Antiretroviral Therapy Does Not Restore Brain Lipids During SIV Infection: Regional Analysis of Metabolic Homeostasis and Depletion

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ABSTRACT

Human immunodeficiency virus infection (HIV) continues to promote neurocognitive impairment, mood disorders, and brain atrophy even in the modern era of viral suppression. Brain lipids are vulnerable to HIV-associated energetic strain and contribute to HIV-associated neurologic dysfunction due to alterations in lipid breakdown and structural lipid composition. HIV neuropathology is region dependent, yet there has not been a comprehensive spatial evaluation of brain lipids during infection that may impact neurologic function. To address this gap, we evaluated brain lipid distribution using matrix laser desorption/ionization imaging mass spectrometry (MALDI-IMS) across four brain regions (parietal cortex, midbrain, thalamus, and temporal cortex), as well as kidney for a peripheral tissue control, in a virally suppressed simian immunodeficiency virus (SIV)-infected rhesus macaque. We assessed lipids indicative of fat breakdown (acylcarnitines) and critical structural lipids (phosphatidylcholines and phosphatidylethanolamines) across fatty acid chain lengths and degrees of unsaturation. Acylcarnitines with very long-chain, polyunsaturated fatty acids were more abundant across all brain regions than shorter chain, saturated or monounsaturated species. We observed two distinct brain lipid distribution patterns for acylcarnitines and phosphatidylcholines. However, no clear expression patterns emerged for phosphatidylethanolamines. Surprisingly, acylcarnitines were largely missing in kidney, while common phosphatidylcholines and phosphatidylethanolamines were absent in kidney. Overall, our study provides substantial evidence for persistent bioenergetic changes to the brain despite viral suppression, including region-dependent mobilization of acylcarnitines for oxidation and disparities in the presence of key phospholipids necessary for maintaining proper brain structure and function. These data indicate that region-specific interventions to restore proper lipid metabolism are essential for treating HIV neurologic disease in the era of ART.
INTRODUCTION

Human immunodeficiency virus (HIV) continues to pose a significant risk to neurologic health, despite increased life expectancy and quality of life afforded by antiretroviral therapy (ART)-mediated viral suppression (1). While the severity of neurologic disease has decreased in the ART era, virally suppressed people with HIV are living long enough to age with the virus and are more susceptible to neurocognitive impairment, mood disorders, and brain atrophy compared to uninfected individuals (2, 3). HIV induces considerable bioenergetic strain to the brain, which is a substantial driver of the neurologic dysfunction that persists despite viral suppression. Lipids are particularly vulnerable to this energetic strain, due to their high abundance and unique composition in the brain, which contributes substantially to neurologic decline when homeostasis of structural lipids are disrupted or the capacity for lipid utilization is decreased (2, 3). Therefore, it was not surprising when strong associations were found between the development of neurocognitive and mood disorders in people with HIV and disruptions in brain lipid metabolism (4–18). For example, post-mortem brain tissues from people with HIV that experienced cognitive decline were enriched in sphingomyelin containing very long-chain polyunsaturated fatty acids in parietal cortex, medial frontal gyrus, and cerebellum (18). In primary astrocyte cultures, brain cholesterol biosynthesis gene expression and brain cholesterol abundance were both decreased by the HIV viral protein, Tat (19). Further, Tat increased fatty oxidation gene expression in primary astrocytes (20). Another viral protein, gp120, had a similar effect in hippocampal neurons, where it increased sphingolipids containing very long-chain polyunsaturated fatty acids (18). These same very long-chain polyunsaturated fatty acid containing phospholipids were also enriched in the brain of transgenic rats that overexpressed regulatory and accessory HIV proteins (21, 22). These studies clearly demonstrate the contribution of lipid dysregulation to central nervous system dysfunction during HIV. However, previous in vivo and ex vivo studies focused exclusively on profiling total lipid abundance using brain homogenate, without consideration of the spatial differences inherent in the lipid profile.
This is a major limitation as it is well described that lipid distribution is not uniform across brain regions (23, 24), which likely occurs due to the region-specific expression of lipid breakdown genes and regional differences in structural lipid composition (25, 26). Similarly, region-dependent neuropathological hallmarks of HIV are well recognized to occur, where certain brain regions are preferentially vulnerable to HIV-induced damage and others remain spared (4–15). For example, areas of the cortex and the basal ganglia are more prone to white matter atrophy while areas of cortex and thalamus are prone to gray matter loss due to infection (4, 15). Despite strong evidence of region-dependent HIV neuropathology and sub-anatomic heterogeneity inherit in lipid metabolism, there have been no studies to date that characterized brain lipid distribution across multiple regions after infection.

Therefore, our goal was to characterize lipid distribution in brain utilizing a virally suppressed model reflective of the modern landscape of HIV treatment to evaluate the direct contribution of ART in restoring HIV-induced lipid alterations. We evaluated key lipids indicative of fatty acid breakdown and structural membrane lipid homeostasis using a virally suppressed SIV-infected rhesus macaque model using matrix assisted laser desorption/ionization imaging mass spectrometry (MALDI-IMS). Of importance, this model allows for the direct evaluation of the capacity for ART to restore HIV-induced brain lipid dysregulation in the absence of comorbid disease or dietary and activity differences, which is a substantial confounding factor when performing human studies. We also evaluated lipids in kidney to serve as a peripheral control with known HIV pathology as altered lipid utilization is associated with kidney injury during HIV (27). We first assessed acylcarnitines, fatty acid derivatives transiently generated during long-chain fatty acid β-oxidation (Fig. 1A) (23). We also characterized phosphatidylcholines (Fig. 1B) and phosphatidylethanolamines (Fig. 1C), prominent phospholipids that are integral in comprising membranes necessary for maintaining cellular composition, integrity, and function.
Species from these lipid classes contain fatty acids with a wide range of chain lengths and degrees of unsaturation that impact physical properties and signaling roles.

In this study, we determined that viral suppression afforded by ART was not sufficient to restore brain lipid deposition to levels anticipated in the absence of HIV/SIV. Further, we identified distinct lipid distribution patterns across diverse brain regions that varied according to fatty acid chain length and degree of unsaturation. Very long chain acylcarnitines were more abundant in midbrain, thalamus, and temporal cortex. Similar trends occurred for long-chain saturated and monounsaturated phosphatidylcholines, which had the greatest enrichment in the same three brain regions. Surprisingly, parietal cortex was depleted in both phosphatidylcholines and phosphatidylethanolamines. Unexpectedly, we identified prominent gaps in lipid enrichment across multiple lipid species in diverse brain regions, wherein lipids were completely depleted from the brain tissue and essentially undetectable. Of note, this did not occur throughout the entirety of the tissue section, and lipid enrichment often occurred in an area directly adjacent to the region unpredictably devoid of lipids. These findings demonstrate that lipid dysregulation continue to occur in people with HIV and suggest that altered abundance of these lipids critical to normal brain function contribute to HIV CNS persistence in current the ART era. Overall, this study demonstrates the need for targeted therapeutics to restore region-specific changes in lipid metabolism linked to neurologic decline in people living with HIV.
MATERIALS AND METHODS

Animal Ethics Statement All procedures were performed in accordance with the NIH’s Guide for the Care and Use for Laboratory Animals, the US Department of Agriculture Animal Welfare Act, and under the approval of the Johns Hopkins Medical School Animal Care and Use Committee. The rhesus macaque used for this study was housed in a cage that was appropriate for both size and weight in accordance with federal animal welfare regulation; 1 occupied a 9.0-ft² stainless-steel perk bars cage. This animal was included in other studies that included blood and cerebrospinal fluid (CSF) collections and ART injections performed between 8 and 12 a.m (28). In those studies, the macaques was anesthetized with ketamine hydrochloride to induce sedation while retaining physiological homeostasis for blood and CSF collections. The macaque was monitored to ensure there were no signs of pain or distress. Criteria for human euthanasia prior to planned endpoint if any of the following were observed: a) weight loss of greater than 15%, b) CD4+ T-lymphocytes count less than 5% baseline level, c) clinical signs of neurological disease, d) intractable diarrhea, and opportunistic infection.

SIV-infected Rhesus Macaque Model
We used a well-established SIV-infected rhesus macaque (Macaca mulatta) model optimal for studying brain pathology, immunological compromise, viral replication, and the impact of therapeutics on HIV outcome (28–43). This study was conducted using historical samples originally harvested from one juvenile 4-year-old, 6.1 kg SIV-infected, ART-treated rhesus macaque male (28). The macaque was inoculated intravenously using SIVmac251 as previously described (28, 29). Two weeks after SIV infection, the rhesus macaque was treated with daily intraperitoneal injections of combined ARTs consisting of TFV (20 mg/kg), DTG (25 mg/kg), and FTC (50 mg/kg). ARTs were donated from Gilead, ViiV Healthcare, Bristol-Meyers Squibb, Merck, Abbvie, Janssen, and Roche.
Quantitation of viral load in plasma and CSF

SIV was assessed in plasma and CSF as previously described \((28, 44–47)\). Briefly, RNA was isolated from 140 \(\mu\)L of plasma or CSF using the QIAamp Viral RNA Mini kit (Qiagen) in accordance with the manufacturer's protocol with the modification of on-column DNase digestion (RNase free DNase kit; Qiagen) using RQ1 RNase free DNase (Promega) in the enzyme mix. Quantification of SIV RNA was performed by reverse transcription polymerase chain reaction (qRT-PCR) using the following primers and probes: FWD-(SGAG21) 5′-GTCTGCCTCATCTGGTGCATTC-3′; REV-(SGAG22) 5′-CACTAGGTGTCTCTGACTATCTGTGTTTG-3′, probe (pSGAG23) 5′-(FAM)CTTCCTCAGTGTGTTTCACTTTCTCTTCTG-(BH1)-3′.

Necropsy and Harvest

At approximately 130 days post inoculation, the juvenile SIV-infected, ART-treated rhesus macaque male was sacrificed in accordance with federal guidelines and institutional policies \((28)\). Necropsy protocols were performed in accordance with previous work routinely performed within the Retrovirus Laboratory of the Department of Molecular and Comparative Pathobiology in accordance with the American Veterinary Medical Association guidelines for euthanasia of animals \((28, 29, 46, 48)\). Euthanasia occurred with an overdose of sodium pentobarbital while under ketamine sedation (15- to 20- mg/kg intramuscular injection) before perfusion with phosphate-buffered saline (PBS) (Gibco) to remove blood from tissues as described \((28, 29, 46, 48)\). After harvest, the whole brain was placed in cold 2.5% agarose, and 4mm coronal sections were obtained; fresh kidney was cut into four sections horizontally. Brain regions (parietal cortex, temporal cortex, thalamus, and midbrain) and kidney were fresh frozen using liquid nitrogen in optimum cutting temperature medium (OCT) (Sakura Finetek Inc, Torrance, CA) and stored at -80°C until cryosectioning \((29)\).
**Materials & Chemicals for MALDI**

MALDI matrix, a-cyano-4-hydroxycinnamic acid (CHCA) was obtained from MilliporeSigma (St. Louis, MO). The CHCA matrix is widely suitable for lipids (49). All solvents and other chemicals used were either reagent or high-performance liquid chromatography (HPLC) grade and purchased from Fisher Scientific (Hampton, NH), unless otherwise specified.

**Tissue Sections & Matrix Application**

OCT-embedded brain and kidney were sectioned to 10 µm onto Superfrost Plus Microscope Slides (Fisher Scientific, Pittsburgh, Pennsylvania) at -20°C using a Leica CM3050S cryostat (Leica Biosystems, Buffalo Grove, IL). Matrix [CHCA, 10 mg/ml in ACN:H_{2}O (50:50, v/v)] was applied to each slide using a TM-Sprayer (HTX Technologies, LLC, Chapel Hill, NC) at a flow rate of 100 mL/min. The TM-Sprayer was operated at an air pressure (N_{2}) of 10 psi, spray nozzle velocity of 1200 mm/min, track spacing at 2 mm, and spray nozzle temperature at 80°C. Sixteen passes (matrix deposition cycles) were employed for homogeneous deposition of matrix onto each slide.

**MALDI Imaging**

MALDI-MSI data were acquired using a LTQ Orbitrap XL (Thermo Fisher Scientific, Bremen, Germany), equipped with a Fourier transform mass spectrometer (FTMS) with the MALDI ion source fitted with a direct beam N_{2} laser (l 5 337.7 nm). Data acquisition was performed using the positive ion mode of the MS instrument at a mass range of m/z 100–1000 Da. Mass resolution of the FTMS analyzer was 60,000. CHCA matrix was used at 10 mg/ml for ionization of lipids and the laser energy of 7.8 mJ was used. The camera from the MALDI instrument was used to define regions of interest and generate MALDI position files. Ion images had a spatial resolution of 50 µm. Mass spectrometry data processing and analysis were done using Xcalibur
3.0 (ThermoFisher Scientific) and ion images were generated using ImageQuest 1.0.1 software (Thermo Fisher Scientific, San Jose, CA).

**Selection of Ion Images**

Representative lipid ion images were selected for known m/z values of multiple species for the following lipid classes: acylcarnitines, phosphatidylcholines, and phosphatidylethanolamines using Xcalibur 3.0 (ThermoFisher Scientific) (50). Ion values were determined for H⁺, K⁺, and Na⁺ adducts from each neutral lipid using LIPID MAPS Lipidomics Gateway mass (m/z) calculation tool for lipid classes. Representative images were selected upon determination of uniform expression patterns from all three adducts.
RESULTS

Virally suppressed, SIV-infected macaque model

We utilized a virally suppressed rhesus macaque model because of the experimental control it facilitates that limit confounding factors inherent with postmortem human brain (28, 51–54). One juvenile male rhesus macaque was infected with SIVmac251 for 14 days, followed by 90 days of viral suppression. We showed that this model achieved peak viremia at 14 days post inoculation and accomplished complete viral suppression by day 42 post inoculation (28 days after ART initiation) (28). Upon necropsy, perfusion was performed, and the brain and kidney were harvested for lipid analysis via MALDI-IMS (18).

Determination of lipid spatial distribution in a virally suppressed, SIV-infected macaque

Brain lipids are necessary for maintaining structure, propagating action potentials, use as signaling molecules, and as a source of energy (3, 23, 55). Numerous neurocognitive disorders, mood disorders, and neurovirulent diseases, including HIV, are linked to dysregulated lipid metabolism (17, 56–58). HIV viral proteins decrease brain cholesterol and sphingolipid abundance and the expression of enzymes necessary for their biosynthesis (18, 19). HIV viral proteins also disrupted membrane lipid homeostasis when overexpressed in rats (21). However, previous studies failed to examine a component critical to lipid abundance and function: region-specific metabolic profiles necessary to meet energetic demands, supply structural needs, and maintain metabolic homeostasis.

To address this gap in knowledge, we evaluated brain lipid distribution by MALDI-IMS from HIV-relevant brain regions (midbrain, parietal cortex, thalamus, temporal cortex) (4, 28). We also performed MALDI-IMS on kidney as a point of reference for lipid distribution in peripheral tissues with known HIV-relevance as renal disorders remain prevalent even among virally suppressed people living with HIV (59). To examine lipid regional distribution, 10-µm coronal slices of parietal cortex, thalamus, temporal cortex, midbrain, and kidney were imaged.
with a 50-µm resolution (Fig. 1A; Fig. 1B) using a m/z acquisition range of 100-1000 Da (Fig 1A; Fig. 1B). We focused on three main classes of lipids of key importance to lipid utilization (acylcarnitines) and cellular structure/homeostasis (phosphatidylcholines and phosphatidylethanolamines).

**Carnitines are abundant in brain, variably expressed in differing regions, and minimally present in kidney**

We first evaluated acylcarnitines, which have diverse chain lengths that range from two to over twenty carbons with zero to over six degrees of unsaturation (60). There was a striking pattern wherein very long-chain acylcarnitines were highly expressed in brain, but minimally present in kidney, which was consistent across all imaged brain regions from our SIV-infected, ART-treated rhesus macaque. This is evidenced by the very long chain acylcarnitine, C22:4, that was highly expressed in brain but marginally detected in kidney (Fig. 2). Interestingly, C18:3 was the only acylcarnitine species prominently observed in kidney (Fig. 2). Surprisingly, this acylcarnitine was minimally represented in brain.

We determined that, while prominently represented, very long-chain acylcarnitines had a high level of regional specificity across brain regions (Fig. 2). C20:0 and C22:3 were highly expressed and homogenously distributed in parietal cortex. Yet, they were sparsely abundant in midbrain, localizing only to the perimeter of the tissue (Fig. 2). These same acylcarnitines had a distinct localization in thalamus where they were almost uniformly present with incomplete, but prevalent, representation. Similar trends existed for C22:5, which was not present in parietal cortex, but was abundant in both midbrain and thalamus. Interestingly, C22:5 had a unique localization where it had the highest abundance in the innermost portion of tissue but was absent at the outermost edges. C20:1 showed a similar trend in regional specificity where it was homogenously present in parietal cortex but localized only to the central portions of midbrain and thalamus (Fig. 2).
Phosphatidylcholines of longer-chain lengths are more prominent in brain than in kidney

We next evaluated phosphatidylcholines, focusing on species with common medium-, long-, and very long-chain acyl groups (Fig. 3). Parietal cortex had the lowest enrichment of analyzed phosphatidylcholines, where those of all chain lengths were minimally detected (Fig. 3A). Conversely, phosphatidylcholines were highly expressed and uniformly present in midbrain. Thalamus and temporal cortex had high expression of medium-chain phosphatidylcholines, but lower abundance of the longer chain lengths. Interestingly, phosphatidylcholines had a comparable spatial distribution as some of the acylcarnitines in thalamus, wherein the greatest expression was restricted to the uniquely patterned internal zone (Fig. 3A; Fig. 2). The temporal cortex had the most unique and variable phosphatidylcholine localization patterns of all examined brain regions. PC(32:0) and PC(32:1) were abundant in a large, central portion of the temporal cortex, while PC(40:4) localized only to the top portion of the tissue. In contrast, PC(44:6) was primarily only at the bottom portion of the temporal cortex (Fig. 3A). Of interest, there was one notable exception that occurred uniformly across brain regions. Very-long chain phosphatidylcholines were lowly expressed in brain (Fig. 3A). No clear trend in phosphatidylcholines occurred in kidney, where some species were highly and uniformly expressed, while others were present to a lower extent (Fig. 3B).

Phosphatidylethanolamines are heterogeneously present in brain and kidney

Phosphatidylethanolamines were the last lipid of investigation. Overall, the spatial distributions of phosphatidylethanolamine species showed less consistent patterning than that which occurred for acylcarnitines and phosphatidylcholines in both brain and kidney (Fig. 4). Like phosphatidylcholines, smaller chain phosphatidylethanolamines were more prominent across all brain regions and in kidney, while larger phosphatidylethanolamines were less represented across both tissues (Fig. 4). Interestingly, phosphatidylethanolamines were not visible in parietal cortex (Fig. S1).
DISCUSSION

Our study is among the first to evaluate the spatial distribution of major lipid classes related to lipid utilization (acylcarnitines) and membrane phospholipid homeostasis (phosphatidylcholines and phosphatidylethanolamines) in brain and kidney by MALDI-IMS in a virally suppressed non-human primate model of HIV infection. We identified distinct lipid distribution patterns lipid distribution for acylcarnitines and phosphatidylcholines, but no well-defined patterns for phosphatidylethanolamines. Acylcarnitines comprised of long-chain polyunsaturated fatty acids were more abundant across all brain regions compared to kidney. However, the distribution pattern within each brain region differed by species, where some species were present along the peripheries of regions and others were restricted to the interior portions. Phosphatidylcholines containing common saturated and monounsaturated long-chain fatty acids were most abundant across brain regions and in kidney. Surprisingly, the parietal cortex was particularly vulnerable to lipid depletion and lacked both phosphatidylcholines and phosphatidylethanolamines. Our findings demonstrate distinct patterns of lipid distribution, including enrichment of very long chain polyunsaturated acylcarnitines across brain regions and limited phosphatidylcholines in parietal cortex. Further, they indicate that, despite viral suppression, ART is not sufficient to restore HIV-induced dysregulations in brain lipid abundance and composition, where some brain regions are at heightened risk for lipid depletion. This has profound implications for the persistence of HIV CNS disease in the era of ART, identifies a previously unrecognized marker of HIV neurologic dysfunction, and indicates that therapeutically targeting lipid metabolic processes in a region-specific manner will be integral in restoring appropriate neurologic function in people living with HIV. Our innovative approach in evaluating lipid distribution across multiple brain regions was imperative to understand HIV-induced changes in the brain lipidome and demonstrates the importance of spatially profiling lipids, rather than exclusively quantifying lipid concentrations in tissue homogenate (23, 25).
HIV viral proteins, which are expressed despite suppression of viral replication, greatly contribute to neurologic dysfunction. These viral proteins contribute to neurotoxicity manifesting as dendritic injuries (61–63), neurogenic deficits (64), and neuroendocrine defects (65). Neurotoxic HIV proteins include gp120, gp41, Tat, Nef, Rev, and Vpr (66, 67). Viral proteins alter brain bioenergetics in numerous ways, including Ca^{2+} dysregulation (68–70), modulated fatty acid oxidation (20), and electron transport chain complex abnormalities (71). They also disrupt metabolic coupling in the brain (20), elevate reactive oxygen species (72, 73), and exacerbate neuroinflammation (61, 74). Previous studies showed that HIV viral proteins increased fatty acid oxidation genes in vitro and disrupted the balance of membrane lipids (20, 21). Our findings in this study identify the high abundance of many acylcarnitines during viral suppression. This is of major significance because acylcarnitines are fatty acid oxidation intermediates, which is often contested to occur within brain (23). Our studies strongly implicate that the HIV-infected brain undergoes a considerable amount of fatty acid oxidation and is enriched in lipid species associated with countering HIV-associated neuroinflammation. Further, while phosphatidylcholines containing polyunsaturated very long-chain fatty acids are less prominent than phosphatidylcholines containing common saturated and unsaturated long-chain fatty acids, they are still well represented across most brain regions. Previous work determined brain tissue from people with HIV that experienced severe cognitive decline had increased very long-chain fatty acids in sphingolipids, a class of structural membrane lipids, despite viral suppression (18). It is possible that elevations in very long-chain, polyunsaturated fatty acids contribute to, or are indicative of, cognitive decline. Determining the mechanisms by which HIV/SIV and viral suppression impact these fatty acids is necessary to identify therapeutic strategies to restore changes to brain lipid homeostasis that contribute to HIV-associated cognitive decline.

Chronic inflammation is another major contributor to CNS dysfunction that persists in people living with HIV, even with successful ART (75–77). Inflammation in the periphery,
perivascular space, and within the brain parenchyma contribute to CNS dysfunction during HIV (78–82). Chronic inflammation promotes neuropathology, including accelerated brain aging, increased risk of developing psychiatric disorders, and cognitive decline (83). In the short term, the neuroimmune response is beneficial by promoting pathogen clearance, inducing angiogenesis, and increasing wound healing (84). In addition to canonical cytokines and chemokines, classes of specialized pro-resolving lipid mediators, including lipoxins, protectins, resolvins, and meresins, also promote neuroinflammation (85). Our study identified high abundance of the C22:5 fatty acid prominently found in specialized proresolving mediators known to alleviate neuroinflammation in specific areas of midbrain, thalamus, and temporal cortex (Fig. 2). This is important because previous studies determined that HIV viral proteins increased the abundance of C22:5 acid in total lipid, phosphatidylethanolamine, and triglycerides (21). Our findings indicate that lipid dysregulation in brain that contributes to persistent neuroimmune activation during HIV/SIV-infection, even with adequate viral suppression. It is likely that the high abundance of C22:5 exists to counter the chronic neuroinflammation that occurs after HIV infection.

ART may also play a direct role in lipid dysregulation in brain as its adverse impact on metabolism is well characterized. Systemically, it is well established that ART causes a mean weight gain of 3-7 kg within the first year of initiating treatment (86–89). Further, specific ART drugs or ART classes can have varying impacts on circulating lipid levels, blood pressure, and risks for developing metabolic syndromes, including diabetes mellitus. For example, classical non-nucleoside reverse transcriptase inhibitors and protease inhibitors were known for greatly increasing the likelihood of developing dyslipidemia (90). Even though newer antiretrovirals do not elevate circulating lipids to the same extent, tenofovir prodrugs, including tenofovir alafenamide, increase cholesterol species (91). This occurs despite the known impact of HIV on diminishing total cholesterol levels (86, 92–95). Conversely other ARTs, including raltegravir, dolutegravir, and bictegravir, have either a beneficial or minimal impact on the cholesterol profile.
after changing regimens from older protease inhibitors (86, 96). Hypertension, another disorder related to lipid dysregulation, is also more prevalent in individuals receiving ART. There is a 1.68-fold higher risk of developing hypertension in people living with HIV on ARTs in comparison to those not on ART (86, 97). However, it is not well characterized whether many of these changes to metabolic parameters occur due to the antiretrovirals directly, or because of the weight gain associated with many ARTs (86). With the clear role of ART in promoting lipodystrophy and metabolic dysfunction systemically, it is likely that similar perturbations also occur in brain.

Indices of kidney injury due to HIV are common as virally suppressed people living with HIV are aging. However, ART also poses a direct insult to kidney physiology. For example, tenofovir and its adenosine analog induced mitochondrial dysfunction and glycogen accumulation in human kidney cells that may contribute to nephrotoxicity (98). While less is understood about the impact ART on lipid metabolism in kidney, tenofovir decreased levels of low-density lipoprotein and increased the rate of change in urine-liver-type fatty acid binding protein/creatinine. These are known markers of elevated risk of hyperlipidemia and chronic kidney disease respectively (99), demonstrating a precedence for ART promoting lipid dysregulation in kidney. The contribution of ART to lipid perturbations in kidney has profound implications for people with HIV as lipid metabolism is highly relevant to kidney health, as it is the organ that preferentially oxidizes fatty acids used for energy. Further, lipid metabolism dysfunction is a well-established risk factor for kidney disease (27). Despite the clear importance and relevance of lipids in kidney function and normal physiology, we detected only a single acylcarnitine species in kidney (C18:3) (Fig. 2). It is possible that the acylcarnitine species we did not detect were exhausted to generate ATP, or that defective mitochondrial metabolism occurred that prevented other acylcarnitines from being produced (98). Our findings demonstrate that the kidney remains vulnerable to lipid dysfunction, even in the setting of ART-
mediated viral suppression, and HIV/SIV and ART may contribute to increased propensity for kidney injury due to lipid dysregulation.

HIV poses a major risk to brain lipid metabolic homeostasis that contributes to neurologic decline and brain pathology that continue to occur, despite viral suppression (4–18). Characterizing region-dependent changes in lipid profiles is critical for determining region-specific interventions that will restore proper brain function and metabolism disrupted due to HIV CNS disease. We utilized advancements in lipid imaging by MALDI-IMS combined with our virally suppressed SIV rhesus macaque model for an innovative approach to address this gap. Here, we demonstrated that, despite viral suppression, lipids indicative of increased fatty acid oxidation, dysregulated structural lipids, and activated neuroimmune response were prominent in a SIV-infected non-human primate. These metabolic changes occurred in a region-dependent manner, and it is imperative to fully understand HIV-induced changes to the brain lipidome that may contribute to HIV-associated neurologic decline in order to develop therapeutics that target spatially distinct areas of brain metabolic dysfunction.
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Images in figures were created using BioRender.

Conflict of Interest

The authors report no conflicts of interest.

Author Contributions

D.W.W. and C.J.W. performed experiments, A.M.G. and C.J.W. analyzed data, D.W.W. and C.J.W. were responsible for conceptualization of the study design, H.K.S. and N.N.B. developed the methodologies, C.J.W. and D.W.W. wrote the original draft with review and editing from all authors.
FIGURE LEGENDS

Figure 1. MALDI MS Imaging of SIV-infected, ART-treated macaque tissues using CHCA matrix and positive mode of the LTQ Orbitrap XL instrument. Representative full scan spectra and images of total ion counts of SIV-infected, ART-treated rhesus macaque A) brain regions: (parietal cortex, midbrain, thalamus, and temporal cortex) and B) kidney. Red signifies the highest intensity and blue the lowest of each m/z. C) Acylcarnitine (red), phosphatidylcholine (blue), and phosphatidylethanolamine (gray) structures. R = acyl (fatty chain) group.

Figure 2. Very long-chain acylcarnitines are prevalent in the brain, but not kidney, where spatial distribution varies by species composition. Representative MALDI-IMS of SIV-infected, ART-treated rhesus macaque brain regions [parietal cortex (P. ctx), midbrain, thalamus, and temporal cortex (T. ctx)] and kidney of A) very long-chain polyunsaturated AC C22:4 species, B) polyunsaturated C18:3 AC species, C) varying very long-chain un-, mono-, and polyunsaturated AC species demonstrating differences in spatial distribution. Red signifies the highest intensity and blue the lowest of each m/z.

Figure 3. Lower chain-length phosphatidylcholines are more abundant in both kidney and across brain regions. Representative MALDI-IMS of SIV-infected, ART-treated rhesus macaque brain regions of A) m/z values shared by representative PCs of similar chain length in A) the following brain regions: parietal cortex (P. ctx), thalamus, temporal cortex (T. ctx), and midbrain as well as B) in kidney. Red signifies the highest intensity and blue the lowest of each m/z.
Figure 4. Patterns in phosphatidylethanolamine spatial distribution across brain regions
or within a respective tissue are less clear. Representative MALDI-IMS of SIV-infected, ART-
treated rhesus macaque brain regions of A) m/z values shared by representative PEs of similar
chain length in A) the following brain regions: thalamus, midbrain, and temporal cortex (T. ctx)
as well as B) in kidney. Red signifies the highest abundance and blue the lowest of each m/z.

Figure S1. Phosphatidylethanolamines were not observed within parietal cortex.
MALDI-IMS of SIV-infected, ART-treated rhesus macaque parietal cortex of m/z values shared
by PEs in linked PowerPoint file. Red signifies the highest abundance and blue the lowest of
each m/z.
References


Figure 1.
Figure 2.
Figure 3.
Figure 4.