Randomized placebo-controlled trial to investigate the effects of eplerenone in patients with HIV-associated abdominal fat accumulation

Official Title of the Study: Eplerenone in HIV Associated Abdominal Fat Accumulation

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I. Background and Significance

Metabolic abnormalities and body composition changes are associated with combination antiretroviral therapy (cART) in HIV-infection. Recent studies have shown that hypertension [1], impaired glucose tolerance [2], Type 2 diabetes [3, 4], increased carotid intima-media thickness (cIMT) [5] as well as 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 HDL [11], increased systemic inflammatory markers [12-14], and increased subclinical atherosclerosis [15]. At this time there is no standard of care to adequately treat these abnormalities.

The etiology of body composition changes, metabolic abnormalities, and increased cardiovascular risk is multifactorial and likely includes perturbation of multiple endocrine axes. In non-HIV-infected individuals, recent data has shown that aldosterone, a mineralocorticoid hormone, is increased in association with increased VAT [16] and decreased insulin sensitivity [17]. We have demonstrated increased 24-hour urine aldosterone in HIV-infected women with increased visceral fat accumulation, compared to age and BMI-matched healthy controls [18]. Urinary aldosterone secretion was strongly related to VAT in this study. In addition, we also observed an independent relationship between increased urinary aldosterone and hemoglobin A1c, which was independent of BMI or visceral adiposity. Furthermore, emerging animal and human studies have suggested effects of mineralocorticoid blockade on insulin sensitivity, inflammation, and hepatosteatosis [19, 20].

Physiology of aldosterone and the mineralocorticoid receptor

Recent data suggest that aldosterone and mineralocorticoid receptor (MR) activation may play a role in cardiovascular and metabolic disease [21, 22]. *In vitro* studies demonstrate effects of aldosterone on adipocyte differentiation, adipocytokine production, lipid metabolism, and insulin signaling. The physiologic relevance of these *in vitro* effects is unclear based on our current understanding of aldosterone's mechanism of action. The mineralocorticoid receptor, which is the only identified receptor for aldosterone to date, has equal affinity for aldosterone and cortisol. Certain tissues, including kidney, colonic epithelium, vascular smooth muscle [23], and endothelium [24], highly express 11β hydroxysteroid dehydrogenase 2 (11βHSD2), which inactivates cortisol and facilitates aldosterone's specificity of action at the MR. In other tissues, including cardiac and adipose tissue, MR expression is present, but significant expression of 11βHSD2 has not been identified [25]. As cortisol circulates in concentrations approximately 1000-fold higher than aldosterone under normal physiologic conditions, cortisol is thought to be the primary agonist for the MR in these tissues, with changes in aldosterone concentration presumably of little physiologic significance. However, emerging evidence challenges this paradigm. First, numerous effects of aldosterone occur too rapidly to attribute to the classic genomic model of transcription regulation [26, 27]. These actions, which include stimulating proliferation of vascular smooth muscle cells [28] and inducing arterial vasoconstriction [29], may be mediated by non-genomic signaling through the MR or may involve a separate receptor for aldosterone that has yet to be identified. Second, animal studies suggest that

glucocorticoid may act as an antagonist rather than an agonist of the MR in some tissues [30-32]. In a rodent model, infusion of aldosterone alone caused hypertension and cardiac fibrosis, whereas concomitant infusion of 30-fold corticosterone significantly attenuated the development of hypertension and fibrosis [31]. Moreover, transgenic mice that overexpress 11βHSD2 in cardiomyocytes, causing decreased glucocorticoid occupancy of the MR and increased signaling by aldosterone, developed cardiac fibrosis, dilated cardiac hypertrophy, and heart failure [32]. Taken together, these data suggest that aldosterone may have physiological relevance even in tissues without significant 11βHSD2 activity. Increasing evidence suggests that aldosterone may be increased in individuals with increased VAT and may play a significant role in lipid metabolism, glucose homeostasis, and inflammation.

Interactions between aldosterone, mineralocorticoid receptor, and adipose tissue

Abundant evidence suggests a bidirectional relationship between adipose tissue and aldosterone, with adipose tissue potentially increasing aldosterone levels, and aldosterone increasing adipogenesis and lipid storage in adipocytes. Regarding the effect of adipose tissue on aldosterone production, angiotensinogen production has been demonstrated in both differentiated 3T3-L1 adipocytes [33] and in human abdominal subcutaneous (SC) adipose tissue [34]. Angiotensin converting enzyme (ACE) mRNA is also present in human SC adipose tissue [34]. Further, Ehrhart-Bornstein et al. have demonstrated that secretory products from human adipocytes can stimulate mineralocorticoid steroidogenesis in human adrenocortical cells even in the presence of angiotensin receptor blockade with valsartan, suggesting that other "mineralocorticoid releasing factors" in addition to angiotensinogen may be produced by adipocyte tissue [35]. Extending on this work, this group has subsequently demonstrated that adipocyte-derived factors induce Wnt signaling and increase StAR promoter activity, increasing production of cortisol and aldosterone [36]. Further, adipocyte-derived factors also appear to sensitize human adrenocortical cells to angiotensin II, also contributing to increased aldosterone production [37]. These mechanisms may underlie the numerous reports of increased systemic aldosterone levels with increasing body fat. Twenty-four hour urine aldosterone/creatinine is higher in animal models of obesity (db/db mice) compared to lean (db/+) mice [19], and, in non-HIV populations, both 24-hour urine aldosterone and angiotensin II-stimulated aldosterone are higher in overweight and obese normotensive adults compared to lean controls [38]. Some reports suggest that the relationship of aldosterone and body fat may be largely mediated by increased VAT. Goodfriend et al. demonstrated that plasma aldosterone correlates with VAT in normotensive women [16], and, as above, we have demonstrated a positive relationship of 24-hour urinary aldosterone with VAT in HIV-infected women [18].

In addition to evidence that adipose tissue increases aldosterone production, recent studies suggest that MR activation may play an important role in adipogenesis and lipid metabolism. MRs have been identified in adipocytes from several fat depots, including both VAT and SAT from humans [25] and murine brown adipocytes [39]. Furthermore, several groups have shown that aldosterone stimulates adipocyte differentiation and increases triglyceride accumulation [40-42]. In adrenalectomized male rats, aldosterone infusion over 12 days resulted in body weight gain correlating to epididymal fat pad mass, but not to percent tissue water, suggesting that aldosterone stimulates increase in adipose tissue [43]. Notably, as 11βHSD2 is not highly expressed in adipocytes [25], MR activation, by either cortisol or aldosterone, may be a more relevant focus in adipose tissue than aldosterone levels per se. In this respect, MR

appears to play a crucial role in adipocyte differentiation and lipid accumulation. In 3T3-L1 cells, MR but not glucocorticoid receptor (GR) downregulation by siRNAs inhibited adipocyte differentiation and decreased cell triglyceride content [44]. Furthermore, whereas murine adipocytes lacking GR show mild impairment in lipid accumulation during early differentiation, adipocytes lacking MR demonstrate failure to accumulate lipid throughout differentiation [39]. These observations are supported by *in vivo* models of ob/ob and db/db mice, in which MR blockade with eplerenone significantly decreased the number of hypertrophic adipocytes in epididymal fat [45]. Blockade of MR, which we propose to study in this grant application, would block MR activation by cortisol as well as aldosterone in adipose tissue, and may decrease lipid accumulation and ultimately affect body composition. There is also some evidence to suggest that MR blockade may affect hepatosteatosis, which is prevalent in HIV-infected individuals in association with increased abdominal adiposity [46, 47]. In a murine model of diet induced obesity, Wada et al. demonstrate that spironolactone significantly decreases hepatic triglyceride accumulation as well as decreasing serum triglyceride, total cholesterol, and free fatty acid [20]. In humans, Fallo et al. demonstrate an increased prevalence of hepatosteatosis in patients with primary aldosteronism (PA) compared to normotensive controls, but the prevalence in PA (57.5%) was not different from the prevalence found in a matched cohort with low-renin essential hypertension (55.8%) [48]. Further studies are clearly needed to determine whether mineralocorticoid activity affects hepatic triglyceride accumulation. Aim I of the current proposal will investigate relationships between aldosterone level and body fat distribution, including ectopic fat storage in liver.

Aldosterone, Mineralocorticoid Receptor, and Insulin Resistance

Multiple epidemiologic studies have found positive associations between aldosterone and markers of insulin resistance in overweight and obese patients [16, 17, 38, 49], hypertensive patients [49], and patients with heart failure [50]. Moreover, a high prevalence of impaired glucose tolerance has been recognized in primary aldosteronism (PA) for several decades [51]. In recent series, patients with PA are generally reported to have impaired glucose homeostasis in comparison to both controls and patients with essential hypertension [52, 53], although one recent study refutes this association [54]. Further, treatment of PA with either surgery or aldosterone antagonism improves glucose homeostasis in this population [52]. Genetic polymorphisms in aldosterone synthase have also demonstrated an association with both fasting glucose and glucose following OGTT, which further suggests a possible relationship between aldosterone and glucose homeostasis [55].

Although impaired insulin secretion secondary to hypokalemia is one likely cause of impaired glucose homeostasis in hyperaldosteronism, *in vitro* and animal studies have demonstrated additional mechanisms linking aldosterone directly to glucose homeostasis. Hitomi et al., using vascular smooth muscle cells from rat aorta, demonstrate that aldosterone dose-dependently decreases insulin receptor substrate-1 (IRS-1), prevents insulin-induced Akt phosphorylation, and reduces insulin-stimulated glucose uptake [56]. Notably, all of these effects are reversed with addition of eplerenone to achieve MR blockade [56]. Wada et al. demonstrate similar effects of aldosterone to reduce IRS-1 and IRS-2, decrease Akt phosphorylation, and decrease glucose uptake in 3T3-L1 adipocytes [57]. In their model, however, glucocorticoid antagonism with RU486 but *not* MR antagonism with eplerenone reversed the effects of aldosterone, suggesting possible action through the GR rather than the MR [57]. Another potential mechanism has been demonstrated *in vivo* using Wistar rats, in which

aldosterone treatment significantly decreased glucose uptake in muscle and liver in conjunction with decreased GLUT4 protein expression in muscle and decreased GLUT2 protein expression in liver [58], suggesting that aldosterone may directly contribute to insulin resistance at the level of the liver and the muscle. *In vivo* rodent models also demonstrate beneficial effects of mineralocorticoid blockade on glucose homeostasis. Hirata et al. demonstrate that eplerenone treatment of ob/ob and db/db mice significantly reduced glucose and HOMA-IR [45], and Guo et al. also describe decrease in HOMA-IR in db/db mice treated with eplerenone [19]. Wada et al. demonstrate similar results in a murine model of diet induced obesity, with improved response to both glucose tolerance test and insulin tolerance test following treatment with spironolactone [20]. Taken together, these data strongly support a relationship between mineralocorticoid activity and glucose homeostasis and suggest that mineralocorticoid blockade may be a useful strategy to improve glucose homeostasis in models of increased adiposity.

Mineralocorticoid receptor and subclinical inflammation

HIV-infection is accompanied by increased markers of systemic inflammation [12-14], and levels of inflammatory cytokines predict atherosclerosis [6, 59] and myocardial infarction [60] in HIV-infected individuals. In addition to the recognized role of angiotensin II to stimulate production of reactive oxidant species [61, 62], 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 produced significant 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 [63]. These changes were accompanied by increased gene expression of cyclooxygenase-2 (COX-2), macrophage chemoattractant protein-1 (MCP-1), osteopontin, and ICAM-1. Concomitant eplerenone treatment significantly ameliorated inflammatory changes and attenuated alterations in gene expression [63]. Although a role of hypertension cannot be excluded in this model, a model of atherosclerosis-prone apoE deficient mice supports the association between MR activation and vascular inflammation, independent of blood pressure. 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, TNFα, MCP-1 [64]. Eplerenone treatment did not alter total cholesterol levels or blood pressure, but significantly reduced atherosclerotic lesion area and decreased superoxide production as well as expression of NADPH oxidase, TNFα and MCP-1 [64]. Moreover, although treatment with valsartan alone also had beneficial effects to decrease lesion area and reduce oxidative stress, the combination of valsartan and eplerenone was significantly more effective than valsartan alone to reduce atherosclerotic area and oxidative stress, suggesting an effect of MR blockade distinct from that of ATII blockade [64]. Similar results have been reported in a rabbit model, with eplerenone treatment significantly improving endothelial function and decreasing superoxide production and NADPH oxidase activity in aorta of animals fed a high-lipid diet [65]. Taken together, these animal data suggest a direct effect of MR activation on oxidative stress, endothelial function, and atherosclerotic changes in the vasculature.

Effects of aldosterone on adipocyte inflammation and on systemic levels of inflammation have also been demonstrated. Aldosterone has also been shown to contribute to angiotensin-II induced increase in C-reactive protein (CRP) [66], and aldosterone stimulation of adipose tissue increases mRNA expression of IL-6 and PAI-1

[39]. Further, aldosterone increases LDL-oxidation in human vascular smooth muscle cells [67]. Evidence that MR blockade ameliorates inflammation confirms that inflammatory actions of aldosterone occur via the MR. Eplerenone treatment in angiotensin II-infused, hypertensive rats decreases both serum and urinary CRP [66]. As described further in our preliminary data, MR blockade by treatment with eplerenone in obese db/db mice markedly reversed obesity related increases in mRNA expression of TNF-α and other inflammatory markers locally in retroperitoneal adipose tissue and increased adiponectin mRNA expression [19]. Similarly, Hirata et al. describe numerous effects of eplerenone in adipose tissue of ob/ob and db/db mice [45]. Significantly, they report that MR mRNA levels in adipose tissue were significantly increased in ob/ob and db/db mice compared to lean controls, supporting a key role for MR in adipocyte biology. Eplerenone treatment in these mice significantly reduced reactive oxygen species and decreased mRNA expression of NADPH oxidase subunits [45]. Further, in ob/ob mice, eplerenone reduced mRNA expression of MCP-1, IL-6, and TNFα [45]. Similar effects were reported in a diet-induced obesity mouse model, in which spironolactone resulted in significant decreases in hepatic expression of TNFα, IL-6, and MCP-1 [20]. Given this abundant animal data, research is greatly needed into the effects of aldosterone and MR blockade on systemic and local inflammation in humans. In this study we will carefully study the effects of MR blockade on systemic inflammation and endothelial function in HIV-infected individuals, with the hypothesis that MR blockade will have beneficial effects to reduce systemic inflammatory markers and improve endothelial function in this population with increased risk of atherosclerosis and CV morbidity.

Conclusion

Despite the critical need to reduce CVD risk in HIV-infected patients, a comprehensive and effective strategy has not yet been developed to accomplish this purpose. Indeed, numerous strategies have been tried with varying degrees of success to improve the metabolic and cardiovascular abnormalities in HIV-infected patients. Lifestyle modification (LSM) strategies have demonstrated a substantial benefit to reduce CVD risk factors among HIV-infected patients. We have previously demonstrated positive effects of a well designed, achievable LSM program on HDL, waist circumference and HgbA1c [68]. Based on our data, we maintain that LSM should be the foundation of any treatment strategy. As shown in our preliminary data and in other studies using this strategy, however, LSM is unlikely to reverse completely the metabolic abnormalities associated with HIV [68], [69], [70], [71].

In summary, epidemiologic studies have established that aldosterone is increased in association with increased BMI, and some evidence suggests that VAT in particular may be related to increased aldosterone. *In vitro* and animal data demonstrate a cross-talk between aldosterone/MR and adipose tissue, such that increased adiposity may increase aldosterone, and MR activation may, in turn, increase adipocyte differentiation and triglyceride accumulation. Further, increasing research demonstrates a role for MR activation in glucose homeostasis, inflammation, and atherosclerosis. Lo et al. [18] demonstrated increased aldosterone levels in association with increased VAT in HIV-infected women. MR activation may be a mediator of some of the detrimental effects of excess visceral adiposity in HIV, and MR blockade may provide to be an important element of a larger strategy to reverse endocrine abnormalities, improve insulin sensitivity, and decrease inflammation in HIV.

Evidence suggests that MR blockade may have beneficial effects on cardiovascular and metabolic parameters, and the selective MR blocker eplerenone has

proven safe and effective in non-HIV infected populations with cardiovascular disease. Our preliminary data suggest increased aldosterone in HIV-infected individuals with increased abdominal adiposity, a population with significant risk of insulin resistance and cardiovascular morbidity and for whom no specific treatment is currently available. MR blockade with eplerenone may be one beneficial strategy to address impaired glucose homeostasis and cardiovascular risk in this group, but the effects of eplerenone in HIV-infected individuals with abdominal fat accumulation and impaired glucose tolerance are not known. We hypothesize that by blocking the effects of increased aldosterone secretion, eplerenone treatment will significantly improve insulin sensitivity in this population and may also decrease visceral adipose tissue, and improve dyslipidemia and inflammation, thereby improving vascular function. In a 6-month, randomized, placebo-controlled study followed by 6-month open-label extension, we will test for the first time the effects of the selective mineralocorticoid blocker, eplerenone, on markers of insulin sensitivity, dyslipidemia, body composition, inflammation and vascular function in this population.

Eplerenone was chosen for this study instead of an ACE-I or Angiotensin II Receptor Blocker (ARB) because we want to specifically test the effects of MR blockade, including in tissues in which cortisol may active the MR due to lack of significant 11βHSD2 expression. Evidence suggests that MR activation in adipose tissue may affect adipocyte differentiation and triglyceride accumulation, and cortisol may act similarly to aldosterone at the MR in this regard. As we aim to determine the effects of MR blockade on cardiometabolic parameters and body composition, specifically VAT accumulation, we have chosen eplerenone in order to block the effects of both aldosterone and potentially cortisol at the MR. Eplerenone was chosen rather than spironolactone because eplerenone is more selective for the MR, does not affect adrenal steroid synthesis as spironolactone does, and has a better side effect profile.

II. Specific Aims

To determine if mineralocorticoid receptor blockade with eplerenone among HIV-infected patients with increased waist circumference and impaired glucose tolerance will ameliorate cardiovascular risk parameters, we will perform a randomized double-blind study of eplerenone vs. placebo for 6 months, with a 6 month safety extension. We will provide all subjects with lifestyle modification throughout the study. We hypothesize that addition of eplerenone to lifestyle modification will significantly:

A. Improve insulin sensitivity as measured by euglycemic hyperinsulinemic clamp.
B. Improve endothelial function and adipokines.
C. Reduce critical measures of adiposity, including liver fat and IMCL

III. Subject Selection

Inclusion/Exclusion:

Inclusion criteria:

1. Increased waist circumference based on NCEP guidelines (>102cm in men and >88cm in women) and impaired glucose tolerance (either IFG > 100 mg/dL but < 126 mg/dL or 2hr glucose > 140 mg/dl but < 200 mg/dL, or fasting insulin >12 uIU/mL)
2. HIV positive for 5 years and on a stable ART regimen for at least 12 months
3. Age ≥ 30 and ≤ 65 years of age

4. Stable CD4 count and HIV viral load in the past year

Exclusion criteria:

1. ACE Inhibitor, ARB, verapamil, or spironolactone
2. Potassium supplementation
3. Estimated GFR<60, creatinine > 1.5 mg/dL
4. Serum K > 5.5 mEq/L, ALT > 2.5 times the upper limit of normal, Hgb < 11g/dL
5. Uncontrolled hypertension (SBP ≥ 160 or DBP ≥ 100)
6. Current or prior steroid use within past 6 months
7. Known history of diabetes mellitus or current use of anti-diabetic medications
8. Concomitant use of full dose ritonavir, nelfinavir, clarithromycin and other strong inhibitors of CYP34A
9. Use of St. John's Wart (CYP3A4 inducer)
10. For women: Pregnant or actively seeking pregnancy, breastfeeding, failure to use an acceptable non-hormonal form of birth control, including abstinence, barrier contraceptives, or non-hormonal IUD.
11. Estrogen or progestational derivative use within 3 months
12. Testosterone use for non-physiologic purposes, or physiologic testosterone replacement for < 3 months.
13. Current growth hormone or growth hormone releasing hormone use
14. Current viral, bacterial or other infections (excluding HIV)
15. Current active substance abuse
16. Patients with a significant history of cardiovascular disease, including prior MI or stroke

Source of subjects and recruitment methods:

Participants will be recruited from community flyers and announcements at HIV focused community centers, community newspaper advertising, and referrals from infectious disease physicians and primary care physicians familiar with the study. Recruitment may also be done via RSVP for Health and/or internet announcements such as Partners "All User Broadcast." Potential participants, from all referral sources, will contact the study investigator directly by phone for a pre-study evaluation via telephone to determine eligibility.

IV. Subject Enrollment

Informed consent will be obtained at the screening visit by a licensed physician or nurse practitioner, prior to any procedures or interventions. Potential research subjects will have the entire protocol explained in detail and study investigators will review the consent form item by item, answering any and all questions. Once risks and benefits have been reviewed and subjects have agreed to participate by signing the consent, the screening visit will continue as described in the subject selection section. Once final eligibility has been determined subjects will be scheduled for a baseline visit.

Treatment assignment and randomization:

During the baseline visit randomization will be done. All subjects will receive lifestyle modification as described below. Subjects will be randomized to receive eplerenone 50mg po qd or identical placebo for 6 months. Doses will start at 25mg for the first week to avoid hypotension. The study will be double-blind. Randomization will be stratified by

gender, age (>45 y), and BP (SBP>140 or DBP>90 vs. normotensive). After the first 6 months, open-label eplerenone will be given for 6 months in all patients, and lifestyle modification will continue. During the open-label period all subjects will start eplerenone 25 mg a day for 1 week and dose will be increased to 50 mg a day at the 25 week safety visit to maintain blinding.

Prior to randomization in all cases, the subject's primary care physician will be consulted regarding the appropriateness of the protocol including the lifestyle modification program. Subjects are not eligible if they do not meet all of the enrollment criteria

V. Study Procedures

Data Collection/Study Procedures

Screen Visit (to determine eligibility)

1. Informed consent; history and PE, vital signs including blood pressure, and urine pregnancy test for all female subjects
2. Bionutrition: Height, metabolic weight, and anthropometric measurements, including waist and hip circumferences, distribution of a 4-day food record
3. Fasting blood: potassium, creatinine, CBC, ALT, FSH (women only)
4. 2 hour oral glucose tolerance test

Prior to the baseline, 3, 6 and 12 month visits, subjects will go on a uniform 200 meq/day sodium diet.

Baseline, 6 months, and 12 months:

1. Urine pregnancy test of all female subjects
2. MRI/MRS for abdominal visceral adipose tissue, subcutaneous adipose tissue, liver fat, intramyocellular lipid content
3. Flow mediated vasodilation
4. Bionutrition: DEXA, REE, anthropometric measurements and review of a session in the lifestyle modification workbook
5. Fasting blood will be drawn for CBC, lipid profile, HgbA1c, chem 7, ALT, CD4/8, HIV viral load, aldosterone, plasma renin activity, inflammatory markers and adipokines: MCP-1, CRP, adiponectin, PAI-1, resistin, TNFα, TNFR2, IL-6. SHBG, total and free testosterone, LH, FSH, estradiol (females only).
6. 24 hour urine will be collected for aldosterone, potassium, sodium, cortisol and creatinine
7. Euglycemic hyperinsulinemic clamp
8. Interval medical history and physical exam including blood pressure check
9. Modifiable activity questionnaire
10. Overnight q 20 minute sampling for GH pulsatility analysis.

Interim Safety Visits 1 wk, 2 wks, 4 wks, 2 months and same schedule post 6 month crossover with an additional visit at 10 months

1. Physical exam and interval medical history including blood pressure check
2. Blood: creatinine, K, CBC, ALT
3. Urine pregnancy test of all female subjects

3 Month Visit

1. Urine pregnancy test for all female subjects
2. Physical exam, including blood pressure check, and medical history

3. Euglycemic hyperinsulinemic clamp
4. Bionutrition: DEXA, anthropometric measurements, and review of a session from the lifestyle modification workbook.
5. Fasting blood: lipid profile, HgbA1c, chem. 7, CBC, potassium, creatinine, ALT, CD4/8, viral load, aldosterone, plasma renin activity, inflammatory markers and adipokines: MCP-1, CRP, adiponectin, PAI-1, resistin, TNFα, TNFR2, IL-6. SHBG, total and free testosterone, LH, FSH, estradiol (females only).
6. 24 hour urine will be collected for aldosterone, potassium, sodium, cortisol, and creatinine
7. Optional overnight q 20 minute sampling for growth hormone pulsatility analysis
8. Modifiable activity questionnaire

Oral Glucose Tolerance Test***:*** A standard 75g OGTT will be performed on the morning of the screen visit following a 12 hour fast, with interval measurements of insulin and glucose at 0 and 120 min.

Euglycemic-Hyperinsulinemic Clamp: After 12-h overnight fast, patients will receive a primed infusion of regular insulin mixed with albumin, 80 mU/m^2, for 2 hours, with a variable rate of dextrose administered to maintain blood glucose at 5.0 mmol/L (90 mg/dL). Blood samples will be collected for glucose at time 0 and every 5 min during the 2-hour clamp, and samples for insulin will be collected every 20 min from 0-120 minutes. Insulin sensitivity will be calculated.

Optional GH Pulsatility Analysis: Starting at 2000 until 0740 the next morning, fasting blood will be sampled every 20 minutes. Approximately 1.5cc of blood will be drawn at each time point for a total of 54cc. Mean overnight GH concentration, basal concentration, pulse frequency, pulse amplitude and overall pattern regularity will be determined using the deconvolution analysis and cluster computerized pulsatility program.

^{1}H Magnetic Resonance Spectroscopy & MRI Scanning**:** After 12-hour overnight fast subjects will under go ^{1}H -MRS of the calf muscle and of the liver. For calf muscle ^{1}H -MRS, voxel measuring 15 x 15 x 15 mm (3.4 ml) will be placed on the axial T1-weighted slice with largest muscle cross-sectional area of the tibialis anterior and subsequently the soleus, avoiding visible interstitial tissue, fat or vessels. Single-voxel ^{1}H -MRS data were acquired using point-resolved spatially localized spectroscopy pulse sequence. For liver 1H-MRS, a breath-hold true fast imaging with steady precession sequence will be obtained. A voxel measuring 20 x 20 x 20 mm (8 ml) will be placed within the right lobe of the liver, avoiding vessels or artifact. Breath-hold single-voxel ^{1}H -MRS data will be acquired using point-resolved spatially localized spectroscopy pulse sequence without water suppression with the following parameters: TE of 30 msec, TR of 1500 msec, eight acquisitions, 1024 data points, and receiver bandwidth of 2000 Hz. For abdominal visceral and subcutaneous fat areas, conventional MR images will be acquired for anatomic reference of ^{1}H-MRS overlays. Image series will include Axial T1-weighted localizers. A single axial slice of the abdomen and mid thigh will be obtained with magnetic resonance imaging. Abdominal visceral and subcutaneous fat areas, as well as mid thigh subcutaneous fat area will be determined based on offline analysis of tracings obtained utilizing commercial software. No intravenous contrast will be used. The total amount of time in the magnet will be approximately 1 hour.

Flow Mediated Vasodilation: After a 12 hour overnight fast a blood pressure cuff will be inflated to 50 mmHg above systolic blood pressure (or 250 mmHg, whichever is less) for five minutes. The diameter of the brachial artery will be recorded by ultrasound under no-flow conditions and over a five minute period after the cuff is deflated (reflow). The images will be recorded for analysis of the time-course and extent of dilation of the brachial artery.

Whole Body Dual Energy X-ray Absorptiometry (DEXA)
DEXA (Hologic) will be used to determine whole body and regional fat will be assessed. The technique has a precision error (1 SD) of 3% for whole body fat and 1.5% for lean mass. It estimates body composition from the attenuation of X-rays pulsed in synchrony between 100 and 140 kV with the line frequency for each pixel of the scanned image. (As an external standard for analysis of body composition, a step phantom, with six fields of acrylic and aluminum of varying thickness and known absorptive properties, was scanned periodically for calibration.) The DEXA scan will be used to determine total body fat as well as regional body fat measurements.

Anthropometric Measurements
Subjects' height and weight will be obtained and plotted on standard weight charts to determine percent of ideal body weight. Body mass index will be calculated. Anthropometric measurements of waist to hip ratio, leg circumference, arm circumference and neck circumference will be performed using a standardized technique. Waist-to-hip ratio will be determined from the circumferential measurements of the iliac waist and the hips at the level of the iliac crest taken with the patient in an upright standing position.

Bionutritional Analysis: Protein, carbohydrate, fat, micronutrient, dietary supplements and alcohol intake will be determined from 4-day food records (3 weekdays and 1 weekend day) (Nutrition Data systems).

High Sodium Diet: A high sodium diet will be followed for 6 days prior to the Baseline, 3, 6 and 12 month visits and consists of the subject's normal, ad lib diet supplemented with high sodium meals and/or chicken broth, and snacks to equal approximately 200 mEq of sodium. The 7th day (last day) of the high sodium diet will be completed on the first day of the Baseline, 3, 6 and 12 month visit.

Lifestyle Modification: Goals derived from the AACE and NCEP-ATP III guidelines and the Diabetes Prevention Program are as follows: $\leq$35% calories from fat, < 7% calories from saturated fat, up to 10% calories from polyunsaturated fat, reduction of trans fatty acid intake, up to 20% calories from monounsaturated fat, and 25-35g of fiber per day. 3 hrs of physical activity/week at moderate intensity, >10,000 steps in daily activity, measured by pedometer. The curriculum is modeled after the Diabetes Prevention Program. Subjects will complete lifestyle sessions in the offices of the Program in Nutritional Metabolism, the Clinical Research Center at MGH or by phone conversation with protocol study staff trained to implement the curriculum. The curriculum will be introduced and reinforced with weekly sessions during wks 0-24 (see **Table**), then continued with monthly sessions after open label extension. There will also be $100 per patient allowance that will be used in individualized ways to promote lifestyle goals: For example, subsidizing a gym membership, purchasing of relevant cookbooks or purchase

of appropriate food items from health food stores, etc.

Session	Topic	Initial Session	Reinforcement
1	Welcome to the Lifestyle Balance Program	Week 1	Week 13
2	How to Read a Nutrition Label	Week 2	Week 14
3	The Components of a Healthy Diet	Week 3	Week 15
4	Things to Know Before Becoming Active	Week 4	Week 16
5	Living on Active Lifestyle	Week 5	Week 17
6	Focus on Fiber	Week 6	Week 18
7	Whole Grains	Week 7	Week 19
8	Fats & Cholesterol	Week 8	Week 20
9	Fruit, Vegetables & Milk	Week 9	Week 21
10	Fish Meat, Poultry, Eggs, Peas & Beans	Week 10	Week 22
11	Tips for Health Eating Out	Week 11	Week 23
12	Food Safety	Week 12	Week 24
13	Appendix-printable fact sheets and logs		

VI. Biostatistical Analysis

The primary endpoint will be change in insulin stimulated glucose uptake as measured by euglycemic clamp. Secondary endpoints include change in levels of adipokines and endothelial function. In addition, we will assess eplerenone's effects on hepatic fat and IMCL. VAT and SAT will be measured simultaneously and controlled for in the analyses of hepatic fat and IMCL. Randomization will be stratified by gender, age (< or > 45), and BP (SBP >140 or DBP>90, or normotensive). Wilk-Shapiro test will be used to assess for normality of distribution of all variables, and variables that are not normally distributed will be log-transformed. Baseline variables will be compared between treatment groups by t-test for continuous variables and Wilcoxon Rank Sum test for variables that are not normally distributed. Any baseline variable that is statistically different between treatment groups will be adjusted for in subsequent analysis. We will analyze the entire set of repeated measurements (baseline, 3 and 6 mos) by using the longitudinal mixed effects model, using all available data, including interim data from those who do not complete the study, to determine differences in longitudinal mean changes between epleronone and placebo over 6 mos of treatment (i.e., test for time x group interaction). The analyses will be intention to treat, using all interim data for subjects who do not complete the study. We will control for gender, race, anti-hypertensive and lipid lowering medication use, urine free cortisol, dietary intake (caloric, macronutrient, alcohol, supplements, salt intake), activity, ARV use and smoking. We will analyze the dietary data in terms of overall caloric intake and macronutrient composition, to determine whether there are any differences in diet by study treatment, or whether change in dietary intake patterns, including vitamins, affects the treatment results. Similar analyses will be performed for activity using the Modifiable Activity Questionnaire. In a sensitivity analysis if the dropout rate is higher than expected or differs between groups, we will

use a LOCF approach carrying forward the last available data to use in the analysis, to determine if the dropouts affected the results. All patients cross-over to open label eplerenone from mos 6 to 12. The primary analysis will be the comparison of placebo and eplerenone after 6 months and the purpose of the open label design after 6 mos is to encourage recruitment and retention. Safety data will be collected at all time points and continued treatment over 12 mos will allow us to determine safety and tolerability over one year and whether changes in critical endpoints continue to improve with one year of dosing.

Sample Size Calculation

Assuming 25 evaluable patients in each group, the study will have 80% power to detect a between groups difference of 0.8 SD in the percent change over 6 mos, at P=0.05 by an independent samples t-test. Thus the power of the longitudinal mixed models approach will be greater than the first-and last pair analysis. Based on data from our group, mean±SD of insulin stimulated glucose uptake in this population using our clamp protocol is 7.4±1.7mg/kg/min. The current study will be powered to detect a change in glucose uptake of 1.4mg/kg/min, or approximately 20% change, which would be physiologically relevant.

VII. Risks and Discomforts

Epelerenone: Adverse drug reactions associated with the use of eplerenone include: hyperkalaemia, hypotension, dizziness, headache, stomach pain, cough, breast enlargement, rash, abnormal vaginal bleeding, erectile dysfunction, and altered renal function. Other rare side effects have been reported include fatigue, chest pain, tingling in arms and legs, loss of muscle tone, weakness or heaviness in legs, confusion, lack of energy, cold, gray skin or irregular heartbeat, and diarrhea. These potential side effects will be assessed for throughout the study. Any subject with serum K >5.5 mEq/L at screen will be excluded from the study. During the study, a subject who has an elevated K of > 5.5 mEq/L at any study visit will have a recheck; if the K remains elevated after recheck they will be excluded from the study. Additionally, subjects who experience symptoms of hyperkalemia and have a K > 5.0 mEq/L will be excluded from continued participation in the study. Subjects who have hypotension (SBP<90) at any study visit after titration to full dose eplerenone 50mg/d will be given a dose reduction to 25 mg/d. If a subject remains hypotensive (SBP <90) or symptomatic of hypotension at the reduced dose they will be dropped from the study. Since patients with renal insufficiency may require reduced dosing based on creatinine clearance, subjects with renal insufficiency (serum creatinine > 1.5 mg/dl) will also be excluded from participation. Concomitant use of full dose ritonavir, nelfinavir, clarithromycin and other strong inhibitors of CYP34A (see Appendix A for complete list) will be an exclusion in the study as will the use of St. John's Wart (CYP3A4 inducer).

Oral Glucose Tolerance Test: Subjects may find the 75g glucose beverage distasteful. In addition, there is a possibility of "reactive" hypoglycemia in the latter part of the OGTT or after its conclusion. If subjects develop symptoms of hypoglycemia they will have their blood sugar tested and will be treated appropriately. Subjects will be given a meal immediately following the conclusion of the OGTT.

Radiation
As a result of participation in this study, subjects will be exposed to 4 DEXA scans over 12 months. The radiation risk associated with the whole body DEXA scan is approximately 0.26 mrem of radiation, for a total of approximately 1 mrem during the study. The total radiation exposure associated with this study does not pose excessive risk to subjects.

Magnetic Resonance Imaging: Subjects will be carefully screened for metal implants such as surgical clips or pacemakers prior to MRI scanning. Subjects will be given earplugs due to the loud noises during the test. Subjects who feel uncomfortable in confined spaces may have difficulty in the narrow cylinder of the MRI, and the MRI can be stopped at any time at the patient's request. There are no known hazards for pregnant women.

Euglycemic Hyperinsulinemic clamp/insulin: Administration of insulin infusion can cause hypoglycemia. Blood sugar will be monitored every 5 minutes during the insulin clamp, and 20% dextrose is infused per protocol to achieve target blood glucose of 90mg/dL. In the event of hypoglycemia, 50% dextrose is available at the bedside, and a physician or nurse practitioner is present throughout the insulin clamp procedure. There is a theoretical risk of infection associated with the use of albumin, a protein purified from donated blood. The risk of transmitting disease is reduced by testing blood donors for infections, and by heat treating and purifying the albumin. The albumin that will be used for the insulin mixture is the same formulation that is commonly used for patients in the hospital and is FDA approved. Because of these measures, the risk is considered to be very small; no cases of disease transmission have ever been identified for albumin.

Blood Drawing: The total blood drawn for subjects completing the study is equivalent to approximately 2 3/4 cups during 12 month period. Patients who have the optional overnight blood sampling will have an additional ¾ cup of blood drawn. This quantity of blood drawing over 12 months does not pose excessive risk to patients. Patients with a hemoglobin < 11 mg/dL will be excluded from the study. There will be minimal risk and discomfort associated with blood drawing and IV placement. The risks of these procedures are minor bruising or bleeding at the site of the blood draw or IV catheter.

Flow Mediated Vasodilation: There are no known risks of ultrasound measurements. There is the possibility of discomfort from having the blood pressure cuff inflated for 5 minutes, this includes numbness or tingling of the arm.

VIII. Potential Benefits

The potential benefits of this study include improved body composition, cardiovascular health, and improved glucose and insulin metabolism associated with HIV associated metabolic abnormalities. These benefits may be associated with eplerenone, lifestyle modification or both. In addition, all subjects will be provided with tools to help them meet the prescribed goals, including a core curriculum binder. All of the participant educational handouts have been included as attachments to this document.

IX. Monitoring and Quality Assurance

Written informed consent for this protocol will be obtained from all subjects at the first screening visit, prior to any procedures or interventions. In all cases, consent will be witnessed by an appropriate health care professional (investigator/co-investigator staff). All efforts will be made to insure the privacy rights of the subject. No written or oral communication will be made about any subject with anyone other than the subject, unless the subject so requests. Medical information obtained from the study may become part of the subject's permanent hospital record, subject to the confidentiality and privacy regulations of the Massachusetts General Hospital. Subjects will be closely monitored to insure that study participation is not adversely affecting their quality of life, and any adverse events will be reported in a timely fashion and in accordance with the Partners HRC guidelines for adverse event reporting. Kidney function and potassium levels will be serially monitored. If a subject experiences an opportunistic infection or any acute illness, the lifestyle modification program will be held while the subject is acutely ill.

Hyperkalemia is a potential side effect of eplerenone use, and contraindications for theuse of eplerenone include serum K > 5.5 mEq/L at initiation. Therefore, any subject with serum K >5.5 mEq/L at screen will be excluded from the study. During the study, a subject who has an elevated K of > 5.5 mEq/L at any study visit will have a recheck; if the K remains elevated after recheck they will be excluded from the study. Additionally, subjects who experience symptoms of hyperkalemia and have a K > 5.0 mEq/L will be excluded from continued participation in the study. Subjects who have hypotension (SBP<90) at any study visit after titration to full dose eplerenone 50mg/d will be given a dose reduction to 25 mg/d. If a subject remains hypotensive (SBP <90) or symptomatic of hypotension at the reduced dose they will be dropped from the study. Since patients with renal insufficiency may require reduced dosing based on creatinine clearance, subjects with renal insufficiency (serum creatinine > 1.5 mg/dl) will also be excluded from participation. Other side effects have been reported and include dizziness, fatigue, diarrhea, headache, stomach pain, cough, breast enlargement or breast enlargement, rash, abnormal vaginal bleeding. Rare but serious side effects include chest pain, tingling in arms and legs, loss of muscle tone, weakness or heaviness in legs, confusion, lack of energy, cold, gray skin or irregular heartbeat. These potential side effects will be assessed for throughout the study.

Exclusion criteria during the study include use of diabetic agents; initiation of ACE-I, ARB, or spironolactone; Hgb < 11; ALT >2.5 x ULN; persistent hyperkalemia (K >5.5 mEq/L despite recheck and/or symptoms of hyperkalemia if K >5.0 mEq/L). If a subject remains hypotensive (SBP <90) or symptomatic of hypotension after a dose reduction of eplerenone to 25mg for this same reason during the study, they will be dropped from the study.

Data Safety Monitoring Plan

Prior to the start of study, a data safety monitoring board will be created and this board will consist of a statistician**,** a clinician not directly related to the research project, but one who is familiar with HIV infection and its complications, and a member of the HIV community. This board will meet quarterly to review any potential side effects or adverse events in relation to the use of eplerenone or participation in the study. The DSMB will meet and review adverse events in accordance with the guidelines of the Massachusetts General Hospital Human Research Committee. Reports generated by

the DSMB will be submitted to the Partners Institutional Review Board when otherwise indicated or requested.

The principal investigator and/or co-investigator will be responsible for following patients during the study. Patients will be assessed at every visit during their study participation. The investigators and study staff will assure that all CRFs, source documents and informed consent are accurate and complete after each study visit.

X. References

1. Seaberg, E.C., et al., *Association between highly active antiretroviral therapy and hypertension in a large cohort of men followed from 1984 to 2003.* AIDS, 2005. **19**(9): p. 953-60.
2. Hadigan, C., et al., *Metabolic abnormalities and cardiovascular disease risk factors in adults with human immunodeficiency virus infection and lipodystrophy.* Clin Infect Dis, 2001. **32**(1): p. 130-139.
3. Brown, T.T., et al., *Antiretroviral therapy and the prevalence and incidence of diabetes mellitus in the multicenter AIDS cohort study.* Arch Intern Med, 2005. **165**(10): p. 1179-84.
4. Carr, A., et al., *Diagnosis, prediction, and natural course of HIV-1 protease-inhibitor- associated lipodystrophy, hyperlipidaemia, and diabetes mellitus: a cohort study.* Lancet, 1999. **353**(9170): p. 2093-9.
5. Hsue, P.Y., et al., *Progression of atherosclerosis as assessed by carotid intima-media thickness in patients with HIV infection.* Circulation, 2004. **109**: p. 1603-08.
6. Lo, J., et al., *Increased prevalence of subclinical coronary atherosclerosis detected by coronary computed tomography angiography in HIV-infected men.* Aids, 2010.
7. Triant, V.A., et al., *Increased acute myocardial infarction rates and cardiovascular risk factors among patients with human immunodeficiency virus disease.* J Clin Endocrinol Metab, 2007. **92**(7): p. 2506-12.
8. Joy, T., et al., *Relationship of body composition to body mass index in HIV-infected patients with metabolic abnormalities.* J Acquir Immune Defic Syndr, 2008. **47**(2): p. 174-84.
9. Grunfeld, C., et al., *Association of upper trunk and visceral adipose tissue volume with insulin resistance in control and HIV-infected subjects in the FRAM study.* J Acquir Immune Defic Syndr, 2007. **46**(3): p. 283-90.
10. He, Q., et al., *Insulin resistance, hepatic lipid and adipose tissue distribution in HIV-infected men.* Antivir Ther, 2008. **13**(3): p. 423-8.
11. Wohl, D., et al., *The associations of regional adipose tissue with lipid and lipoprotein levels in HIV-infected men.* J Acquir Immune Defic Syndr, 2008. **48**(1): p. 44-52.
12. Dolan, S.E., et al., *Increased cardiovascular disease risk indices in HIV-infected women.* J Acquir Immune Defic Syndr, 2005. **39**(1): p. 44-54.
13. Samaras, K., et al., *Proinflammatory markers, insulin sensitivity, and cardiometabolic risk factors in treated HIV infection.* Obesity (Silver Spring), 2009. **17**(1): p. 53-9.
14. Reingold, J., et al., *Association of HIV infection and HIV/HCV coinfection with C-reactive protein levels: the fat redistribution and metabolic change in HIV infection (FRAM) study.* J Acquir Immune Defic Syndr, 2008. **48**(2): p. 142-8.

15. Guaraldi, G., et al., *Lipodystrophy and anti-retroviral therapy as predictors of sub-clinical atherosclerosis in human immunodeficiency virus infected subjects.* Atherosclerosis, 2010. **208**(1): p. 222-7.
16. Goodfriend, T.L., et al., *Visceral obesity and insulin resistance are associated with plasma aldosterone levels in women.* Obes Res, 1999. **7**(4): p. 355-62.
17. Garg, R., et al., *Aldosterone production and insulin resistance in healthy adults.* J Clin Endocrinol Metab, 2010. **95**(4): p. 1986-90.
18. Lo, J., et al., *Increased aldosterone among HIV-infected women with visceral fat accumulation.* Aids, 2009. **23**(17): p. 2366-70.
19. Guo, C., et al., *Mineralocorticoid receptor blockade reverses obesity-related changes in expression of adiponectin, peroxisome proliferator-activated receptor-gamma, and proinflammatory adipokines.* Circulation, 2008. **117**(17): p. 2253-61.
20. Wada, T., et al., *Spironolactone improves glucose and lipid metabolism by ameliorating hepatic steatosis and inflammation and suppressing enhanced gluconeogenesis induced by high-fat and high-fructose diet.* Endocrinology, 2010. **151**(5): p. 2040-9.
21. Sowers, J.R., A. Whaley-Connell, and M. Epstein, *Narrative review: the emerging clinical implications of the role of aldosterone in the metabolic syndrome and resistant hypertension.* Ann Intern Med, 2009. **150**(11): p. 776-83.
22. Rossi, G.P., et al., *Primary aldosteronism: cardiovascular, renal and metabolic implications.* Trends Endocrinol Metab, 2008. **19**(3): p. 88-90.
23. Kornel, L., *Colocalization of 11 beta-hydroxysteroid dehydrogenase and mineralocorticoid receptors in cultured vascular smooth muscle cells.* Am J Hypertens, 1994. **7**(1): p. 100-3.
24. Alzamora, R., L. Michea, and E.T. Marusic, *Role of 11beta-hydroxysteroid dehydrogenase in nongenomic aldosterone effects in human arteries.* Hypertension, 2000. **35**(5): p. 1099-104.
25. Urbanet, R., et al., *Analysis of Insulin Sensitivity in Adipose Tissue of Patients with Primary Aldosteronism.* J Clin Endocrinol Metab, 2010.
26. Losel, R., A. Schultz, and M. Wehling, *A quick glance at rapid aldosterone action.* Mol Cell Endocrinol, 2004. **217**(1-2): p. 137-41.
27. Wehling, M., et al., *Rapid cardiovascular action of aldosterone in man.* J Clin Endocrinol Metab, 1998. **83**(10): p. 3517-22.
28. Ishizawa, K., et al., *Aldosterone stimulates vascular smooth muscle cell proliferation via big mitogen-activated protein kinase 1 activation.* Hypertension, 2005. **46**(4): p. 1046-52.
29. Romagni, P., et al., *Aldosterone induces contraction of the resistance arteries in man.* Atherosclerosis, 2003. **166**(2): p. 345-9.
30. Gomez-Sanchez, E.P., et al., *ICV infusion of corticosterone antagonizes ICV-aldosterone hypertension.* Am J Physiol, 1990. **258**(4 Pt 1): p. E649-53.
31. Young, M.J. and J.W. Funder, *The renin-angiotensin-aldosterone system in experimental mineralocorticoid-salt-induced cardiac fibrosis.* Am J Physiol, 1996. **271**(5 Pt 1): p. E883-8.
32. Qin, W., et al., *Transgenic model of aldosterone-driven cardiac hypertrophy and heart failure.* Circ Res, 2003. **93**(1): p. 69-76.
33. Saye, J.A., et al., *Angiotensinogen gene expression in 3T3-L1 cells.* Am J Physiol, 1989. **256**(2 Pt 1): p. C448-51.
34. Karlsson, C., et al., *Human adipose tissue expresses angiotensinogen and enzymes required for its conversion to angiotensin II.* J Clin Endocrinol Metab, 1998. **83**(11): p. 3925-9.

35. Ehrhart-Bornstein, M., et al., *Human adipocytes secrete mineralocorticoid-releasing factors.* Proc Natl Acad Sci U S A, 2003. **100**(24): p. 14211-6.
36. Schinner, S., et al., *Adipocyte-derived products induce the transcription of the StAR promoter and stimulate aldosterone and cortisol secretion from adrenocortical cells through the Wnt-signaling pathway.* Int J Obes (Lond), 2007. **31**(5): p. 864-70.
37. Krug, A.W., et al., *Human adipocytes induce an ERK1/2 MAP kinases-mediated upregulation of steroidogenic acute regulatory protein (StAR) and an angiotensin II-sensitization in human adrenocortical cells.* Int J Obes (Lond), 2007. **31**(10): p. 1605-16.
38. Bentley-Lewis, R., et al., *Body mass index predicts aldosterone production in normotensive adults on a high-salt diet.* J Clin Endocrinol Metab, 2007. **92**(11): p. 4472-5.
39. Hoppmann, J., et al., *The balance between gluco- and mineralo-corticoid action critically determines inflammatory adipocyte responses.* J Endocrinol, 2010. **204**(2): p. 153-64.
40. Rondinone, C.M., D. Rodbard, and M.E. Baker, *Aldosterone stimulated differentiation of mouse 3T3-L1 cells into adipocytes.* Endocrinology, 1993. **132**(6): p. 2421-6.
41. Penfornis, P., et al., *The mineralocorticoid receptor mediates aldosterone-induced differentiation of T37i cells into brown adipocytes.* Am J Physiol Endocrinol Metab, 2000. **279**(2): p. E386-94.
42. Caprio, M., et al., *Pivotal role of the mineralocorticoid receptor in corticosteroid-induced adipogenesis.* Faseb J, 2007. **21**(9): p. 2185-94.
43. Devenport, L.D., K.G. Goodwin, and P.M. Hopkins, *Continuous infusion of aldosterone: correlates of body weight gain.* Pharmacol Biochem Behav, 1985. **22**(5): p. 707-9.
44. Caprio, M., et al., *Pivotal role of the mineralocorticoid receptor in corticosteroid-induced adipogenesis 10.1096/fj.06-7970com.* FASEB J., 2007. **21**(9): p. 2185-2194.
45. Hirata, A., et al., *Blockade of mineralocorticoid receptor reverses adipocyte dysfunction and insulin resistance in obese mice.* Cardiovasc Res, 2009. **84**(1): p. 164-72.
46. Hadigan, C., et al., *Magnetic resonance spectroscopy of hepatic lipid content and associated risk factors in HIV infection.* J Acquir Immune Defic Syndr, 2007. **46**(3): p. 312-7.
47. Guaraldi, G., et al., *Nonalcoholic fatty liver disease in HIV-infected patients referred to a metabolic clinic: prevalence, characteristics, and predictors.* Clin Infect Dis, 2008. **47**(2): p. 250-7.
48. Fallo, F., et al., *Nonalcoholic fatty liver disease in primary aldosteronism: a pilot study.* Am J Hypertens, 2010. **23**(1): p. 2-5.
49. Colussi, G., et al., *Insulin resistance and hyperinsulinemia are related to plasma aldosterone levels in hypertensive patients.* Diabetes Care, 2007. **30**(9): p. 2349-54.
50. Freel, E.M., et al., *Aldosterone status associated with insulin resistance in patients with heart failure--data from the ALOFT study.* Heart, 2009. **95**(23): p. 1920-4.
51. Conn, J.W., R.F. Knopf, and R.M. Nesbit, *Clinical Characteristics of Primary Aldosteronism from an Analysis of 145 Cases.* Am J Surg, 1964. **107**: p. 159-72.
52. Catena, C., et al., *Insulin sensitivity in patients with primary aldosteronism: a follow-up study.* J Clin Endocrinol Metab, 2006. **91**(9): p. 3457-63.

53. Fallo, F., et al., *Prevalence and characteristics of the metabolic syndrome in primary aldosteronism.* J Clin Endocrinol Metab, 2006. **91**(2): p. 454-9.
54. Matrozova, J., et al., *Fasting plasma glucose and serum lipids in patients with primary aldosteronism: a controlled cross-sectional study.* Hypertension, 2009. **53**(4): p. 605-10.
55. Ranade, K., et al., *Genetic variation in aldosterone synthase predicts plasma glucose levels.* Proc Natl Acad Sci U S A, 2001. **98**(23): p. 13219-24.
56. Hitomi, H., et al., *Aldosterone suppresses insulin signaling via the downregulation of insulin receptor substrate-1 in vascular smooth muscle cells.* Hypertension, 2007. **50**(4): p. 750-5.
57. Wada, T., et al., *Aldosterone inhibits insulin-induced glucose uptake by degradation of insulin receptor substrate (IRS) 1 and IRS2 via a reactive oxygen species-mediated pathway in 3T3-L1 adipocytes.* Endocrinology, 2009. **150**(4): p. 1662-9.
58. Selvaraj, J., et al., *Impact of excess aldosterone on glucose homeostasis in adult male rat.* Clin Chim Acta, 2009. **407**(1-2): p. 51-7.
59. Floris-Moore, M., et al., *Association of HIV viral load with monocyte chemoattractant protein-1 and atherosclerosis burden measured by magnetic resonance imaging.* Aids, 2009. **23**(8): p. 941-9.
60. Triant, V.A., J.B. Meigs, and S.K. Grinspoon, *Association of C-reactive protein and HIV infection with acute myocardial infarction.* J Acquir Immune Defic Syndr, 2009. **51**(3): p. 268-73.
61. Brown, N.J., *Aldosterone and vascular inflammation.* Hypertension, 2008. **51**(2): p. 161-7.
62. Griendling, K.K., et al., *Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells.* Circ Res, 1994. **74**(6): p. 1141-8.
63. Rocha, R., et al., *Aldosterone induces a vascular inflammatory phenotype in the rat heart.* Am J Physiol Heart Circ Physiol, 2002. **283**(5): p. H1802-10.
64. Suzuki, J., et al., *Eplerenone with valsartan effectively reduces atherosclerotic lesion by attenuation of oxidative stress and inflammation.* Arterioscler Thromb Vasc Biol, 2006. **26**(4): p. 917-21.
65. Rajagopalan, S., et al., *Mineralocorticoid receptor antagonism in experimental atherosclerosis.* Circulation, 2002. **105**(18): p. 2212-6.
66. Ortiz, R.M., A. Mamalis, and L.G. Navar, *Aldosterone Receptor Antagonism Reduces Urinary C-Reactive Protein Excretion in Angiotensin II-Infused, Hypertensive Rats.* J Am Soc Hypertens, 2009. **3**(3): p. 184-91.
67. Limor, R., et al., *Aldosterone up-regulates 12- and 15-lipoxygenase expression and LDL oxidation in human vascular smooth muscle cells.* J Cell Biochem, 2009. **108**(5): p. 1203-10.
68. Fitch, K.V., et al., *Effects of a lifestyle modification program in HIV-infected patients with the metabolic syndrome.* AIDS, 2006. **20**(14): p. 1843-50.
69. Engelson, E.S., et al., *Body composition and metabolic effects of a diet and exercise weight loss regimen on obese, HIV-infected women.* Metabolism: Clinical & Experimental, 2006. **55**(10): p. 1327-36.
70. Terry, L., et al., *Exercise training in HIV-1-infected individuals with dyslipidemia and lipodystrophy.* Med Sci Sports Exerc, 2006. **38**(3): p. 411-7.
71. Yarasheski, K.E., et al., *Resistance exercise training reduces hypertriglyceridemia in HIV-infected men treated with antiviral therapy.* J Appl Physiol, 2001. 90(1): p. 133-8.

Appendix A

Strong Inhibitors of CYP3A4

- Ketoconazole
- Itraconazole
- Fluconazole
- Ritonavir
- Nelfinavir
- Indinavir
- Clarithromycin
- Telithromycin
- Erythromycin
- Nefazadone
- Bergamottin (in grapefruit juice)
- Quercetin (nutritional supplement)
- Aprepitant
- Verapamil
- Chloramphenicol
- Saquinavir