Cardiometabolic Effects of Eplerenone in HIV Infection

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Principal Investigator:
Colleen Hadigan, MD, MPH
Laboratory of Immunoregulation, NIAID
301-594-5754

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Study Staff Roster

Study Coordinator:

Julia Purdy, MSN, CPNP	Clinical Center (CC)	301-451-9109

Associate Investigators:

Dr. David Bluemke, MD, PhD	CC	301-402-1854
Dr. Chia-Ying Liu, MD	CC	301-402-6278
Dr. Caryn Morse, MD	CC	301-496-6028
Julia Purdy, MSN, RN	CC	301-451-9109

Biostatistician:

Michael Proschan, PhD	Biostatistics Research Branch (NIAID)	240-669-5245

Collaborating Institutions:

Dr. Steven Grinspoon, MD
Massachusetts General Hospital
55 Fruit Street
Boston, MA 02114
Tel: 617-726-3890
Email: sgrinspoon@mgh.harvard.edu

Dr. Takara Stanley, MD
Massachusetts General Hospital
55 Fruit Street
Boston, MA 02114
Tel: 617-726-2909
Email: tstanley@partners.org

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List of Abbreviations

ACE	angiotensin converting enzyme
AE	adverse event
ARB	angiotensin reporter blocker
BMI	body mass index
cART	combination antiretroviral therapy
CBC	complete blood count
CYP	cytochrome P450 isozyme
DEXA	dual-energy x-ray absorptiometry
eGFR	estimated glomerular filtration rate
FSH	follicle-stimulating hormone
GCP	Good Clinical Practice
Hgb	hemoglobin
HIV	Human Immunodeficiency Virus
HRPP	Human Research Protections Program
ICH	International Conference on Harmonisation
IRB	Institutional Review Board
LH	luteinizing hormone
MI	myocardial infarction
MR	mineralocorticoid receptor
MRI	magnetic resonance imaging
OHRP	Office for Human Research Protections
PT/PTT	prothrombin time/partial thromboplastin time
SAE	serious adverse event
SHBG	sex hormone-binding globulin
UP	unanticipated problem
UPnonAE	unanticipated problem that is not an adverse event
VAT	visceral adipose tissue

Protocol Summary

Short Title:	ECHO
Sample Size:	n = 20
Accrual Ceiling:	50
Study Population:	HIV-infected men and women between 20 and 75 years-old
Study Duration:	December 2015-February 2018 Total length of individual patient participation: ~7 months
Study Design:	Open-label pilot study
Study Agent/ Intervention Description:	Inspra®/Eplerenone (Pfizer Inc [New York City, NY, USA]), 25 mg/day for 1 week, then 50 mg/day for 23 weeks
Primary Objective:	To evaluate the effects of eplerenone on cardiac and hepatic steatosis in people with HIV infection who have high central adiposity.
Secondary Objectives:	To evaluate the effects of eplerenone treatment on biomarkers of inflammation and immune activation.
Primary Endpoints:	1. Improvement of cardiac steatosis as measured by magnetic resonance imaging (MRI) of the intraventricular septum 2. Improvement of hepatic steatosis as measured by MRI of the liver and histopathologic examination of liver biopsy.
Secondary Endpoint:	Reduction of biomarkers of inflammation and immune activation.

Précis

HIV-infected individuals are at higher risk than uninfected people for developing cardiovascular disease. Visceral adipose tissue is also increased in HIV-infected people compared to uninfected individuals. Animal studies suggest that blockade of the mineralocorticoid receptor (MR) may have beneficial effects on cardiovascular and metabolic parameters via inhibition of adipocyte differentiation and triglyceride accumulation. We will examine the effects of the MR antagonist eplerenone (50 mg daily) on HIV-infected adults with abdominal fat accumulation in a 24-week, open-label, proof-of-concept study. Magnetic resonance imaging will be conducted at screening and the final study visit to evaluate cardiac and hepatic steatosis. We anticipate that blocking the effects of increased aldosterone secretion with eplerenone will significantly reduce intramyocardial lipid content and hepatic steatosis in this population. These effects may be accompanied by decreases in visceral adipose tissue, and improvements in dyslipidemia and inflammation, thereby improving cardiovascular health.

1. Background Information and Scientific Rationale

1.1. Background Information

Metabolic, cardiovascular, and body composition abnormalities are associated with combination antiretroviral therapy (cART) and chronic HIV infection. Recent studies have shown that hypertension,[1] impaired glucose tolerance,[2] type 2 diabetes,[3,4] increased carotid intima-media thickness,[5] coronary atherosclerosis,[6] and myocardial infarction[7] are more prevalent in HIV-infected individuals than in non-infected controls. Visceral adipose tissue (VAT) is increased in HIV-infected patients compared to non-infected controls[8] and is associated with cardiac risk factors such as decreased insulin sensitivity,[9,10] hypertriglyceridemia and low high-density lipoprotein,[11] increased systemic inflammatory markers,[12-14] increased subclinical atherosclerosis,[15,16] and steatohepatitis. At this time there is no standard of care to adequately treat these abnormalities.

1.1.1. Adiposity in HIV-Infected Individuals

Cardiometabolic disturbances persist in the current era of HIV infection and in the context of contemporary cART practices. We recently conducted a detailed cross-sectional study of HIV-infected adults without known cardiovascular disease (n = 95) and age-, race-, and sex-matched controls (n = 30).[17] Using state-of-the-art magnetic resonance imaging (MRI) techniques, we identified significant increases in intramyocardial lipid content in HIV-infected subjects (HIV-infected = 1.5% ± 1.2%, control = 1.0% ± 1.0%, p = 0.04). In addition, HIV-infected subjects demonstrated significant subclinical impairment in cardiac function as measured by myocardial strain (HIV-infected = 21.7% ± 8.6%, control = 30.5% ± 14.2%, p = 0.004).

Further, the marker of immune activation MCP-1 was markedly elevated in HIV-infected subjects compared to controls (HIV-infected = 473 ± 249 pg/mL, control = 321 ± 221 pg/mL, p = 0.003) and was correlated with the degree of impaired cardiac strain ($r = -0.43$, $p < 0.0001$). MCP-1 is a chemokine important in the regulation of monocyte and macrophage migration. In HIV-infected subjects, increased levels of MCP-1 was strongly associated with impairment in cardiac function, similar to patients with chronic heart failure. These data highlight the importance of and potential underlying relationship between dysregulation in lipid metabolism/storage, immune activation, and downstream organ function. Of note, we also found

that increased VAT was a strong independent predictor of both cardiac and hepatic steatosis in HIV-infected individuals, which further supports the pathophysiologic role of central adiposity in the process of cardiometabolic disturbances in HIV infection.

While VAT appears important, the causes of body composition changes, metabolic abnormalities, and increased cardiovascular risk are multifactorial in HIV infection and likely include perturbation of multiple endocrine axes. In non–HIV-infected individuals, recent data have shown that aldosterone, a mineralocorticoid hormone, is increased in association with increased VAT[18] and decreased insulin sensitivity.[19] Our collaborators, led by Dr. Steven Grinspoon, demonstrated increased 24-hour urine aldosterone in HIV-infected women with increased visceral fat accumulation, compared to age- and body mass index (BMI)–matched healthy controls.[20] In this study, urinary aldosterone secretion was strongly related to VAT. Furthermore, emerging animal and human studies have suggested effects of mineralocorticoid blockade on insulin sensitivity, inflammation, and cardiac and hepatic steatosis, all of which are metabolic problems enriched in persons living with chronic HIV infection.[21-24]

1.1.2. Rationale for Studying the Mineralocorticoid Receptor

The renin-angiotensin-aldosterone system represents a hormonal cascade that maintains blood pressure and salt water balance. Recent data suggest mineralocorticoid receptor (MR) activation also plays a role in promotion of fibrosis and inflammation in various tissues including the vasculature, cardiac muscle, and the liver. Reports speculate that MR activation by aldosterone may play a role in cardiovascular and metabolic disease, as well, which is further supported by animal models.[25,26] For example, in vitro and in vivo data from a mouse model of steatohepatitis suggest that an MR antagonist ameliorates hepatic steatosis and fibrosis,[23] whereas the same approach attenuates cardiac steatosis and diastolic dysfunction in a rat model of type 2 diabetes.[24]

There are reports of increased systemic aldosterone levels associated with high body fat. Twenty-four-hour urine aldosterone/creatinine concentrations were higher in an animal model of obesity (db/db mice) compared to lean (db/+) mice.[21] In another study, non–HIV-infected overweight and obese normotensive adults exhibited higher concentrations of both 24-hour urine aldosterone and angiotensin II-stimulated aldosterone than lean controls.[27] Some reports suggest that the relationship of aldosterone and body fat may be largely mediated by increased VAT. Goodfriend and colleagues demonstrated that plasma aldosterone correlated with VAT in normotensive women,[18] and as described above, we have demonstrated a positive relationship of 24-hour urinary aldosterone with VAT in HIV-infected women.[20]

These observations are supported by in vivo models of ob/ob and db/db mice, in which MR blockade with the aldosterone antagonist eplerenone markedly decreased the number of hypertrophic adipocytes in epididymal fat.[28] There is also evidence to suggest that MR blockade may reduce hepatic steatosis, which is prevalent in HIV-infected individuals and is associated with increased visceral abdominal adiposity.[15,29] Wada and colleagues designed a murine model of metabolic syndrome and non-alcoholic steatohepatitis using transgenic mice that overexpressed liver-specific SREBP-1 and were fed a high-fat diet.[23] They showed that MR blockade with eplerenone resulted in improvements in hepatic triglyceride content, liver transaminase levels, circulating triglycerides, and measures of insulin resistance. Fallo and colleagues demonstrated an increased prevalence of hepatic steatosis in human patients with primary aldosteronism compared to normotensive controls.[30] Together, these observations provide compelling evidence to support investigation of the role of MR blockade in the context of hepatic steatosis and increased visceral adiposity in people with HIV infection. Further studies are clearly needed to determine whether mineralocorticoid activity affects myocardial and hepatic triglyceride accumulation.

HIV infection is accompanied by increased markers of systemic inflammation.[12-14] Levels of inflammatory cytokines predict atherosclerosis[6,31] and myocardial infarction[32] in HIV-infected individuals. As noted in our preliminary data, subclinical impairment in regional cardiac function in adults with HIV infection is associated with markers of inflammation and immune activation such as MCP-1. Recent studies suggest that MR activation may play a distinct role in both cardiovascular and systemic inflammation. In Sprague-Dawley rats, aldosterone administration for 4 weeks resulted in increased medial thickening of coronary arteries as well as perivascular leukocyte infiltration and, in some animals, significant leukocyte infiltration into cardiac tissue with subsequent cardiomyocyte degeneration and necrosis.[33] These changes were accompanied by increased gene expression of COX-2, MCP-1, osteopontin, and ICAM-1. Concomitant eplerenone treatment significantly ameliorated inflammatory changes and attenuated alterations in gene expression.

Although a role of hypertension cannot be excluded in this model, a model of atherosclerosis-prone apolipoprotein E-deficient mice supports the association between MR activation and vascular inflammation, independent of blood pressure.[34] Six weeks of high-cholesterol diet in these mice led to formation of atherosclerotic lesions in the aorta, with increased superoxide production and increased vascular expression of NADPH oxidase, TNF-α, and MCP-1. Eplerenone treatment did not alter total cholesterol levels or blood pressure, but significantly reduced atherosclerotic lesion area, superoxide production, and expression of

NADPH oxidase, TNF-α and MCP-1.[34] Further, in ob/ob mice, eplerenone reduced mRNA expression of MCP-1, IL-6, and TNF-α.[28] Finally, using the non-hypertensive Zucker diabetic rat model, Ramirez and colleagues showed benefits of 16 weeks of eplerenone treatment to attenuate cardiac steatosis, apoptosis, and diastolic dysfunction as measured by E/A ratio, without any untoward effects of hyperkalemia, or renal or hepatic injury.[24] While animal models in mice and rats may suggest a physiologic role for eplerenone in cardiometabolic conditions, these models may not be directly applicable in human conditions of hyperaldosteronism and the benefits of this approach remain to be determined.

1.2. Rationale

Research is needed into the effects of aldosterone and MR blockade on systemic and local inflammation and cardiometabolic parameters in humans. Eplerenone is a MR antagonist which has benefit for patients with aldosterone related hypertension and heart failure, but with considerably greater receptor specificity than spironolactone. Therefore eplerenone is associated with fewer undesirable progestin-like side effects, i.e. mastodynia and disturbance of the menstrual cycle in women. In this study we will carefully study the effects of MR blockade on lipid deposition in the myocardium and liver as well as systemic inflammation and immune activation in HIV-infected individuals.

2. Study Objectives

2.1. Primary Objectives

To evaluate the effects of eplerenone (Inspra®, Pfizer Inc [New York City, NY, USA] or generic) on cardiac and hepatic steatosis in people with HIV infection who have high central adiposity.

2.2. Secondary Objectives

To evaluate the effects of eplerenone treatment on biomarkers of inflammation and immune activation.

3. Study Design

3.1. Description of the Study Design

This is an open-label pilot study of the effects of eplerenone on 20 HIV-infected subjects who have increased central adiposity and evidence of hepatic steatosis. Eplerenone will be administered for 6 months as follows: 25 mg once daily for 1 week and then 50 mg once daily for the remainder of the study. This is a proof of concept study and therefore the sample size will be relatively small and there will be no control arm. If there is a signal for a beneficial effect of eplerenone, then we will consider design of a larger controlled trial in the future.

3.2. Study Endpoints

3.2.1. Primary Endpoint

1. Improvement of cardiac steatosis as measured by MRI of the intraventricular septum
2. Improvement of hepatic steatosis as measured by MRI of the liver and histopathologic examination of liver biopsy

3.2.2. Secondary Endpoint

Reduction of biomarkers of inflammation and immune activation.

4. Study Population

4.1. Recruitment Plan

We will recruit patients with HIV infection from existing NIH cohorts as well as HIV healthcare clinics in the greater Washington, DC community. In addition, we will use the OP8 Clinic recruitment strategies that are in place and include a full-time patient recruiter and community-based outreach within local area clinics specializing in HIV.

Recruitment of NIH Employees

NIH employees and members of their immediate families may participate in this protocol. We will follow the Guidelines for the Inclusion of Employees in NIH Research Studies and will give each employee a copy of the "NIH information sheet on Employee Research Participation."

For NIH employees:

- Neither participation nor refusal to participate will have an effect, either beneficial or adverse, on the subject's employment or work situation.

- The NIH information sheet regarding NIH employee research participation will be distributed to all potential subjects who are NIH employees.
- The employee subject's privacy and confidentiality will be preserved in accordance with NIH CC and NIAID policies, which define the scope and limitations of the protections.
- For NIH employee subjects, consent will be obtained by an individual independent of the employee's team. Those in a supervisory position to any employee and co-workers of the employee will not obtain consent.
- The importance of maintaining confidentiality when obtaining potentially sensitive and private information from co-workers or subordinates will be reviewed with the study staff at least annually and more often if warranted.

4.2. Subject Inclusion Criteria

1. Increased waist circumference on the basis of National Cholesterol Education Program guidelines (> 102 cm in men and > 88 cm in women)
2. Hepatic steatosis established by hepatic MRI $\geq$ 5% and/or liver biopsy within the last 12 months
3. HIV-infected, HIV viral load < 50 copies/mL and no change in ART regimen for at least 3 months
4. Age $\geq$ 18 and $\leq$ 75 years
5. Agree to have samples stored for future research

4.3. Subject Exclusion Criteria

1. Estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73 m^2, serum creatinine > 1.5 mg/dL
2. Serum potassium > 5.5 mEq/L, alanine aminotransferase > 2.5 times the upper limit of normal, hemoglobin (Hgb) < 11 g/dL
3. Uncontrolled hypertension: systolic blood pressure $\geq$ 160 mm Hg or diastolic blood pressure $\geq$ 100 mm Hg
4. A blood pressure < 90mmHg systolic or $\leq$ 50mm Hg diastolic .
5. Screening EKG with a significant heart block (e.g. PR > 300 ms) will trigger a cardiology consult before proceeding with the study. Based on the cardiology consult findings, the participants eligibility will be determined.
6. Current hepatitis C infection, unless there has been a sustained virologic response for at least 12 months

7. Type 2 diabetes with microalbuminuria
8. Current or prior steroid use within past 6 months (except short-course or single-dose administration). Stable use of inhaled or nasal steroids are allowed.
9. Use of angiotensin converting enzyme (ACE) inhibitors, angiotensin reporter blockers (ARBs), potassium-sparing diuretics, and other medications that may increase the risk of hyperkalemia
10. Use of potassium supplementation or other medications known to increase potassium (see Appendix C)
11. Concomitant use of strong inhibitors and/or inducers of cytochrome P450 isozyme (CYP)3A4 (see Appendix C)
12. If receiving testosterone, estrogen or progesterone therapy, must be on a stable dose for at least 3 months
13. Current use of growth hormone or growth hormone-releasing hormone
14. Current serious viral, bacterial, or other infection (excluding HIV)
15. Current active substance abuse/dependence
16. Substantial history of cardiovascular disease, including prior myocardial infarction (MI), congestive heart failure, or stroke
17. Contraindication to MRI
18. Pregnant or planning to become pregnant
19. Breastfeeding
20. Any condition that, in the opinion of the PI, may substantially increase the risk of participation

Co-enrollment Guidelines: Co-enrollment on observational studies or those evaluating the use of a licensed medication are allowed. Study staff should be notified of co-enrollment as it may require the approval of the Investigator.

4.4. Justification for Exclusion of Special Populations

Exclusion of Women:

- Pregnancy: Pregnant subjects are excluded from this study because the effects of eplerenone on the developing human fetus are unknown.
- Breastfeeding: Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with eplerenone.

Exclusion of Subjects ≤ 18 years-old and ≥ 75 years-old

Minors are excluded from this study because there are insufficient data regarding dosing or adverse events (AEs) in patients younger than 18 years-old. The incidence of laboratory-documented hyperkalemia was increased in patients 75 and older as a result of age-related decreases in creatinine clearance.

5. Study Agent/Interventions

5.1. Disposition and Dispensation

Study agent will be distributed via the NIH Pharmacy according to standard pharmacy procedures.

5.1.1. Formulation, Packaging, and Labeling

Each bottle will be individually labeled with the patient ID number, dosing instructions, recommended storage conditions, the name and address of the manufacturer, and that the agent should be kept out of reach of children.

5.2. Study Agent Storage and Stability

Eplerenone tablets should be stored at room temperature (15°C-30°C).

5.3. Preparation, Administration, and Dosage of Study Agent

Eplerenone is provided as 25- or 50-mg tablets that are to be taken orally. Eplerenone can be taken with or without food. Subjects will be dosed at 25 mg daily for 1 week, and then 50 mg daily for 23 weeks. The total duration of dosing for each subject is 24 weeks. Subjects who develop hyperkalemia (potassium > 5.5 mEq/L), eGFR< 60 mL/min/1.73 m^2, or serum creatinine > 1.5 mg/dL will have eplerenone withheld, and will only resume treatment if an alternative cause for these lab abnormalities is identified and the abnormality resolves. Unused study drug will be returned to the NIH Pharmacy at the end of the dosing period.

5.4. Study Product Accountability Procedures

Study agent accountability will be maintained by the pharmacy per standard procedures.

5.5. Assessment of Subject Compliance with Study Agent/Intervention

Medication compliance will be assessed at each visit by assessing pill count.

5.6. Concomitant Medications and Procedures

All concomitant prescription medications taken during study participation will be recorded.

5.7. Prohibited Medications

A member of the study team will screen the medication lists of eligible subjects before they enroll in the study. Subjects are prohibited from taking the following medications while they are taking eplerenone (see Appendix C):

- Strong CYP3A4 inhibitors or inducers
- ACE inhibitors and/or an ARB
- Potassium-sparing diuretics
- Medications that may cause hyperkalemia
- Steroids (other than a single dose or restricted short course)
- Potassium supplementation

6. Study Schedule and Procedures

A table of the study schedule is provided in Appendix B.

6.1. Screening

The following procedures will be conducted at the NIH Clinical Center (CC) to determine if a volunteer is eligible to participate in this study and may occur over a period of several days due to scheduling:

1. Physical exam, medical history, and vital signs, including measurements of blood pressure, height, weight, and BMI
2. Bionutrition: anthropometric measurements, Whole-body dual-energy x-ray absorptiometry (DEXA)
3. Urine pregnancy test (for subjects of childbearing potential only)
4. Blood collection for acute care, hepatic, and mineral panels; complete blood count (CBC); prothrombin time/partial thromboplastin time (PT/PTT); CD4 count and HIV viral load count; hepatitis serology

5. Urine studies: Urinalysis, urine protein/creatinine ratio and albumin/creatinine ratio.
6. Resting electrocardiogram
7. Non-contrast MRI for cardiac and hepatic steatosis and visceral fat volume
8. Transient elastography (FibroScan®, Echosens [Paris, France]) to measure liver tissue stiffness
9. Liver biopsy will be performed according to standard procedures by an experienced interventional radiologist (optional). Patients unwilling to undergo liver biopsy or for whom it is contraindicated (eg, platelets < 75,000) will not have a liver biopsy. Biopsies will be evaluated for biomarkers of inflammation and immune activation.

6.2. Baseline (Day 0)

The following procedures will be conducted at baseline at the NIH CC:

1. Interval medical history and physical exam, including blood pressure check
2. Research blood collection (fasted overnight) for acute care, hepatic, and mineral panels, CBC, fasting lipid profile; HgbA1c, CD4/8 count, HIV viral load, aldosterone, plasma renin activity, inflammatory markers and adipokines (MCP-1, CRP, adiponectin, PAI-1, resistin, TNF-α, TNFR2, IL-6), sex hormone-binding globulin (SHBG), total and free testosterone, luteinizing hormone (LH, female only), follicle-stimulating hormone (FSH, females only), estradiol (females only), and serum, plasma and peripheral blood mononuclear cells (PBMCs) for storage
3. Urine pregnancy test for subjects of childbearing potential
4. Subjects will receive a 1-week supply of eplerenone and instructions for use. Subjects will be dosed with 25 mg once daily for 1 week, and then 50 mg once daily for 23 weeks.

6.3. Study Phase (Weeks 1 to Week 24)

Interim safety visits will be scheduled for Weeks 1, 2, 4, and 8. Subjects will visit the NIH CC as outpatients and the following procedures will be conducted:

1. Physical exam and interval medical history, including blood pressure check
2. Research blood collection (fasted overnight) for acute care, hepatic, and mineral panels, , and serum, plasma, and PBMCs for storage. CD4/8 and HIV viral load counts will be done at week 4.
3. Urine pregnancy test for subjects of childbearing potential
4. Pill count of unused study drug to assess adherence

Another outpatient visit to the NIH CC will be scheduled for Week 12. The following procedures will be conducted:

1. Physical exam, including blood pressure check, and interval medical history
2. Research blood collection (fasted overnight) for acute care, hepatic, and mineral panels, lipid profile, CBC, HgbA1c, CD4/8 count, HIV viral load, aldosterone, plasma renin activity, inflammatory markers and adipokines (MCP-1, CRP, adiponectin, PAI-1, resistin, TNF-α, TNFR2, IL-6), SHBG, total and free testosterone, LH (female only), FSH (females only), estradiol (females only), and serum, plasma and PBMCs for storage
3. Urine pregnancy test for subjects of childbearing potential

At week 18, subjects will return to the NIH CC or can choose to go to their local medical provider (if this does not incur additional burden), for the following procedures:

1. Interval medical history, including blood pressure check
2. Research blood collection (fasted overnight) for acute care, hepatic, and mineral panels.
3. If this visit is done by their local medical provider , we will request the results to include in the patient's NIH record.

6.4. Final Study Visit (Week 24)

Subjects will return to the NIH CC for a final study visit at Week 24, after the full regimen of eplerenone has been completed. The following procedures will be conducted:

1. Interval medical history and physical exam, including blood pressure check
2. Bionutrition: DEXA, anthropometric measurements
3. Research blood collection (fasted overnight) for acute care, hepatic, and mineral panels, CBC, lipid profile; HgbA1c, CD4/8 count, HIV viral load, aldosterone, plasma renin activity, inflammatory markers and adipokines (MCP-1, CRP, adiponectin, PAI-1, resistin, TNF-α, TNFR2, IL-6), SHBG, total and free testosterone, LH (female only), FSH (females only), estradiol (females only), and serum, plasma, and PBMCs for storage
4. Urine pregnancy test for subjects of childbearing potential
5. MRI for cardiac and hepatic steatosis and visceral fat volume
6. FibroScan transient elastography
7. Liver biopsy (optional)

6.5. Follow-up End of Study visit (optional)

Subjects may be asked to return for assessments following their week 24 to determine if there are changes in their health status after withdrawal of study medication. These follow up evaluations could include:

1. Interval medical history, including blood pressure check
2. Research blood collection (fasted overnight) for acute care, hepatic, and mineral panels, CBC, lipid profile; HgbA1c, CD4/8 count, HIV viral load, aldosterone, plasma renin activity, inflammatory markers and adipokines (MCP-1, CRP, adiponectin, PAI-1, resistin, TNF-α, TNFR2, IL-6), SHBG, total and free testosterone, LH (female only), FSH (females only), estradiol (females only), and serum, plasma, and PBMCs for storage
3. Urine pregnancy test for subjects of childbearing potential
4. MRI for cardiac and hepatic steatosis and visceral fat volume
5. FibroScan transient elastography

6.6. Early Termination Visit

If a subject's participation is terminated before the end of the study, then they will be asked to come to the NIH CC for a Termination Visit. Laboratory tests and study evaluations to be conducted at that visit will be determined on the basis of the subject's health, their willingness, and the appropriateness of the test at that time point.

6.7. Pregnancy and Follow-up Visit

If a subject becomes pregnant during the course of participation, then their participation in the study will be stopped. The study team will ask the subject to notify them of the pregnancy outcome.

7. Potential Risks and Benefits

7.1. Potential Risks

Eplerenone: In one clinical trial, the overall incidence of AEs reported in patients treated with eplerenone was similar to those treated with placebo.[35] The AEs that occurred more frequently in eplerenone-treated patients were hyperkalemia (3.4% with eplerenone vs. 2.0% with placebo) and increased creatinine (2.4% with eplerenone vs. 1.5% with placebo). Discontinuation of

treatment as a result of hyperkalemia or abnormal renal function was less than 1.0% in both groups.

In another clinical trial, the overall incidence of AEs reported in patients treated with eplerenone was also similar to those treated with placebo.[35] The AEs occurred at a similar rate in both groups regardless of age, gender, or race. Therapy was discontinued as the result of an AE in 3% of patients treated with eplerenone and 3% of patients given placebo. The most common reasons for discontinuation of eplerenone were headache, dizziness, angina pectoris/MI, and increased concentration of gamma glutamyl transferase. Gynecomastia, mastodynia and abnormal vaginal bleeding were reported with eplerenone but not with placebo. The rates increased slightly with increasing duration of therapy.

Angioneurotic edema and rash have been reported from post-approval use of Inspra.[35]

There may be additional risks of eplerenone that are currently unknown.

DEXA Scan: The total amount of radiation exposure from 2 DEXA scans is 0.00006 rem. The radiation exposure in this study is below the limit of 5 rem per year allowed for adult research subjects by the NIH Radiation Safety Committee. There is no direct evidence that radiation exposure at this level is harmful, but, as with all radiation exposure, there may be a very slight increase in the risk of cancer.

The average person in the United States receives a radiation exposure of 0.3 rem per year from natural sources, such as the sun, outer space, and the earth's air and soil. A copy of the pamphlet, “An Introduction to Radiation for NIH Research Subjects,” will be provided to subjects by request.

Liver Biopsy: Ultrasound-guided percutaneous liver biopsy will be performed by trained interventional radiologists who specialize in gastrointestinal procedures at the NIH. Subjects will be observed as outpatients following liver biopsy per standard of care for liver biopsy and conscious sedation when used. The risks associated with liver biopsy include pain, transient hypotension (vasovagal response), bleeding, and transient bacteremia. Subjects with any contraindication to liver biopsy, including platelets < 75,000, elevated PT or PTT, (except patients with known lupus anticoagulant antibody and aPTT mixing study that is normal at one hour), or chronic aspirin use that cannot be suspended will not undergo biopsy. Individuals on aspirin for primary prevention of MI may undergo biopsy after discontinuation of aspirin for ≥ 7

days. Subjects will receive teaching and instructions for post-biopsy care and will be asked to call or return to clinic with persistent pain or other concerning symptoms.

Conscious Sedation: Conscious sedation will be used in subjects undergoing liver biopsy. Sedative drugs will be provided by experienced physicians of the NIH CC. Side effects from sedative medications include cardiovascular and respiratory depression manifested by bradycardia, hypotension, respiratory acidosis, and apnea. Additional adverse reactions reported with these drugs are stinging or pain at the injection site, hiccoughs, nausea, vomiting, postoperative drowsiness and headache, and hypersensitivity reactions. Patients will be closely monitored and appropriate countermeasures will be taken as necessary.

MRI: For most subjects, the primary risk associated with MRI is discomfort as subjects will be asked to lie still for a long period of time. Additionally, subjects may find the clicking sound caused by the MRI disturbing. Subjects with pacemakers, aneurysm clips, metallic prostheses, shrapnel fragments, welders and metal workers are at risk for injury and will be excluded from the study.

Blood Draw: Collection of blood may be associated with discomfort, bruising, local hematoma formation and, on rare occasions, infections, lightheadedness, and fainting. The amount of blood drawn for research purposes will be within the limits allowed for adult subjects by the NIH CC (Medical Administrative Policy 95-9: Guidelines for Limits of Blood Drawn for Research Purposes in the Clinical Center: http://cc-internal.cc.nih.gov/policies/PDF/M95-9.pdf.

Electrocardiogram: There are no foreseeable risks associated with this procedure.

Transient Elastography: There are no foreseeable risks to these procedures.

7.2. Potential Benefits

Subjects may not receive any benefit from this study. However, there is the potential that a 6-month treatment regimen of eplerenone may result in reductions of intramyocardial lipid content, hepatic steatosis, and visceral adipose tissue, thereby improving overall cardiovascular health. If this study is successful, then we will develop a larger trial to evaluate the clinical utility of eplerenone in HIV-infected individuals with steatosis.

8. Research Use of Stored Human Samples, Specimens, or Data

- **Intended Use:** Samples and data collected under this protocol may be used to study HIV infection and related disorders. No genetic testing will be performed.
- **Storage:** Access to stored samples will be limited using either a locked room or a locked freezer. Samples and data will be stored using codes assigned by the investigators. Data will be kept in password-protected computers. Only investigators will have access to the samples and data.
- **Tracking:** Samples and data acquired as part of this protocol will be tracked using the CRIMSON database system.
- **Disposition at the Completion of the Protocol:**
  - In the future, other investigators (both at NIH and outside) may wish to study these samples and/or data. In that case, Institutional Review Board (IRB) approval must be sought prior to any sharing of samples and/or data. Any clinical information shared about the sample would similarly require prior IRB approval.
  - At the completion of the protocol (termination), samples and data will either be destroyed, or after IRB approval, transferred to another existing protocol.
- **Reporting the Loss or Destruction of Samples/Specimens/Data to the IRB:**
  - Any loss or unanticipated destruction of samples or data (for example, as a result of freezer malfunction) that meets the definition of Protocol Deviation and/or compromises the scientific integrity of the data collected for the study, will be reported to the NIAID IRB.
  - Additionally, subjects may decide at any point not to have their samples stored. In this case, the principal investigator will destroy all known remaining samples and report what was done to both the subject and to the IRB. This decision will not affect the subject's participation in this protocol or any other protocols at NIH.

9. Remuneration Plan for Subjects

Eligible subjects will receive partial remuneration from the NIH for immediate costs associated with study-related expenses (eg, transportation and lodging) according to established NIH/NIAID guidelines. Subjects will also be compensated for the time and inconvenience of study participation according to the attached schedule (see Table 1). Thus, compensation may be up to $1280 for completion of the entire study, commensurate with the time and inconvenience of study participation including blood draws, liver biopsy, and scans. If a subject returns for

additional study related procedures as noted in section 6.5, they will be compensated at a rate consistent with the remuneration table.

Table 1 Remuneration

Visit/Procedure	Hours per Visit (Compensation)	Inconvenience Units (Compensation)	Total Compensation per Visit
Screening visit(s): Screening labs, history/physical, MRI,(brief), anthropometrics transient, elastography	4 ($50)	8 ($80)	$130
Screening visit 2: MRI (full) and DEXA	4($50)	7($70)	$120
Screening optional liver biopsy	7 ($80)	22 ($220)	$300
Day 0: History/physical, labs, education start eplerenone	3 ($30)	2($20)	$50
Weeks 1, 2, 4, 8, 12, 18: History/physical, adherence, labs	2 ($20) per visit 10 ($100) total	1 ($10) per visit 5 ($50) total	$30 per visit $180 total
Week 24: History/physical and labs, DEXA, MRI, elastography, anthropometrics	5 ($60)	12($140)	$200
Week 24 optional liver biopsy	7 ($80)	22 ($220)	$300
TOTAL COMPENSATION FOR COMPLETE STUDY PARTICIPATION			**$1280**

DEXA = dual-energy x-ray absorptiometry; MRI = magnetic resonance imaging;.
Note: Screening visit and final visit at week 24 may be scheduled over multiple days for subject's convenience.

10. Assessment of Safety

10.1. Toxicity Scale

The Investigator will grade the severity of each AE according to the "Division of Aids Table for Grading the Severity of Adult and Pediatric Adverse Events" Version 2.0, November 2014, which can be found at:
http://rsc.techres.com/Document/safetyandpharmacovigilance/DAIDS_AE_GRADING_TABLE_v2_NOV2014.pdf

Some grade 1 lab parameters on the DAIDS Toxicity Table (fibrinogen, potassium [low], uric acid [males only, elevated]) fall within the NIH lab reference range for normal values. These normal values will not be reported as grade 1 AEs. The grade 1 values for these tests will be reported as follows:

- Fibrinogen: 100-176 mg/dL
- Potassium (low): 3.0-3.3 mmol/L

- Uric acid (males): 8.7-10.0 mg/dL
- Magnesium (low): 0.60-0.65 mmol/L

10.2. Recording/Documentation

At each contact with the subject, information regarding AEs will be elicited by appropriate questioning and examinations. All AEs, both expected/unexpected and related/unrelated will be recorded on a source document.

Source documents will include progress notes, laboratory reports, consult notes, phone call summaries, survey tools, and data collection tools. Source documents will be reviewed in a timely manner by the research team. All reportable AEs that are identified will be recorded in CRIMSON. The start date, the stop date, the severity of each reportable event, and the PI's judgment of the AE's relationship and expectedness to the study agent/intervention will also be recorded in CRIMSON.

10.3. Definitions

Adverse Event: Any untoward medical occurrence in a human subject, including any abnormal sign, symptom, or disease, temporally associated with the subject's participation in research, whether or not considered related to the research.

Serious adverse event (SAE): Any AE that results in one or more of the following outcomes:

- death;
- a life-threatening event (places the subject at immediate risk of death from the event as it occurred);
- an inpatient hospitalization or prolongation of existing hospitalization;
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions;
- a congenital anomaly/birth defect;
- on the basis of appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition. (Examples of such events include allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse)

Protocol Deviation: Any change, divergence, or departure from the IRB-approved study procedures in a research protocol. Protocol deviations are designated as serious or non-serious and further characterized as

1. Those that occur because a member of the research team deviates from the protocol
2. Those that are identified before they occur, but cannot be prevented
3. Those that are discovered after they occur

Serious Protocol Deviation: A deviation that meets the definition of an SAE or compromises the safety, welfare, or rights of subjects or others.

Non-compliance: The failure to comply with applicable NIH Human Research Protections Program (HRPP) policies, IRB requirements, or regulatory requirements for the protection of human subjects. Non-compliance is further characterized as

1. Serious: Non-compliance that
    a. Increases risks, or causes harm, to subjects
    b. Decreases potential benefits to subjects
    c. Compromises the integrity of the NIH-HRPP
    d. Invalidates the study data
2. Continuing: Non-compliance that is recurring
3. Minor: Non-compliance that, is neither serious nor continuing.

Unanticipated Problem (UP): Any incident, experience, or outcome that meets all three of the following criteria would be considered a serious UP:

1. unexpected in terms of nature, severity, or frequency in relation to
    a. the research risks that are described in the IRB-approved research protocol, informed consent document, package insert, or other study documents; and
    b. the characteristics of the subject population being studied
2. possibly, probably, or definitely related to participation in the research
3. suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Unanticipated problem that is not an Adverse Event (UPnonAE): An unanticipated problem that does not fit the definition of an AE, but that may, in the opinion of the investigator, involve risk to the subject, affect others in the research study, or substantially impact the integrity of

research data. These events may involve a greater risk of social or economic harm to subjects or others rather than physical/psychological harm. Such events would be considered a non-serious UP. Examples of a UPnonAE include a breach of confidentiality, accidental destruction of study records, or unaccounted-for study drug.

10.4. Reporting Procedures

10.4.1. Expedited Reporting to the NIAID IRB

Serious and non-serious UPs, deaths, serious deviations, and serious or continuing non-compliance will be reported within 7 calendar days of investigator awareness. SAEs that are possibly, probably, or definitely related to the research will be reported to the NIAID IRB within 7 calendar days of investigator's awareness, regardless of expectedness.

10.4.2. Waiver of Reporting Anticipated Protocol Deviations, Expected non-UP AEs, and Deaths

Anticipated deviations in the conduct of the protocol will not be reported to the IRB unless they occur at a rate greater than anticipated by the study team. Expected AEs will not be reported to the IRB unless they occur at a rate greater than that known to occur in HIV-infected patients. If the rate of these events exceeds the rate expected by the study team, then the events will be classified and reported as though they are UPs. Deaths related to the natural history of HIV infection will be reported at the time of continuing review.

10.4.3. Annual Reporting to the NIAID IRB

The following items will be reported to the NIAID IRB in summary at the time of Continuing Review:

- Serious and non-serious unanticipated problems
- Expected SAEs that are possibly, probably, or definitely related to the research
- SAEs that are not related to the research
- All AEs, except expected AEs granted a waiver of reporting
- Serious and non-serious protocol deviations
- Serious, continuing, and minor non-compliance
- Any trends or events which in the opinion of the investigator should be reported

10.5. Reporting of Pregnancy

In the event of pregnancy:

- Discontinue the study agent and procedures but continue to follow-up over the phone for safety and outcomes
- Report to the IRB
- Advise research subject to notify the obstetrician of study participation and study agent exposure

10.6. Type and Duration of the Follow-up of Subjects after Adverse Events

AEs that occur from the time of enrollment of the subject are followed until the final outcome is known or until the end of the study follow-up period.

SAEs that have not resolved by the end of the follow-up period are followed until final outcome is known. If it is not possible to obtain a final outcome for an SAE (eg, the subject is lost to follow-up), then the reason a final outcome could not be obtained will be recorded by the investigator.

10.7. Pausing Rules for an Individual Subject

Pausing is the suspension of administration of study agent to a single subject until a decision is made whether or not to resume administration of the study agent.

The pausing criteria for a single subject in this study include any of the following:

- A subject experiences an SAE that is unexpected and possibly, probably, or definitely related to eplerenone
- A subject develops a potassium level > 5.5 meq/L, eGFR< 60 mL/min/1.73 m^2, or serum creatinine > 1.5 mg/dL
- Any safety issue that the investigator determines should pause administration of a study agent to a single subject

10.7.1. Resumption Following a Pause for a Single Subject

The PI will determine whether or not it is safe to resume administration of the study agent to the subject.

- Subjects who develop a potassium level > 5.5 meq/L, eGFR< 60 mL/min/1.73 m^2, or serum creatinine > 1.5 mg/dL will be asked to return to clinic in 24-48 hours for repeat lab testing.
- Subjects will only continue/resume treatment if an alternative cause for these lab abnormalities is identified and the abnormality resolves.

A subject who does not resume eplerenone will continue to be followed for safety.

10.8. Halting Rules for the Protocol

Halting the study requires immediate discontinuation of study agent administered for all subjects and suspension of enrollment until a decision is made whether or not to continue enrollment and study agent administration.

The halting rules are:

- Two or more subjects experience the same or similar SAEs that are unexpected and are possibly, probably, or definitely related to eplerenone

OR

- any safety issue that the PI determines should halt the study

The PI will determine if the study should be halted. The NIAID IRB may also halt the study.

10.8.1. Reporting a Study Halt

If a halting rule is met, then a description of the AE(s) or safety issue must be reported by the PI, within one business day, to the IRB by fax or email.

10.8.2. Resumption of a Halted Study

The PI will determine if it is safe to resume the study. The PI will notify the IRB of the decision on resumption of the study.

10.9. Withdrawal of an Individual Subject

An individual subject will be withdrawn for any of the following:

- An individual subject's decision (the investigator will attempt to determine the reason for the subject's decision.)
- Non-compliance with study procedures to the extent that it is potentially harmful to the subject or to the integrity of the study data.
- The investigator determines that continued participation in the study would not be in the best interest of the subject.

10.10. Replacement of a Subject Who Discontinues Study Treatment

If a subject withdrawals after fewer than 12 weeks of study treatment, then they will be replaced. If a subject is replaced, then all of the data will still be included for the safety assessment.

10.11. Safety Monitoring Plan

The data gathered during this study will be monitored by the PI for safety and compliance with protocol-specified requirements.

11. Statistical Considerations

The proposed study is designed as a proof-of-concept study and will employ paired comparison of each subject pre- and post-treatment. With a total of 20 subjects we will have 90% power to detect a 0.1% unit change in intramyocardial lipid content based on an estimated within subject standard deviation of MRS intramyocardial lipid of 0.07% reported by O'Connor and colleagues[36]. In our prior work, HIV-infected subjects had a mean intramyocardial lipid content of 1.5% whereas controls were 1.0%. Therefore, with this sample size we will have ample power to detect a minimal clinically relevant reduction of 0.3% units or greater following eplerenone therapy.

11.1. Statistical Analysis Plan

As previously stated, the primary endpoints for this study will be change in cardiac and hepatic steatosis from initial measurement (pre-treatment) to end of study measurement. This will be tested using a paired t-test, and a two sided p value of < 0.05 will be used for detection of

significance. In addition, we will conduct exploratory analyses to determine if eplerenone therapy is associated with changes in biomarkers of inflammation (eg, MCP-1 and IL-6). We also plan to evaluate potential changes in visceral fat volume with treatment, but these will be exploratory analyses, primarily descript, and which may not be sufficiently powered in this small preliminary trial. Finally we will construct descriptive tables summarizing reported side effects and any observed lab abnormalities observed during the course of study participation in all subjects.

12. Ethics/Protection of Human Subjects

12.1. Informed Consent Process

Informed consent is a process where information is presented to enable persons to voluntarily decide whether or not to participate as a research subject. It is an ongoing conversation between the human research subject and the researchers which begins before consent is given and continues until the end of the subject's involvement in the research. Discussions about the research will provide essential information about the study and include: purpose, duration, experimental procedures, alternatives, risks and benefits. Subjects will be given the opportunity to ask questions and have them answered.

The subjects will sign the informed consent document prior to undergoing any research procedures. The subjects may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the subjects for their records. The researcher will document the signing of the consent form in the subject’s medical record. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

12.1.1. Non–English-Speaking Subjects

If a non–English-speaking subject is unexpectedly eligible for enrollment, the subject will be provided with the CC Short Written Consent Form for Non–English-Speaking Research Subjects in the subject’s native language and a verbal explanation of the purpose, procedures, and risks of the study as described in MAS Policy M77-2, NIH HRPP SOP 12, and 45 CFR 46.117(b)(2). If the CC Short Written Consent Form is used three times or more for the same language, this will be reported to the IRB immediately.

12.2. Subject Confidentiality

All records will be kept confidential to the extent provided by federal, state and local law. The study monitors and other authorized representatives of the Sponsor may inspect all documents and records required to be maintained by the Investigator, including but not limited to, medical records. Records will be kept locked and all computer entry and networking programs will be done with coded numbers only. Clinical information will not be released without written permission of the subject, except as necessary for review by IRB or the OHRP.

13. Data Handling and Record Keeping

13.1. Data Capture and Management

Study data will be maintained in CRIMSON and collected directly from subjects during study visits and telephone calls, or will be abstracted from subjects' medical records. Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary to confirm the data abstracted for this study. Data entry into CRIMSON will be performed by authorized individuals. The Investigator is responsible for assuring that the data collected are complete, accurate, and recorded in a timely manner.

13.2. Record Retention

The investigator is responsible for retaining all essential documents listed in the ICH GCP Guideline. Study records will be maintained by the PI for a minimum of 5 years and in compliance with institutional, IRB, state, and federal medical records retention requirements, whichever is longest. All stored records will be kept confidential to the extent required by federal, state, and local law.

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Appendix B: Schedule of Procedures/Evaluations

Evaluation	Screening	Baseline Day 0	Safety Visits (Weeks 1, 2, 4, 8, 18)	Interim Visit (Week 12)	Final Study Visit (Week 24)
Physical exam, medical history	X[a]	X	X	X	X
Vital signs[b]	X	X	X	X	X
Urine pregnancy test[c]	X	X	X	X	X
Research blood collection	X	X	X	X	X
Urine collection	X				
Cardiac and hepatic MRI	X				X
Transient elastography[d]	X				X
Liver biopsy[e]	X				X
Bionutrition[f]	X				X

Appendix C: Prohibited Medications

Strong CYP3A4 Inhibitors	• Boceprevir • Clarithromycin • Conivaptan • Grapefruit juice • Indinavir • Itraconazole • Ketoconazole	• Lopinavir/ritonavir • Mibefradil • Nefazodone • Nelfinavir • Posaconazole • Ritonavir	• Saquinavir • Seville oranges • Suboxone • Telaprevir • Telithromycin • Voriconazole
Strong CYP3A4 Inducers	• Avasimibe • Carbamazepine • Phenytoin	• Rifampin • Rifabutin	• Rifapentine • St. John's wort
Potassium-Sparing Diuretics	• Amiloride • Spironolactone • Triameterene		
Agents that May Cause Hyperkalemia	• Aliskerin • Cyclosporine • Digoxin	• Penicillin G • Pentamidine	• Tacrolimus • Verapamil

CYP = cytochrome P450 isozyme; NSAID = nonsteroidal anti-inflammatory drug.