of Clinical Endocrinology & Metabolism. 2009;94(1):261-7. doi: 10.1210/jc.2007-2579.
86. Gabriella O, Giovanni G, Stefano Z, Federica C, Pietro B, Marianna M, Stefania C, Riccardo S, Guido L, Paolo R. Ectopic Fat is Linked to Prior Cardiovascular Events in Men With HIV. JAIDS J Acquired Immune Defic Syndromes. 2012;59(5).
87. Guaraldi G, Scaglioni R, Zona S, Orlando G, Carli F, Ligabue G, Besutti G, Bagni P, Rossi R, Modena MG. Epicardial adipose tissue is an independent marker of cardiovascular risk in HIV-infected patients. AIDS. 2011;25(9):1199-205.
88. McComsey GA, Kitch D, Sax PE, Tebas P, Tierney C, Jahed NC, Myers L, Melbourne K, Ha B, Daar ES. Peripheral and central fat changes in subjects randomized to abacavir-lamivudine or tenofovir-emtricitabine with atazanavir-ritonavir or efavirenz: ACTG Study A5224s. Clin Infect Dis. 2011;53(2):185-96.
89. Haubrich RH, Riddler SA, DiRienzo AG, Komarow L, Powderly WG, Klingman K, Garren KW, Butcher DL, Rooney JF, Haas DW. Metabolic outcomes in a randomized trial of nucleoside, nonnucleoside and protease inhibitor-sparing regimens for initial HIV treatment. AIDS (London, England). 2009;23(9):1109.
90. Iacobellis G, Pellicelli AM, Sharma AM, Grisorio B, Barbarini G, Barbaro G. Relation of subepicardial adipose tissue to carotid intima-media thickness in patients with human immunodeficiency virus. The American journal of cardiology. 2007;99(10):1470-2.
91. Crum-Cianflone NF, Kathiria N, Shauger S, Love K, Boswell G. The association of epicardial adipose tissue with coronary artery calcification among HIV-infected men. AIDS. 2012;26(12):1573-6.
92. Machann J, Thamer C, Schnoedt B, Haap M, Haring HU, Claussen CD, Stumvoll M, Fritsche A, Schick F. Standardized assessment of whole body adipose tissue topography by MRI. J Magn Reson Imaging. 2005;21(4):455-62.
93. Shahzad R, Bos D, Metz C, Rossi A, Kirişli H, van der Lugt A, Klein S, Witteman J, de Feyter P, Niessen W. Automatic quantification of epicardial fat volume on non-enhanced

cardiac CT scans using a multi-atlas segmentation approach. Med Phys. 2013;40(9):091910.
94. Bertaso AG, Bertol D, Duncan BB, Foppa M. Epicardial fat: definition, measurements and systematic review of main outcomes. Arq Bras Cardiol. 2013;101(1):e18-e28.
95. Sacks HS, Fain JN. Human epicardial adipose tissue: a review. Am Heart J. 2007;153(6):907-17.
96. Ishii T, Asuwa N, Masuda S, Ishikawa Y. The effects of a myocardial bridge on coronary atherosclerosis and ischaemia. The Journal of pathology. 1998;185(1):4-9.
97. Möhlenkamp S, Hort W, Ge J, Erbel R. Update on myocardial bridging. Circulation. 2002;106(20):2616-22.
98. Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, Lloyd JK, Deanfield JE. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. Lancet. 1992;340(8828):1111-5. PubMed PMID: 1359209.
99. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial arterya report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol. 2002;39(2):257-65.
100. McKibben RA, Margolick JB, Grinspoon S, Li X, Palella FJ, Kingsley LA, Witt MD, George RT, Jacobson LP, Budoff M. Elevated levels of monocyte activation markers are associated with subclinical atherosclerosis in men with and those without HIV infection. J Infect Dis. 2014:jiu594.
101. Hürlimann D, Chenevard R, Ruschitzka F, Flepp M, Enseleit F, Béchir M, Kobza R, Muntwyler J, Ledergerber B, Lüscher TF. Effects of statins on endothelial function and lipid profile in HIV infected persons receiving protease inhibitor-containing anti-retroviral combination therapy: a randomised double blind crossover trial. Heart. 2006;92(1):110-2.
102. Gupta SK, Johnson RM, Saha C, Mather KJ, Greenwald ML, Waltz JS, Rehman J, Dubé MP. Improvement in HIV-related endothelial dysfunction using the anti-inflammatory agent salsalate: a pilot study. AIDS. 2008;22(5):653-5.
103. Wassmann S, Faul A, Hennen B, Scheller B, Böhm M, Nickenig G. Rapid effect of 3-hydroxy-3-methylglutaryl coenzyme a reductase inhibition on coronary endothelial function. Circul Res. 2003;93(9):e98-e103.
104. Takase H, Toriyama T, Sugiyama M, Nakazawa A, Hayashi K, Goto T, Sugiura T, Ikeda K, Sato K, Ueda R. Effect of Nifedipine on C-Reactive Protein Levels in the Coronary Sinus and on Coronary Blood Flow In Response to Acetylcholine in Patients With Stable Angina Pectoris Having Percutaneous Coronary Intervention. The American Journal of Cardiology. 2005;95(10):1235-7.
105. Egashira K, Hirooka Y, Kai H, Sugimachi M, Suzuki S, Inou T, Takeshita A. Reduction in serum cholesterol with pravastatin improves endothelium-dependent coronary vasomotion in patients with hypercholesterolemia. Circulation. 1994;89(6):2519-24.
106. Bergholm R, Leirisalo-Repo M, Vehkavaara S, Mäkimattila S, Taskinen M-R, Yki-Järvinen H. Impaired responsiveness to NO in newly diagnosed patients with rheumatoid arthritis. Arterioscler Thromb Vasc Biol. 2002;22(10):1637-41.
107. John S, Schneider MP, Delles C, Jacobi J, Schmieder RE. Lipid-independent effects of statins on endothelial function and bioavailability of nitric oxide in hypercholesterolemic patients. Am Heart J. 2005;149(3):473. e1-. e10.
108. O'Driscoll G, Green D, Taylor RR. Simvastatin, an HMG–coenzyme A reductase inhibitor, improves endothelial function within 1 month. Circulation. 1997;95(5):1126-31.
109. Dupuis J, Tardif J-C, Cernacek P, Théroux P. Cholesterol Reduction Rapidly Improves Endothelial Function After Acute Coronary Syndromes The RECIFE (Reduction of

Cholesterol in Ischemia and Function of the Endothelium) Trial. Circulation. 1999;99(25):3227-33.

110. Mancini GJ, Henry GC, Macaya C, O'Neill BJ, Pucillo AL, Carere RG, Wargovich TJ, Mudra H, Lu TF, Klibaner MI. Angiotensin-converting enzyme inhibition with quinapril improves endothelial vasomotor dysfunction in patients with coronary artery disease The TREND (Trial on Reversing Endothelial Dysfunction) Study. Circulation. 1996;94(3):258-65.
111. Mukerjee R, Wu CFJ. A modern theory of factorial design: Springer; 2006.
112. Young F, FDA. Magnetic Resonance Diagnostic Device; Panel Recommendation and Report on Petitions for MR Reclassification. . Federal Register. 1988;53:7575-9. .
113. Kivowitz C, Parmley WW, Donoso R, Marcus H, Ganz W, Swan HJ. Effects of isometric exercise on cardiac performance. The grip test. Circulation. 1971;44(6):994-1002. PubMed PMID: 4331419.
114. Weiss RG, Bottomley PA, Hardy CJ, Gerstenblith G. Regional myocardial metabolism of high-energy phosphates during isometric exercise in patients with coronary artery disease. N Engl J Med. 1990;323(23):1593-600. PubMed PMID: 2233948.
115. Deftereos S, Giannopoulos G, Kossyvakis C, Efremidis M, Panagopoulou V, Kaoukis A, Raisakis K, Bouras G, Angelidis C, Theodorakis A, Driva M, Doudoumis K, Pyrgakis V, Stefanadis C. Colchicine for prevention of early atrial fibrillation recurrence after pulmonary vein isolation: a randomized controlled study. J Am Coll Cardiol. 2012;60(18):1790-6. Epub 2012/10/09. doi: S0735-1097(12)03033-1 [pii]10.1016/j.jacc.2012.07.031. PubMed PMID: 23040570.