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Significant Association of Aldosterone and Liver Fat Among HIV-Infected Individuals With Metabolic Dysregulation

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Objective: Fatty liver disease is increased among individuals with HIV. We sought to explore how aldosterone, a key hormone linked to insulin resistance and inflammation, relates to liver fat in the large population of individuals with HIV and metabolic abnormalities.

Methods: Forty-six individuals with HIV and increased waist circumference and dysglycemia were assessed for liver fat using proton magnetic resonance spectroscopy. Serum aldosterone level was obtained following strictly controlled posture conditions and a standardized sodium diet and was related to liver fat.

Results: Among the entire group [median (interquartile range) liver fat: 5% (3%, 12%) and homeostatic model assessment of insulin resistance: 1.74 (1.21, 2.83)], serum aldosterone significantly correlated with liver fat (r = 0.31; P = 0.049). Liver fat level was significantly higher in those with aldosterone above vs below the median [8% (3%, 20%) vs 4% (2%, 10%); P = 0.02]. In the presence of metabolic syndrome, individuals with aldosterone levels above vs below the median had markedly elevated liver fat values [14% (9%, 23%) vs 5% (3%, 12%); P = 0.005] and increased presence of fatty liver disease (FLD; 92% vs 50%; P = 0.02). Controlling for metabolic syndrome, hepatitis C virus, and alcohol use, aldosterone was a significant and independent predictor of liver fat (β estimate: 0.6038, P = 0.01; overall model r^2 = 0.41, P = 0.0005) and FLD (OR: 1.38, P = 0.02; overall model r^2 = 0.28, P = 0.002).

Conclusion: These data highlight a robust association between aldosterone and liver fat among individuals with HIV and metabolic dysregulation. Increased aldosterone may be a risk factor for liver fat accumulation among the population with HIV.

Freeform/Key Words: aldosterone, fatty liver disease, HIV, liver fat, metabolic syndrome

Abbreviations: ^{1}H-MRS, proton magnetic resonance spectroscopy; ART, antiretroviral therapy; DBP, diastolic blood pressure; FLD, fatty liver disease; HCV, hepatitis C virus; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; MR, mineralocorticoid receptor; MS, metabolic syndrome; NIH, National Institutes of Health; PAI-1, plasminogen activator inhibitor-1; PRA, plasma renin activity; RAAS, renin-angiotensin-aldosterone system; SBP, systolic blood pressure; VAT, visceral adipose tissue.

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The prevalence of fatty liver disease (FLD) varies by report and is generally increased in the population with HIV [1, 2], between 35% and 42% compared with 30% in the general population [3, 4]. Fortunately, FLD is a reversible process; however, without clinical attention, progression to more severe inflammation and irreversible disease can occur. Although uncontrolled viremia, antiretroviral therapy (ART) use, alcohol use, hepatitis C virus (HCV) coinfection, and chronic inflammation may all contribute to FLD, some of these factors do not entirely account for the increased risk of FLD in HIV [5, 6]. In this regard, elucidating other mechanisms leading to FLD in HIV is critical.

Excess visceral adipose tissue (VAT), a dysfunctional fat depot with altered capacity for lipid storage compared with the subcutaneous depot, may contribute to a higher burden of FLD in HIV [7, 8]. In the presence of VAT accumulation, lipid may subsequently deposit in the liver via the portal system. We previously reported data demonstrating aldosterone dysregulation in individuals with HIV [9, 10], particularly those with increased VAT. Although data have shown the association of the renin-angiotensin-aldosterone system (RAAS) to liver fat in animal models, the relationship of fatty liver to aldosterone has never previously been investigated in HIV. We sought to test the hypothesis that aldosterone is a key hormone related to FLD in HIV among a group chosen for metabolic dysregulation.

1. Methods

A. Participants

Forty-six individuals between the ages of 30 and 65 years with a known history of HIV >5 years and ART use >12 months were recruited to participate in a longitudinal study investigating a novel strategy to block the RAAS in HIV [11]. In the current study, we used baseline data from the previously completed interventional study to report relationships among key metabolic variables, specifically related to liver fat and aldosterone. These data highlighting baseline relationships were not included in the prior analyses [11]. Individuals with increased waist circumference based on National Cholesterol Education Program guidelines [>102 cm (males), >88 cm (females)] were included. An oral glucose tolerance test was performed to select for those with abnormal glucose homeostasis: impaired fasting glucose (glucose >100 mg/dL and <126 mg/dL), impaired glucose tolerance (2-hour glucose >140 mg/dL and <200 mg/dL), or fasting insulin >12 μIU/mL. Uncontrolled hypertension [systolic blood pressure (SBP) ≥160 mm Hg or diastolic blood pressure (DBP) ≥100 mm Hg], pregnancy, or a known history of diabetes mellitus, cardiovascular disease, and cirrhosis were exclusionary. Current medications affecting the RAAS pathway, potassium supplements, steroids, estrogen or progestin derivatives, growth hormone and growth hormone–releasing hormone, and supraphysiologic testosterone replacement were not permitted. Individuals demonstrating serum potassium level >5.5 mEq/L, alanine aminotransferase level >2.5 times the upper limit of normal, creatinine level >1.5 mg/dL, or estimated glomerular filtration rate <60 mL/min/1.73 m^2 were excluded. This study was approved by the Partners Human Research Committee, and all individuals provided informed consent for participation.

B. Standardized Sodium Diet to Characterize Aldosterone

Individuals were directed by a registered dietician to supplement their usual diet with broth packets (47.8 mEq Na^+ per packet) for 6 days to achieve a dietary sodium intake of >200 mEq per day.

C. Laboratory Assessment

After the 6-day diet, individuals were admitted overnight to an inpatient research facility. Individuals were asked to fast for 12 hours and lie supine overnight. Plasma renin activity

(PRA) and aldosterone and other metabolic parameters were assessed on the following morning while individuals maintained the fasting state and supine posture. PRA was assessed using the GammaCoat [^{125}I] RIA kit (sensitivity, 0.01 ng/mL/h; #CA1533; DiaSorin; RRID:AB_2736926 [12]). Serum aldosterone (sensitivity, 2.5 ng/dL) and urine cortisol levels were measured by solid-phase RIA by the Coat-A-Count method (#TKAL2; Diagnostics Products Corp; RRID:AB_2737007 [13]). ELISA was used to measure markers of inflammation and immune activation from plasma.

D. Liver Fat Quantification

Proton magnetic resonance spectroscopy (^{1}H-MRS) of the liver was performed after a 12-hour fast. Breath-hold single-voxel data of the right hepatic lobe were acquired using a point-resolved spatially localized spectroscopy pulse sequence without water suppression [14]. A fitting algorithm for water and lipid resonances (0.9 to 2.2 ppm) was performed using an LCModel (version 6.3-0K; Stephen Provencher), scaled to the unsuppressed water peak (4.7 ppm) and expressed as the fat fraction (lipid/lipid + water). ^{1}H-MRS correlates well and is a comparable noninvasive diagnostic surrogate for the more invasive liver biopsy [15, 16]. Individuals were identified as having FLD if the fat fraction was ≥5% on the basis of established criteria for steatosis [1]. MRI was used to concurrently measure VAT and subcutaneous adipose tissue at the level of the L4 vertebral body using an axial T1-weighted fat-suppressed pulse sequence. Manual tracing of the fat compartments was assessed using commercial software (Vitrak; Merge e/Film).

E. Statistical Analysis

Normality of variables was tested using the Shapiro-Wilk test. Normally and nonnormally distributed variables are presented as mean ± SEM and median [interquartile range], respectively. Categorical variables are represented by proportions. One individual was withdrawn from the analysis because of inability to maintain the controlled posture before the blood draw. Liver fat could not be quantified in three individuals. Variables were log-transformed to achieve a normal distribution. Relationships were assessed by Pearson correlation. Individuals were stratified by the presence or absence of metabolic syndrome (MS). MS was characterized by the presence of any three criteria: (1) waist circumference ≥102 cm (men) or ≥88 cm (women); (2) triglyceride level ≥150 mg/dL; (3) high-density lipoprotein (HDL) level <40 mg/dL (men) or <50 mg/dL (women); (4) blood pressure ≥130/85 mm Hg or use of antihypertensive medication; and (5) fasting plasma glucose level ≥100 mg/dL. Multivariate and logistic regressions were performed to assess independent effects of aldosterone on liver fat and FLD, respectively, controlling for alcohol use and HCV and other variables determined to be related to liver fat in the presence of MS ($P < 0.10$). In an exploratory analysis using multivariate regression, we sought to elucidate which specific components (triglycerides, HDL, SBP, DBP) unique to the group with presence of MS were predictors of liver fat separate from those components (waist circumference and dysglycemia) required for entry into the study. Statistical significance was determined to a two-sided $P < 0.05$. Analyses were performed using SAS JMP (version 12).

2. Results

A. Baseline Characteristics

Individuals with HIV were aged 50 ± 1 years; 51% of individuals were white, and 67% were male. The mean durations of HIV infection and ART use were 17 ± 1 years and 10 ± 1 years, respectively. Study participants demonstrated good immunological control with CD4^{+} count of 608 ± 35 cells/μL and log HIV viral load of 1.50 ± 0.07 copies/mL. A CD4^{+} nadir count of 217 ± 28 cells/μL was reported. Eleven percent of individuals had a known diagnosis of HCV,

and 40% reported current alcohol use. Criteria for MS were met in 56% of individuals, permitting relatively similar proportions of individuals with and without MS to be evaluated (Table 1). No individuals demonstrated evidence of hypokalemia. Of the women included, median FSH level was 10.3 [5.3, 54.1] mIU/mL, and 47% reported postmenopausal status by history. Although we did not time the assessment of the RAAS to the menstrual cycle, FSH levels drawn at screen did not correlate with aldosterone levels among all women ($r = 0.07$; $P = 0.81$).

B. Body Composition and Metabolic Indices

Body composition measures were consistent with increased abdominal fat accumulation [waist circumference, 111 (105, 117) cm; VAT, 235 ± 14 cm^2]. Median liver fat was 5% [3%, 12%] in the entire group. Fifty percent of individuals with HIV had evidence of FLD on ^{1}H-MRS, a proportion of FLD similar to that demonstrated in other studies of HIV [6]. The group as a whole demonstrated abnormal glucose homeostasis, with an hemoglobin A1c (HbA1c) level of 5.7% ± 0.1% and a homeostatic model assessment of insulin resistance of 1.74 [1.21, 2.83]. PRA and aldosterone levels measured under standardized diet and posture techniques were 0.20 [0.09, 0.40] ng/mL/h and 3.73 [2.49, 8.39] ng/dL among the entire group, respectively (Table 1).

C. Relationships to Liver Fat

Among the entire group, increased triglyceride ($r = 0.50$; $P = 0.0007$), aldosterone ($r = 0.31$; $P = 0.049$), plasminogen activator inhibitor-1 (PAI-1) ($r = 0.56$; $P = 0.0001$), and high-sensitivity C-reactive protein (hsCRP) ($r = 0.33$; $P = 0.03$) levels and reduced HDL level ($r = -0.54$; $P = 0.0003$) correlated with liver fat. In addition, $CD4^+$ T cell nadir ($r = 0.33$; $P = 0.07$), waist circumference ($r = 0.29$; $P = 0.06$), VAT ($r = 0.28$; $P = 0.07$), adiponectin ($r = -0.30$; $P = 0.06$), and monocyte chemoattractant protein-1 ($r = 0.30$; $P = 0.06$) tended to be related to liver fat. In contrast, PRA ($r = -0.13$; $P = 0.40$) and 24-hour urine cortisol ($r = 0.18$; $P = 0.26$) did not correlate with liver fat. Upon further stratification, aldosterone ($r = 0.47$; $P = 0.02$), PAI-1 ($r = 0.51$; $P = 0.01$), and hsCRP ($r = 0.53$, $P = 0.007$) were significantly correlated, and $CD4^+$ T cell nadir ($r = 0.42$; $P = 0.07$) tended to be correlated with liver fat among those with evidence of MS. In contrast, HDL ($r = -0.69$; $P = 0.002$) and PAI-1 ($r = 0.48$; $P = 0.05$) were significantly related to liver fat among those witho\ut evidence of MS (Table 2).

D. Relationship of Aldosterone to FLD

Upon stratification by median aldosterone level, liver fat [8% (3%, 20%) vs 4% (2%, 10%); $P = 0.02$] was significantly higher and FLD (64% vs 35%; $P = 0.06$) tended to be higher among the HIV group with above vs below median aldosterone levels. More significant relationships were seen upon stratification for MS. Among individuals with HIV and MS, those with above vs below median aldosterone had increased liver fat [14% (9%, 23%) vs 5% (3%, 12%); $P = 0.005$) and more FLD (92% vs 50%; $P = 0.02$) (Fig 1; Table 3).

E. Aldosterone as an Independent Predictor of Liver Fat and FLD

In multivariate modeling controlling for MS, alcohol use, and HCV, aldosterone was a significant and independent predictor of liver fat (β estimate: 0.6038, $P = 0.01$; overall model $r^2 = 0.41$, $P = 0.0005$) and FLD (OR: 1.38, $P = 0.02$; overall model $r^2 = 0.28$, $P = 0.002$). In secondary models controlling for additional variables related to liver fat among those with MS ($CD4^+$ T cell nadir, PAI-1, and hsCRP), aldosterone remained significantly and independently associated with liver fat (β estimate: 0.4644, $P = 0.03$; overall model $r^2 = 0.75$, $P \leq 0.0001$) and FLD (OR: 1.45, $P = 0.04$; overall model $r^2 = 0.47$, $P = 0.005$) (Table 4).

Table 1. Baseline Demographics and Clinical Characteristics (n = 45)

Demographics	
Age, y	50 ± 1
Race, %	
White	51
African American	44
Other	5
Male sex, %	67
Current alcohol use, %	40
Current tobacco use, %	27
HIV Parameters	
$CD4^+$ T cell nadir count, cells/μL	217 ± 28
$CD4^+$ T cell count, cells/μL	608 ± 35
$CD8^+$ T cell count, cells/μL	840 ± 46
Log HIV RNA viral load, copies/mL	1.50 ± 0.07
Undetectable viral load, %	76
Duration HIV, y	17 ± 1
Duration ART use, y	10 ± 1
Current PI use, %	42
Current NRTI use, %	96
Current NNRTI use, %	51
Current HCV, %	11
Body Composition and Ectopic Fat	
Waist circumference, cm	111 [105, 117]
BMI, kg/m^2	32 [29, 35]
VAT area, cm^2	235 ± 14
SAT area, cm^2	386 ± 23
Liver fat, %	5 [3, 12]
Current fatty liver disease, %	50
Metabolic Parameters	
SBP, mm Hg	131 ± 2
DBP, mm Hg	83 ± 1
Current hypertension %	29
ALT, U/dL	22 [16, 33]
Total cholesterol, mg/dL	174 ± 5
Triglycerides, mg/dL	162 ± 11
HDL cholesterol, mg/dL	42 [33, 52]
Current dyslipidemia, %	29
Fasting glucose, mg/dL	97 ± 2
Hemoglobin A1c, %	5.7 ± 0.1
HOMA-IR	1.74 [1.21, 2.83]
Current metabolic syndrome, %	56
Serum aldosterone, ng/dL	3.73 [2.49, 8.39]
Plasma renin activity, ng/mL/h	0.20 [0.09, 0.40]
Serum potassium, mmol/L	4.1 ± 0.0
Urine cortisol, μg/24 h	27 [15, 40]
Markers of Inflammation and Immune Activation	
IL-6, pg/mL	8.78 [5.84, 17.66]
Adiponectin, pg/mL	4491 [3353, 5520]
PAI-1, ng/mL	37.4 ± 2.7
hsCRP, mg/L	3.5 [1.3, 9.6]
MCP-1, pg/mL	196 ± 10

Data are reported as mean ± SEM, percentage, or median [interquartile range].
Abbreviations: ALT, alanine aminotransferase; BMI, body mass index; HOMA-IR, homeostatic model assessment of insulin resistance; hsCRP, high-sensitivity C-reactive protein; MCP-1, monocyte chemoattractant protein-1; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside/nucleotide reverse transcription inhibitor; PAI-1, plasminogen activator inhibitor-1; PI, protease inhibitor; SAT, subcutaneous adipose tissue.

Table 2. Univariate Correlations With Liver Fat

	All Individuals		Absence of Metabolic Syndrome (n = 18)		Presence of Metabolic Syndrome (n = 24)	
	r	*P* Value	*r*	*P* Value	*r*	*P* Value
Demographics						
Age, y	0.06	0.72	−0.04	0.88	0.10	0.64
Alcohol use, average/wk	−0.02	0.89	0.06	0.82	−0.03	0.91
HIV parameters						
$CD4^+$ T cell nadir count, cells/μL	0.33	0.07	0.46	0.14	0.42	0.07
Log HIV RNA viral load, copies/mL	−0.14	0.37	−0.03	0.90	−0.35	0.10
Body composition and ectopic fat						
Log waist circumference, cm	0.29	0.06	−0.03	0.90	0.16	0.46
VAT, cm^2	0.28	0.07	0.26	0.30	0.14	0.50
Metabolic parameters						
SBP, mm Hg	−0.09	0.57	−0.33	0.19	−0.04	0.85
DBP, mm Hg	0.12	0.44	−0.09	0.74	0.05	0.81
Log ALT, U/dL	0.08	0.62	−0.20	0.43	0.09	0.69
Triglycerides, mg/dL	0.50	0.0007	0.34	0.16	0.26	0.21
Log HDL, mg/dL	−0.54	0.0003	−0.69	0.002	−0.08	0.71
Fasting glucose, mg/dL	−0.07	0.66	−0.16	0.54	−0.07	0.75
Log HOMA-IR	0.21	0.19	0.22	0.39	−0.002	0.99
Log serum aldosterone, ng/dL	0.31	0.049	0.34	0.16	0.47	0.02
Markers of inflammation and immune activation						
Log IL-6, pg/mL	0.10	0.54	−0.14	0.59	0.20	0.34
Log adiponectin, pg/mL	−0.30	0.06	−0.27	0.30	−0.04	0.89
PAI-1, ng/mL	0.56	0.0001	0.48	0.05	0.51	0.01
Log hsCRP, mg/L	0.33	0.03	0.03	0.91	0.53	0.007
MCP-1, pg/mL	0.30	0.06	0.22	0.39	0.20	0.36

Relationships are determined by Pearson correlation coefficient. Performed under conditions of standardized sodium intake for both groups as described in the text.
Abbreviations: ALT, alanine aminotransferase; HOMA-IR, homeostatic model assessment of insulin resistance; MCP-1, monocyte chemoattractant protein-1

F. Aldosterone and Components of MS as Predictors of Liver Fat

In multivariate modeling controlling for specific components (triglycerides, HDL, SBP, and DBP) unique to the group with presence of MS, aldosterone (β estimate: 0.7848; $P = 0.001$), triglycerides (β estimate: 0.0022; $P = 0.02$), and HDL (β estimate: −1.3714; $P = 0.006$) were significant and independent predictors of liver fat (overall model $r^2 = 0.52$; $P \leq 0.0001$). SBP and DBP were not predictors of liver fat in this model (Table 5).

3. Discussion

These data suggest that greater aldosterone level in the setting of metabolic dysregulation is associated with an increased prevalence of FLD among well-treated individuals with HIV. Indeed, we show that each 1 ng/dL rise in aldosterone level is associated with a 38% increase in the prevalence of FLD in the population studied, after controlling for other pertinent etiologies of FLD such as alcohol use and HCV coinfection. Individuals with MS and above-median aldosterone level demonstrated the highest liver fat value; more than 90% of these individuals had FLD, which was almost double the prevalence among individuals who had MS and below-median aldosterone level. Our data were derived from a group of individuals with metabolic dysfunction, approximately half of whom demonstrated MS and FLD, closely representative of the prevalence of these disorders among other studies of HIV [17]. These

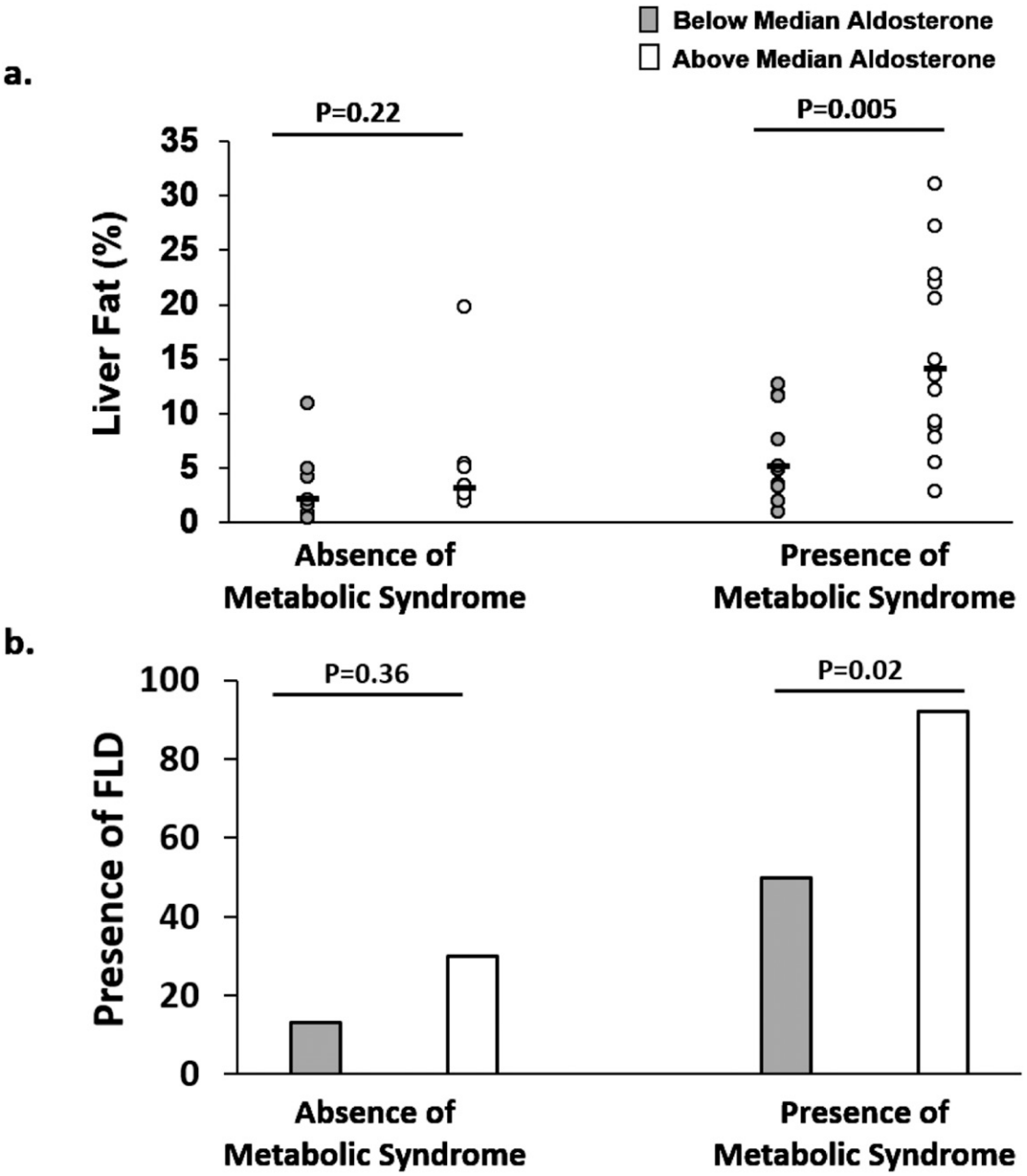

Figure 1. Comparisons of (a) percentage of liver fat and (b) presence of FLD (fat fraction >5%) in individuals with HIV stratified by absence/presence of metabolic syndrome and above-median/below-median aldosterone level. For percentage of liver fat, results are reported in a scatter plot with medians (horizontal lines). To represent the data in a clinically relevant manner, percentages of liver fat are reported as values before log-transformation, and the *P* values reported are based on the appropriate statistical test applied to the log-transformed values.

data allow us to gain further insight into a potentially unique mediator of liver fat accumulation in HIV.

Large cohorts have demonstrated that MS is a critical risk factor for the development of liver fibrosis and subsequent progression of FLD [17]. However, the mechanisms accounting for liver fat accumulation in the population with HIV remain largely unknown. This highlights the need to discern risk factors of FLD in HIV to account for the increased prevalence compared with that of the general population. In this regard, we showed that aldosterone is a

Table 3. Assessment of Liver Fat Parameters by Median Serum Aldosterone Level

	All Individuals			Absence of Metabolic Syndrome			Presence of Metabolic Syndrome		
	Below-Median Serum Aldosterone (n = 20)	Above-Median Serum Aldosterone (n = 22)	*P* Value	Below-Median Serum Aldosterone (n = 8)	Above-Median Serum Aldosterone (n = 10)	*P* Value	Below-Median Serum Aldosterone (n = 12)	Above-Median Serum Aldosterone (n = 12)	*P* Value
Liver Fat Parameters									
Liver fat, %	4 [2, 10]	8 [3, 20]	0.02	2 [1, 5]	3 [2, 5]	0.22	5 [3, 12]	14 [9, 23]	0.005
Presence of fatty liver disease, %	35	64	0.06	13	30	0.36	50	92	0.02

For purposes of representing the data in a clinically relevant manner, nonnormally distributed variables are reported as values before log-transformation, and the *P* value are based on the appropriate statistical test applied to the log-transformed values. Performed under conditions of standardized sodium intake for both groups as described in the text.

potentially important factor independent of other well-known traditional risk factors (alcohol use and HCV) associated with liver fat among the population with HIV and MS. Indeed, ectopic fat accumulation, such as FLD, may develop as a sequela of chronic inflammation [18, 19]. With simultaneous controlling for significant traditional and nontraditional risk factors (inflammation), aldosterone appeared to remain a relevant variable. Indeed, multiple factors contribute to excess liver fat in HIV, and aldosterone may have additional unique hormone-mediated effects on FLD. Further research is needed to discern the mechanism by which aldosterone relates so highly to liver fat in the population with HIV and MS. In the current study, we did not see an association of PRA with liver fat, suggesting the link between aldosterone and liver fat is independent of PRA. Further studies are needed to confirm this finding.

Table 4. Multivariate Model to Assess Determinants of Liver Fat

	Initial Model		Extended Model	
Log Liver Fat	**β Estimate**	***P* Value**	**β Estimate**	***P* Value**
	(r^2 = 0.41; P = 0.0005)		(r^2 = 0.75; $P \leq$ 0.0001)	
Log serum aldosterone	0.6038	0.01	0.4644	0.03
Presence of metabolic syndrome	0.2557	<0.0001	0.1753	0.009
Alcohol use, avg/wk	−0.0075	0.71	0.0542	0.20
Presence of HCV	−0.0665	0.46	0.0361	0.65
$CD4^+$ T cell nadir count			0.0007	0.03
PAI-1			0.0103	0.001
Log hsCRP			0.1307	0.28
	Initial Model		**Extended Model**	
Presence of Fatty Liver Disease	**OR**	***P* Value**	**OR**	***P* Value**
	(r^2 = 0.28; P = 0.002)		(r^2 = 0.47; P = 0.005)	
Serum aldosterone	1.38	0.02	1.45	0.04
Presence of metabolic syndrome	15.37	0.0005	14.53	0.03
Alcohol use, avg/wk	0.86	0.26	0.99	0.98
Presence of HCV	1.21	0.87	2.67	0.60
$CD4^+$ T cell nadir count			1.00	0.41
PAI-1			1.06	0.07
hsCRP			1.00	0.72

r^2 represents the coefficient of determination and the proportion of variance explained by the model. *P* value represents significance by the whole model. Performed under conditions of standardized sodium intake as described in the text.

Table 5. Multivariate Model to Assess Determinants of Liver Fat Related to Metabolic Syndrome

Log Liver Fat		
	β Estimate	**P Value**
	(r^2 = 0.52; $P < 0.0001$)	
Log serum aldosterone	0.7848	0.001
Triglycerides	0.0022	0.02
Log HDL cholesterol	−1.3714	0.006
SBP	−0.0020	0.65
DBP	0.0011	0.88

r^2 represents the coefficient of determination and the proportion of variance explained by the model. *P* value represents significance by the whole model. Performed under conditions of standardized sodium intake as described in the text.

RAAS activation has been associated with liver fat in other populations. Individuals with primary hyperaldosteronism, a disease model of autonomous aldosterone production, had a higher prevalence of FLD than controls, which is predicted by the degree of aldosterone elevation [20]. In contrast, aldosterone synthase–deficient mice, a model of aldosterone deficiency, demonstrated reduced fasting glucose level, hepatic fat content, and liver triacylglycerol level compared with wild-type mice [21]. Aldosterone's actions are mediated by the mineralocorticoid receptor (MR). Although MR is typically found in epithelial tissues, evidence suggests that MR is also expressed in unique tissues and cell types, such as adipose tissue [22–24] and inflammatory cells [25]. As such, liver tissue could be affected by MR signaling via cross-talk with the adipose depot or through monocyte and macrophage activation.

A small open-label study evaluating the effect of MR antagonism in HIV reported increases in liver fat [26]. We recently performed a larger placebo-controlled randomized clinical trial evaluating MR antagonism in HIV and demonstrated no treatment effect on liver fat compared with placebo [11]. Because few data are available, our primary aim in the current study was to explore the fundamental relationship of aldosterone to liver fat in HIV. Our data suggest that future investigations of MR blockade on liver fat in sufficiently sized randomized clinical trials may still be useful to investigate among individuals with HIV and evidence of metabolic complications. A plausible alternative explanation for greater circulating aldosterone levels could be altered metabolism by the liver [27]. In this regard, we were careful to exclude those with a history of cirrhosis, and the degree to which transaminases were elevated was modest. Regardless, a strategy to block aldosterone, whether this is mechanistically related to hormone synthesis or clearance, may be an effective clinical treatment approach.

There are limitations to this study. A non-HIV group was not included. Some data suggest that PRA and aldosterone levels are elevated in population with obesity [28, 29], suggesting that total adiposity could be a confounding factor in the current study directed toward the population with HIV. We chose to study the population with HIV because our prior data from a rigorous physiologic study demonstrated that RAAS dysregulation was associated with excess visceral adiposity (reflecting a redistribution of total adiposity compared with other, more general indices of total adiposity, such as body mass index) and metabolic disease and was independently related to HIV serostatus [9]. Nonetheless, by using ^{1}H-MRS, the gold standard of noninvasive imaging techniques, to assess liver fat, we demonstrated a relationship between aldosterone and liver fat, particularly among individuals with HIV and MS. This finding has clinical relevance for a large subpopulation of individuals with HIV and metabolic dysregulation. Because this was meant to be a hypothesis-generating study, we did not exclude individuals with alcohol use and HCV, but rather controlled for these variables in regression modeling, further confirming the independent relationship between aldosterone and fatty liver in HIV. However, because this study was cross-sectional, causality cannot be inferred, and longitudinal studies designed specifically to control for traditional risk factors (*e.g.,* HCV and alcohol use) should evaluate whether increased aldosterone level contributes

to FLD progression or *vice versa* and whether those with elevated aldosterone levels are more prone to metabolic dysregulation and therefore are at higher risk for FLD.

In this study, we demonstrated a robust association between aldosterone and liver fat in HIV. Future studies are needed to understand the mechanisms and potential clinical consequences of this relationship with respect to the development of FLD and potential treatment strategies.

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