Influence of maternal human immunodeficiency virus (HIV) and antiretroviral (ARV) drugs on neonate neurometabolism.

by

Nosipho Busisiwe Masina

(MSNNOS002)

In fulfilment of the requirements for the degree Master of Science (Medicine) in Neuroscience.

Supervisor: Dr Martha Holmes

Co-supervisor: Dr Frances Robertson

Division of Biomedical Engineering

Department of Human Biology

Faculty of Health Sciences

University of Cape Town

February 2022

Declaration

I, *Nosipho Busisiwe Masina*, hereby declare that the work on which this dissertation is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

I empower the university to reproduce for the purpose of research either the whole or any portion of the contents in any manner whatsoever.

Signature: Signed by candidate

Date: 13 February 2022

Acknowledgements

To my supervisor, Dr. Martha Holmes, I thank you for your continuous guidance, support, and encouragement throughout my Master's degree. For the multiple consultations and always availing yourself whenever I needed your help. I am profoundly grateful. Without your supervision, this dissertation would not have been possible.

To my co-supervisor, Dr. Frances Robertson, though your supervision was brief, thank you for your input and advice.

Thank you to the National Research Foundation (NRF) of South Africa and the University of Cape Town Biomedical Engineering Division for providing funding for my Master's degree.

To my parents, thank you for your commitment and investment in my education. For supporting and encouraging me throughout this journey.

uMdali, thank you for placing me in spaces that my family and ancestors never had the opportunity to be in.

To my dear ancestors, those that come with light, *Ngiyabonga.*

Makwande, kukhanye, kube mhlophe.

Dedication

This one’s for me

Abstract

The human immunodeficiency virus (HIV) can be vertically transmitted from a woman living with HIV to her child during pregnancy, childbirth, and breastfeeding. Antiretroviral therapy (ART) prevents vertical transmission of HIV by providing prophylaxis to the fetus or infant and lowering the mother's viral load (VL). Due to the prevention of vertical HIV transmission, there is a decrease in infants acquiring HIV vertically. Correspondingly, there is an increasing population of HIV-exposed and uninfected (HEU) infants and children, with the current global estimate of HEU infants and children at around 15 million.

While results have been inconsistent, some studies show that HEU children have an increased risk of morbidity and mortality, as well as neurodevelopmental delays across language, motor and cognitive domains, when compared to their HIV-unexposed and uninfected (HUU) counterparts. Several factors have been associated with the higher morbidity and mortality rates exhibited by HEU children. These factors include perinatal and postnatal (during breastfeeding) exposure to maternal HIV and ART, a pro-inflammatory state in the mother, and a compromised maternal immune system.

This study aimed to see if the metabolic brain abnormalities seen in older HEU children could be detected in neonates. Based on previous results, we hypothesize lower ratios of glutamate (Glu) to total creatine (creatine plus phosphocholine) (Glu/Cr+PCr), N-acetyl-aspartate (NAA) to Cr+PCr (NAA/Cr+PCr), and choline-containing compounds phosphocholine plus glycerophosphocholine (GPC+PCh) to Cr+PCr (GPC+PCh/Cr+PCr) in the basal ganglia (BG) of HEU neonates compared to HUU neonates, as seen previously for absolute concentrations of these metabolites in 9-year-old HEU children. Furthermore, we hypothesized lower Glu/Cr+PCr and NAA/Cr+PCr ratios in the midfrontal gray matter (MFGM) of HEU neonates compared to HUU neonates, as previously observed for absolute concentrations of Glu and NAA in 11-year-old HEU children.

Using proton magnetic resonance spectroscopy (MRS), metabolite/Cr+PCr ratios were measured in the BG (83 HEU neonates and 45 HUU neonates) and MFGM (65 HEU neonates and 31 HUU neonates) of neonates at a mean gestational age (GA) equivalent of 41.56 weeks (range 39-45 weeks). Linear regression models were used to compare HIV and ART exposure group differences in metabolite/Cr+PCr ratios in HEU neonates and HUU neonates, as well as HEU neonates who have been exposed to ART since conception (pre-conception) and HEU neonates who have been exposed to ART after 5 weeks of GA to HUU neonates. NAA/Cr+PCr, GPC+PCh/Cr+PCr, Glu/Cr+PCr, and myo-inositol (Ins)/Cr+PCr were the metabolite/Cr+PCr ratios measured.

In the MFGM, no differences in metabolite/Cr+PCr ratios relative to HUU neonates were observed. However, in the BG, metabolite/Cr+PCr ratios were similar across neonates, with only one exception: GPC+PCh/Cr+PCr. When compared to HUU neonates, HEU neonates had lower mean GPC+PCh/Cr+PCr ratios (B = -0.03, p = **0.004**). In comparison to HUU neonates, both groups of HEU neonates exposed to ART since conception (pre-conception) (B = -0.02, p = **0.02**) and those exposed to ART after 5 weeks of GA (post-conception) (B = -0.03, p = **0.01**) had lower mean GPC+PCh/Cr+PCr ratios. NAA/Cr+PCr ratios were negatively correlated with maternal Harvard Trauma Scores during pregnancy across all neonates (r = -0.19, p = **0.04**). In addition, we also found significant positive correlations between NAA/Cr+PCr ratios and maternal cluster of differentiation (CD) 4^+ ($CD4^+$) T lymphocytes (T cell) count across all HEU neonates, independent of ART duration in pregnancy (r = 0.30, p = **0.01**).

The observed differences in GPC+PCh/Cr+PCr ratio between HEU and HUU neonates may be a consequence of maternal dietary choline deficiency (along with other micronutrients such as vitamin B_{12} and folate) and/or increased choline-consuming gut microbiota in mothers living with HIV. In HEU neonates, reductions in choline may lead to compensatory pathways that free other sources of choline for development. These mechanisms may prevent neuroinflammation as well as neuronal and oligodendrocyte (non-neuronal cell) loss and/or damage, explaining why only differences in GPC+PCh/Cr+PCr ratios were observed. Lastly, we reported NAA/Cr+PCr ratios in the BG that were influenced by maternal immune measures in HEU neonates and maternal trauma scores across all neonates. These associations suggest the neonate's BG is vulnerable to maternal health factors during fetal gestation.

Table of Contents

List of figures

List of tables

List of abbreviations

ACh:	Acetylcholine
ACTH:	Adrenocorticotropic hormone
AD	Alzheimer's disease
AGAT:	Arginine:glycine amidino transferase
Angpt2:	Angiopoietin 2
ANOVA:	Analysis of variance
ART:	Antiretroviral therapy
ARV:	Antiretroviral
BBB:	Blood-brain-barrier
BG:	Basal ganglia
CCR5+:	C-C chemokine receptor type 5
CD:	Cluster of differentiation
CDP:	Cytidine diphosphate
CI:	Confidence interval
CM:	Centimeter
CMV:	Cytomegalovirus
CNS:	Central nervous system
CRH:	Corticotropin-releasing hormone
Cr+PCr:	Creatine plus phosphocreatine
DNA:	Deoxyribonucleic acid
EBV:	Epstein-Barr virus
E.g.:	Example
FWHM:	Full width half maximum
G:	Grams
GALT:	Gut-associated lymphoid tissue
GA:	Gestational age
GAMT:	Guanidinoacetate methyltransferase
GI:	Gastrointestinal
Glu:	Glutamate
GPC+PCh:	Glycerolphoshocholine plus phosphocholine
gp120	Glycoprotein 120
HEU:	HIV-exposed and uninfected
HIV:	Human immunodeficiency virus
HMO:	Human milk oligosaccharides
HPA:	Hypothalamic-pituitary-adrenal
HTS:	Harvard Trauma Questionnaire for Posttraumatic Stress Symptoms
HUU:	HIV-unexposed and uninfected
IDO1:	Indoleamine 2,3-dioxygenase 1
IFNs:	Interferons
IL:	Interleukin
Ins:	Myo-Inositol
IQ:	Intelligence quotient
IP3:	Inositol-1,4,5-triphosphate
IUPM:	Infectious units per million
LCModel:	Linear Combination Model
LI:	Latent inhibition
LPS:	Lipopolysaccharide
MFGM:	Midfrontal gray and white matter
mg/day:	Milligrams per day

mL:	Milliliter
mRNA:	Messenger RNA
MRI:	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
N:	Number
NAA:	N-acetyl-aspartate
Nef:	Negative regulatory factor
NK:	Natural killer
NMDA:	N-methyl-D-aspartate
NO:	Nitric oxide
Oz:	Ounces
PCR:	Polymerase chain reaction
PEMT:	Phosphatidylethanolamine N-methyltransferase
PI:	Protease inhibitor
Poly I:C:	Polyriboinosinic– polyribocytidilic acid
PTSD:	Post-traumatic stress disorder
PPI:	Pre-pulse inhibition
PWM:	Peritrigonal white matter
rDNA:	Ribosomal deoxyribonucleic acid
RNA:	Ribonucleic acid
ROS:	Reactive oxygen species
sCD14:	Soluble CD14
SD:	Standard deviation
SIV:	Simian immunodeficiency virus
SMIT:	Sodium-myo-inositol cotransporters
SNR:	Signal-to-noise ratio
TCA:	Tricarboxylic acid
TE:	Echo time
TH:	T helper
TI:	Inversion time
TLR:	Toll-like receptor
TMA:	Trimethylamine
TMAO:	Trimethylamine N-oxide
TNF:	Tumor necrosis factor
TR:	Repetition time
Vegfc:	Vascular endothelial growth factor C
VL:	Viral load
α:	Alpha
β:	Beta
γ:	Gamma
µL:	Microliter
µM:	Micrometer
<:	Less than
>:	Greater than
+:	Plus
%:	Percentage
χ^2:	Chi-square

1. Introduction

The human immunodeficiency virus (HIV) can be vertically transmitted from a woman living with HIV to her child during pregnancy, childbirth, and breastfeeding. Antiretroviral therapy (ART) prevents vertical transmission of HIV by providing prophylaxis to the fetus or infant and lowering the mother's viral load (VL) (1, 2). The World Health Organization estimated the number of pregnant women living with HIV at 1.3 million, with 81% receiving ART (3). Due to the prevention of vertical HIV transmission, there is a decrease in infants acquiring HIV vertically. Correspondingly, there is an increasing population of HIV-exposed and uninfected (HEU) infants and children, with the current global estimate of infants and children who are HEU at around 15 million (4).

While findings have been inconsistent, some studies indicate that HEU infants and children are at an increased risk of impaired growth and development (5,6). Preterm birth and low birth weight are substantially more common in HEU infants than in HIV-unexposed and uninfected (HUU) infants (7). According to several studies, exposure to specific antiretroviral (ARV) drugs has been associated with adverse birth outcomes (8). Nucleoside reverse transcriptase inhibitors and non-nucleoside reverse transcriptase inhibitors (NNRTI) have both been linked to fetal malformations such as neural tube defects and mitochondrial dysfunction, respectively (9).

Although HEU children are not infected with HIV, evidence suggests that they have a higher frequency of hospitalizations and are at a higher risk of severe infections such as anemia, respiratory tract infections, tuberculosis, and other infections (10–12). Occasionally, severe infections may be followed by clinical evidence of immune impairment. Several factors have been associated with higher morbidity and mortality rates in HEU children. These factors include perinatal and postnatal (during breastfeeding) exposure to maternal HIV and ART, a pro-inflammatory state in the mother, and a compromised maternal immune system (11,13).

When compared to their HUU counterparts, the growing HEU population is at a higher risk of neurocognitive and neurodevelopmental delays (14–16). Several foundational processes occur during the *in utero* period and the first two years of life, including neurulation, neurogenesis, microglial entry, synaptogenesis, apoptosis, gliogenesis, and myelination (17). These processes are accompanied by changes in brain volume and cortical thickness (18,19). Perinatal and postnatal (during breastfeeding) exposure to maternal HIV and ART may have an impact on fetal brain development. Perturbations in coordinated brain development processes caused by these factors can disrupt early development and have long-term consequences (20,21).

Neurodevelopmental studies show that HEU children are more likely to develop neurological symptoms such as expressive language delay and subtle cognitive and motor impairment (14,22), whereas other studies found no neurodevelopmental differences between HEU and HUU children (23–26). In a longitudinal study, neurocognitive performance in HEU children was initially similar to that of their HUU peers but started to fall behind in the late pre-school to early school-age years (27). Some studies have found that HEU children in their pre-school and school-aged years have poor school performance, grade repetition, and a lower working memory profile (28–31). In other studies of school-aged HEU, researchers discovered subtle deficits in language-related cognitive performance when compared to control children (30–32).

Magnetic resonance imaging (MRI) complements cognitive testing by providing possible insight into abnormalities or developmental delays responsible for cognitive impairments. Proton magnetic resonance spectroscopy (MRS) is a non-invasive technique that measures concentrations of specific metabolites. These biochemicals provide information about brain health and development as well as pathological consequences. Altered metabolite concentrations can provide insight prior to or even in the absence of clinical features (33–36). The metabolites commonly studied include: total creatine (creatine plus phosphocreatine) (Cr+PCr) which reflects energy metabolism, N-acetyl-aspartate (NAA), a neuronal integrity marker, glutamate (Glu), a primary excitatory neurotransmitter, in addition, choline-containing compounds phosphocholine plus glycerophosphocholine (GPC+PCh) and myo-inositol (Ins). GPC+PCh reflects myelination and myelin breakdown as well as cell membrane turnover, whereas Ins is a glial marker (36,37).

1.1 Background

In order to understand and discuss the possible effect of HIV and ART exposure on regional brain metabolism in newborns, we present background on the influential factors. Fetal brain development is vulnerable to maternal health factors, in particular maternal infection, nutrition, and stress (38). While the neonate is less dependent on the health of the mother after birth, the infant brain may still be vulnerable to maternal health factors through breastfeeding. The composition of human milk is also susceptible to changes caused by maternal infection, nutrition, and stress (39–41).

1.1.2 The effects of HIV on the maternal immune system

1.1.2.1 The gut-associated lymphoid tissue as an anatomical reservoir for HIV

The gut-associated lymphoid tissue (GALT) is the largest immune organ in the human body. It contains a high concentration of C-C chemokine receptor type 5 ($CCR5^+$) expressing cluster of differentiation (CD) 4 $^+$ ($CD4^+$) T lymphocytes (T cells) that are susceptible to HIV. It also contains dendritic cells and macrophages, making it ideal for HIV acquisition as well as replication (42–44). It is one of the first tissues to become infected following an HIV infection. In non-human primate studies, severe and/or near-complete depletion of $CD4^+$ T cells was observed as early as 4 days after simian immunodeficiency virus (SIV) infection (45,46). The severe $CD4^+$ T cell depletion is caused by the viral infection's direct cytotoxic effect and cytotoxic T lymphocyte killing of the $CD4^+$ T-infected cells due to high viral replication rates (47). Furthermore, uninfected $CD4^+$ T cell bystander apoptosis contributes (47). Depletion of $CD4^+$ T cells is associated with increases in $CD8^+$ T cell percentages as well as pro-inflammatory chemokines and cytokine markers (48–50). The majority of $CD4^+$ T cells are found in the lymph nodes, particularly in the mucosal lymphoid tissues such as the gastrointestinal (GI) tract (51).

According to research, HIV replication persists in the GALT despite ART treatment (52,53). The GALT is an anatomical HIV reservoir. It is a latent reservoir, which means it contains long-lived memory $CD4^+$ T cells bearing replication-competent proviruses. When activated $CD4^+$ T cells are infected by HIV, viral ribonucleic acid (RNA) is reverse transcribed into viral deoxyribonucleic acid (DNA) (known as a provirus) and integrated into the host genome. During the reversion of activated $CD4^+$ T cells to a resting memory state, where HIV gene expression is limited, these cells are virologically quiescent and lack the ability to produce viral particles (54,55). This then generates a pool of long-lived memory $CD4^+$ T cells with HIV proviruses. These long-lived latent $CD4^+$ T cell reservoirs are unaffected by ART and unrecognised by the immune system. These cells can resume viral reproduction (known as viral rebound) upon reactivation if ART is discontinued, which is a major obstacle to HIV eradication (55,56). The case of the Mississippi baby suggested that very early ART initiation (as early as 30 hours of age) continued for 18 months (upon confirmed HIV infection at week 1 of age) reduced the latent reservoirs, which were adequate to permit a period of 27 months of virological control when ART was discontinued. However, virological rebound was observed at 45 months of age, illustrating how even treatment started 30 hours after birth was not able to prevent the establishment of a latent reservoir (57).

A study conducted by Perelson et al. examining the decay of the replication-competent latent reservoir identified the half-life of latent reservoirs to be approximately 44 months (58). Based on this,

it would take 73 years of ART alone to fully eradicate HIV from the body (59). The mechanisms of low but persistent levels of ongoing HIV replication explain how the virus persists despite ART, even in individuals on uninterrupted ART with undetectable plasma viral loads for 5 years (53,60). Initiation of ART during the acute phase of infection reduces the size of the latent reservoirs but does not eliminate them. Persaud et al. discovered that, irrespective of the early lopinavir/ritonavir-based ART initiation for continuous 96 weeks, replication-competent viruses were detected in a significant proportion of infants (60%). The median frequency of the replication-competent reservoir was 1.88 infectious units per million (IUPM) $CD4^+$ T cells at 24 weeks and 0.33 IUPM at 96 weeks (61). Clinical evidence suggests that HIV latent reservoirs are established early in infection (within days after infection) before ART can be initiated or the development of antiviral immunity (HIV-specific T cells and antibody responses) (62,63). It is noteworthy that, before an antiviral immune response develops, there are increased numbers of new viral particles produced, and viral plasma levels rise to more or less 100 million copies per milliliter (mL) (64). Apart from the fact that ARV drug concentrations are unable to fully suppress viral replication in lymphoid tissue compartments, Fletcher et al. discovered that drug penetration for commonly used ARV drugs was lower in mononuclear cells isolated from the lymph nodes, ileum, and rectum than in peripheral blood mononuclear cells (65). These factors enable the persistence of HIV replication as well as the development of drug mutations and resistance (66).

1.1.2.2 HIV and microbial translocation

Early loss of GALT $CD4^+$ T cells represents the loss of over 90% of lymphocytes within the body as compared with the peripheral blood, which contains only 2 to 5% of all lymphocytes (46,47). The biggest loss to the T cell population are the $CD4^+$ cell subsets, T helper (T_H) 17 cells (T_H17), which are responsible for the production of pro-inflammatory cytokines like interleukin (IL) 17 and 22. These pro-inflammatory cytokines (IL-17 and IL-22) are important for antimicrobial immunity (67) because they promote the recruitment of neutrophils to areas of bacterial infection and the production of antimicrobial peptides such as defensins (68–70). Furthermore, they are critical for immune function and epithelial integrity through the induced proliferation of enterocytes (71). The loss of T_H17 cells leads to microbial overgrowth and epithelial injury. This is evidenced by a loss of epithelial cells (enterocytes), a decreased expression of genes that promote epithelial repair, and a disruption of tight junctions between cells (72–76). With disease progression, compromised mucosal integrity allows commensal microbes and/or microbial products to translocate (microbial translocation) from the intestinal lumen into the lamina propria and eventually into the systemic circulation in the absence of overt bacteraemia (77). These microbial products may include lipopolysaccharide (LPS) (78), peptidoglycan (79), ribosomal DNA (rDNA) (80), lipoteichoic acid (81), unmethylated CpG-containing

DNA (82), and flagellin (78). Furthermore, microbial products have both local and systemic effects after passing through the liver due to their stimulation of innate immune cells (43,83).

LPS is found in the outer membrane of gram-negative bacteria. It is an example of the many microbial products that elicit potent pro-inflammatory cytokine responses. Others include nucleotide-binding oligomerization domains 1 and 2, along with Toll-like receptor (TLR) 4, TLR9, TLR6, TLR2 and TLR5. LPS correlates with measures of immune activation (77). LPS is recognized by TLR4 on immune cells such as macrophages, monocytes, and dendritic cells. Upon binding, a signalling cascade is activated, leading to the production of pro-inflammatory cytokines including IL-1 beta (β), IL-6, type I interferons (IFNs, including IFN-α and IFN-β), and tumor necrosis factor alpha (TNF-α) (77,84,85). The production of cytokines is highly essential for the host. However, excessive levels of systemic pro-inflammatory cytokines may contribute to immune activation, inflammation, and the apoptosis of immune cells. This can lead to an impaired immune system's ability to sustain normal levels of lymphocytes that are responsible for the production of homeostatic effector cytokines such as IL-17 and IL-22 (86,87). These cytokines are essential for the maintenance of epithelial barrier integrity in mucosal tissues.

Soluble CD14 (sCD14) is a marker of monocyte and macrophage activation that is positively correlated with LPS levels in individuals living with HIV (88,89). sCD14 can bind to LPS and deliver it to a variety of cell types for further LPS-induced activation (90). Microbial translocation is an essential factor driving inflammation and immune activation in individuals with chronic HIV infection. In an SIV-infected animal study, Kristoff et al. demonstrated that blocking microbial translocation drastically reduced T cell activation and the production of inflammatory cytokines (91). Additionally, as a direct result of T cell activation, intracellular nuclear factor kappa B levels are increased and enhance the transcription of integrated proviruses, resulting in the production of new viral particles (92). Appay et al. suggested a vicious cycle in which HIV replication promotes immune activation (example, T cell activation) and immune activation promotes HIV replication (43).

An upregulation of tryptophan catabolism further decreases the T_H17 cell population by altering the balance of T_H17 to regulatory T cells (Treg cells). Briefly, upon activation of macrophages and dendritic cells by LPS, indoleamine 2,3-dioxygenase 1 (IDO1) is produced by these cells, which is responsible for the conversion of tryptophan to tryptophan catabolites like kynurenine. A T cell shift from the T_H17 phenotype to Treg cells is observed when tryptophan catabolites simultaneously inhibit RAR-related orphan receptor C expression (responsible for inducing the production of IL-17 and IL-22 secreting T_H17 cells) and induce forkhead box protein P3 expression (responsible for Treg differentiation). An endless loop is established in which the loss of T_H17 cells results in the translocation of microbial products, such as LPS, that stimulate macrophages and dendritic cells and activate the IDO1 pathway,

which further results in the loss of T_H17 cells. In addition, these tryptophan catabolites result in decreased natural killer (NK) cell production of IL-17 and IL-22 (93,94). A positive correlation was reported between IDO1 activity and increased levels of sCD14 and LPS, increased $CD8^+$ T cell activation, and decreased $CD4^+$ T cell counts in individuals living with HIV in a study conducted by Favre et al. (95).

1.1.2.3 Decreased clearance of bacterial products

Microbial products normally colonize the colon, with strong mechanisms in place to prevent entry into the systemic circulation. These mechanisms include the physical barrier (mucus and epithelial cells) and innate immune factors (secreted immunoglobulin A, phagocytic activity, antigen-specific T cells, and intraepithelial lymphocytes) (96). Microbial products pass through the portal vein from the gut mucosa into the liver. The liver plays a critical role in the clearance of microbial products. In particular, either hepatocytes or Kupffer cells recognize microbial components through TLR, whose stimulation leads to the clearance of microbial products (mostly LPS) through the release of pro-inflammatory cytokines and oxygen free radicals (97). In animal studies, LPS intravenously administered is cleared from the circulation within a few minutes, with the majority traced in the liver (98,99). However, Kupffer cells can be infected by HIV, which may impair their ability to clear microbial products and mitigate the consequences of microbial translocation. According to studies of people living with HIV who have liver fibrosis and cirrhotic portal hypertension, HIV alone may be associated with blood diverted away from the Kupffer cells, impaired hepatic structure and function, and decreased synthesis of proteins involved in LPS clearance (94,100,101). This then allows microbial products access to the peripheral circulation. Furthermore, during HIV infection, the levels of endotoxin core antibodies (EndoCAb), which play an important role in the clearance of LPS, are reduced in the peripheral circulation (89).

1.1.2.4 Other sources of immune activation

HIV-mediated microbial translocation is not the only driving force of immune activation and inflammation. HIV replication contributes directly to T cell activation through antigenic stimulation by the virus. And stimulation of innate cells by viral RNA binding to TLR7 and TLR8 leads to a strong T cell response, with up to 50% of certain $CD8^+$ T subsets activated and persisting during the chronic infection phase (102). The percentage (%) of the $CD4^+$ T cell responses is low; this is likely due to their depletion or cell death by the virus (103,104). Viral RNA is also sensed by the pattern recognition receptors TLR7 and TLR8 as well as TLR9 on immune cells such as NK cells and plasmacytoid dendritic cells, respectively, which trigger the production of pro-inflammatory cytokines (105,106). Moreover,

HIV DNA present in the cytoplasm of the targeted cells has been reported to activate caspase-1, and the release of pro-inflammatory cytokines, including IL-1β, ensues (107). However, the degree of immune activation observed throughout the period of HIV infection through the stimulation of HIV antigens alone is not the consequence of the overall observed immune activation. In vitro research studies demonstrate that HIV gene products, like the envelope protein glycoprotein 120 (gp120) (108–110) and the accessory protein negative regulatory factor (Nef) (111–113), can induce direct activation of lymphocytes, monocytes, and macrophages in the absence of direct infection and give rise to pro-inflammatory cytokines and chemokines (114). HIV infection can indirectly cause immune activation and inflammation, mostly through co-infections. Influenza and common viruses such as Epstein-Barr virus (EBV) and Cytomegalovirus (CMV) contribute to high T cell activation. Studies have found that during primary HIV infection, $CD8^+$ T cells and $CD4^+$ T cells specific for CMV, EBV, and influenza show a significant degree of activation (115–118).

1.1.2.5 The effects of ART and HIV on the body

Though studies have been inconsistent, ART might temper the process of microbial translocation through the restoration of mucosal $CD4^+$ T cells when initiated early and during primary HIV infection. But its effect is incomplete and delayed when compared to $CD4^+$ T cells in the peripheral blood. As a result, microbial translocation continues long after peripheral $CD4^+$ T cell restoration. A study conducted by Guadalupe et al. revealed that one of two patients treated during primary HIV infection had incomplete restoration of the intestinal $CD4^+$ and $CD8^+$ T cells in GALT despite 5 years of ART (49). A study conducted by Mehandru et al. found similar outcomes, despite 1–7 years of ART, 70% of the patients in this study retained roughly 50% to 60% depletion of lamina propria lymphocytes (119). The effector sub-compartment of the GI mucosa of individuals living with HIV continues to severely lack $CD4^+$ T cells despite continuous ART treatment (120). The potential root cause of the lack of complete restoration and the ongoing loss of $CD4^+$ T cells during ART has not been fully determined, but several studies have suggested different mechanisms as contributing factors. Potential mechanisms include: 1) the lack of complete viral suppression in the mucosal tissue, 2) decreased expression of genes that are responsible for the repair and regeneration of the mucosal, 3) dysregulation of T cell homeostasis in intestinal mucosal lymphoid tissue, 4) the severe damage that occurs in GALT very early during the acute phase of HIV infection, and 5) the lack of homing of $CCR9^+\beta7^{hi}$ $CD4^+$ T cells (which includes the gut-homing of T_H17 cells) to the mucosal tissue (48,121–123). Several studies of ART-treated patients living with HIV found that plasma measures of microbial translocation such as LPS, sCD14, bacterial 16S rDNA, zonulin (a biomarker of intestinal permeability that decreases in response to epithelial barrier disruption), and intestinal fatty acid binding protein (gut epithelial cell apoptosis marker) were

associated with disease progression (89,124–126). Incomplete restoration of mucosal $CD4^+$ T cells may account for the ongoing microbial translocation in treated patients living with HIV. Despite the fact that the women in the studies were of childbearing age. These studies, however, predominately enrolled men with a median age of 44 years. Therefore, future research is needed to determine whether these findings can be replicated in women living with HIV during pregnancy.

1.1.3 Consequences of maternal immune activation

Compelling evidence demonstrates that an infection during pregnancy leads to direct physiological changes in the fetal environment, which can disrupt prenatal brain development. This can result in negative, long-lasting effects on the brain and behavioural development, accompanied by structural and functional brain abnormalities (127). During pregnancy, maternal bacterial and viral infections increase the risk of neurological disorders such as depression, schizophrenia (SZD), anxiety, learning disabilities, and autism spectrum disorders for the fetus (128–132). Although the mechanisms underlying this epidemiological relationship remain unclear, these disorders have been associated with maternal inflammation or maternal immune activation (133,134). Cytokines may be involved because infection during pregnancy activates the maternal immune system, generating cytokines. It has been demonstrated that increased levels of pro-inflammatory cytokines, including TNF-α , IL-6 and IL-1β are associated with the neurological disorders mentioned above (128–132).

Irving et al. carried out a study to determine whether maternal influenza virus infection resulted in viral infection and maternal antibodies being transmitted through the placenta. The study, however, found no evidence of transmission of the influenza virus or antibodies through the placenta (135). This was supported by other animal studies that found no viral RNA in the offspring's brains where the mother was infected with influenza (136,137). A study injected pregnant rats with 0.5 mg/kg of LPS, a frequently used experimental model to induce activation of the immune system in the absence of infection, to simulate an inflammatory response on gestation day 16 and to mimic bacterial infection. They discovered significantly higher levels of TNF-α, IL-6 and IL-1β in the placenta, as well as significantly higher levels of IL-6 in the amniotic fluid. In the same study, pregnant rats injected with a higher dose of LPS (2.5 mg/kg) on gestation day 16 had a small but significant decreased levels of TNF-α ($p = 0.035$) in the fetal brain, demonstrating how maternal infection had a direct impact on the cytokine levels of the developing fetal brain (138). In agreement with this phenomenon, Bell et al. found increased fetal brain TNF-α and IFN-γ (5 and 10 fold, respectively) and slightly decreased IL-10 following the cervical administration of LPS to the pregnant rat on gestation day 15 (139). A study conducted by Gilmore et al. administered the synthetic analogue of double-stranded RNA polyriboinosinic–polyribocytidilic acid (poly I:C), another experimental model for triggering immune

system responses in the absence of infection, to pregnant rats to model maternal viral infection. They discovered significantly increased TNF-α protein levels ($p = 0.0498$) in the placenta and a trending increase ($p = 0.0925$) in the amniotic fluid (140). Meyer et al. found that maternal viral-like (poly I:C) immune activation resulted in increased fetal brain IL-6 protein levels without increases in endogenously synthesized fetal IL-6 (141). These studies show that it is not the virus (or infection) that directly causes fetal brain infection, but rather the indirect release of cytokines by either the maternal or placental compartments that enter the fetal circulation and subsequently result in the alterations of the fetal brain's development.

The literature is inconsistent as to whether maternal inflammatory cytokines are capable of crossing the placenta. Some studies, such as those described above by Gilmore et al., Urakubo et al., and Bell et al., support this with observations of cytokines in the fetal brain, amniotic fluid, and the placenta (138,140). Pro-inflammatory cytokines IL-2 and IL-6 were detected in the amniotic fluid and fetal tissues, respectively, of pregnant dams injected directly with these pro-inflammatory cytokines (142,143). Interestingly, there was a bi-directional transfer of IL-6 in the healthy term human placental perfusion model, indicating the ability of cytokines to cross the placenta in a non-inflammatory condition (144). Other cytokines capable of crossing the placenta include granulocyte colony-stimulating factor and transforming growth factor-β 1 (145,146). Other in vitro studies, however, indicate that pro-inflammatory cytokines such as TNF-α, IL-β or IL-α are transferred minimally or not at all (144,147).

Pasca et al. and Hsiao et al. proposed that the placenta can produce its own array of cytokines (including IL-10, IL-8, IL-6, IL-2, and IL-1, IFNs and TNF-α) that may be a second source for entry into the fetal circulation and subsequently the fetal brain (148,149) . Cytokines are normal constituents of the placenta that are responsible for preserving the integrity of the structure and functions of the placenta (134). Cells of the placental barrier, including chorionic villi, uterine epithelial cells, and trophoblasts, express a variety of TLRs. After the initial maternal infection response, these cells may recruit additional cytokines (150–153). This is supported by a study conducted by Holmlund et al. that found strong immunoreactivity (analysed with immunohistochemistry) for TLR4 and TLR2 in the trophoblast that covers the peripheral chorionic villi of the placenta. This study also showed that upon TLR2 and TLR4 stimulation with zymosan and LPS from fresh placental explants or incubated with medium alone, they readily induced IL-6 and IL-8 cytokine production (150). Abraham et al. also found that human trophoblast cells acquired in the early pregnancy stages produced type 1 interferons upon TLR3 activation with LPS that have the ability to enter the fetal circulation (153).

Additionally, studies suggest that HIV infection contributes to the inflammatory response in the placenta. Placental mononuclear cells, which are susceptible to HIV infection, from women living with

HIV secreted higher levels of the pro-inflammatory cytokines IL-1β and IL-6 as compared to women living without HIV (154). Placental trophoblastic cells (epithelial cells of fetal origin) from women living with HIV expressed significantly higher levels of IL-1β, IL-6, and TNF-α compared to placental trophoblastic cells from women living without HIV in a study conducted by Lee et al (155). In another study conducted by Lee et al., placental trophoblasts from women living with HIV significantly produced higher median levels of IFN-β protein. Macrophages in the placenta of women living with HIV have increased production of granulocyte-macrophage colony stimulating factors and IL-2 and decreased production of IL-4 and IL-10 (156).

Moreover, the maternal–fetal interface is responsible for the shift of the maternal T_H response from a cell-mediated (T_H1) to a humoral (T_H2) dominant response to sustain pregnancy and prevent allograft rejection (157,158). The T_H2 response promotes successful fetal outcomes, whereas the T_H1 response induces adverse pregnancy outcomes such as spontaneous miscarriages (159). T_H1 is characterized by the recruitment and activation of NK cells and cytotoxic $CD8^+$ T cells, the production of pro-inflammatory cytokines such as TNF-α and IFN-γ, and the stimulation of TLRs. Increased production of T_H1- associated cytokines such as TNF-α has been linked to adverse pregnancy outcomes such as spontaneous miscarriages, pre-eclampsia and preterm birth (160) and can be prevented by T_H1 cytokine inhibitors or the administration of anti-inflammatory T_H2 cytokines such as IL-4 and IL-10 (161). In comparison to peripheral blood mononuclear cells from women with a history of normal pregnancies, which did not produce IFN-γ but did produce IL-10, women with unexplained recurrent miscarriages produced high levels of IFN-γ but not IL-4 or IL-10 (162). Increased IFN-γ levels were found to be higher in the plasma and placental explant cultures of women living with HIV on ART who were virologically suppressed (156,163). T_H2 cells either migrate to the maternal–fetal interface or can be derived from naïve T cells (converted to T_H2 cells). Infection or pathogens disrupt this shift, resulting in an increased T_H1 response and a decreased T_H2 response (with decreased IL-10-producing Tregs). In the case of CMV infection during pregnancy, T_H1 induction leads to spontaneous miscarriages. Similarly, studies have reported higher risks of miscarriages and stillbirths in women living with HIV (164). In a study conducted by Mikyas et al., serum levels of TNF-α were significantly increased at all time points throughout the three trimesters in pregnant women living with HIV, with 67% of women treated with ART in comparison to pregnant women living without HIV. However, this study was not clear on whether these women's plasma viral loads were suppressed (165).

It is still a matter of debate whether other pro- and anti-inflammatory cytokines and IFNs can also cross the placenta or whether it is only limited to certain cytokines. As much as the maternal and fetal blood circulations are separated, HEU infants have been reported to have phenotypically antigen-experienced $CD8^+$ and $CD4^+$ T cells (even though these are found in small numbers), with increased

expression of HLA-DR, CD69, CD45R0 and CD40 (166–169). The peripheral blood of HEU children has been reported to have $CD8^+$ immune responses to HIV proteins such as Nef, Gag, and Env (170). More importantly, HIV gene products like the envelope protein gp120 from the mother (108–110) can cross the placental barrier even in the absence of fetal infection (171,172). This can lead to the activation of lymphocytes, monocytes, and macrophages, subsequently producing pro-inflammatory cytokines and chemokines (114). Moreover, HIV-specific $CD8^+$ IFN-γ responses have been found in the peripheral blood of HEU infants (173). IL-8 and IL-1β produced by macrophages and dendritic cells were detected and significantly higher in the plasma of HEU infants born to mothers with undetectable VL compared to HUU infants (174). At 2 weeks of age, HEU infants' monocytes and classical dendritic cells produced more pro-inflammatory cytokines, such as TNF-α, IL-6 and IL-2, per cell in response to specific pathogen-associated molecular patterns (PAMPs) and TLRs stimulation. In response to specific TLR stimulation, HEU infants' plasmacytoid dendritic cells produced more TNF-α and IFN-α per cell within the first 6 months of life. However, all differences were normalized by 12 months of age in a study conducted by Reikie (175). A study reported decreased and increased levels of IL-4 and IFN-γ, respectively, in the cord blood mononuclear cells of HEU newborns born to mothers who were virologically unsuppressed (176). TNF-α and IFN-γ levels were significantly higher in the cord blood of HEU infants born to mothers with both detectable and undetectable plasma VL than in HUU infants (177). This study also found significantly lower anti-inflammatory IL-10 production in HEU infants born to mothers who had detectable plasma VL (177). Clerici et al. found significantly increased IL-7 in HEU infants at birth and later in life compared to HUU infants (169).

Once cytokines enter the fetal circulation, they may enter the fetal brain through the blood-brain barrier (BBB). The BBB (which consists of endothelial cells, pericytes, and astrocytes) is a physical barrier that separates the peripheral blood and the extracellular fluid from the brain. In order to prevent solutes in the peripheral circulation and the extracellular fluid from crossing into the extracellular fluid of the brain. The vascular BBB is made up of endothelial cell tight junctions and the blood-cerebrospinal fluid, which is situated at the choroid plexus (178). Cytokines are too large and hydrophobic to pass through the BBB by membrane diffusion. However, because the BBB has selective cytokine binding sites, systemic pro-inflammatory cytokines (such as IL-1β, IL-1α, IL-6 and TNF-α) can cross the BBB via saturable transport (179–182). Cytokines such as ILs are able to bind to the cerebral vascular endothelium and cause endothelial cell metabolism alterations (183). Furthermore, cytokines freely diffuse at circumventricular organs, where the BBB is permeable or leaky (184). In addition, cytokines have the ability to increase BBB permeability, thereby increasing the likelihood of cytokines and/or other inflammatory mediators' access to the brain (185,186). Moreover, microglia and astrocyte cells secrete cytokines on the brain side of the BBB that can induce neuroinflammation.

However, the BBB is more permeable in infancy, though tight junctions are present early in development. Lipid-insoluble molecules are more likely to enter the infant brain as compared to the developed brain of adults (187–189). Cytokines that cross the brain or are produced within the brain cause damage and/or loss to cells (astrocytes, neurons) and may ultimately affect neural function and behaviour.

The majority of research has focused on the maternal pro-inflammatory cytokine (as opposed to anti-inflammatory cytokine) response to infection during pregnancy and the increased risk for neurological disorders in offspring later in life. Less is known about the assumed roles of anti-inflammatory cytokines, prenatal exposure to maternal infection, and the emergence of brain and behavioural dysfunctions. Meyer et al. compared the neuropathological outcomes of wild-type mice born to dams that received a single injection of the viral mimic poly(I:C) (2 mg/kg) (prenatally exposed to pro-inflammatory cytokines) and transgenic mice constitutively overexpressing IL-10 (macIL-10tg) in macrophages. IL-10 is an anti-inflammatory cytokine that is released in order to limit the excess production of pro-inflammatory cytokines. The authors proposed that if postnatal brain abnormalities resulted from prenatal infection and/or inflammation exposure due to elevated levels of pro-inflammatory cytokines in the fetal brain, then higher levels of anti-inflammatory cytokines such as IL-10 at the maternal-fetal interface may be capable of reducing the effects of pro-inflammatory cytokine exposure during the perinatal period. Their study supported this prediction, finding increased levels of IL-10 during pregnancy prevented numerous behavioural abnormalities in adult offspring who were exposed to prenatal immune challenge by poly(I:C). They also discovered that elevated IL-10 levels during prenatal development were associated with abnormal behaviours in adult offspring even in the absence of prenatal exposure to pro-inflammation (190). This is similar to cytokine antagonists, cytokine inhibitors, and antibodies. TNF-α in LPS, for example, caused skeletal development retardation, fetal death, and intra-uterine growth restriction, demonstrating that injecting anti- TNF-α (pentoxifylline, an inhibitor of TNF-α synthesis or antibodies) significantly inhibited TNF-α production caused by LPS administration. This then resulted in a decreased overall number of fetal deaths and reversed LPS-induced skeletal development and fetal intrauterine growth restriction (191,192). Adult offspring born to mice that were injected with IL-6 during pregnancy had pre-pulse inhibition (PPI) and latent inhibition (LI) deficits. The elimination of IL-6 from the maternal immune response using genetic methods or co-administrated with an anti-IL-6 antibody, maternal poly(I:C) treatment during pregnancy, can prevent PPI and LI, as well as exploratory and social deficits in the adult offspring caused by poly(I:C). Similarly, when a poly(I:C) is given to a pregnant IL-6 knockout mouse, the poly(I:C) is incapable of causing behavioural deficits and transcriptional changes in the adult offspring (193).

All of this points to the importance of an equilibrium between pro- and anti-inflammatory cytokines in the prenatal period for healthy brain development. An equilibrium between pro- and anti-inflammatory cytokines in the fetal brain may undo the long-term consequences of both classes of cytokines on the brain and behavioural functions. Researchers hypothesized that a shift towards either an excess in pro- or anti-inflammatory cytokines during an infectious response would therefore result in behavioural dysfunctions in adult offspring (194). For instance, offspring of pregnant rodents with either an overexpression of the anti-inflammatory cytokine IL-10 (190) or the pro-inflammatory cytokine IL-6 (193) had similar behavioural and cognitive abnormalities. However, an overproduction of anti-inflammatory molecules may result in less pathology compared to an increase in pro-inflammatory cytokines. Meyer et al. pointed out extensive behavioural abnormalities following excessive pro-inflammatory cytokine exposure in the fetal brain, as opposed to the restricted pathological phenotype of specific behavioural abnormalities after increased anti-inflammatory molecules in the fetal brain (190).

1.1.3.1 The effect of cytokines on glia cells

Peripheral macrophages are rapid responders to cell homeostasis disruptions. Macrophage activation recruits immune cells and effector molecules for the restoration of tissue damage as well as the killing of pathogens. In the brain, the resident tissue macrophages, the microglia, have a similar function to peripheral macrophages (195,196). They regulate innate immunity and participate in adaptive immune responses in the brain. Moreover, these cells express numerous surface and nuclear receptors needed for the binding of complement proteins, major histocompatibility molecules, cytokines, chemokines, cell adhesion molecules, TLRs, and many others (197,198).

During embryogenesis and early postnatal development, microglia (derived from a subset of $CD45^{+}$ monocytic cells) infiltrate the brain, where they differentiate from the ameboid phenotype to a unique ramified morphology (resting state) (195,199). Ramified morphology is characterised by long branching processes that constantly survey the brain for any disruptions of homeostasis or immune challenge due to the presence of injured cells and toxins associated with infection (198,200). In this “resting” state, microglia have low expression and/or activation of surface markers as well as a small cell body. However, resting microglia are not dormant or inactive, and the term “activation” markers is somewhat of a misnomer. Nimmerjahn et al. found that these microglia cells are highly active in their presumed resting state, surveying their local microenvironment by continually extending and retracting their branching process (201).

Until challenged by a stimulus or immune pathogen, microglia are said to stay in a ramified state. Upon a range of stimuli associated with tissue injury or inflammation, the ramified microglia are transformed into amoeboid microglia. If the ramified processes are withdrawn, the central cell body region grows, and surface markers are upregulated to adopt full immune effector functions (202–204). In this state (amoeboid or activated), microglia are capable of responding to a range of stimuli associated with pathological changes by secreting various inflammatory molecules such as cytokines (such as IL-1β, IL-6 and TNFα) and free radicals such as reactive oxygen species (ROS) and nitric oxide (NO) (197). Increased numbers of activated microglia were found in the brains of adult offspring treated prenatally with LPS (205) and poly(I:C) (206–209). These cells are highly phagocytic, meaning they clear apoptotic cells and cellular debris in the brain. Released inflammatory mediators also recruit more microglia to the site of activation, which impairs the BBB permeability, thus allowing an influx of immune cells from the peripheral (most notably activated monocytes and lymphocytes). Furthermore, secreted cytokines and prostaglandins send signals to astrocytes to amplify their inflammatory response, resulting in the accumulation and production of NO and iNOS (isoform of NO synthase) (198,210). Thus, reduced microglial activation protects the brain from injuries induced by excessive inflammation (211,212).

1.1.3.2 Glial priming

After prenatal exposure to infection and/or inflammation, studies have suggested that the amoeboid microglia in the fetal brain do not change but remain in this state (for example, do not differentiate from the ameboid phenotype to a unique ramified morphology) into postnatal life (213). Moreover, microglia that differentiate from the ameboid phenotype to a unique ramified morphology can be activated by inflammatory cytokines that gain entrance to the fetal brain and become activated or amoeboid microglia. The way microglia respond to stimuli is critically dependent on whether they have recently been activated or ‘‘primed’’ previously. Primed microglia cells have a large cell body, short and thick processes, and are hyperresponsive to secondary stimuli, similar to activated or amoeboid cells. However, when compared to cells in an activated state, primed glia cells do not produce cytokines and other pro-inflammatory mediators. As a result, studies have hypothesized that in response to subsequent infection or injury in the periphery (example, “second hit”), primed microglia cause an over-production of pro-inflammatory cytokines (an exacerbated response) along with free radicles within the brain (214). The overproduction of pro-inflammatory cytokines can cause neuroinflammation as well as neuronal and oligodendrocytes (non-neuronal cells that can potentially be demyelinated) damage and/or loss (neurodegeneration) (178), which can lead to deficits in cognitive and motor function as well as other impairments (215,216).

Microglia priming was first described by Cunningham et al. in a model of prion disease. In this study, microglia from pre-symptomatic prion disease mice displayed dramatically increased production of the pro-inflammatory cytokine IL-1β, neutrophil infiltration, inducible NO synthase expression, and neuronal apoptosis in the brain in comparison to non-prion mice, ensuing central and systemic LPS challenges. This study provided direct evidence of an exacerbated inflammatory response to a "second hit" inflammatory challenge (215). Studies have shown how peripheral inflammation can stimulate central inflammatory cytokine messenger RNA (mRNA) and protein synthesis. For instance, an early study by Pitossi et al. demonstrated that following peripheral injection of LPS, the BBB was disrupted. As a result, IFN-γ, IL-6, TNF-α and IL-1β mRNAs were expressed in six brain regions (cerebellum, hypothalamus, brain stem, thalamus, striatum, hippocampus, and cortex). The onset of transcription ranged from 45 minutes (IL-1β, TNF-α) to 4 hours (IFN-γ), and the peak of mRNA accumulation occurred at various times depending on the cytokines and brain regions. Furthermore, LPS administration led to the accumulation of cytokine transcripts, observed at 45 minutes for IL-1β, TNF-α, and IFN-γ and at 2 hours for IL-6 in the pituitary gland (217).

The literature regarding priming has largely focused on microglia (218,219). Astrocytes (the major glial population of the brain) are essential for neuronal development, repair, and synapse formation during brain development (220). Astrocytes are components of the BBB and play a role in controlling molecules that are transported in and out of the brain (221). Astrocytes have also been shown to have altered function as a result of a previous inflammatory challenge. For example, given that astrocytes express receptors for IL-1β and TNF-α (among others) and are activated by these cytokines, with microglia being the primary producers of these factors. In a study conducted by Henn et al., astrocytes showed a primed response to subsequent TLR2 ligands after being cultured with a cytokine mix of IL-1β and TNF-α or with media conditioned by activated microglia (222).

1.1.3.3 The effects of cytokines on the hypothalamic-pituitary-adrenal (HPA) axis

Cytokines that gain access to the fetal central nervous system (CNS) as a result of a maternal infection may activate the HPA axis, which is responsible for generalized stress responses. Activation of the HPA axis by cytokines releases corticotropin-releasing hormone (CRH) and arginine vasopressin from the fetal hypothalamus. Subsequently, these neuropeptides trigger the release of the adrenocorticotropic hormone (ACTH) from the anterior pituitary gland. Consequently, ACTH prompts adrenal cortex glucocorticoids production and systemic releases. Released glucocorticoids promote the production of anti-inflammatory cytokines while stimulating the inhibition of pro-inflammatory cytokine production. Glucocorticoids are essential for normal brain development, particularly in initiating dendrite maturation and the remodelling of axons, as well as for cell survival (223). Excessive exposure

to glucocorticoids in the fetal brain may result in delayed maturation of neurons (ultimately affecting the structure of neurons and synapse formation between neurons), glia cells, and myelination. These changes may have long-lasting negative effects on the brain's development in utero as well as the function of the HPA axis in the adult offspring's (224,225). Additionally, the release of CRH and ACTH, which secrete adrenal-derived glucocorticoids such as dehydroepiandrosterone and cortisol, are important in neuroprotective mechanisms and in maintaining intrauterine homeostasis. However, excessive CRH and ACTH levels can be detrimental to fetal development (226).

Pro-inflammatory cytokines are often produced in a cascade because it takes one cytokine to stimulate its target cells in order to produce more cytokines. Pro-inflammatory cytokines that have gained access to the fetal circulation as a result of maternal infection can activate or stimulate specific antigen-presenting cells and non-antigen-specific cells. These can further produce and release a variety of pro-inflammatory cytokines, depending on the cell type (227). Pro-inflammatory cytokines are sensed by the afferent vagus nerve fibres, which convey signals to the brain. The vagus nerve afferents as well as the glomus cells in the paraganglia surrounding afferent vagus nerve endings express pro-inflammatory cytokine receptors (228). The efferent and afferent parts of the inflammatory reflex are integrated by the medullary nucleus tractus solitarius. Nucleus tractus solitarius neurons project to the dorsal motor nucleus of the vagus nerve, which houses the majority of the efferent vagal preganglionic fibres (229). Subsequently, the efferent arm of the inflammatory reflex, known as the ''cholinergic anti-inflammatory pathway'', releases acetylcholine (ACh). ACh is the primary vagus nerve neurotransmitter that inhibits pro-inflammatory cytokine production through the use of the α7 nicotinic ACh receptor subunit. Macrophages and other cytokine-producing cells (such as endothelial, dendritic, and T cells, monocytes, and other non-neuronal cells) expressing α7 nicotinic ACh receptors are inactivated when exposed to ACh (230,231). Borovikova et al. discovered that ACh significantly reduced the release of cytokines including TNF-α, IL-1β, IL-6, and IL-18 at the post-transcriptional stage but not the release of the anti-inflammatory cytokine IL-10 in endotoxin (LPS) activated human macrophage cultures (232). Systemic humoral anti-inflammatory responses are induced when information is relayed to the hypothalamus and the dorsal vagal complex. This happens when inflammatory mediators activate afferent vagus nerve fibres, causing an increase in ACTH release from the anterior pituitary glands (233).

1.1.3.4 Cytokines and neurotransmitters

There is strong evidence that the pro-inflammatory cytokine IL-2 can impact or regulate neurotransmitters in the developing brain, altering their concentration or availability. The pyramidal cell layers of the hippocampus and frontal cortex contain IL-2 receptors (234). Other cytokines,

including IL-1α, IL-6 and IFN-α failed to modulate ACh release (235). Animal studies have shown how IL-2 inhibits the release of ACh in certain parts of the brain only, like the hippocampus and frontal cortex. (235,236). Moreover, in an animal study, the release of ACh was also inhibited by IL-2 in the hippocampus (237). In old mice treated with IL-2, Nemni et al. observed neuronal cell loss and degenerative changes in the hippocampus, as well as impairment of mnesic functions (238). Inflammatory cytokines such as IL-6, IL-1β, and TNF-α, as well as IFN-γ and IFN-α, can activate the enzyme IDO1, which in turn converts tryptophan into kynurenine (239,240). Kynurenine preferentially converts quinolinic acid in microglia and kynurenic acid in astrocytes. Kynurenic acid inhibits the release of Glu and reduces the astrocytic expression of Glu transporters in astrocytes. In contrast, quinolinic acid promotes the release of Glu through the activation of astrocyte N-methyl-D-aspartate (NMDA) receptors (239,241,242). Also, quinolinic acid has been shown to promote glutamate (released from astrocytes) binding and activation of extra-synaptic NMDA receptors on neuronal cells. This has been shown to lead to decreased brain-derived neurotrophic factors, which are important in neurogenesis. Activation of extra-synaptic NMDA receptors causes loss of mitochondrial membrane potential and cell death (243). Moreover, quinolinic acid induces oxidative stress, which along with Glu release, contributes to the brain's excitotoxicity (244,245). Furthermore, increased glutamate release can potentially lead to increased glutamate excitotoxicity due to insufficient glutamate reuptake by astrocytes (246–248).

1.1.4 Impact of HIV and ART on gut microbiome

The foundations of the brain are put in place during the first 1000 days, which requires an adequate supply of nutrients, including key vitamins and minerals (249). Potential gut microbiome changes in mothers living with HIV and HEU infants may influence infant nutrition (250,251). For example, the enzymes necessary for vitamin B_{12} – which is involved in myelination – synthesis come from bacteria (252). As a result, the maternal microbiome may indirectly contribute to infant brain growth.

1.1.4.1 Maternal gut microbiome

There is mounting evidence that changes in gut immunity and integrity in HIV infection are associated with dysbiosis (an imbalance in the composition of the microbiota) in the gut microbial community. HIV disease disrupts the normal microbiota of the gut, and several studies have found altered gut microbiomes in people living with HIV who were treated or untreated with ART (253,254). Dysbiosis was found to be associated with plasma inflammatory soluble factors as well as increased gut and peripheral T cell activation (253,255). HIV-induced dysbiosis is typically characterized by a reduction in overall microbial gut diversity (256). Peptostreptococcus, Porphyromonas, Anaerococcus, and

Fusobacteria enrichment was found in people living with HIV who were not receiving ART, while Alistipes, Coprococcus, Roseburia, and Ruminococcus were depleted. Treated individuals living with HIV showed similar trends, but to a lesser extent; however, they were not statistically significant (257). Individuals living with HIV on ART had a high abundance of Prevotella and Succinivibrio, whereas HIV seronegative controls had a high abundance of Bacteroides and Faecalibacterium (258). Interestingly, gut resident bacteria in individuals on ART were able to catabolise tryptophan through the kynurenine pathway, which may contribute to the ongoing loss of IL-17 and IL-22 secreting T_H17 cell populations. This study also found an association between dysbiosis in individuals on ART and plasma concentrations of IL-6, both of which are established markers of disease progression (253). Gammaproteobacteria, Enterobacteriaceae, and Enterobacteriales from the Proteobacteria phylum were enriched in individuals living with HIV (receiving suppressive ART and with undetectable HIV loads) compared to controls. The researchers also discovered significant positive correlations between the relative abundances of Enterobacteriaceae and Enterobacteriales and the IL-1β, IFN-γ and sCD14 levels (259). According to a recent study, HIV status during pregnancy was associated with lower serum levels of inflammatory markers in mothers (GM-CSF and MMP-9) and HEU infants (IFN-γ, IL-1β). Furthermore, they also found associations between inflammatory markers (including GM-CSF, IFN-γ, IL-1β) in infants at 6 – 10 weeks and poorer motor function at 2 years (260).

In comparison with people living with HIV, HIV seronegative individuals had increased Bacteroidaceae (Bacteroides), Porphyromonadaceae (Parabacteroides), and Rikenellaceae (Alistipes) in a study conducted by Lozupone et al. (261). Interestingly, Bacteroides fragilis, which produces polysaccharide A, is responsible for the increased IL-10 and memory $CD4^+$ T cell populations. However, in this study, it was discovered to be lower in people living with HIV (262). In the same study, people living with HIV on long-term ART could still cluster with chronically untreated individuals living with HIV, indicating that ART does not restore the microbiota composition to an HIV-negative phenotype (262). Supporting evidence found that Bacteroides and Odoribacter genera, as well as an operational taxonomic unit (OTU) classified as Parabacteroides distasonis, significantly decreased with HIV infection, and within most individuals on ART, these low levels were sustained. Similarly, an OTU classified as Eubacterium biforme, the Prevotella genus, and the Paraprevotellaceae family significantly increased with HIV infection. However, in individuals living with HIV on ART, the abundances vary, though they do not decrease toward the low levels observed in individuals living without HIV (261).

ARV drugs themselves may contribute to dysbiosis. Long-term ART, in particular a protease inhibitor (PI)-based ART regimen, was associated with reduced Roseburia and F. *prausnitzii from the* Clostridiales order in a study conducted by Pinto-Cardoso et al. Both Roseburia and F. *prausnitzii* have the ability to produce butyrate, which is required for maintaining a healthy gut homeostasis. The PI-

based ART regimen is associated with significantly higher levels of sCD14 in plasma and decreased endothelial integrity when compared to both NNRTI-based ART and individuals living without HIV (263).

Notably, these are observational studies that do not provide evidence that HIV and/or ART cause bacterial changes; rather, they show associations. More research is needed to determine causation.

1.1.4.2 HEU infant gut microbiome

The maternal gut microbiome can be vertically transferred to the neonate via breastfeeding (264,265). The infant microbiome is primarily seeded via the feto-maternal interface (266). Jimenez et al. demonstrated that bacteria can be detected in the meconium of healthy neonates (267). Other studies detected the presence of bacteria in the umbilical cord blood, placenta, amniotic fluid, and fetal membranes (267–272). Collado et al. found shared features between microbiota compositions identified in the placenta, amniotic fluid and meconium, supporting the transfer of microbiota at the feto-maternal interface (266).

The bacterial changes involved in dysbiosis in mothers living with HIV may be passed on to uninfected infants. ART may contribute to the disruption of the gut microbiome of HEU children (273). A substantial proportion of bacteria found in an HEU infant's stool can be detected in the mother's breast milk (274). Dysbiosis in HEU infants may result in the disruption of the intestinal barrier, allowing gut microbiota composition or metabolic products to be translocated from the intestinal lumen to the systemic circulation (254,275) (which, as described above, can further lead to immune activation).

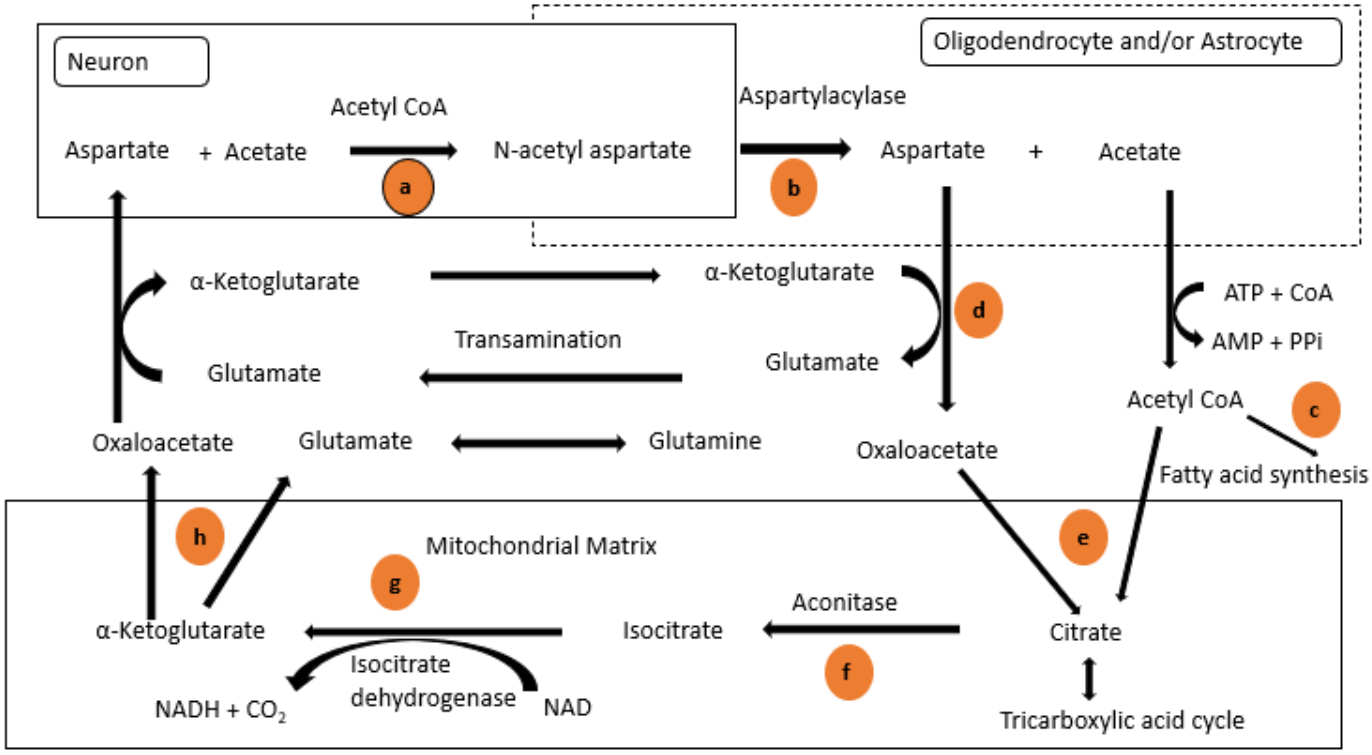
1.1.5 Metabolites analyzed

1.1.5.1 Neuronal integrity

N-acetyl-aspartate is a neuronal marker that is involved in energy production as well as a source of the acetate moiety required for lipid synthesis (276). It is synthesized in neurons from acetyl coenzyme-A (CoA) and aspartate (**Figure 1 (a)**) (277) and further catabolized to aspartate and acetate in oligodendrocytes (**Figure 1 (b)**) (278). During CNS development, acetate is converted to acetyl CoA for fatty acid production, which is essential for myelin sheath synthesis (**Figure 1 (c)**) (276).

Postnatal myelination shows reduced but significant integration of NAA-derived acetate into the brain lipids, suggesting NAA is involved in myelin lipid turnover (279,280) or the degradation of acetyl CoA to carbon dioxide through the tricarboxylic acid (TCA) cycle for energy production (281). Aspartate is converted to oxaloacetate through transamination (**Figure 1 (d)**). Oxaloacetate and acetyl CoA

combine to generate citrate, which is subsequently converted to isocitrate and then to α-ketoglutarate (**Figure 1 (e-g)**). Alpha-ketoglutarate (an essential component of energy and signaling metabolism) is either converted to oxaloacetate or glutamate (**Figure 1 (h)**) (278). Reduced NAA concentrations are associated with neuronal and axonal damage (282).



Adopted from (278)

Figure 1: Biosynthesis of N-acetyl-aspartate from aspartate and acetyl coenzyme A and its involvement in energy production and lipid synthesis.

Abbreviations: α-Ketoglutarate= alpha-Ketoglutarate; AMP= adenosine monophosphate; ATP=adenosine triphosphate; CoA= coenzyme-A; NAD+= nicotinamide adenine dinucleotide; NADH= nicotinamide adenine dinucleotide plus hydrogen; PPi= pyrophosphate. (d) It must be noted that the glutamate produced here is not the end point but rather an acceptor for the amino group removed from aspartate.

In the mammalian brain, Glu is the most abundant excitatory neurotransmitter. It is involved in many brain functions, such as memory and learning, sensory and motor processing, cognition, and homeostasis (283–287). It is produced from glutamine using the enzyme glutaminase within neurons (**Figure 2 (a)**) and then stored in synaptic vesicles. It is then released into the synaptic cleft from the

presynaptic neuron terminal, where it then binds to postsynaptic neuron receptors for synaptic transmission (**Figure 2 (b)**). Excess extracellular Glu is taken up by astrocytes (**Figure 2 (c)**) (288,289).

Within astrocytes, Glu is metabolically stored via its conversion to glutamine (**Figure 2(d)**) (290). Then it is released from the astrocytes into the extracellular fluid, where it is taken up by neurons to produce glutamate (**Figure 2 (e)**). This mechanism demonstrates the recycling of Glu through the glutamate-glutamine cycle. Glu is alternately synthesized de novo from α-ketoglutarate in the TCA cycle (**Figure 3**) (291,292). Excess Glu generated by pathogenic processes induces cellular injury and/or death in neurons and oligodendrocytes via glutamate-receptor-mediated excitotoxicity pathways (293).

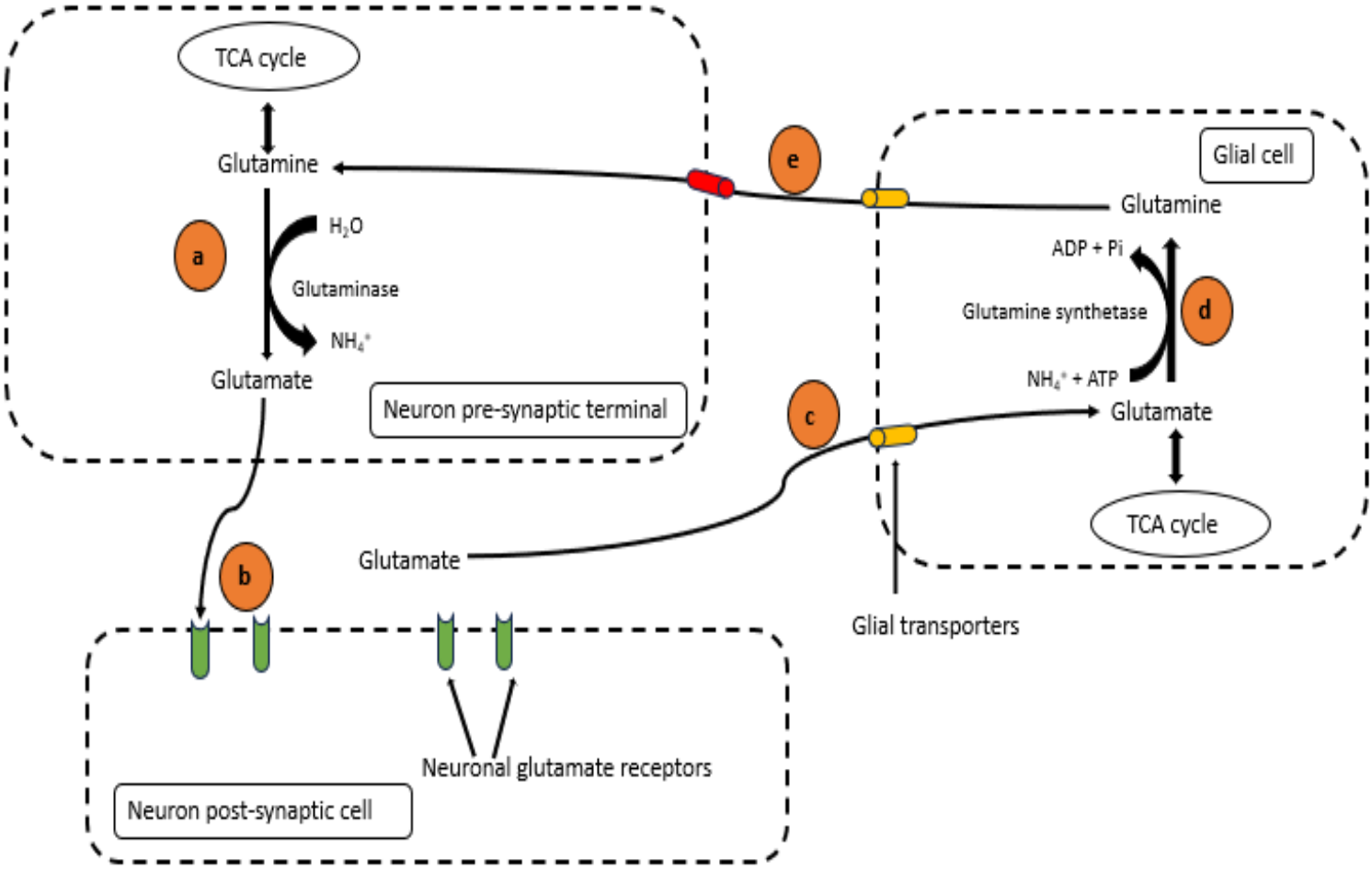


Figure 2: Schematic representations outlining the glutamate-glutamine cycle in a glutamatergic synapse.

For synaptic transmission, glutamate produced from pre-synaptic neurons binds to post-synaptic neuron receptors. Excess extracellular glutamate is taken up into astrocytes, where it is amidated to glutamine by glutamine synthetase using free ammonia and returned to the neurons. The glutaminase reaction regenerates the glutamate and produces ammonia. Glutamate might, to some extent, be metabolized within the TCA cycle of both neurons and astrocytes.
Abbreviations: ADP= adenosine diphosphate; ATP= adenosine triphosphate; H_2O= dihydrogen monoxide; NH_4^+=ammonium; Pi= phosphate; TCA= tricarboxylic acid.

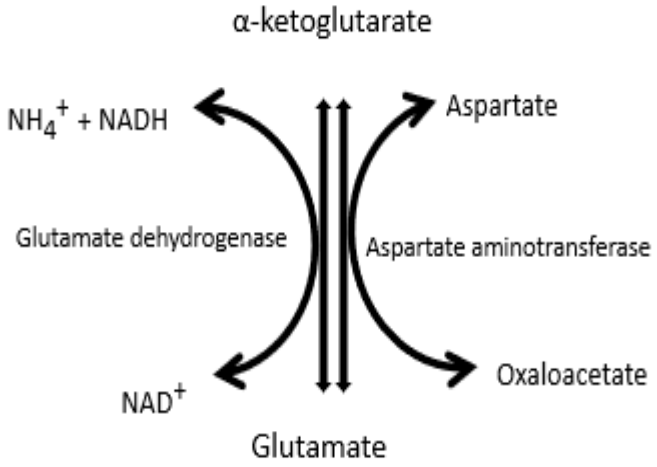


Figure 3: Biosynthesis of glutamate from α-ketoglutarate in the tricarboxylic acid cycle.

Abbreviations: NAD^+= nicotinamide adenine dinucleotide; NADH= nicotinamide adenine dinucleotide plus hydrogen; NH_4^+= ammonium.

1.1.5.2 Energy metabolism

Creatine plays an important role in adenosine triphosphate regeneration through the creatine/phosphocreatine/creatine kinase system (**Figure 4**) (294,295). It is either acquired through dietary sources or endogenously synthesized by the liver, kidney, pancreas, and brain from arginine and glycine (**Figure 5**). Creatine acquired through dietary intake enters the circulatory system and combines with peripherally synthesized creatine for delivery to high-energy demanding cells in the brain. It enters the brain through the microcapillary endothelial cells of the BBB, which contain creatine transporters. However, the astrocytes surrounding these microcapillaries do not contain creatine transporters (296,297). As a result, reduced creatine is taken up by the brain, and the brain is dependent on synthesizing its own creatine (298). Although arginine:glycine amidino transferase (AGAT) and guanidinoacetate methyltransferase (GAMT) are expressed in neurons, oligodendrocytes, and astrocytes, they are rarely co-expressed in brain areas such as the cortex and basal ganglia. As a result, GAA must be transferred from AGAT-expressing cells to GAMT-expressing cells in order to complete the creatine synthesis pathway (299).

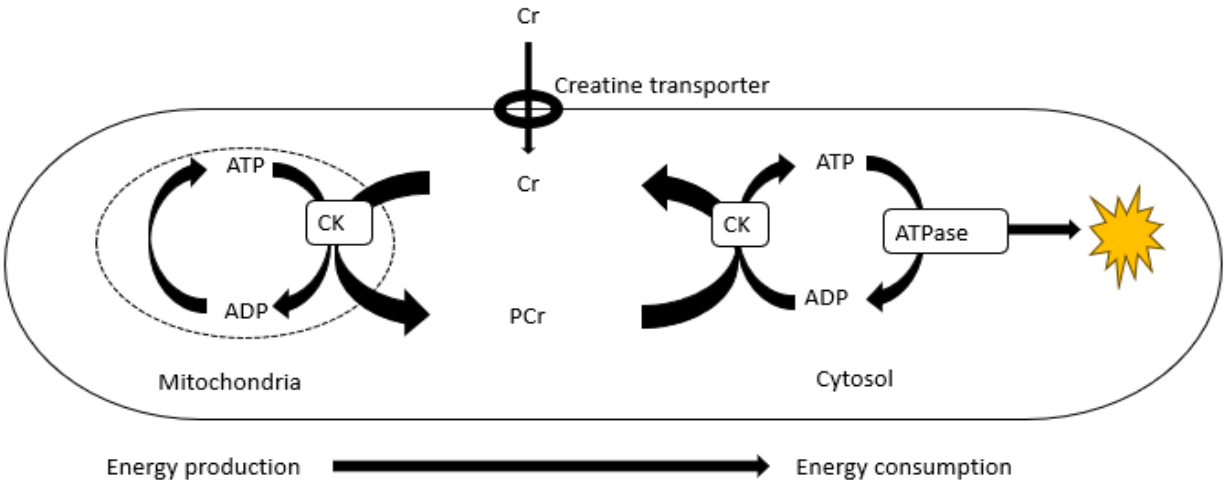


Figure 4: Systematic representation of the creatine/phosphocreatine/creatine kinase system in cells.

Creatine is taken up into cells by creatine transporters and transformed to the high-energy compound phosphocreatine by mitochondrial creatine kinase before leaving the mitochondria for local ATP consumption.
Abbreviations: ADP= adenosine diphosphate; ATP= adenosine triphosphate; ATPase= adenosine triphosphatase; CK= creatine kinase; Cr= creatine; PCr= phosphocreatine.

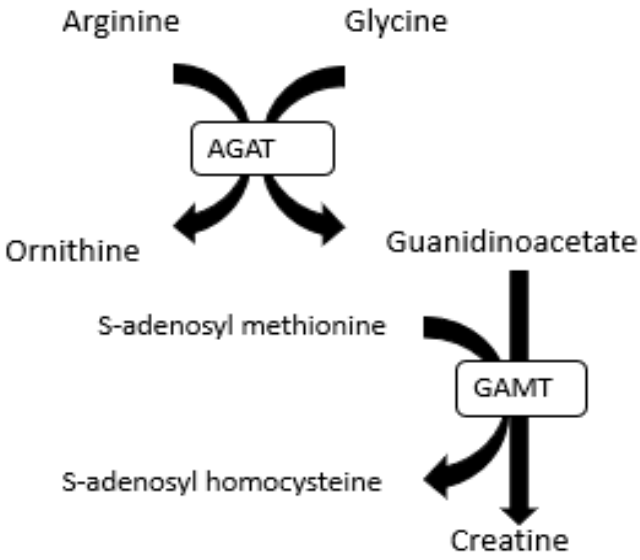


Figure 5: Creatine synthesis in a two-step enzymatic pathway involving arginine:glycine amidino transferase and guanidinoacetate methyltransferase.

Abbreviations: AGAT= arginine:glycine amidino transferase; GAMT= guanidinoacetate methyltransferase.

1.1.5.3 Membrane integrity

Choline is a precursor for phospholipids such as phosphatidylcholine and sphingomyelin. These choline-containing phospholipids are essential for the structural components of cellular membranes and the myelination of nerve cells, respectively (300,301). Phosphatidylcholine accounts for approximately 95% of the total choline in tissues. The liver and, to a lesser extent, the brain synthesize phosphatidylcholine from phosphatidylethanolamine through the phosphatidylethanolamine N-methyltransferase (PEMT) pathway (**Figure 6**) or from pre-existing choline through the cytidine diphosphate (CDP)-choline pathway (**Figure 7**) (301,302). Sphingomyelin, which is synthesized from phosphatidylcholine (**Figure 8**), is a constituent of the myelin sheath that covers the axons of nerve cells and enables efficient nerve signal transmission (303). Furthermore, choline is also a precursor for the neurotransmitter ACh (**Figure 9**), which plays an important role in cholinergic signaling within the brain (304).

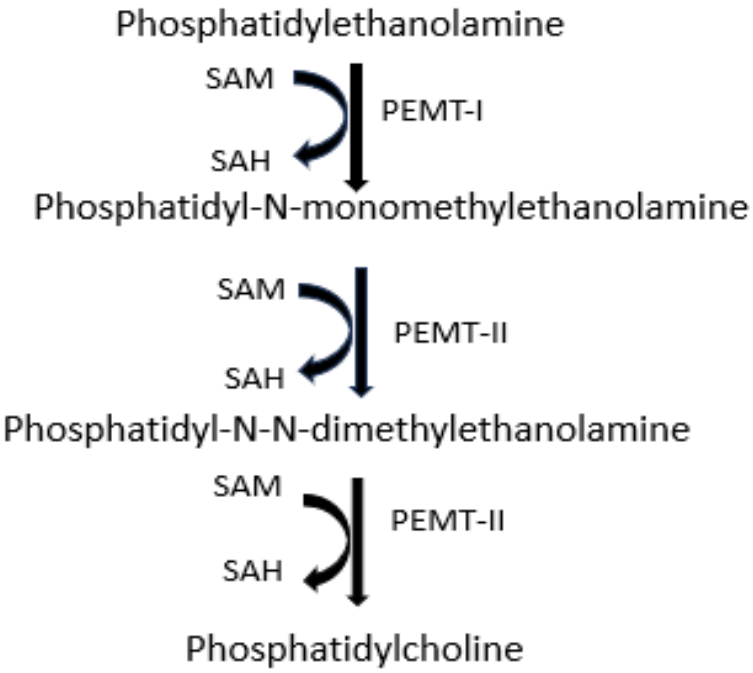


Figure 6: Biosynthesis of phosphatidylcholine from phosphatidylethanolamine through the PEMT pathway.

Abbreviations: PEMT-I= phosphatidylethanolamine-N-methyltransferase; PEMT-II= phosphatidyl-N-methylethanolamine-N-methyltransferase; SAM= S-adenosylmethionine; SAH= S-adenosylhomocysteine.

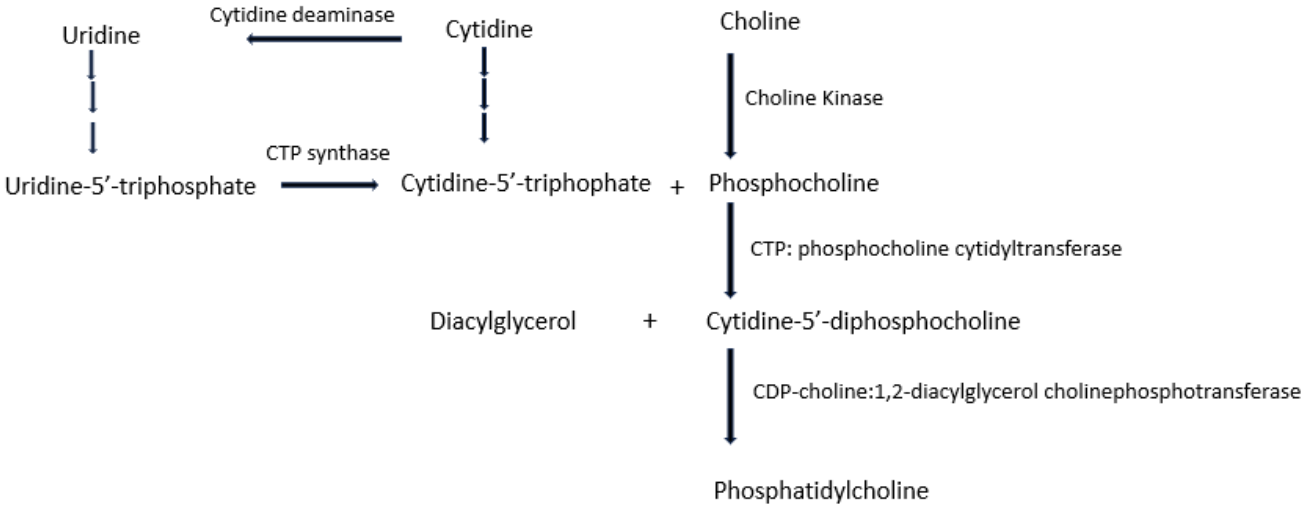


Figure 7: Biosynthesis of phosphatidylcholine from preexisting choline through the CDP-choline cycle.

Abbreviations: CTP= cytidine-5'-triphosphate.

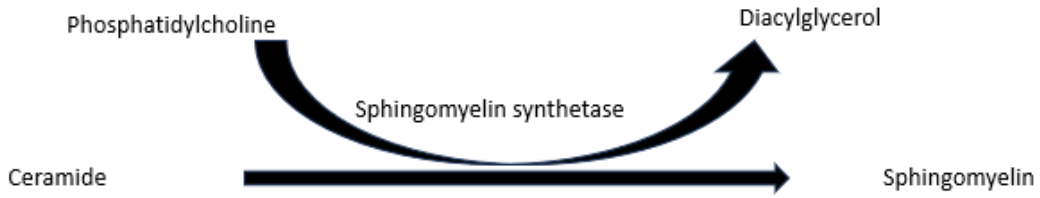


Figure 8: Biosynthesis of sphingomyelin from phosphatidylcholine and ceramide.

Figure 9: Acetylcholine biosynthesis from choline and acetyl coenzyme-A.

When choline is not endogenously synthesized by the liver, it is acquired through dietary intake as a free base or as a constituent of phospholipid molecules (for example, sphingomyelin, phosphatidylcholine, glycerophosphocholine, and phosphocholine, which can be broken down to liberate choline) from foods such as eggs, liver, wheat germ, and fish (305). In an irreversible reaction within the liver, much of the dietary free choline is oxidized to betaine, lowering choline bioavailability (**Figure 10 (a)**) (306–308). Betaine is essential in the remethylation of homocysteine to methionine, which is then converted to S-adenosylmethionine (**Figure 10 (b)**) (309). S-adenosylmethionine is a major methyl donor for methylation pathways such as DNA methylation and phosphatidylethanolamine methylation (**Figure 10 (c)**), which are responsible for regulating gene expression and phosphatidylcholine synthesis, respectively (309–312). Regardless of the source, choline is released into the systemic circulation and is transported to the brain for further metabolism (313).

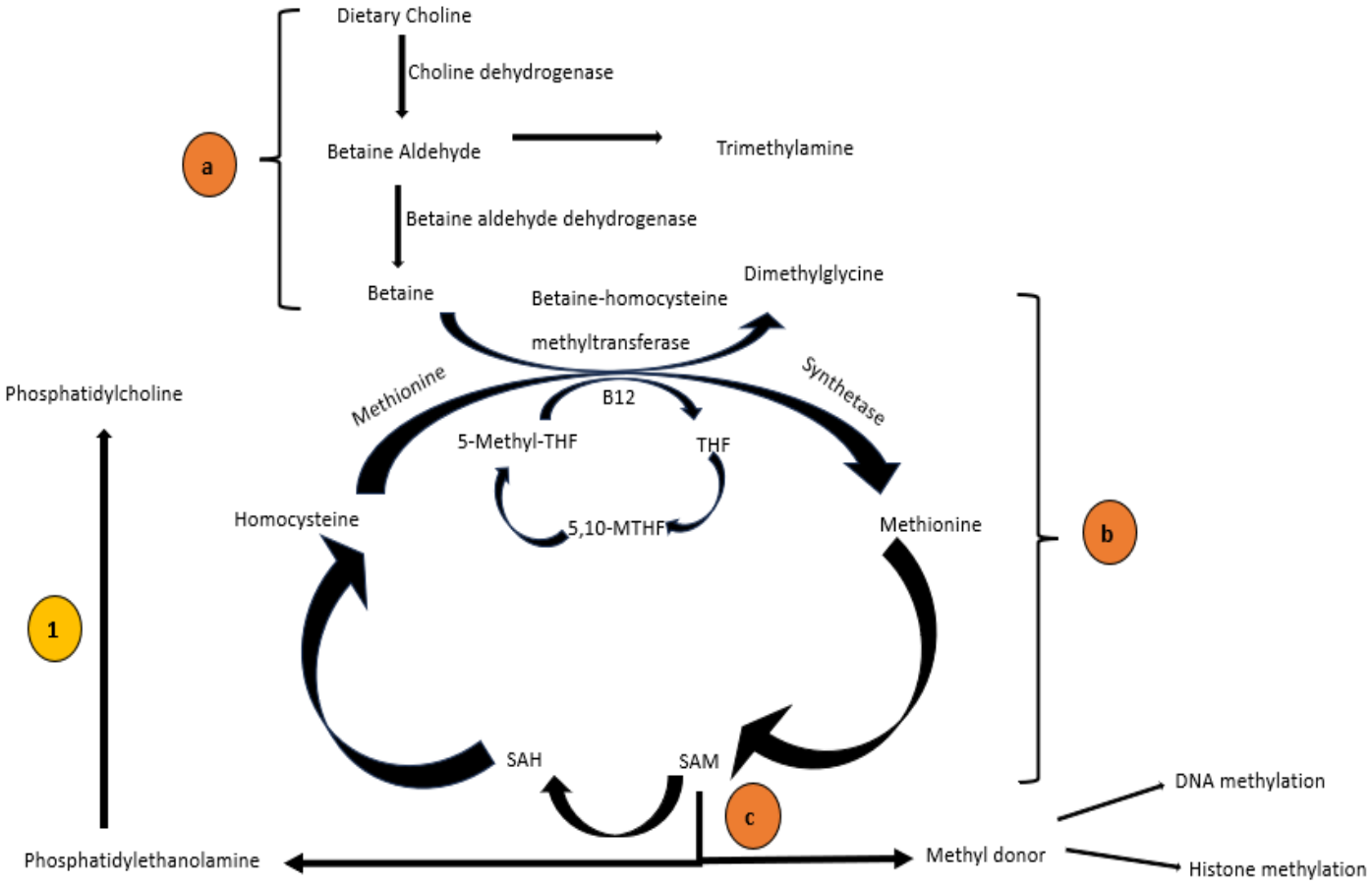


Figure 10: Metabolism of choline to betaine, methionine and, S-adenosylmethionine.

(1) See figure 6 for sequential methylation of phosphatidylethanolamine to phosphatidylcholine.
Abbreviations: 5,10-MTHF= 5,10-methylene-tetrahydrofolate; 5-methyl-THF= 5-methyl-tetrahydrofolate; B12= vitamin B_{12}; SAH= S-adenosylhomocysteine; SAM= S-adenosylmethionine; THF= tetrahydrofolate.

Numerous studies have found increased choline levels in the brain following choline administration, demonstrating how the brain efficiently extracts choline from the plasma. Babb et al. observed an increase in GPC+PCh/Cr+PCr in the left putamen of participants using MRS after ingestion of capsules of choline bitartrate (314). Furthermore, Stoll et al. discovered significant and remarkably increased GPC+PCh/Cr+PCr resonance in the subjects' brains after a single oral dose of free choline (in capsules of choline bitartrate) (315).

Maternal choline availability during pregnancy is critical for fetal brain development processes such as neurogenesis, neural connectivity, and neuroplasticity, all of which are associated with learning, cognitive, and memory functions throughout the lifespan (316). At 16 weeks of gestation, there was a significant positive association between maternal plasma free choline and its derivative betaine and infant cognitive developmental scores at 18 months of age. At 16 weeks of gestation, there was a strong trend between maternal plasma free choline and its derivative betaine and infant gross motor development at 18 months of age (317).

Choline is synthesized endogenously in small quantities, so it is made available to the fetus through the placenta during maternal pregnancy, and maternal breast milk is a rich source of choline for the developing neonate after birth (318,319). Choline is delivered to the fetus and/or infant through processes that deplete both maternal plasma choline (320) and choline tissue stores (**Figure 11 (a-c)**) (321,322).

Furthermore, gut microbes may influence the amount of choline delivered to the fetus and/or infant by the mother (323). According to research, trimethylamine (TMA) can be produced by certain gut microbiota. More importantly, these choline-consuming gut microbes compete with the host for choline to produce TMA. TMA, produced in the intestinal lumen, is then absorbed into the blood circulation and further metabolized in the liver to trimethylamine N-oxide (TMAO) (324,325). Ultimately, TMAO is distributed throughout the body, where it acts as an osmolyte (326).

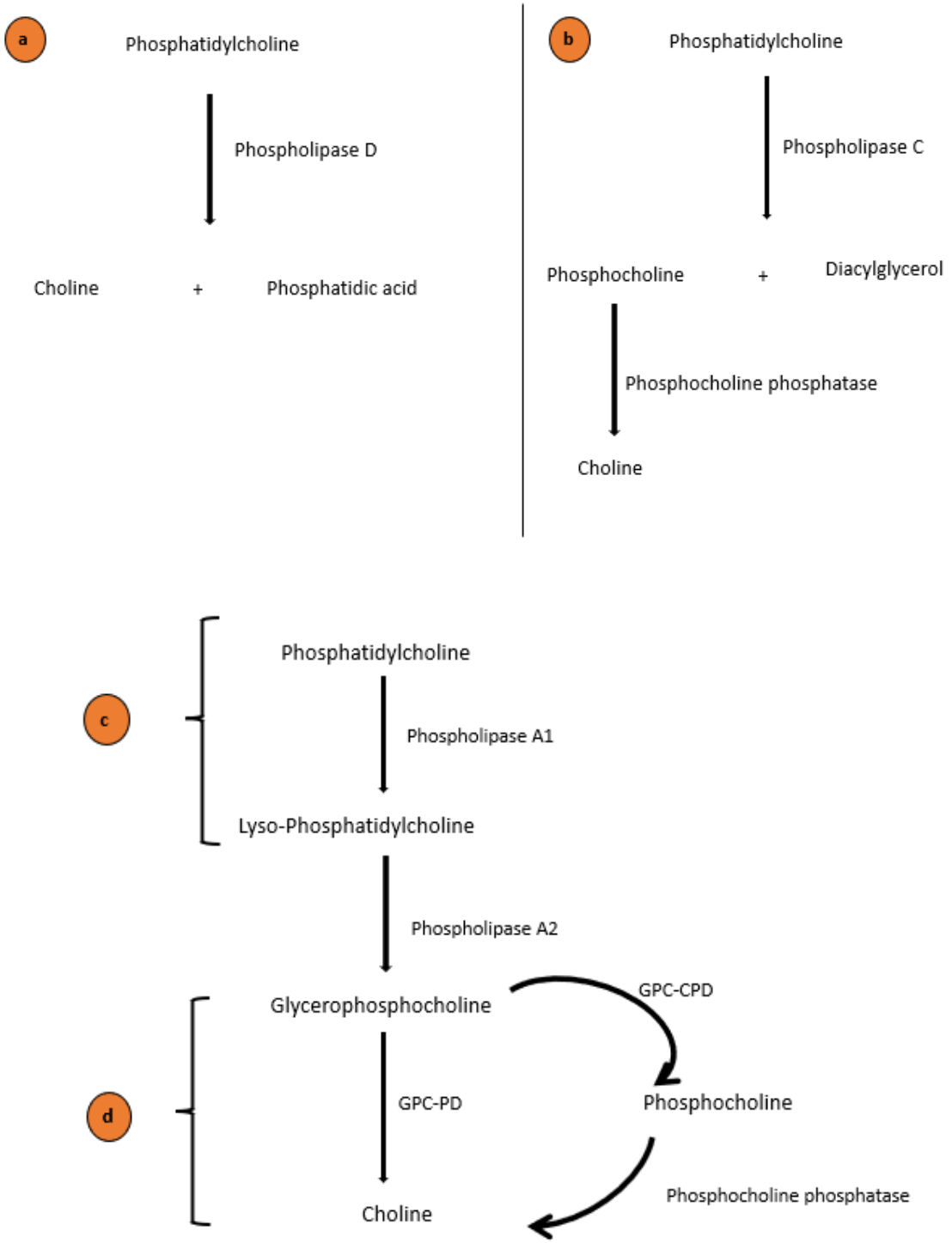


Figure 11: Choline is liberated from phosphatidylcholine and from phosphatidylcholine molecules formed from preexisting choline by a family of enzymes.

The various phospholipases convert phosphatidylcholine into a) choline (phospholipase D), b) phosphocholine (phospholipase C), or c) lysophosphatidylcholine (phospholipase A1). The enzyme d) glycerophosphocholine phosphodiesterase also releases choline from glycerophosphocholine.
Abbreviations: GPC-CPD= glycerophosphocholine choline-phosphodiesterase; GPC-PD= glycerophosphocholine phosphodiesterase.

1.1.5.4 Signal transduction

Myo-inositol (Ins) is observed in higher concentrations in glial cells compared to neurons (327,328). The brain derives Ins from two sources: (1) de novo synthesis from glucose and (2) dietary Ins. Grains and plant fibers are particularly high in inositol as inositol hexakis-phosphate. Ins can be synthesized endogenously from glucose in the brain, kidney and liver (329). Glucose-6-phosphate, formed from glucose, is converted to inositol-1-phosphate, which undergoes dephosphorylation within the brain to form free Ins (**Figure 12 (a)**). Ins is taken up by cells in the brain through a hydrogen-myo-inositol symporter (HMIT) and two sodium-myo-inositol cotransporters, SMIT1 and SMIT2 (**Figure 12 (b)**). These sodium-myo-inositol cotransporters are expressed on both neurons and astrocytes. SMIT1 is primarily expressed on astrocytes, whereas SMIT2 is primarily expressed on neurons. Hydrogen-myo-inositol symporters are only expressed in astrocytes.

A portion of intracellular Ins is involved in the phosphatidylinositol cycle, which generates the signaling molecule inositol-1,4,5-triphosphate (IP3), which is responsible for the release of calcium from endoplasmic reticulum stores (**Figure 13**). Ins is also an osmolyte. In response to the swelling of cells, neurons and glial cells release Ins. Hypo-osmolality is the cellular response to the brain's osmotic stress, and sustained stress results in the loss of Ins to regulate tissue osmolarity (327).

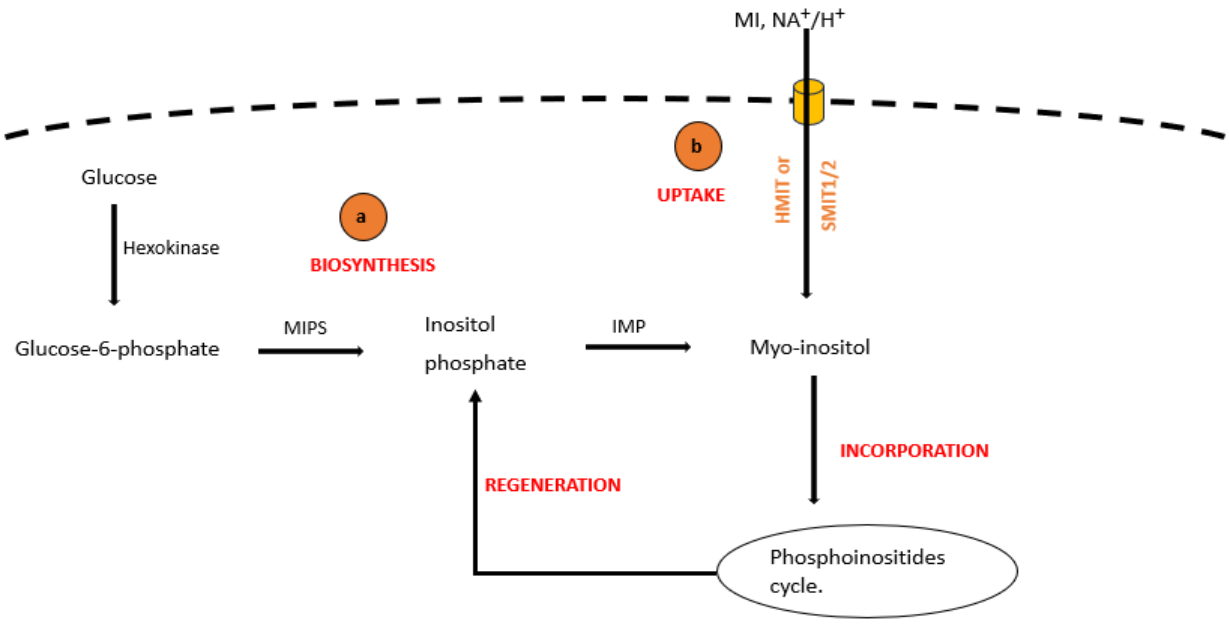


Figure 12: Myo-inositol biosynthesis, uptake, regeneration, and entrance into the phosphoinositide cycle.

Abbreviations: HMIT= hydrogen-myo-inositol symporter; IMP= inositol monophosphatase; MI= myo-inositol; MIPS=myo-inositol-phosphate synthase; SMIT1/2= sodium-myo-inositol transporters 1/2.

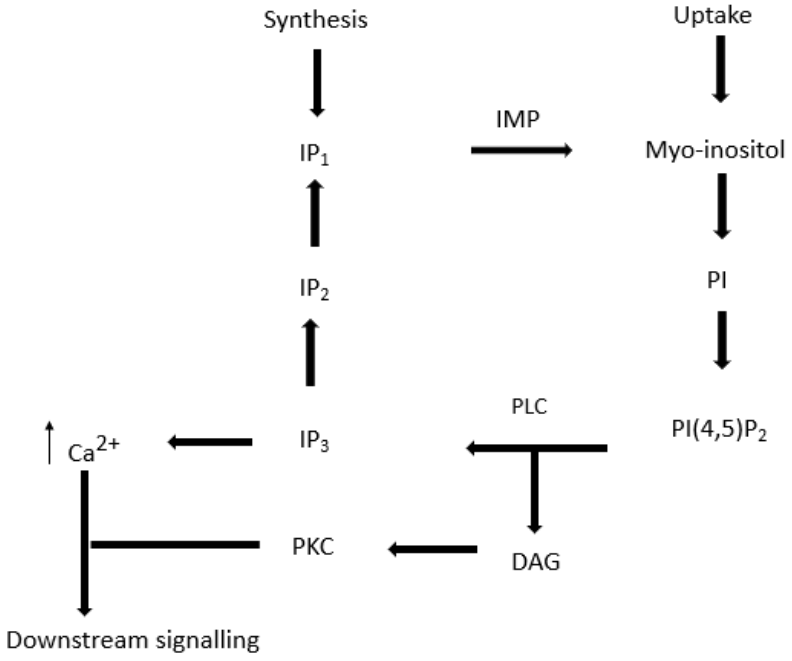


Figure 13: Formation of intracellular signaling molecules from myo-inositol through the phosphatidylinositol cycle.

Abbreviations: Ca^{2+}= calcium; DAG= diacylglycerol; IMP= inositol monophosphatase; IP_1= inositol-1-phosphate; IP_2= inositol-4,5-bisphosphate; IP_3 = inositol-1,4,5-trisphosphate; PI= phosphatidylinositol; $PI(4,5)P_2$ = phosphatidylinositol-4,5-bisphosphate; PKC= protein kinase C; PLC= phospholipase.

1.1.6 Previous MRS studies

1.1.6.1 MRS studies in healthy infants

Studies of healthy infants allow for the establishment of typical metabolite levels in the developing brain, providing a baseline to identify important differences due to age or pathology. A study by Blüml et al. presented growth profiles of metabolite levels across the brain in infancy. They found that the first 3 months of life are critical for rapid metabolic changes in the developing brain. Within the first 3 months, absolute metabolite concentrations of Cr+PCr, Glu, and NAA increased rapidly. Myo-inositol decreased rapidly during this period, whereas GPC+PCh remained stable. These results were observed in both the cerebral white and gray matter of infants aged from birth to 3 months. Between 3 months and 2 years, NAA increased, but it was not significant; Cr+PCr, GPC+PCh, Ins, and Glu declined significantly in the parietal white matter. In the gray matter, Glu increased after 3 months but stabilized after approximately 1 year. NAA and Cr+PCr increased, and Ins and GPC+PCh decreased between 3 months and 2 years (330).

1.1.6.2 MRS studies in HEU children

In an earlier study, Cortey et al. conducted a study of 10 newborns within the first 10 days of life. Five infants were born to HIV-seropositive mothers (the HEU group), and the other five were not exposed to HIV. HEU infants had significantly higher NAA/Cr+PCr and GPC+PCh/Cr+PCr ratios in white matter than the control group (331).

A study of children living with and without HIV at the age of 5 was conducted by Mbugua et al. The study reported lower levels of NAA and GPC+PCh metabolites in the BG of children living without HIV compared to children living with HIV who initiated ART before 12 weeks of age. According to Mbugua et al., the unexpected results in children living without HIV were driven by HEU children, who accounted for 80% of the uninfected children (35).

Holmes et al. found age-related increases in several metabolites in a longitudinal study of young HEU and HUU children. In the MFGM, the study found NAA and Cr+PCr metabolite levels increased significantly with age. Glu increased strongly with age in the BG and NAA, Cr+PCr, Glu, and GPC+PCh increased with age in the peritrigonal white matter (PWM). Interestingly, these results were independent of HIV exposure. No significant differences were found between HEU and HUU children across regions and metabolites (332).

Robertson et al. reported no differences in metabolite levels in the BG between HEU and HUU children at the age of 7, but at the age of 9, the study found lower metabolite levels of Glu, NAA, Cr+PCr, and GPC+PCh in HEU children compared to HUU children. Robertson et al. suggest that the lower metabolite levels of NAA, GPC+PCh, Glu, and Cr+PCr in HEU children at 9 years of age reflect loss of neurons, reduced cell density, and reductions in normal neurotransmission and energy metabolism, respectively (37). Robertson et al. also suggested that the lack of group differences observed at age 7 as opposed to the group differences observed at age 9 may be due to the different scanners used for the two groups. Contrary to the Allegra scanner used to scan children at 7 years of age, which has a single head coil, the Skyra scanner used to scan children at 9 years of age has 32 head coils that provide better quality.

In a follow-up study of the same cohort conducted by Graham et al., there were no differences in metabolite levels between HEU and HUU children at the age of 11 within the BG, suggesting the negative consequences of perinatal exposure to maternal HIV and/or ART were resolved by age 11 (333). Graham et al. also presented MRS findings in additional brain regions as compared to Robertson et al (37). In HEU children in comparison to HUU children, the study found lower metabolite levels of Glu as well as NAA in the MFGM. The study mentioned that the results observed demonstrate neuronal damage and impaired Glu production through the TCA cycle due to ART-driven mitochondrial

toxicity and/or the impaired uptake of Glu from the synaptic gap by the astrocytes, as previously suggested by Ernst et al (334). In addition, NAA levels were also lower in HEU children in comparison to HUU children in the PWM (333).

Table 1: A table summarizing the MRS studies including HEU children.

Title, First author, year	Region of interest	Sample	Selected findings
Proton MR Spectroscopy of Brain Abnormalities in Neonates Born to HIV-Positive Mothers, Cortey A, 1994	White matter	5 infants born to HIV-seropositive mothers 5 infants born to HIV-negative mothers.	↑ [NAA]/[Cr+PCr] and [GPC+PCh]/[Cr+PCr] in HIV-exposed uninfected infants.
HIV-associated CD4/8 depletion in infancy is associated with neurometabolic reductions in the basal ganglia at age 5 years despite early antiretroviral therapy, Mbugua K, 2016	BG	38 children living with HIV age range 5-6.3 years. 12 on late ART and 26 on early ART. 15 uninfected controls age range 5.1-6.4 years. 12 HEU and 3 HUU	↓ in NAA and GPC+PCh in children living without HIV in comparison to children living with HIV who initiated ART at or before 12 weeks of age.
Longitudinal increases of brain metabolite levels in 5-10-year-old children, Holmes M, 2017	MFGM, PWM and the right BG	35 HUU and 29 HEU. Ages 5, 7 and 9 years.	In the MFGM; NAA and Cr+PCr ↑ with age. In the PWM; GPC+PCh, Cr+PCr, Glu and NAA ↑ with age In the BG; no significant changes in GPC+PCh, Cr+PCr and NAA levels were found with ↑ age. Glu ↑ with age. Across regions and metabolites, no significant interactions between HIV-exposure and age were observed.
Perinatal HIV infection or exposure Is associated with low N-Acetyl aspartate and Glutamate in basal ganglia at age 9 but not 7 years, Robertson F, 2018	BG	7-year-old; 45 children living with HIV, 14 HEU and 12 HUU children. 9-year-old; 67 children living with HIV, 15 HEU and 21 HUU children.	7-year-old; there were no difference in the metabolite levels between the groups. 9-year-old; HEU children had ↓ Glu, NAA, Cr+PCr and GPC+PCh than HUU children.
MRS suggests multi-regional inflammation and white matter axonal damage at 11 years following perinatal HIV infection, Graham A, 2020	BG, MFGM and PWM	11-year-old; 76 children living with HIV, 30 HEU and 30 HUU children	In the BG; there were no difference in the metabolite levels between the groups. In the MFGM; ↓NAA and Glu in HEU children than HUU children In the PWM; HEU children had ↓ NAA than HUU children

Abbreviations: BG= basal ganglia; Cr+PCr= creatine plus phosphocreatine; Glu= glutamate; GPC+PCh= choline-containing compounds (glycerolphoshocholine plus phosphocholine); HEU= HIV-exposed and uninfected; HUU= HIV-unexposed and uninfected; Ins= myo-inositol; MFGM= midfrontal gray matter; NAA= N-acetyl aspartate; PWM= peritrigonal white matter.

1.1.7 The impact of maternal HIV on the neurodevelopment of HEU children

There are a growing number of studies that demonstrate an association between HIV exposure without infection and neurodevelopmental impairment across cognitive, motor, and/or language domains, although the manifestations are more subtle compared to children living with HIV. A review conducted by Le Doaré established that HEU children aged 3-5 years had subtle deficits in motor functions, expressive and receptive language, cognition, and behaviour (14). Moreover, another review by McHenry et al. found that HEU children had lower motor and cognitive scores compared to HUU children (16). Compared to HUU controls, a study conducted by Le Roux et al. in South Africa found increased odds of motor and cognitive delays in 12-month-old HEU infants, but they did not find a delay in language (335). Van Rie et al. found moderate to severe delays in motor and language development in a substantial proportion of HEU children (aged 18 to 72 months) when compared to HUU children from Kinshasa, Democratic Republic of the Congo (32). HEU children aged 2 years in the Drakenstein Child Health Study in South Africa and Zimbabwe were found to have receptive and expressive language delays and poor motor and language development, respectively, compared to HUU children (336,337).

A study from China reported lower cognitive and adaptive behaviour scores in HEU children aged 6 to 36 months. This study reported lower neurodevelopmental levels in the motor domain in older children in the HEU group (30-35 months old) than younger HEU children from the same study (338). This was similar to a longitudinal study that reported that neurocognitive performance in HEU children was initially similar to that of their HUU peers but started to fall behind in the late pre-school to early school-age years (27). In addition, a study by Adclok et al. discovered declining language abilities in older (aged 16–30 months vs. 8–15 months) HEU children in Kenya (339). Other studies suggest that cognition and behaviour may be affected at older ages. Poorer school mathematics performance was found in HEU children (6-12 years) in Zambia (29), while grade repetition in HEU children with an age range of 7-11 years was reported by Mitchell et al (28). Kerr et al. reported lower verbal intelligence quotient (IQ), full-scale IQ, and Binet Bead Memory scores among HEU children compared to HUU children. The mean differences were −6.13 (95% Cl:−10.3 to −1.96), p = 0.004; −4.57 (95% Cl:−8.80 to −0.35), p = 0.03; and −3.72 (95% Cl:−6.57 to −0.88), p = 0.01, respectively (31).

In contrast, other studies found no significant neurodevelopmental outcomes between HEU and HUU children. A study by Springer et al. reported cognitive, language (receptive and expressive), and motor (gross motor and fine motor) composite scores within the normal range between HEU and HUU infants (340). In a study from Thailand, no substantial differences were found in IQ scores between HEU and HEU children with a mean (standard deviation (SD)) age of 10.3 (2.8) years. A study by Chaudhury et

al. found HEU children at 24 months performed equally well on neurodevelopmental assessments in comparison to HUU children (341).

In order to make conclusions about a question or topic in the literature, one must consider the quality, study design, and methodology differences, as well as the strengths and weaknesses of the studies examined.

1.1.7.1 Weaknesses of the studies examined

It is possible that developmental delays in HEU children may be due to other factors rather than any direct biological consequences of maternal HIV exposure during fetal gestation. One study discovered that the living conditions of HEU children had a significant impact on neurodevelopmental outcomes (31). Analyses by McHenry et al. revealed that child malnutrition and anemia, as well as a mother's low education, were risk factors for one or more domains of neurodevelopmental delay (338).

When compared to HUU children, the Van Rie et al. and Adclok et al. studies included a relatively small number of HEU children, making it difficult to control for potential confounders and draw statistical conclusions, respectively (32,339). In addition, HEU and HUU children in the study conducted by Van Rie et al. differed significantly across a range of socioeconomic factors (32). A review conducted by McHenry et al. also included studies that had a relatively small sample size, which had a high risk of confounding. Furthermore, six studies from outside the United States were classified as "low quality" (16).

A study by Springer et al. reported that HEU infants performed equally well on neurodevelopmental assessments as HUU infants. However, a greater proportion of HEU than HUU children had some evidence of developmental delay (composite score <85) in the cognitive (HEU (9%) vs. HUU (0%)), motor (HEU (6.9%) vs. HUU (5.2%)) and language (HEU (28%) vs. HUU (18%)) domains. The precision was limited due to the relatively small sample size (340).

Confounders that could affect the neurodevelopment of HEU children were not taken into account in some studies. Jahanshad et al.'s study lacked maternal history, such as mental health history and drug and alcohol use during pregnancy (342). In a study conducted by Kerr et al., no data on maternal antiretroviral drug exposure or pregnancy information (such as a preterm birth) was recorded (31). Furthermore, because mothers in a study by Chandna et al. were not randomized to ART use or regimen, it is difficult to determine whether any associations were due to ART or confounding factors (337).

Nicholson et al. found poorer school mathematics performance in HEU children from different schools,

and differences in grade recording by different teachers and schools may have increased variability, potentially increasing the likelihood of finding differences. Some of the children's reports were also missing. Lower mathematics scores, according to the study, could be accurate, but it could also be a coincidental finding as a result of numerous comparisons. The study did not explain why students performed worse in mathematics at school (29). According to Mitchell et al., the primary causes of grade repetition in HEU children were issues with school readiness and disruptions at school rather than the biological effects of maternal HIV exposure (28).

1.1.7.2 Strengths of the studies examined

A variety of factors were considered in studies with larger sample sizes. The Drakenstein Child Health Study in South Africa enrolled 732 children and included an appropriate control group from a similar socioeconomic environment and socio-demographic risk factors (336). Another study conducted by Chaudhury et al. had a large sample size of 732 children (168 HEU and 564 HUU children) and the ability to test and control for a large number of potential environmental (electricity, housing, water, and sanitation) and socioeconomic (maternal age, education, income, depression, as well as alcohol and tobacco use during pregnancy) confounders (341).

The study by Smith et al. was the only longitudinal study that measured neurodevelopment at two timepoints, allowing an assessment of how neurocognitive function evolves over time in HEU children. The most important aspect is how it worsens with age (27).

The majority of data comes from non-breastfeeding populations or populations with a small percentage of HEU children who are breastfed. In the latter case, studies by Chaudhury et al. (341) and Springer et al. (340), for example, discovered that 9% of HEU children and 40% of HEU children (by 2 weeks of age) were breastfed, respectively. A study conducted by Le Roux included HUU and HEU children who were all breastfed (335).

In a study conducted by McHenry et al., older children in the HEU group (30-35 months) had lower mean composite scores in the motor domain than younger children (age groups: 6-11, 12-17, 18-23, and 24-29 months) (338). Adclok et al. discovered worsening language outcomes at 16-30 months rather than 8-15 months (339). These studies showed that these changes may reflect a slowing in motor development rather than a loss of skills and that more subtle language impairments may be difficult to detect at 8–15 months before explicit verbal communication develops, respectively (336,338,339).

1.1.8 The impact of maternal ARV drugs on neurodevelopment in HEU children

ART prevents vertical transmission of HIV by lowering the maternal VL as well as providing prophylaxis through breastfeeding and transplacental exposure. Although ART protects the fetus and infants from infection, ART exposure may have unexpected consequences for the fetus and infants' brains. In support of this, numerous animal studies find exposure to ART alone is related to morphological and functional alterations in the brain (343–347).

Early developmental stages in infants can be measured by tests focused on sensorimotor or cognitive domains. The most commonly used test for identifying neurodevelopmental delays in children between the ages of 1 and 42 months is the Bayley Scales of Infant and Toddler Development (Bayley), which has multiple subsets including cognitive, language, motor, social-emotional, and adaptive behaviour (348).

The mean Bayley scores among HEU children with prenatal ARV drug exposure in comparison with those without (HUU) were slightly lower in a study conducted by William et al (349). Lower development and adaptive behaviour scores were found in HEU children exposed to multiple ARTs in utero compared to HUU children (ages 18 to 36 months). However, when maternal substance use during pregnancy was controlled, these differences were no longer observed (350). Sirois et al. found no associations between *in utero* exposure to ART and mean scores for any of the five Bayley-III domains in HEU infants with and without perinatal ART exposure ranging from 9-15 months, supporting the overall safety of ARV drugs. However, based on specific ARV drugs, atazanavir was associated with lower mean scores on the receptive communication subtest of the language domain (351). Supporting evidence was provided by a study conducted by Rice et al., though this study used the MacArthur–Bates Communicative Development Inventory, which measures language development between 8 and 30 months of age. At 12 months of age, atazanavir-exposed infants had increased odds of late language emergence (delays) than atazanavir-unexposed infants (352). The scores of infants exposed to tenofovir in utero were related to reduced social-emotional performance; those exposed to nelfinavir were associated with reduced cognitive performance; and finally, those exposed to lopinavir/atazanavir were related to reduced language and adaptive behavioural performance when compared to HEU infants unexposed to the specific ARV medications (351). Children who were prenatally exposed to either lamivudine or lopinavir/atazanavir performed significantly better in terms of social emotional performance and language performance than HEU children who were not exposed to either lamivudine or lopinavir/atazanavir (350).

The Wechsler Preschool and Primary Scale of Intelligence measures changes in later stages of development. In a study of children aged 5 to 13, the mean cognitive and academic outcome scores

in HEU children exposed to in utero ART were slightly lower than population norms. However, they were not statistically significant after controlling for confounding variables (353). In a longitudinal study conducted by Smith et al., in comparison with population norms for their ages. HEU children prenatally exposed to ART had significantly lower adaptive behaviour and socialization scores at the age of 3.5 years. However, when HEU children were 5.5 years old, they did not differ significantly from the population norms in terms of adaptive behaviour and socialization, despite having significantly lower verbal IQ scores (27). A study comparing the long-term effects of an individual ARV drug, zidovudine, to a placebo in children aged 5.6 years found no significant differences in cognitive function (354).

1.1.9 Aim and hypothesis

The aim of this study is to see if the metabolic brain abnormalities found in older HEU children are present in neonates, as well as to identify any additional abnormalities that may exist.

Based on previous studies in older children, we hypothesize lower levels of Glu/Cr+PCr and NAA/Cr+PCr in the BG and MFGM and GPC+PCh/Cr+PCr in the BG in HEU compared to HUU neonates. Additionally, we hypothesize no ART duration exposure differences in metabolite/Cr+PCr ratios.

1.1.9.1 Motivation

(1) There is no published MRS work on neonates exposed to HIV and ART with varying ART duration in utero, and

(2) Several confounders, such as feeding practices, socioeconomic status, parenting, and education, that have the potential to emerge over time are minimized when neuroimaging is performed within the first 35 days of life.

1.1.9.2 Main questions

(1) Does maternal HIV exposure impact early infant brain development?

(2) Does the length of exposure to ART in utero influence the developing brain?

2. Methods and Materials

2.1 Participants

This study is a sub-study of a larger cohort study referred to as the Healthy Baby Study (HBS). The HBS study included an MRI protocol for the acquisition of structural, functional and metabolic data in neonates, and this study focuses on the MRS data only. Between 2017 and 2021, HBS recruited pregnant women attending the antenatal clinic at the Michael Mapongwana Community Health Centre (MMCHC) in Khayelitsha, Cape Town, where HIV sero-prevalence approaches 30% (355). The study included pregnant women living with and without HIV who were 18 years of age or older before 7 weeks of gestation. Pregnant women living with HIV either initiated ART treatment pre-conception or at their first prenatal clinical visit after 5 weeks of gestational age (GA). Exposing the fetus to ART since conception (pre-conception) or after 5 weeks of GA (post-conception), respectively. The date of the last menstrual period, fundal height, and an early antenatal ultrasound performed at the clinic were used to calculate the GA. Following delivery, the GA was adjusted at antenatal time points based on the GA estimate at birth, taking into account the aforementioned factors as well as a repeat ultrasound examination.

Exclusion criteria for pregnant women included a history of recurrent premature deliveries, chronic disorders (tuberculosis, hypertension, epilepsy, diabetes), known tuberculosis contact, being on medications other than necessary pregnancy supplements and using drugs, or drinking more than minimally (>7 drinks per week), or binge drinking (4 or more drinks per occasion). Pregnant women living with HIV who were non-adherent to medication, not on the Tenofovir/Efavirenz/Emtricitabine-containing regimen, or who had not disclosed their HIV status to their households were excluded.

At enrollment, demographic information was recorded. All pregnant women had monthly study visits at similar times as routine antenatal care visits at MMCHC to monitor their health during pregnancy. At the study visit, information was captured related to possible viral illnesses and trauma using the Harvard Trauma Questionnaire. All pregnant women were also interviewed regarding their alcohol and drug use using the timeline follow-back approach (356,357). At study visits closest to 20 and 33 weeks of gestation, all pregnant women's urine was tested for recreational drug use (cannabis, methamphetamine and methaqualone).

The HIV status of all pregnant women was confirmed using an HIV rapid test as per standard care for all pregnant women in South Africa at the first antenatal clinic visit, at 32 weeks of gestation, in labour and every 3 months if breast-feeding. The $CD4^+$ T cell count and VL within 6 months of pregnancy and

at delivery of pregnant women living with HIV were obtained from the antenatal clinic records. Pregnant women living with HIV were on a daily fixed dose combination containing Tenofovir, Efavirenz and Emtricitabine. At each study visit, an adherence counselor administered an ART adherence questionnaire.

The Harvard Trauma Questionnaire for Posttraumatic Stress Symptoms (HTS) was developed specifically to screen for the presence of post-traumatic stress disorder (PTSD) in cross-cultural populations (358). The HTS is a valid and reliable measure for screening PTSD when cross-cultural sensitivity is required (359). Research addresses post-traumatic stress disorder based on traumatic experiences such as sexual and/or physical abuse, emotional abuse, neglect and loss of family (360–363). Pregnancy may trigger PTSD, with studies demonstrating how mothers who have experienced traumatic events and have developed PTSD are more likely to exhibit hostile parenting behaviours or more severely aggressive parenting practices (364,365). Withdrawn and emotionally unavailable behaviours of traumatized mothers are experienced by their infants during daily routines such as feeding and breastfeeding (366).

Questionnaires were administered in the mothers' home language by trained research staff. They were translated into isiXhosa, then back into English, and reconciled with the original version in accordance with psychological assessment standards. Questions were read to the pregnant women, and options for answers were given. All pregnant women were asked if they had experienced any difficult or stressful events in the previous 6 months, whether they had witnessed it happen to someone else or heard about it happening to someone they knew. If they identified an event, then the full questionnaire was administered, with questions read to the participant to answer about the most upsetting event.

As per standard of care, neonates born to mothers living with HIV received a polymerase chain reaction (PCR) test for HIV-1 DNA at birth, 10 weeks postpartum and 18 weeks postpartum (if at high-risk of transmission) and an ELISA rapid test at 9 and 18 months. Although neonates did not receive HIV-1 DNA PCR testing on the day of the MRI scan, the results were consistent with those taken subsequent to scanning, and we are confident that there were no neonates living with HIV on the day of the MRI scan.

HEU neonates were classified as low-risk or high-risk according to maternal parameters (high-risk if maternal VL >1000 copies/mL at 32 weeks of gestation). Low-risk neonates were given Nevirapine for 6 weeks, whereas high-risk neonates were started on a combination of Nevirapine and Zidovudine for 6 weeks.

Neonates' exclusionary criteria included birth weight <2500 grams (g), preterm delivery <36 weeks GA, being positive on HIV-1 PCR, or conditions such as persistent hypoglycaemia, neonatal asphyxia, severe neonatal jaundice, chromosomal abnormalities, or congenital malformations that could influence neurodevelopmental outcomes.

2.2 Ethics Approval

The study was approved by the Human Research Ethics Committees of the University of Cape Town and Stellenbosch University. All women enrolled provided written informed consent for themselves and their neonates in their preferred language before participating in the study.

2.3 MRI data acquisition

MRI scans were acquired at the Cape Universities Body Imaging Centre (CUBIC), Groote Schuur Hospital, Cape Town. Neonates were scanned using a 3T Skyra scanner (Siemens, Erlangen, Germany) without sedation at a mean GA equivalent of 41.56 weeks (range 39-45 weeks). Around one hour before the scheduled scan, a paediatrician weighed, examined, and performed the Dubowitz Infant Neurological Examination while blind to HIV and ART exposure status (367). Neonates were then fed, had sponge ear plugs inserted into their ears, and had neonatal foam ear pads placed over their ears, held in place by a knitted cap. They were then gently rocked to sleep while lying supine on an MRI-compatible vacuum cushion containing styrofoam beads (VacFix®, S&S Par Scientific, Houston, TX) that fit snugly around their entire body, including the head, and imaged using the Siemens 16-channel paediatric head and neck coil.

Neonates had three MRI acquisitions (structural, diffusion-weighted imaging, and MRS) on the day of the scan (**Figure 14**). If neonates woke up or couldn't fall asleep again within the allocated 2-hour period, they could not continue with the MRI scan.

The protocol included a high-resolution T1-weighted multiecho magnetisation prepared rapid gradient echo acquisition (MEMPRAGE) (368) (FOV 192 mm x 192 mm, repetition time (TR) 2540 ms, inversion time (TI) 1450, echo times TE's = 1.69/3.55/5.41/7.27 ms, bandwidth 650 Hz/px, 144 slices, a resolution of 1.0 x 1.0 x 1.0 mm3 with flip angle of 7 degrees) and a single voxel proton MRS (Point Resolved Spectroscopy (PRESS): 1.5 cm × 1.5 cm × 1.5 cm3 voxel; TR 2000 ms, TE 30 ms, 64 averages, bandwidth 1300 Hz, vector size 1024)) in the BG and the MFGM with chemical shift selective (CHESS) water suppression. A water reference was acquired in the same voxel without water suppression (TR 4000 ms, TE 30 ms).

2.4 Processing the spectra

Eddy current correction (369) and spectral fitting were carried out on the scanner-averaged data using linear combination model software (LCModel) (version 6.3-1) as described previously by Provencher et al. (370) to calculate ratios of metabolites to total creatine (metabolite/Cr+PCr) (371,372). The LCModel basis set included creatine (Cr), taurine, glucose, phosphocholine, glycerophosphocholine, glutamine (Gln), scyllo-inositol, Glu, Ins, L-alanine, lactate, NAA, negative creatine methylene (−CrCH2), phosphocreatine (PCr), N-acetylaspartylglutamate (NAAG), aspartate, and g-aminobutyric acid (GABA). Lipid and macromolecule resonances were modelled using additional 5 macromolecule and 4 lipid Gaussian basis functions (LCModel User's Manual).

To ensure quality data, spectra with a full width half maximum (FWHM) greater than (>) 0.07 parts per million (ppm) and a signal-to-noise ratio (SNR) less than (<) 7 were considered to be of low quality and were discarded from analysis (as in the Holmes et al. study (332)). To demonstrate that all metabolites reported were well fitted by LCModel, we report the range of % SD for all metabolites used in the final analysis.

2.5 Statistical analysis

R software version 3.1.1, together with RStudio software version 0.98.50751, was used for all statistical analyses as well as visualisation of the data. To compare demographic and clinical characteristics between groups of neonates or mothers, student t-tests or ANOVA (F) and chi-square (χ^2) tests were used for continuous and categorical variables, respectively. Linear regression models were used to examine HIV exposure group differences in metabolite to total creatine ratios. To look at the possible influence of the duration of ART exposure on the developing fetus, we divided HEU neonates into two groups based upon treatment initiation; - pre-conception or post-conception (at the first prenatal clinical visit).

To determine potential confounding factors for each model, we calculated Pearson correlation coefficients (*r*) between potential confounders and metabolite/Cr+PCr ratios. Possible confounders included: FWHM, SNR, feeding method (exclusively breastfed, exclusively formula fed, or both breast and formula fed), GA at the MRI scan, and neonatal weight, length, and head circumference. The GA at the MRI scan was used instead of the age at the MRI scan. Maternal variables such as maternal age at delivery, highest education level, monthly income, ounces (oz) of absolute alcohol consumed per day averaged across pregnancy (1 oz of absolute alcohol is equivalent to 2 standard drinks) and Harvard Trauma Scores during pregnancy were also considered as potential confounders. Potential confounders related to the outcomes at $p < 0.05$ were included in the model. Significant confounders

of interest, such as Harvard Trauma Scores and clinical measures in mothers living with HIV, were further explored graphically and with interaction terms.

3. Results

3.1 Participant's demographic and clinical data

MRS data was successfully acquired from the BG for 135 neonates and from the MFGM for 117 neonates. The data from the BG for 10 neonates was excluded due to either low quality data (SNR < 7 and/or FWHM > 0.07 ppm), low birth weight (<2500 g), or preterm delivery (<36 weeks GA). The data from the MFGM for 21 neonates was excluded due to either low quality data (SNR < 7 and/or FWHM > 0.07 ppm), low birth weight (<2500 g), or preterm delivery (<36 weeks GA) (**Figure 14**). After exclusion, MRS data was acquired from 125 neonates in the BG voxel and 96 neonates in the MFGM voxel (**Figure 14 and Table 4**). The MRS data was acquired for 128 neonates in total (**Figure 14 and Table 4**). The data comprised 83 HEU neonates and 45 HUU neonates. Of the 83 HEU neonates, 44 were exposed to ART since conception (pre-conception) and 39 were exposed to ART after 5 weeks of GA (post-conception). No differences were found in the neonates' sex, birth and MRI indices with regards to weight, length, head circumference, and GA. Due to the study design, ART exposure duration (in weeks) was significantly different between HEU neonates who were exposed to ART pre-conception and those who were exposed to ART post-conception (t = 16.43, p **< 2.2e-16**). The ART exposure duration ranged from 37.00 to 42.14 weeks in the pre-conception group (median 40.07 weeks) and from 14.00 to 35.57 weeks in the post-conception group (median 26.14 weeks). Sample demographic and clinical characteristics are summarized in **Table 2**.

In **Table 3**, the demographic and clinical characteristics of pregnant women living with and without HIV are presented. Pregnant women living with HIV who initiated treatment pre-conception were significantly older at the time of delivery (mean ± SD = 30.25 ± 5.07, p = **0.01**). Pregnant women living with HIV who initiated treatment post-conception had a significantly lower mean $CD4^+$ T cell count (mean ± SD = 435.49 ± 202.15, p = **0.001**) in comparison to pregnant women living with HIV who initiated treatment pre-conception. A higher median [interquartile range] HIV VL was observed in pregnant women living with HIV who initiated treatment post-conception; however, this was not statistically significant (117.50 [203.50], p = **0.09**). Substance use in pregnancy was low and similar across all women. Pregnant women had similar education and income levels, as well as similar exposure to trauma. After birth, most mothers across all groups exclusively breastfed their neonates.

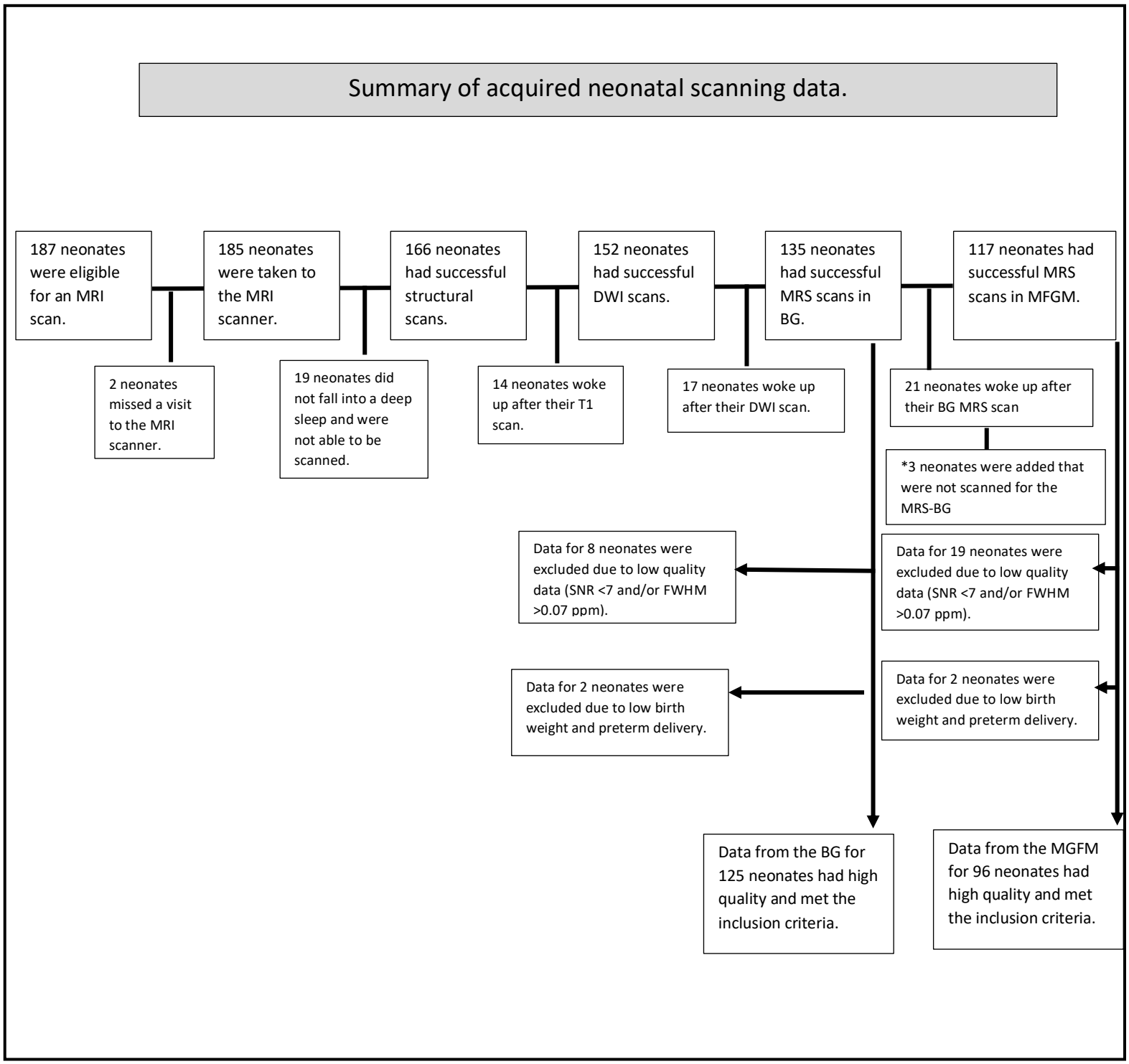


Figure 14: Summary of acquired neonatal scanning data.

Abbreviations: BG= basal ganglia; DWI= diffusion weighted imaging; FWHM= full width half maximum; MFGM= midfrontal gray matter; MRI= magnetic resonance imaging; SNR= signal-to-noise ratio; MRS= magnetic resonance spectroscopy; T1= T1-weighted scan.
*3 neonates that woke up after their DWI scan and could not be scanned for the MRS-BG fell asleep again and were scanned for the MRS-MFGM.

Table 2: Demographic and clinical characteristic of 128 neonates.

N = 128	HUU	HEU (pre-conception)	HEU (post-conception)	χ^2 or t or F	p-value
n =	45	44	39		
Sex (n (%) Male)	24 (53.33)	24 (54.55)	20 (51.28)	0.09	0.96
Age at scan (weeks)	1.72 ± 1.08	1.52 ± 0.90	1.75 ± 1.00	0.87	0.35
Exclusively breastfed (n, %)	44 (97.78)	35 (79.55)	35 (89.74)	8.40	0.08
Exclusively formula fed (n, %)	0 (0)	5 (11.36)	3 (7.69)		
Breast and formula fed (n, %)	1 (2.22)	4 (9.09)	1 (2.56)		
Birth indices:					
Weight ((g) Male)	3391.21 ± 400.24	3289.38 ± 439.60	3250.00 ± 450.13	0.68	0.41
Weight ((g) Female)	3206.76 ± 391.03	3235.75 ± 322.15	3210.79 ± 305.11	0.07	0.79
Length ((cm) Male)	50.54 ± 3.31	50.29 ± 1.88	50.50 ± 2.72	0.10	0.75
Length ((cm) Female)	49. 52 ± 2.36	49.05 ± 2.32	49.61 ± 2.85	0.36	0.55
Head circumference (cm)	33.98 ± 1.34	33.98 ± 1.39	33.55 ± 1.44	0.00	0.99
ART exposure duration (weeks)	N/A	40.06 ± 1.25	24.75 ± 6.04	16.43	**<2.2e-16**
Indices at the MRI scan:					
Weight ((g) Male)[1]	3560.42 ± 468.28	3504.17 ± 541.12	3526.32 ± 318.58	0.18	0.67
Weight ((g) Female)[2]	3400.00 ± 456.24	3292.37 ± 346.00	3355.26 ± 442.18	0.66	0.42
Length ((cm) Male)	51.52 ± 2.05	51.62 ± 2.14	52.45 ± 1.75	0.03	0.87
Length ((cm) Female)[3]	51.07 ± 2.22	50.79 ± 1.73	51.97 ± 1.70	0.17	0.68
Head circumference (cm)[4]	35.09 ± 1.30	35.48 ± 1.26	34.83 ± 1.19	2.10	0.15
Gestational age (weeks)	41.65 ± 0.91	41.56 ± 1.14	41.44 ± 1.13	0.14	0.71

Values are mean ± standard deviation with exception to χ^2, t-test, F values, p-values, number (n) and percentages (%).
The p values for group comparisons are derived from χ^2 or F or a t-test.
Bold font values indicate significance at $p < 0.05$.
[1,2]Weight data at the MRI scan is missing for 1 male and 2 females. [3]Length data at the MRI scan is missing for 2 participants.
[4]Head circumference data at the MRI scan is missing for 4 participants.
Abbreviations: ART= antiretroviral therapy; cm= centimeter; g= grams; GA= gestational age; HEU= HIV-exposed and uninfected; HUU= HIV-unexposed and uninfected; MRI= magnetic resonance imaging; N/A= not applicable; Post-conception= neonates who have been exposed to ART after 5 weeks of GA; Pre-conception= neonates who have been exposed to ART since conception.

Table 3: Maternal demographic and clinical characteristics.

	Pregnant women living without HIV	**Pregnant women living with HIV who initiated ART pre-conception**	**Pregnant women living with HIV who initiated ART post-conception**	**χ^2 or t or F**	**p-value**
n =	45	44	39		
Maternal Indices:					
Age at delivery (years)	27.47 ± 5.30	30.25 ± 5.07	28.15 ± 4.99	6.52	**0.01**
GA at enrolment (weeks)	20.03 ± 5.16	21.58 ± 5.91	20.92 ± 5.92	1.68	0.20
$CD4^+$ T cell count within 6 months of enrolment (cells/µL)[1]	N/A	573.51 ± 146.85	435.49 ± 202.15	3.51	**0.001**
VL within 6 months of enrolment:[2]					
Undetectable (n, %)	N/A	36 (81.82)	26 (66.67)	1.32	0.25
Detectable (n, %)	N/A	8 (18.18)	12 (30.77)		
VL (copies/mL) (median [interquartile range])	N/A	29.00 [12.00]	117.50 [203.50]	-1.78	0.09
Substance use across pregnancy:					
Alcohol use (n, %)	24 (53.33)	18 (40.91)	24 (61.54)	3.61	0.16
Absolute alcohol consumption/day (oz)	0.04 ± 0.02	0.03 ± 0.01	0.04 ± 0.02	2.19	0.15
Cannabis use (n, %)	3 (6.67)	1 (2.27)	0 (0)	3.23	0.20
Methamphetamine use (n, %)	2 (4.44)	0 (0)	2 (5.13)	2.20	0.33
Methaqualone use (n, %)	1 (2.22)	0 (0)	0 (0)	1.86	0.39
Highest school level completed (n, %)					
Grade 6	0 (0)	2 (4.55)	0 (0)		
Grade 8	2 (4.44)	1 (2.27)	0 (0)		
Grade 9	0 (0)	2 (4.55)	1 (2.56)	15.11	0.13
Grade 10	3 (6.67)	3 (6.82)	3 (7.69)		
Grade 11	9 (20.00)	19 (43.18)	13 (33.33)		
Grade 12	31 (68.89)	17 (38.64)	22 (56.41)		
Monthly Income (n, %)					
Less than R2000	7 (15.56)	15 (34.09)	7 (17.95)		
R2000 to less than R5000	24 (53.33)	21 (47.73)	26 (66.67)	9.74	0.14
R5000 to R10 000	10 (22.22)	7 (15.91)	4 (10.26)		

More than R10 000	4 (8.89)	1(2.27)	1 (2.56)		
Unknown[3]	0 (0)	0 (0)	1 (2.56)		
Harvard Trauma Scores [4]	36.40 ± 11.76	39.74 ± 13.20	39.42 ± 16.18	1.25	0.27

Values are mean ± standard deviation with exception to χ^2, t-test, F values, p-values, number (n), percentages (%) and median [interquartile range].

The p values for group comparisons are derived from χ^2 or F or a t-test.

Bold font values indicate significance at $p < 0.05$.

[1]$CD4^+$ T cell count within 6 months of enrolment (cells/µL) data is missing for 3 participants. [2]VL within 6 months of enrolment (either detected or undetected) data is missing for 3 participants. [3]Monthly income data is missing for 1 participant. [4]Harvard Trauma Scores data is missing for 5 participants.

Abbreviations: ART= antiretroviral therapy; $CD4^+$= cluster of differentiation 4 +; GA= gestational age; HIV= human immunodeficiency virus; mL= milliliter; N/A= not applicable; oz= ounces; VL= viral load; µL= microliter.

3.2 Basal ganglia metabolite/Cr+PCr ratios in HEU and HUU neonates

The following were the ranges of the metabolites' %SDs found in the BG: NAA %SD range 5-20; Glu %SD range 7-14; GPC+PCh %SD range 2-6; Ins %SD range 3-7; and Cr+PCr %SD range 3-5.

Table 4 summarizes the final number of subjects with MRS data within the BG voxel by HIV/ART exposure grouping. **Tables 5 and 6** summarize associations between metabolite/Cr+PCr ratios and potential confounders for model building. In the BG, metabolite/Cr+PCr ratios were similar across neonates, with only one exception: GPC+PCh/Cr+PCr. As shown in **Table 7 and Figure 16**, HEU neonates had a lower mean GPC+PCh/Cr+PCr ratio (B = -0.03, p = **0.004**) compared to HUU neonates. Further analysis based on ART duration grouping found GPC+PCh/Cr+PCr ratios were significantly lower in both pre-conception (B = -0.02, p = **0.02**) and post-conception (B = -0.03, p = **0.01**) HEU neonates, compared to HUU neonates (**Table 8 and Figure 17)**. There were no differences in other metabolite/Cr+PCr ratios between HUU and HEU (pre-conception) or between HUU and HEU (post-conception). The ART duration analysis results are shown in **Table 8**.

We found NAA/Cr+PCr ratios negatively correlated with maternal Harvard Trauma Scores across all neonates (*r* = -0.19, p = **0.04**) (**Table 6 and Figure 15**). Using an interaction term, the slopes of the regression lines were not statistically significantly different between HEU and HUU neonates (B = -0.0004, p = 0.63). **Table 9** presents correlations between metabolite/Cr+PCr ratios and maternal clinical variables. We found significant positive correlations between NAA/Cr+PCr and maternal $CD4^+$ T cell count (*r* = 0.30, p = **0.01**) (**Table 9 and Figure 18**). Using an interaction term, this was observed across all HEU neonates independent of ART duration in pregnancy (B = 0.0001, p = 0.54).

Table 4: Sample number in each region of interest.

	Basal ganglia	**Midfrontal gray matter**	**Both basal ganglia and midfrontal gray matter**
HEU (pre-conception)	42	36	34
HEU (post-conception)	38	29	28
HUU	45	31	31
Total	125	96	93

Abbreviations: ART= antiretroviral therapy; GA= gestational age; HEU= HIV-exposed and uninfected; HUU= HIV-unexposed and uninfected; Post-conception= neonates who have been exposed to ART after 5 weeks of GA; Pre-conception= neonates who have been exposed to ART since conception.

Table 5: Correlations between all neonates' basal ganglia metabolite/Cr+PCr ratios and potential neonatal confounding variables.

Metabolite ratios	FWHM	SNR	Weight (g) at the MRI scan	Length (cm) at the MRI scan	Head circumference (cm) at the MRI scan	Gestational age (weeks) at the MRI scan	Feeding method
NAA/Cr+PCr	-0.06 (0.54)	0.05 (0.59)	0.13 (0.14)	0.004 (0.97)	**0.18 (0.04)**	**0.26 (0.004)**	0.13 (0.15)
Glu/Cr+PCr	**0.24 (0.01)**	**-0.18 (0.04)**	-0.10 (0.28)	**-0.24 (0.01)**	0.04 (0.65)	0.06 (0.48)	-0.11 (0.20)
GPC+PCh/Cr+PCr	0.16 (0.07)	**0.22 (0.01)**	**-0.20 (0.02)**	**-0.19 (0.03)**	-0.14 (0.12)	-0.08 (0.38)	-0.03 (0.77)
Ins/Cr+PCr	0.08 (0.36)	0.08 (0.39)	**-0.36 (0.0001)**	**-0.35 (0.0001)**	**-0.23 (0.01)**	**-0.26 (0.003)**	0.06 (0.54)

The values are the Pearson correlation coefficients and p-values. Bold font values indicate significance at $p < 0.05$.
Abbreviations: cm= centimeter; Cr+PCr= total creatine (creatine plus phosphocreatine); FWHM= full width half maximum; g=grams; Glu= glutamate; GPC+PCh= choline-containing compounds (glycerolphoshocholine plus phosphocholine); Ins= myo-inositol; MRI= magnetic resonance imaging; NAA= N-acetyl aspartate; SNR= signal-to-noise ratio.

Table 6: Correlations between all neonates' basal ganglia metabolite/Cr+PCr ratios and potential maternal confounding variables.

Metabolite ratios	Maternal age at delivery (years)	Maternal education	Maternal monthly income	Maternal absolute alcohol consumption /day	Maternal Harvard Trauma Scores
NAA/Cr+PCr	0.07 (0.41)	0.08 (0.36)	0.09 (0.30)	-0.07 (0.59)	**-0.19 (0.04)**
Glu/Cr+PCr	0.005 (0.96)	-0.15 (0.10)	**-0.21 (0.02)**	-0.10 (0.43)	-0.04 (0.66)
GPC+PCh/Cr+PCr	-0.09 (0.32)	0.03 (0.78)	-0.05 (0.60)	0.06 (0.65)	-0.04 (0.63)
Ins/Cr+PCr	0.06 (0.50)	0.09 (0.33)	-0.08 (0.37)	0.16 (0.20)	-0.05 (0.60)

The values are the Pearson correlation coefficients and p-values. Bold font values indicate significance at $p < 0.05$.
Abbreviations: Cr+PCr= total creatine (creatine plus phosphocreatine); Glu= glutamate; GPC+PCh= choline-containing compounds (glycerolphoshocholine plus phosphocholine); Ins= myo-inositol; NAA= N-acetyl aspartate.

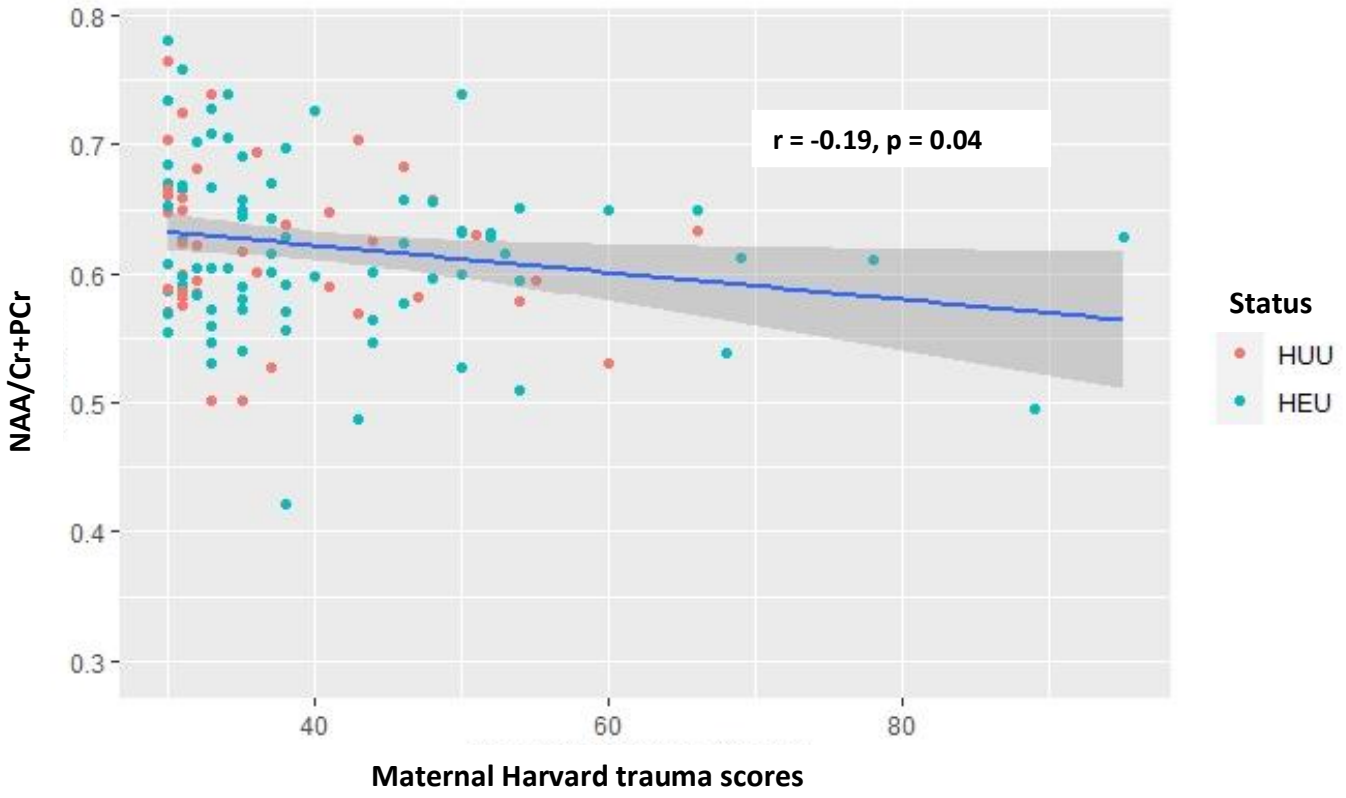


Figure 15: A plot showing an association between NAA/Cr+PCr ratios and maternal Harvard Trauma Scores in HIV-unexposed-uninfected and HIV-exposed-uninfected (pre-conception and post-conception) neonates.

The values are the Pearson correlation coefficient and p-value. Significant at $p < 0.05$.
Abbreviations: ART= antiretroviral therapy; GA= gestational age; HEU= HIV-exposed and uninfected; HUU= HIV-unexposed and uninfected; NAA= N-acetyl aspartate; Cr+PCr= total creatine (creatine plus phosphocreatine); Post-conception= neonates who have been exposed to ART after 5 weeks of GA; Pre-conception= neonates who have been exposed to ART since conception.

Table 7: Unstandardised regression coefficients (B), standard errors (SE) and p-values from linear regression analyses comparing metabolite/Cr+PCr ratios in the basal ganglia of HEU neonates (who have been exposed to ART since conception (pre-conception) and after 5 weeks of GA (post-conception)) to HUU neonates. Potential confounding variables reported in Tables 5 and 6 have been adjusted for.

	HUU (n = 45)		
	HEU (n = 80)		
	B	SE	p
NAA/Cr+PCr[1,2,3]	-0.002	0.01	0.85
Glu/Cr+PCr[4,5,6,7]	-0.03	0.02	0.21
GPC+PCh/Cr+PCr[5,6,8]	-0.03	0.01	**0.004**
Ins/Cr+PCr[1,2,6,8]	-0.02	0.03	0.43

Linear regression models include potential confounders related to the outcome at $p < 0.05$, as reported in **Tables 5 and 6**. Bold font values indicate significance at $p < 0.05$.
1= head circumference (cm) at the MRI scan; 2= GA (weeks) at the MRI scan; 3= maternal Harvard Trauma Scores; 4= FWHM; 5=SNR; 6= length (cm) at the MRI scan; 7= maternal income; 8= weight (g) at the MRI scan.
Abbreviations: ART= antiretroviral therapy; cm= centimeter; Cr+PCr=total creatine (creatine plus phosphocreatine); FWHM: full width half maximum; g= grams; GA= gestational age; Glu= glutamate; GPC+PCh= choline-containing compounds (glycerolphosphocholine plus phosphocholine); HEU= HIV-exposed and uninfected; HUU= HIV-unexposed and uninfected; Ins= myo-inositol; MRI= magnetic resonance imaging; NAA= N-acetyl aspartate; SNR: signal-to-noise ratio.

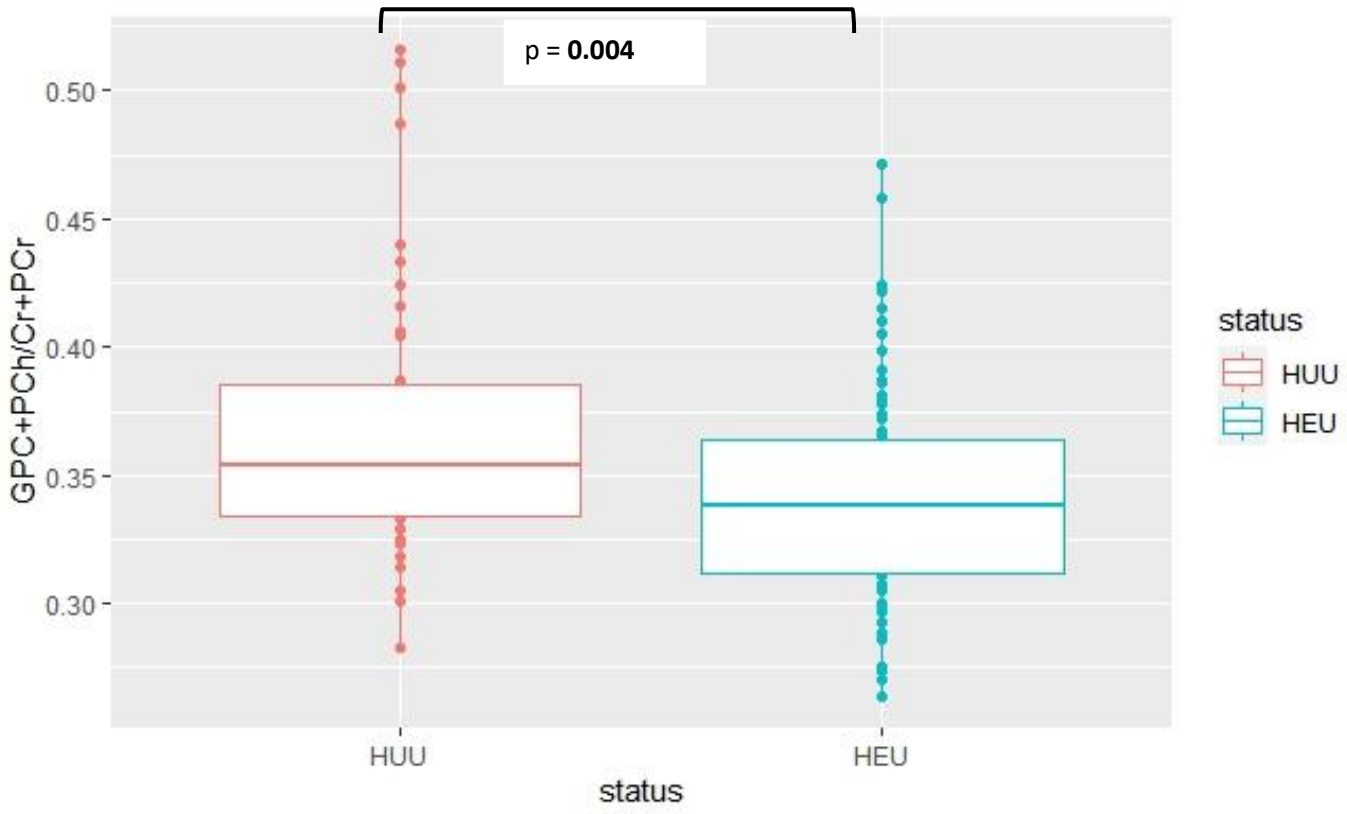


Figure 16: A plot of the basal ganglia choline showing the means, confidence intervals and statistically significant differences between HIV-unexposed-uninfected and HIV-exposed-uninfected neonates at p < 0.05.

Abbreviations: Cr+PCr= total creatine (creatine plus phosphocreatine); GPC+PCh= choline-containing compounds (glycerolphoshocholine plus phosphocholine); HEU= HIV-exposed uninfected; HUU= HIV-unexposed uninfected. Significant at p < 0.05.

Table 8: Unstandardised regression coefficients (B), standard errors (SE) and p-values from linear regression analyses comparing metabolite/Cr+PCr ratios in the basal ganglia of HEU neonates (who have been exposed to ART since conception (pre-conception) and after 5 weeks of GA (post-conception)) to HUU neonates. Potential confounding variables reported in Tables 5 and 6 have been adjusted for.

	HUU (n = 45)					
	HEU (pre-conception) (n = 42)			**HEU (post-conception) (n = 38)**		
	B	SE	p	B	SE	p
NAA/Cr+PCr[1,2,3]	0.01	0.01	0.62	-0.01	0.01	0.38
Glu/Cr+PCr[4,5,6,7]	-0.02	0.03	0.53	-0.04	0.03	0.12
GPC+PCh/Cr+PCr[5,6,8]	-0.02	0.01	**0.02**	-0.03	0.01	**0.01**
Ins/Cr+PCr[1,2,6,8]	-0.01	0.03	0.80	-0.04	0.04	0.25

Linear regression models include potential confounders related to the outcome at $p < 0.05$, as reported in **Tables 5 and 6**. Bold font values indicate significance at $p < 0.05$.
1= head circumference (cm) at the MRI scan; 2= GA (weeks) at the MRI scan; 3= maternal Harvard Trauma Scores; 4= FWHM; 5=SNR; 6= length (cm) at the MRI scan; 7= maternal income; 8= weight (g) at the MRI scan.
Abbreviations: ART= antiretroviral therapy; cm= centimeter; Cr+PCr=total creatine (creatine plus phosphocreatine); FWHM: full width half maximum; g= grams; GA= gestational age; Glu= glutamate; GPC+PCh= choline-containing compounds (glycerolphosphocholine plus phosphocholine); HEU= HIV-exposed and uninfected; HUU= HIV-unexposed and uninfected; Ins= myo-inositol; MRI= magnetic resonance imaging; NAA= N-acetyl aspartate; Post-conception= neonates who have been exposed to ART after 5 weeks of GA; Pre-conception = neonates who have been exposed to ART since conception; SNR: signal-to-noise ratio.

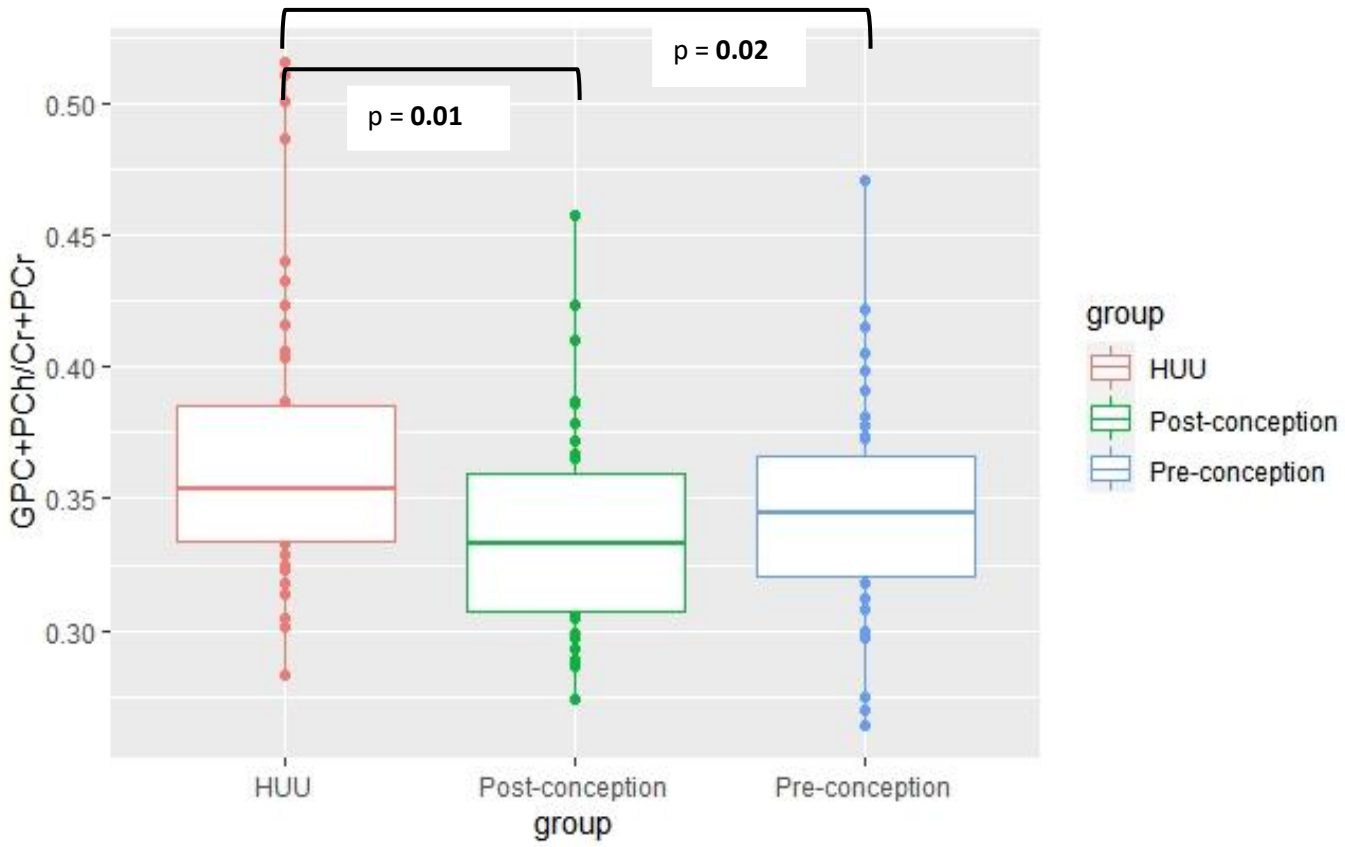


Figure 17: A plot of the basal ganglia choline showing the means, confidence intervals and statistically significant differences in groups (HIV-unexposed-uninfected neonates and HIV-exposed-uninfected neonates who were subdivided into those who were exposed to ART pre- and post-conception) at p < 0.05.

Abbreviations: ART= antiretroviral therapy; Cr+PCr= total creatine (creatine plus phosphocreatine); GA= gestational age; GPC+PCh= choline-containing compounds (glycerolphosphocholine plus phosphocholine); HEU= HIV-exposed and uninfected; HUU= HIV-unexposed and uninfected; Post-conception= neonates who have been exposed to ART after 5 weeks of GA; Pre-conception = neonates who have been exposed to ART since conception.
Significant at p < 0.05.

Table 9: Correlations between basal ganglia metabolite/Cr+PCr ratios in HEU neonates and maternal variables (maternal $CD4^+$ T cell count and VL within 6 months of enrolment).

Metabolite ratios	Maternal $CD4^+$ T cell count	Maternal VL within 6 months of enrolment [a]	Maternal VL within 6 months of enrolment [b]
NAA/Cr+PCr	**0.30 (0.01)**	-0.20 (0.39)	-0.20 (0.08)
Glu/Cr+PCr	0.15 (0.19)	-0.17 (0.48)	-0.11 (0.32)
GPC+PCh/Cr+PCr	0.14 (0.21)	-0.10 (0.67)	-0.23 (0.81)
Ins/Cr+PCr	0.05 (0.65)	-0.22 (0.36)	-0.09 (0.45)

The values are the Pearson correlation coefficients and p-values. Bold font values indicate significance at $p < 0.05$.
[a]Pearson correlation coefficients of women who had detectable viral load (VL) (copies/mL) only. [b]Pearson correlation coefficients of all mothers of HEU neonates; VL set to zero of women who had undetectable VL.
Abbreviations: $CD4^+$= cluster of differentiation 4 +; Cr+PCr= total creatine (creatine plus phosphocreatine); Glu= glutamate; GPC+PCh= choline-containing compounds (glycerolphoshocholine plus phosphocholine); HEU= HIV-exposed and uninfected; Ins= myo-inositol; mL= milliliter; NAA= N-acetyl aspartate; VL= viral load.

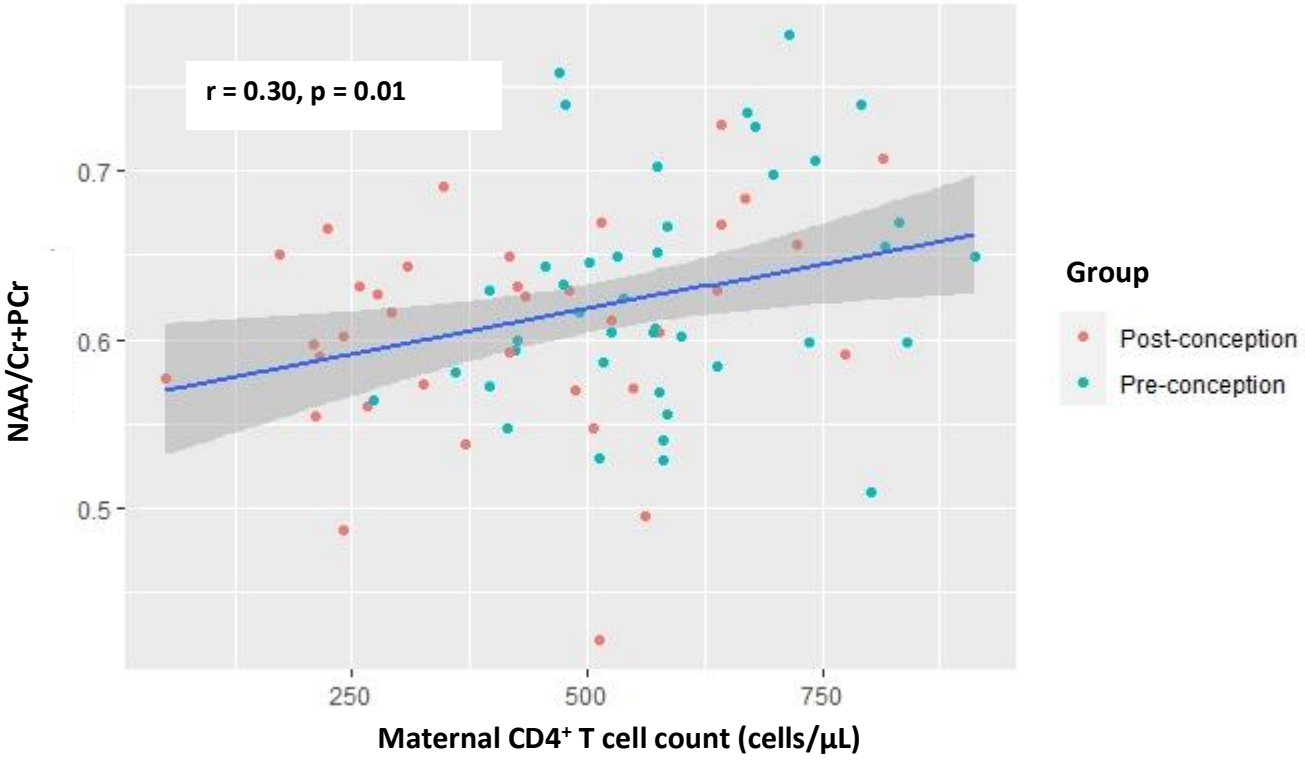


Figure 18: A plot showing an association between the NAA/Cr+PCr ratios of HIV-exposed and uninfected neonates and the maternal CD4⁺ T cell count.

The values are the Pearson correlation coefficient and p-value. Significant at $p < 0.05$.
Abbreviations: CD4⁺= cluster of differentiation 4 +; Cr+PCr= total creatine (creatine plus phosphocreatine); GA= gestational age; NAA= N-acetyl aspartate; µL= microliter.

3.3 Midfrontal gray matter metabolite/Cr+PCr ratios in HEU and HUU neonates

The following were the ranges of the metabolites' %SDs found in the MFGM: NAA %SD ranges from 6-42; Glu %SD ranges from 7-14; GPC+PCh %SD ranges from 2-5; Ins %SD ranges from 2-5 and Cr+PCr %SD ranges from 3-7.

Table 4 summarizes the final number of subjects with MRS data within the MFGM by HIV/ART exposure grouping. **Tables 10 and 11** summarize associations between metabolite/Cr+PCr ratios and potential confounders for model building.

The metabolite/Cr+PCr ratios between HUU and HEU neonates were similar (**Table 12**). Further analysis based on ART duration grouping found no metabolite/Cr+PCr ratio differences between HUU and HEU (pre-conception) or between HUU and HEU (post-conception) (**Table 13**).

No significant correlations between metabolite/Cr+PCr ratios and maternal clinical variables were observed among HEU neonates who have been exposed to ART since conception as well as those exposed to ART post-conception (**Table 14**).

Table 10: Correlations between all neonates' midfrontal gray matter metabolite/Cr+PCr ratios and potential neonatal confounding variables.

Metabolite ratios	FWHM	SNR	Weight (g) at the MRI scan	Length (cm) at the MRI scan	Head circumference (cm) at the MRI scan	Gestational age (weeks) at the MRI scan	Feeding method
NAA/Cr+PCr	**-0.22 (0.03)**	-0.07 (0.51)	0.15 (0.15)	-0.03 (0.80)	**0.28 (0.01)**	0.13 (0.19)	0.17 (0.09)
Glu/Cr+PCr	**0.24 (0.02)**	**0.57 (9.572e-10)**	-0.04 (0.70)	-0.19 (0.07)	0.12 (0.24)	-0.07 (0.47)	-0.03 (0.77)
GPC+PCh/Cr+PCr	**0.34 (0.001)**	0.13 (0.22)	-0.04 (0.73)	-0.09 (0.40)	-0.03 (0.80)	-0.17 (0.11)	-0.14 (0.17)
Ins/Cr+PCr	0.12 (0.23)	0.12 (0.25)	-0.19 (0.07)	**-0.21 (0.04)**	-0.09 (0.40)	**-0.42 (0.00002)**	0.03 (0.78)

The values are the Pearson correlation coefficients and p-values. Bold values indicate significance at $p < 0.05$.
Abbreviations: cm= centimeter; Cr+PCr= total creatine (creatine plus phosphocreatine); FWHM= full width half maximum; g=grams; Glu= glutamate; GPC+PCh= choline-containing compounds (glycerolphoshocholine plus phosphocholine); MRI= magnetic resonance imaging; NAA= N-acetyl aspartate; Ins= myo-inositol; SNR= signal-to-noise ratio.

Table 11: Correlations between all neonates' midfrontal gray matter metabolite/Cr+PCr ratios and potential maternal confounding variables.

Metabolite ratios	Maternal age at delivery (years)	Maternal education	Maternal income	Maternal absolute alcohol consumption /day	Maternal Harvard Trauma Scores
NAA/Cr+PCr	-0.19 (0.07)	-0.05 (0.66)	-0.19 (0.07)	-0.11 (0.46)	0.20 (0.06)
Glu/Cr+PCr	0.11 (0.27)	**-0.22 (0.03)**	-0.18 (0.08)	0.05 (0.77)	-0.08 (0.44)
GPC+PCh/Cr+PCr	-0.13 (0.21)	0.02 (0.85)	-0.08 (0.41)	-0.03 (0.84)	-0.01 (0.91)
Ins/Cr+PCr	0.08 (0.45)	0.12 (0.24)	-0.14 (0.17)	0.26 (0.09)	0.03 (0.80)

The values are the Pearson correlation coefficients and p-values. Bold font values indicate significance at $p < 0.05$.
Abbreviations: Cr+PCr= total creatine (creatine plus phosphocreatine); Glu= glutamate; GPC+PCh= choline-containing compounds (glycerolphoshocholine plus phosphocholine); Ins= myo-inositol; NAA= N-acetyl aspartate.

Table 12: Unstandardised regression coefficients (B), standard errors (SE) and p-values from linear regression analyses comparing metabolite/Cr+PCr ratios in the midfrontal gray matter of HEU neonates (who have been exposed to ART since conception (pre-conception) and after 5 weeks of GA (post-conception)) to HUU neonates. Potential confounding variables reported in Tables 10 and 11 have been adjusted for.

	HUU (n = 31)		
	HEU (n = 65)		
	B	SE	p
NAA/Cr+PCr[1,2]	0.04	0.02	0.11
Glu/Cr+PCr[1,3,4]	0.02	0.04	0.69
GPC+PCh/Cr+PCr[1]	-0.02	0.01	0.10
Ins/Cr+PCr[5,6]	-0.03	0.05	0.60

Linear regression models include potential confounders related to the outcome at $p < 0.05$, as reported in **Tables 10 and 11**.
1= FWHM; 2= head circumference (cm) at the MRI scan; 3= SNR; 4= maternal education; 5= length (cm) at the MRI scan; 6= GA (weeks) at the MRI scan.
Abbreviations: ART= antiretroviral therapy; cm= centimeter; Cr+PCr= total creatine (creatine plus phosphocreatine); FWHM: full width half maximum; GA= gestational age; Glu= glutamate; GPC+PCh= choline-containing compounds (glycerolphoshocholine plus phosphocholine); HEU= HIV-exposed and uninfected; HUU= HIV-unexposed and uninfected; Ins= myo-inositol; MRI= magnetic resonance imaging; NAA= N-acetyl aspartate; SNR: signal-to-noise ratio.

Table 13: Unstandardised regression coefficients (B), standard errors (SE) and p-values from linear regression analyses comparing metabolite/Cr+PCr ratios in the midfrontal gray matter of HEU neonates (who have been exposed to ART since conception (pre-conception) and after 5 weeks of GA (post-conception)) to HUU neonates. Potential confounding variables reported in Tables 10 and 11 have been adjusted for.

	HUU (n = 31)					
	HEU (pre-conception) (n = 36)			HEU (post-conception) (n = 29)		
	B	SE	p	B	SE	p
NAA/Cr+PCr[1,2]	0.02	0.02	0.33	0.05	0.03	0.06
Glu/Cr+PCr[1,3,4]	0.02	0.05	0.65	0.01	0.05	0.81
GPC+PCh/Cr+PCr[1]	-0.02	0.01	0.08	-0.01	0.01	0.27
Ins/Cr+PCr[5,6]	-0.03	0.06	0.61	-0.03	0.07	0.70

Linear regression models include potential confounders related to the outcome at $p < 0.05$, as reported in **Tables 10 and 11**. 1= FWHM; 2= head circumference (cm) at the MRI scan; 3= SNR; 4= maternal education; 5= length (cm) at the MRI scan; 6= GA (weeks) at the MRI scan.

Abbreviations: ART= antiretroviral therapy; cm= centimeter; Cr+PCr= total creatine (creatine plus phosphocreatine); FWHM: full width half maximum; GA= gestational age; Glu= glutamate; GPC+PCh= choline-containing compounds (glycerolphoshocholine plus phosphocholine); HEU= HIV-exposed and uninfected; HUU= HIV-unexposed and uninfected; Ins= myo-inositol; MRI= magnetic resonance imaging; NAA= N-acetyl aspartate; Post-conception= neonates who have been exposed to ART after 5 weeks of GA; Pre-conception = neonates who have been exposed to ART since conception; SNR: signal-to-noise ratio.

Table 14: Correlations between midfrontal gray matter metabolite/Cr+PCr ratios in HEU neonates and maternal variables (maternal $CD4^+$ T cell count and VL within 6 months of enrolment).

Metabolite ratios	Maternal $CD4^+$ T cell count	Maternal VL within 6 months of enrolment [a]	Maternal VL within 6 months of enrolment [b]
NAA/Cr+PCr	-0.11 (0.38)	-0.04 (0.89)	-0.01 (0.92)
Glu/Cr+PCr	0.06 (0.64)	0.23 (0.35)	0.20 (0.11)
GPC+PCh/Cr+PCr	0.10 (0.45)	0.16 (0.51)	0.10 (0.42)
Ins/Cr+PCr	-0.08 (0.53)	0.07 (0.78)	0.003 (0.98)

The values are the Pearson correlation coefficients and p-values. [a]Pearson correlation coefficients of women who had detectable viral load (VL) (copies/mL) only. [b]Pearson correlation coefficients of all mothers of HEU neonates; VL set to zero of women who had undetectable VL.

Abbreviations: $CD4^+$= cluster of differentiation 4 +; Cr+PCr= total creatine (creatine plus phosphocreatine); Glu= glutamate; GPC+PCh= choline-containing compounds (glycerolphoshocholine plus phosphocholine); HEU= HIV-exposed and uninfected; Ins= myo-inositol; mL= milliliter; NAA= N-acetyl aspartate; VL= viral load.

4. Discussion

The aim of this study was to determine whether the metabolic brain abnormalities found in older HEU children are already detected in neonates. Based on previous results, we hypothesized lower ratios of Glu/Cr+PCr, NAA/Cr+PCr, and GPC+PCh/Cr+PCr in the BG of HEU neonates compared to HUU neonates, as seen previously in 9-year-old HEU children (37). Additionally, we hypothesized lower ratios of Glu/Cr+PCr and NAA/Cr+PCr in the MFGM of HEU neonates compared to HUU neonates, as observed previously in 11-year-old HEU (333).

4.1 Lower GPC+PCh/Cr+PCr ratios in HEU neonates compared to HUU neonates

First, in accordance with our hypothesis, we found lower mean GPC+PCh/Cr+PCr ratios in HEU neonates in comparison to HUU neonates in the BG. Possible explanations for this finding include (1) maternal dietary choline deficiency as well as other micronutrients such as vitamin B_{12} and folate (373–376) and (2) increased choline-consuming gut microbiota, which metabolize choline to TMA in mothers living with HIV (323,377). These mechanisms result in reduced choline bioavailability delivered to the fetus and neonate during pregnancy and lactation (80.72% of HEU neonates in this study were exclusively breastfed). Furthermore, membrane breakdown occurred as a compensatory mechanism to liberate choline for the synthesis of ACh, which is required to suppress the production and release of pro-inflammatory cytokines from peripheral macrophages and other cytokine-producing cells like microglia (313).

4.1.1 Maternal choline deficiency

On average, approximately 15% of the daily requirement of choline is synthesized endogenously, with the remainder derived from the diet (306). A significant number of adults have been reported to consume choline at levels below the recommended daily intake levels, particularly pregnant and lactating women (378). Pregnant and lactating women are at increased risk of choline deficiency due to the increased delivery of choline to the fetus and infant, respectively. After assessing gestational choline intake in nearly 900 women, Boeke et al. discovered that only 15% of mothers achieved adequate consumption levels during the first and second trimesters of pregnancy (379). Furthermore, studies reveal that pregnant women only achieve an average of 400 mg per day (mg/day) of choline, whereas the recommended adequate intake of choline for pregnant women is 450 mg/day and 550 mg/day for lactating women. According to the evidence, this is insufficient for pregnant women.

Studies have shown that women consuming choline <300 mg/day have a higher risk of having a baby with birth defects such as neural tube defects than those consuming >500 mg/day (380). Supporting this was a study from the same group that found low serum total choline concentrations to be risk factors for neural tube defects (381). In contrast, more recent studies have failed to find associations between lower maternal choline intake and the risk of neural tube defects (382,383). Additionally, a study including 71 neural tube defect-affected pregnancies and 214 pregnancies with non-neural tube defect malformations found no associations between maternal mean plasma concentrations of choline during pregnancy and the risk of neural tube defects (384).

Although maternal daily intake levels of choline were not measured and none of the neonates in this study had neural tube defects, it is plausible that mothers in this study did not meet the recommended daily intake levels. In addition to pregnant and lactating women being at increased risk of choline deficiencies, lower levels of choline are common in individuals living with HIV. In a study of people living with HIV, ranging from asymptomatic to AIDS-diagnosed, the total mean plasma choline concentrations in people living with HIV were significantly lower ($p < 0.002$) than in people living without HIV (385). In another study of individuals living with HIV on ART, the absolute concentration of choline showed significant annual decreases in frontal white matter (2.61%, $p = 0.0001$) and midfrontal cortical gray matter (2.19%, $p = 0.0123$). Analyses of GPC+PCh/Cr+PCr ratios showed similar patterns of change in the frontal white matter (1.74%, $p = 0.0123$) (386). Given that the brain efficiently extracts choline from the plasma, changes in brain choline levels may reflect changes in plasma choline levels (387,388).

Based on studies reporting reduced choline levels in plasma and the brain in individuals living with HIV. We suggest decreased amounts of choline may be delivered to the fetus and infant during pregnancy and lactation. However, further research is needed to confirm reduced choline levels in women living with HIV during pregnancy and within the context of the fetus.

4.1.2 Effects of maternal dietary choline deficiency on the fetal brain

Maternal diet during fetal gestation is one of many environmental factors that influence fetal brain development. Maternal dietary choline deficiency can compromise the integrity of fetal cell membranes, leading to cell death due to decreased choline-containing phospholipids such as phosphatidylcholine and phosphatidylethanolamine. It may also result in decreased biosynthesis of the neurotransmitter ACh, a function that is often dysregulated in neurodegenerative diseases such as Alzheimer's disease (AD) (389), which is characterised by the gradual degeneration of cholinergic neurons and decreased choline acetyltransferase activity in the hippocampus and cerebral cortex. The loss of cholinergic neurons contributes to the memory and learning deficits associated with AD (390).

Sufficient concentrations of choline in the brain protect the brain from age-related cognitive decline and impairments in certain aspects of memory formation in specific memory-related tasks because sufficient concentrations of choline potentially conserve neurons (391,392). Synapses, which are critical for neuronal connection and transmission, could also be impaired by compromised membrane integrity. The variety of ion channels and receptors on the cell membrane translates an external signal into downstream intracellular signalling (313). Hence, a dietary supplement or sufficient concentrations of choline could potentially preserve neuronal signal transmission (391,392). Given that choline is also a precursor for sphingomyelin, its deficiency will result in impaired myelination of nerve cells, a process that is frequently required for proper neuronal signalling and efficient transmission of nerve signals in the brain (393).

During choline deficiency, S-adenosylmethionine is decreased by 50% (394), which then results in hypomethylation in DNA. DNA methylation occurs at cytosine bases that are followed by guanosine (5'-cytosine-phosphate-guanine-3'; 5'-CpG-3' sites) (395). It influences cellular events such as gene transcription, genomic stability, and imprinting. About 90-98% of CpG sites in DNA are methylated in mammals (396), however, some CpG-rich sites in DNA are not methylated and are referred to as CpG islands (397). When modifications occur and these CpG islands are methylated, especially in promoter regions, gene expression is usually suppressed or silenced (397). Within the neuroepithelial layer of the hippocampus in fetal rodent brains from mothers fed choline-deficient diets, DNA methylation was decreased. Specifically, decreases were observed in the gene encoding the cyclin-dependent kinase (Cdkn3) promoter and in cells in culture where choline was deficient. This hypomethylation resulted in the overexpression of this gene, consequently inhibiting cell proliferation as well as neuronal and glial differentiation (398). A study conducted by Mehedint et al. demonstrated how a diet low in choline in pregnant dams resulted in decreased DNA methylation in the fetal brain in two genes regulating angiogenesis (CpG islands in the proximity of the areas of hippocampal vascular endothelial growth factor C (Vegfc) and angiopoietin 2 (Angpt2)). Hypomethylation of genes is usually associated with increased gene expression, and *Vegfc* and *Angpt2* were overexpressed in the fetal brain. This leads to hippocampal endothelial cell differentiation, decreased cell proliferation, and ultimately decreased blood vessels in the hippocampus of the fetal brain (399). Therefore, reduced levels of choline and its derivatives during maternal pregnancy may alter fetal gene expression, resulting in long-term effects on the developing brain and its function.

Choline, folic acid, vitamin B_{12}, and methionine metabolism interact at the point where homocysteine is converted to methionine. Methionine is also synthesized from homocysteine using methyl groups donated by methyl-tetrahydrofolate in a vitamin B_{12} dependent reaction (400). The formation of methionine results in lower homocysteine concentrations (401). A disturbance in one of these

metabolic pathways, due to deficiency of these nutrients or single nucleotide polymorphisms in the genes for the enzymes, can result in an elevated plasma homocysteine concentration (402,403). An animal study in which rats were fed a choline deficient diet showed doubled plasma homocysteine concentrations and reduced tissue concentrations of methionine and *S*-adenosylmethionine, as well as total folate. Elevated plasma homocysteine concentrations have been shown to be related to both cognitive impairments in non-demented samples and an increased risk of AD (404,405). A study by Hobbs et al. found an association between elevated maternal homocysteine concentrations and increased cases of birth defects (406). Interestingly, studies have also found elevated homocysteine concentrations in women with a history of adverse pregnancy outcomes, such as recurrent early miscarriages (407), which occur frequently among women living with HIV (164).

Since methionine can be formed through two pathways, a disturbance in the availability of one nutrient (either choline, folate, or vitamin B_{12}) may result in compensatory changes in the others (408). When animals and humans were fed a diet low in choline, the requirements for dietary folate and vitamin B_{12} (required for folate recycling) increased, causing methyl-tetrahydrofolate to become the primary methyl group donor (409). Vitamin B_{12} deficiency is more common in people living with HIV in comparison with people living without HIV (374–376). According to the different stages of HIV infection, the prevalence of vitamin B_{12} deficiency in people living with HIV ranges from 10% to 35%. Studies found people living with HIV had a vitamin B_{12} deficiency even in the acute and asymptomatic phases (410–412). Singhal et al. suggested that the increased lymphocyte turnover in HIV infection may deplete vitamin B_{12}, just as fetal growth can deplete vitamin B_{12} (413). Maternal vitamin B_{12} deficiency causes a secondary deficiency in infants who are exclusively breastfed in the first few months of life, leading to neurodevelopmental and growth delays (414). A study conducted by White et al. revealed that food insecurity was associated with an inadequate intake of vitamin B_{12} among mothers who were unable to afford balanced meals and were experiencing food shortages. Despite the fact that the likelihood of food insecurity among mothers living with HIV and those living without HIV was comparable (415). It is plausible that mothers living with HIV in this study experienced food shortages or were unable to afford balanced meals, given that more than 50% of mothers living with HIV had a monthly income within the range of R2000 to R5000.

Folate is another micronutrient commonly found to be deficient in people living with HIV. Studies suggest that increased neopterin as a result of chronic stimulation of the macrophage by y-interferon inhibits folate metabolism (416–418). Prabha et al. report the prevalence of folic acid deficiency in patients living with HIV as 23.4% (373). Another study found significantly low serum and erythrocyte folate levels in 64% and 57% of 74 individuals living with HIV at all stages of the infection, respectively (416). A folate deficient diet along with vitamin B_{12} increases the demand for choline (419), which is

low in people living with HIV. Micronutrient deficiencies may occur due to factors including gut infection, inadequate dietary intake of micronutrients, malabsorption, and altered gut barrier function and metabolism (413,420). Ideally, pregnant women living with HIV in our study should have been screened for micronutrient deficiencies. However, this was not part of the scope of our study, and it is not always feasible.

4.1.3 Intestinal microbiota modulates choline bioavailability from the diet

The amount of TMA produced is influenced by the composition of the microbiota present (377). According to previous research, TMA-producing gut microbiota can reduce choline bioavailability (323). Increased TMA and TMAO levels have been found to be positively correlated with higher activity of anaerobic bacteria of the phylum Firmicutes and Proteobacteria (323,377). TMA and TMAO levels have also been linked to an increased ratio of the gut microbial phylum Firmicutes to Bacteroidetes, with increased levels of Firmicutes and decreased levels of Bacteroidetes (421,422). Furthermore, the microbial enzyme glycine betaine reductase, which is found in the microbial taxon Firmicutes, reduces betaine (which is derived from dietary choline) (377). Ling et al. discovered increased Firmicutes/Bacteroidetes ratios in individuals living with HIV who were treated and untreated with ART (423). Dinh et al. found that the gut microbiota of individuals living with HIV who received ART and had undetectable HIV VL were enriched in the Proteobacteria phylum when compared to individuals living without HIV (259) .

Furthermore, 16S rRNA gene analysis revealed that participants with high levels of TMAO (after consuming dietary eggs) had a higher abundance of Firmicutes, whereas those with lower levels of TMAO had a higher abundance of Bacteroidetes (422). TMAO was discovered in the serum of mice fed a choline supplemented diet (including choline chloride) in an animal study. Such results, however, were not observed in those fed a choline-deficient diet (323). It is possible that the increased choline-consuming gut microbiota, such as the Firmicutes/Bacteroidetes ratio and the Proteobacteria phylum, that were found in individuals living with HIV in studies conducted by Ling et al. (423) and Dinh et al. (259) were also present in mothers living with HIV in this study. These increased choline-consuming gut microbiota may have competed with the host mechanisms involved in the synthesis of either acetylcholine, betaine, or phospholipids (such as phosphatidylcholine) from dietary choline to produce TMA (424). As a result, the bioavailability of choline required by the mother to be delivered to the fetus and/or infant is reduced.

4.1.4 Peripheral inflammatory and ACh

Maternal pro-inflammatory cytokines that enter the fetal circulation activate both specific-antigen and non-antigen specific cells. This can further produce and release a variety of other pro-inflammatory cytokines, which are sensed by the afferent vagus nerve fibres. The afferent vagus nerve then communicates through the medulla to the hypothalamus, which subsequently communicates through the efferent vagus nerve (228) for the release of ACh (from splenic T cells) to inhibit or decrease the production of peripheral pro-inflammatory cytokines (230). The rate of ACh synthesis and release is most likely determined by choline availability together with acetyl-CoA and the enzyme choline acetyltransferase (304). Free choline is needed for this process, however, because the quantity of free choline is relatively small (36-44 micrometer (μM)) in comparison to the quantity of choline from its reservoir, phosphatidylcholine (2000–2500 μM) (425,426). The breakdown of membranes occurs when different phospholipases (phospholipase A1, A2, C, and D) release free choline from the cellular membrane (427), decreasing the total amount of choline that would have been made available to the brain from the peripheral. Hence the low choline levels observed within HEU neonates' brains. Given that phosphatidylcholine is also required for the synthesis of sphingomyelin, this implies that little phosphatidylcholine is made available for the synthesis of sphingomyelin, affecting neuronal signalling and the efficiency of transmission of nerve signals in the brain (393). A longitudinal study found lower levels of pro-inflammatory markers like IFN-γ and IL-1β in the serum of HEU infants at 6-10 weeks compared to HIV-unexposed infants. At 24-28 months, these children's serum levels of the pro-inflammatory markers IFN-γ, IL-1β, and IL-2 were also lower than in HIV-unexposed children (260). This may imply that membrane phosphatidylcholine is broken down to liberate free choline to be used for the synthesis of ACh in order to inhibit pro-inflammatory cytokines from peripheral macrophages and other cytokine producing cells (such as monocytes, dendritic cells, T cells, endothelial cells, and other non-neuronal cells) (230,231).

4.2 Lower GPC+PCh/Cr+PCr ratios in HEU neonates regardless of ART duration

We found lower GPC+PCh/Cr+PCr ratios in the BG in both HEU groups, those that were exposed to ART pre-conception and post-conception. This reveals that the effects of HIV exposure on GPC+PCh/Cr+PCr ratios in the BG are independent of the timing of maternal ART initiation.

4.3 Similar NAA/Cr+PCr and Glu/Cr+PCr ratios between HEU and HUU neonates

Our second finding is contrary to our hypothesis. In both the BG and MFGM, we observed no differences in mean NAA/Cr+PCr and Glu/Cr+PCr ratios between HEU and HUU neonates.

A possible explanation for these findings in the BG could be an increase in the release of ACh, which may be protective. Ach acts on the microglial α7 nAChRs, leading to decreases in the production and release of pro-inflammatory cytokines within the brain. The ACh needed to modulate pro-inflammatory cytokines is acquired from free choline. A small portion of dietary choline is typically acetylated to acetyl-CoA to generate ACh (316). However, if there is a dietary choline deficiency, choline stored as membrane phosphatides (such as phosphatidylcholine) is used for ACh biosynthesis, resulting in cellular membrane breakdown (427). As a result, choline levels in the brain are reduced in order to control pro-inflammatory cytokines. Evidence shows that microglia α7 nAChRs are necessary for the reduction of TNF-α and downstream IL-1β production in the brain (428). Thus, preserving neuronal membrane integrity and conserving neuronal excitatory neurotransmissions. If the HEU infants in this cohort have a choline deficiency, this could explain the lower GPC+PCh/Cr+PCr ratios found in HEU neonates as a compensatory mechanism to liberate choline from phosphatidylcholine for the synthesis of ACh. As the BG is closer to the ventricles than the MFGM, it is possible that its proximity may allow pro-inflammatory cytokines to penetrate the BG (429) making it more vulnerable.

Alternatively, it is possible that HIV and ART exposure does not influence NAA/Cr+PCr and Glu/Cr+PCr ratios in early infancy in this cohort. In the BG, HIV exposure related reductions in GPC+PCh/Cr+PCr ratios may occur though mechanisms that do not affect other metabolite levels. Further, our hypothesis was based on results from a cohort of children, which may not translate to infant populations.

4.4 Positive correlation between NAA/Cr+PCr ratios and maternal $CD4^+$ T cell count

Independent of ART duration in pregnancy, the study found a positive correlation between NAA/Cr+PCr and maternal $CD4^+$ T cell count in HEU neonates. Lower NAA/Cr+PCr levels in HEU neonates were associated with a lower maternal $CD4^+$ T cell count. Several studies found a correlation between a lower $CD4^+$ T cell count and higher levels of markers of plasma microbial translocation such as LPS (430–432) and sCD14 (433,434) in individuals living with HIV, regardless of whether they were taking ART or not. This allows LPS to elicit potent pro-inflammatory cytokines that can cross the placenta and enter the fetal circulation and subsequently the fetal brain. As a result, pro-inflammatory cytokines can cause neuronal and axonal damage within the brain of HEU neonates, leading to lower NAA levels. Furthermore, low circulating $CD4^+$ T cells within the mother may cause incomplete and delayed GALT restoration due to a decreased number of T cells that could travel to the gut (430,435,436). This would allow ongoing microbial translocation despite ART treatment.

The effects of microbial translocation are not the sole cause of the observed immune activation. HIV antigens and HIV gene products, like the envelope protein gp120 (108–110) and the accessory protein

Nef (111–113), also contribute to the direct activation of lymphocytes and macrophages (437). Studies find an association between low $CD4^+$ T cells and higher viral loads. This also contributes to the increased trafficking of pro-inflammatory cytokines across the placenta into the fetal circulation and brain, which could influence neurometabolites.

4.5 Negative correlation between NAA/Cr+PCr ratios across all neonates and maternal Harvard Trauma Scores

We found a negative correlation between NAA/Cr+PCr ratios and maternal Harvard Trauma Scores during pregnancy across all neonates. According to our findings, higher maternal Harvard Trauma Scores resulted in lower NAA/Cr+PCr ratios in all neonates. Wu et al. found an association between elevated maternal depression and decreased NAA levels in the fetal brain, which is in accordance with our findings (438). Furthermore, adult rats exposed to prenatal stress showed reduced NAA levels in the frontal cortex and hypothalamus (439–441). Given that NAA is a neuronal marker, lower levels suggest neuronal and axonal loss and/or damage. Since a variety of ion channels and receptors are embedded in the membranes of neurons that are responsible for translating external signals to downstream intracellular signalling (313), such loss and/or damage would impact neuronal connection and transmission. Cholinergic neurons, for example, would be implicated and are essential for cognitive, learning, and memory functions. Therefore, loss and/or damage of these neurons may contribute to the poor social and cognitive (such as language and IQ) outcomes (442), impaired psychological functioning, anxiety, and poor school performance (443,444) found in infants whose mothers have PTSD.

4.6 Conclusion

High levels of HIV antigens and HIV gene products like the envelope protein gp120 (108–110) and the accessory protein Nef (111–113), along with increased microbial translocation, result in chronic immune activation and inflammation (43,77,83,437). A direct consequence is the increased secretion of pro-inflammatory cytokines that can gain access to the fetal circulation and the brain via the placenta. Pro-inflammatory cytokines in the fetal brain result in microglia activation, leading to the release of more pro-inflammatory cytokines. However, like peripheral macrophages, microglia express α7 nAChRs, and upon activation, they inhibit the production and release of pro-inflammatory cytokines. As a result, they prevent neuroinflammation along with neuronal and oligodendrocyte (non-neuronal cells) loss and/or damage (428,445). Hence, we found no differences in certain metabolite/Cr+PCr ratios between HEU and HUU neonates. However, in response to subsequent infection or injury in the periphery, frequently primed microglia may cause an overproduction of pro-inflammatory cytokines (an exacerbated response) along with free radicals within the brain (214) which may result in altered neurometabolism at older ages. This mechanism may explain the reduced levels of NAA and Glu previously reported in 9- year-old HEU children (37).

Differences observed in GPC+PCh/Cr+PCr ratios between HEU and HUU neonates may be a consequence of maternal dietary choline deficiency (along with other micronutrients such as vitamin B_{12} and folate (373–376)) and increased choline-consuming gut microbiota, which metabolize choline to TMA in mothers living with HIV (323,377). These mechanisms result in reduced choline bioavailability delivered to the fetus and neonate during maternal pregnancy and lactation (80.72% of HEU neonates in this study were exclusively breastfed). Furthermore, membrane breakdown occurred as a compensatory mechanism to liberate choline for the synthesis of ACh, which is required to suppress the production and release of pro-inflammatory cytokines from peripheral macrophages and other cytokine-producing cells like microglia (313).

4.7 Strengths and limitations

The strengths of the study include the age of the population, which minimizes potential confounding effects such as feeding practices (introduction of solid food), socioeconomic status, parenting, and education that have the potential to emerge when the MRS is performed after the first 35 days of life. Secondly, the study recruited maternal-neonatal pairs from the same community with a homogenous socioeconomic background.

A limitation of the current study is that the number of HUU neonates was relatively small in comparison to HEU neonates. Secondly, pro-inflammatory markers within the plasma (from both the mothers and neonates) and brain were not collected and assessed, nor were the plasma choline concentrations. Thirdly, the current study is cross-sectional and only examines the differences of metabolite/Cr+PCr ratios between HUU and HEU neonates, as well as HEU (pre-conception) and HEU (post-conception) to HUU neonates in a single age bracket. Longitudinal analysis at different ages would aid in determining the long-term consequences, if any, of altered metabolite/Cr+PCr ratios.

4.8 Future

The results in this study suggest there are quantifiable consequences of HIV exposure on the uninfected infant's developing basal ganglia, independent of maternal ART. While we speculate on possible long-term consequences and underlying mechanisms, future work is needed for confirmation.

Within this cohort, future work would investigate links between metabolite ratios and infant gut microbiome outcomes to explore the proposed links to the microbiome. While we do not have blood samples from mothers and cannot establish maternal choline levels, we have human milk samples. Future work may explore the link between human milk composition and infant metabolite levels. In addition, future work quantifying choline levels in human milk and infant blood at this point may help better understand the mechanisms involved in this finding. Once additional outcomes are available, advanced statistical modelling to identify the relationships between these variables is needed, as there are potentially multiple mechanisms involved.

In order to better understand the potential consequences of this finding, work studying the links between metabolite/Cr+PCr ratios and later cognitive performance may be investigated. In addition, analysis of MRS data at later time points is needed to establish if this finding is unique to neonates or persists in infancy and childhood.

5. References

1. Gilbert EM, Darin KM, Scarsi KK, McLaughlin MM. Antiretroviral pharmacokinetics in pregnant women. Pharmacotherapy [Internet]. 2015 Sep 1 [cited 2020 Feb 23];35(9):838–55. Available from: http://doi.wiley.com/10.1002/phar.1626
2. Pariente G, Leibson T, Carls A, Adams-Webber T, Ito S, Koren G. Pregnancy-Associated Changes in Pharmacokinetics: A Systematic Review. Chappell LC, editor. PLOS Med [Internet]. 2016 Nov 1 [cited 2020 Feb 23];13(11):e1002160. Available from: https://dx.plos.org/10.1371/journal.pmed.1002160
3. Global HIV Programme [Internet]. [cited 2023 Jan 26]. Available from: https://www.who.int/data/gho/data/themes/hiv-aids/data-on-the-hiv-aids-response
4. Prendergast AJ, Evans C. Children who are HIV-exposed and uninfected: evidence for action. AIDS. 2023;37(2):205–15.
5. Omoni AO, Ntozini R, Evans C, Prendergast AJ, Moulton LH, Christian PS, et al. Child Growth According to Maternal and Child HIV Status in Zimbabwe. Pediatr Infect Dis J [Internet]. 2017 Sep 1 [cited 2021 Nov 28];36(9):869–76. Available from: https://pubmed.ncbi.nlm.nih.gov/28198792/
6. Evans C, Chasekwa B, Ntozini R, Humphrey JH, Prendergast AJ. Head circumferences of children born to HIV-infected and HIV-uninfected mothers in Zimbabwe during the preantiretroviral therapy era. undefined. 2016 Sep 24;30(15):2323–8.
7. Heidari PhD S, Mofenson MD L, Cotton MMed, PhD MF, Marlink MD R, Cahn MD, PhDI P, Katabira MD E. Antiretroviral Drugs for Preventing Mother-to-Child Transmission of HIV: A Review of Potential Effects on HIV-Exposed but Uninfected Children. J Acquir Immune Defic Syndr [Internet]. 2011;57(4):290. Available from: https://www.lib.uwo.ca/cgi-bin/ezpauthn.cgi?url=http://search.proquest.com/docview/875111397?accountid=15115%5Cnhttp://vr2pk9sx9w.search.serialssolutions.com/?ctx_ver=Z39.88-2004&ctx_enc=info:ofi/enc:UTF-8&rfr_id=info:sid/ProQ%3Apqrl&rft_val_fmt=info:ofi/
8. Desmonde S, Goetghebuer T, Thorne C, Leroy V. Health and survival of HIV perinatally exposed but uninfected children born to HIV-infected mothers. Curr Opin HIV AIDS. 2016;11(5):465–76.
9. Foster C, Lyall H. HIV and mitochondrial toxicity in children. J Antimicrob Chemother. 2008;61(1):8–12.
10. Slogrove AL, Cotton MF, Esser MM. Severe infections in HIV-exposed uninfected infants: Clinical evidence of immunodeficiency. J Trop Pediatr. 2009;56(2):75–81.
11. Filteau S. The HIV-exposed, uninfected African child. Trop Med Int Heal. 2009;14(3):276–87.
12. Sperling RS, Shapiro DE, McSherry GD, Britto P, Cunningham BE, Culnane M, et al. Safety of the maternal-infant zidovudine regimen utilized in the Pediatric AIDS Clinical Trial Group 076 study. Aids. 1998;12(14):1805–13.
13. Afran L, Garcia Knight M, Nduati E, Urban BC, Heyderman RS, Rowland-Jones SL. HIV-exposed uninfected children: A growing population with a vulnerable immune system? Vol. 176, Clinical and Experimental Immunology. Wiley-Blackwell; 2014. p. 11–22.
14. Le Doaré K, Bland R, Newell ML. Neurodevelopment in children born to HIV-infected mothers by infection and treatment status [Internet]. Vol. 130, Pediatrics. American Academy of Pediatrics; 2012 [cited 2020 Feb 23]. p. e1326–44. Available from: http://pediatrics.aappublications.org/cgi/doi/10.1542/peds.2012-0405
15. Slogrove A, Reikie B, Naidoo S, Beer C De, Ho K, Cotton M, et al. HIV-exposed uninfected

infants are at increased risk for severe infections in the first year of life. J Trop Pediatr [Internet]. 2012 [cited 2020 Feb 24];58(6):505. Available from: http://m.tropej.oxfordjournals.org/content/53/2/103.short

16. McHenry MS, McAteer CI, Oyungu E, McDonald BC, Bosma CB, Mpofu PB, et al. Neurodevelopment in Young Children Born to HIV-Infected Mothers: A Meta-analysis. Pediatrics [Internet]. 2018 Feb 1 [cited 2020 Feb 24];141(2). Available from: http://www.ncbi.nlm.nih.gov/pubmed/29374109

17. Stiles J, Jernigan TL. The basics of brain development. Vol. 20, Neuropsychology Review. 2010. p. 327–48.

18. Knickmeyer RC, Gouttard S, Kang C, Evans D, Wilber K, Smith JK, et al. A structural MRI study of human brain development from birth to 2 years. J Neurosci [Internet]. 2008 Nov 19 [cited 2020 Feb 28];28(47):12176–82. Available from: http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.3479-08.2008

19. Blanquie O, Liebmann L, Hübner CA, Luhmann HJ, Sinning A, Genetics H, et al. Longitudinal development of cortical and subcortical gray matter from birth to 2 years. 2017 [cited 2020 Feb 28];(February):1644–59. Available from: https://www.ncbi.nlm.nih.gov/m/pubmed/25953770/?i=4&from=/20807747/related

20. Bale TL. Epigenetic and transgenerational reprogramming of brain development [Internet]. Vol. 16, Nature Reviews Neuroscience. Nature Publishing Group; 2015 [cited 2020 Feb 28]. p. 332–44. Available from: http://www.nature.com/articles/nrn3818

21. Shallie PD, Naicker T. The placenta as a window to the brain: A review on the role of placental markers in prenatal programming of neurodevelopment [Internet]. Vol. 73, International Journal of Developmental Neuroscience. Elsevier Ltd; 2019 [cited 2020 Feb 28]. p. 41–9. Available from: http://doi.wiley.com/10.1016/j.ijdevneu.2019.01.003

22. Boivin MJ, Green SDR, Davies AG, Giordani B, Mokili JKL, Cutting WAM. A Preliminary Evaluation of the Cognitive and Motor Effects of Pediatric HIV Infection in Zairian Children. Heal Psychol [Internet]. 1995 [cited 2020 Feb 23];14(1):13–21. Available from: http://doi.apa.org/getdoi.cfm?doi=10.1037/0278-6133.14.1.13

23. Drotar D, Olness K, Wiznitzer M, Guay L, Marum L, Svilar G, et al. Neurodevelopmental outcomes of Ugandan infants with human immunodeficiency virus type 1 infection. Pediatrics [Internet]. 1997 Jul 1 [cited 2020 Feb 23];100(1):e5–e5. Available from: http://pediatrics.aappublications.org/cgi/doi/10.1542/peds.100.1.e5

24. Bagenda D, Nassali A, Kalyesubula I, Sherman B, Drotar D, Boivin MJ, et al. Health, neurologic, and cognitive status of HIV-infected, long-surviving, and antiretroviral-naive Ugandan children. Pediatrics [Internet]. 2006 Mar 1 [cited 2020 Feb 23];117(3):729–40. Available from: http://pediatrics.aappublications.org/cgi/doi/10.1542/peds.2004-2699

25. Msellati P, Lepage P, Hitimana DG, Van Goethem C, Van de Perre P, Dabis F. Neurodevelopmental testing of children born to human immunodeficiency virus type 1 seropositive and seronegative mothers: a prospective cohort study in Kigali, Rwanda. Pediatrics [Internet]. 1993 Dec [cited 2020 Feb 23];92(6):843–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8233747

26. Laughton B, Cornell M, Grove D, Kidd M, Springer PE, Dobbels E, et al. Early antiretroviral therapy improves neurodevelopmental outcomes in infants. AIDS. 2012 Aug 24;26(13):1685–90.

27. Smith M Lou, Puka K, Sehra R, Read SE, Bitnun A. Longitudinal development of cognitive, visuomotor and adaptive behavior skills in HIV uninfected children, aged 3–5 years of age,

exposed pre- and perinatally to anti-retroviral medications. AIDS Care - Psychol Socio-Medical Asp AIDS/HIV [Internet]. 2017 Oct 3 [cited 2020 Feb 23];29(10):1302–8. Available from: https://www.tandfonline.com/doi/full/10.1080/09540121.2017.1325436

28. Mitchell JM, Rochat TJ, Houle B, Stein A, Newell ML, Bland RM. The effect of maternal and child early life factors on grade repetition among HIV exposed and unexposed children in rural KwaZulu-Natal, South Africa.

29. Nicholson L, Chisenga M, Siame J, Kasonka L, Filteau S. Growth and health outcomes at school age in HIV-exposed, uninfected Zambian children: follow-up of two cohorts studied in infancy. BMC Pediatr 2015 151 [Internet]. 2015 Jun 6 [cited 2021 Aug 11];15(1):1–10. Available from: https://bmcpediatr.biomedcentral.com/articles/10.1186/s12887-015-0386-8

30. Milligan R, Cockcroft K. Working memory profiles in HIV-exposed, uninfected and HIV-infected children: A comparison with neurotypical controls. Front Hum Neurosci. 2017 Jul 7;11.

31. Kerr SJ, Puthanakit T, Vibol U, Aurpibul L, Vonthanak S, Kosalaraksa P, et al. Neurodevelopmental outcomes in HIV-exposed-uninfected children versus those not exposed to HIV. AIDS Care - Psychol Socio-Medical Asp AIDS/HIV. 2014 Nov 2;26(11):1327–35.

32. Van Rie A, Mupuala A, Dow A. Impact of the HIV/AIDS epidemic on the neurodevelopment of preschool-aged children in Kinshasa, democratic Republic of the Congo. Pediatrics. 2008 Jul;122(1).

33. Avison MJ, Nath A, Berger JR. Understanding pathogenesis and treatment of HIV dementia: A role for magnetic resonance? [Internet]. Vol. 25, Trends in Neurosciences. 2002 [cited 2020 Feb 17]. p. 468–73. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0166223602022348

34. Tucker KA, Robertson KR, Lin W, Smith JK, An H, Chen Y, et al. Neuroimaging in human immunodeficiency virus infection. In: Journal of Neuroimmunology [Internet]. 2004 [cited 2020 Feb 17]. p. 153–62. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0165572804003467

35. Mbugua KK, Holmes MJ, Cotton MF, Ratai EM, Little F, Hess AT, et al. HIV-associated CD4+/CD8+ depletion in infancy is associated with neurometabolic reductions in the basal ganglia at age 5 years despite early antiretroviral therapy. AIDS. 2016;30(9):1353–62.

36. Keller MA, Venkatraman TN, Thomas A, Deveikis A, LoPresti C, Hayes J, et al. Altered neurometabolite development in HIV-infected children: Correlation with neuropsychological tests. Neurology [Internet]. 2004 May 25 [cited 2020 Feb 17];62(10):1810–7. Available from: http://www.neurology.org/cgi/doi/10.1212/01.WNL.0000125492.57419.25

37. Robertson FC, Holmes MJ, Cotton MF, Dobbels E, Little F, Laughton B, et al. Perinatal HIV infection or exposure is associated with low n-acetylaspartate and glutamate in basal ganglia at age 9 but not 7 years. Front Hum Neurosci [Internet]. 2018 May 7 [cited 2020 Feb 17];12. Available from: http://journal.frontiersin.org/article/10.3389/fnhum.2018.00145/full

38. Fitzgerald E, Hor K, Drake AJ. Maternal influences on fetal brain development: The role of nutrition, infection and stress, and the potential for intergenerational consequences. Early Hum Dev [Internet]. 2020 Nov 1 [cited 2023 Jan 18];150. Available from: https://pubmed.ncbi.nlm.nih.gov/32948364/

39. Amaral Y, Silva L, Soares F, Marano D, Nehab S, Abranches A, et al. What Are the Maternal Factors that Potentially Intervenes in the Nutritional Composition of Human Milk? Nutrients [Internet]. 2021 [cited 2023 Jan 18];13(5). Available from: https://pubmed.ncbi.nlm.nih.gov/34068716/

40. Juncker HG, Naninck EFG, Schipper L, Lucassen PJ, van Goudoever JB, de Rooij SR, et al. Maternal stress in the postpartum period is associated with altered human milk fatty acid composition. Clin Nutr [Internet]. 2022 Nov 1 [cited 2023 Jan 18];41(11):2517–28. Available from: https://pubmed.ncbi.nlm.nih.gov/36223713/

41. Bravi F, Wiens F, Decarli A, Dal Pont A, Agostoni C, Ferraroni M. Impact of maternal nutrition on breast-milk composition: a systematic review. Am J Clin Nutr [Internet]. 2016 Sep 1 [cited 2023 Jan 18];104(3):646–62. Available from: https://pubmed.ncbi.nlm.nih.gov/27534637/

42. Mzingwane ML, Tiemessen CT. Mechanisms of HIV persistence in HIV reservoirs. 2017 [cited 2021 Aug 12]; Available from: https://doi.org/10.1002/rmv.1924

43. Appay V, Sauce D. Immune activation and inflammation in HIV-1 infection: causes and consequences. J Pathol J Pathol [Internet]. 2008 [cited 2021 Aug 12];214:231–41. Available from: www.interscience.wiley.com

44. A I. Mucosal dendritic cells. Annu Rev Immunol [Internet]. 2007 [cited 2021 Aug 13];25:381–418. Available from: https://pubmed.ncbi.nlm.nih.gov/17378762/

45. Veazey RS, DeMaria M, Chalifoux LV, Shvetz DE, Pauley DR, Knight HL, Rosenzweig M, Johnson RP, Desrosiers RC LA. Gastrointestinal tract as a major site of CD4+ T cell depletion and viral replication in SIV infection. Science [Internet]. 1998 Apr 17 [cited 2021 Aug 12];280(5362):427–31. Available from: https://pubmed.ncbi.nlm.nih.gov/9545219/

46. Mattapallil JJ, Douek DC, Hill B, Nishimura Y, Martin M RM. Massive infection and loss of memory CD4+ T cells in multiple tissues during acute SIV infection. Nature [Internet]. 2005 Apr 28 [cited 2021 Aug 12];434(7037):1093–7. Available from: https://pubmed.ncbi.nlm.nih.gov/15793563/

47. Smit-McBride Z, Mattapallil JJ, McChesney M, Ferrick D, Dandekar S. Gastrointestinal T Lymphocytes Retain High Potential for Cytokine Responses but Have Severe CD4+ T-Cell Depletion at All Stages of Simian Immunodeficiency Virus Infection Compared to Peripheral Lymphocytes. J Virol [Internet]. 1998 Aug [cited 2021 Aug 14];72(8):6646. Available from: /pmc/articles/PMC109855/

48. Reinhart TA, Fallert BA, Pfeifer ME, Sanghavi S, Capuano S 3rd, Rajakumar P, Murphey-Corb M, Day R, Fuller CL ST. Increased expression of the inflammatory chemokine CXC chemokine ligand 9/monokine induced by interferon-gamma in lymphoid tissues of rhesus macaques during simian immunodeficiency virus infection and acquired immunodeficiency syndrome. Blood [Internet]. 2002 May 1 [cited 2021 Aug 14];99(9):3119–28. Available from: https://pubmed.ncbi.nlm.nih.gov/11964273/

49. Guadalupe M, Reay E, Sankaran S, Prindiville T, Flamm J, McNeil A, et al. Severe CD4+ T-Cell Depletion in Gut Lymphoid Tissue during Primary Human Immunodeficiency Virus Type 1 Infection and Substantial Delay in Restoration following Highly Active Antiretroviral Therapy. J Virol [Internet]. 2003 Nov [cited 2021 Aug 12];77(21):11708. Available from: /pmc/articles/PMC229357/

50. Puleston J, Cooper M, Murch S, Bid K, Makh S, Ashwood P, Bingham AH, Green H, Moss P, Dhillon A, Morris R, Strobel S, Gelinas R, Pounder RE PA. A distinct subset of chemokines dominates the mucosal chemokine response in inflammatory bowel disease. Aliment Pharmacol Ther [Internet]. 2005 Jan 15 [cited 2021 Aug 19];21(2):109–20. Available from: https://pubmed.ncbi.nlm.nih.gov/15679760/

51. Brenchley JM, Schacker TW, Ruff LE, Price DA, Taylor JH, Beilman GJ, et al. CD4+ T Cell Depletion during all Stages of HIV Disease Occurs Predominantly in the Gastrointestinal Tract. J Exp Med [Internet]. 2004 Sep 20 [cited 2021 Aug 13];200(6):749. Available from: /pmc/articles/PMC2211962/

52. Chun T-W, Nickle DC, Justement JS, Meyers JH, Roby G, Hallahan CW, et al. Persistence of HIV in Gut-Associated Lymphoid Tissue despite Long-Term Antiretroviral Therapy. 2008;

53. Chun T-W, Nickle DC, Justement JS, Large D, Semerjian A, Curlin ME, et al. HIV-infected individuals receiving effective antiviral therapy for extended periods of time continually replenish their viral reservoir. J Clin Invest [Internet]. 2005 Nov [cited 2021 Aug 12];115(11):3250. Available from: /pmc/articles/PMC1265878/

54. Hermankova M, Siliciano JD, Zhou Y, Monie D, Chadwick K, Margolick JB, et al. Analysis of Human Immunodeficiency Virus Type 1 Gene Expression in Latently Infected Resting CD4+ T Lymphocytes In Vivo. J Virol [Internet]. 2003 Jul [cited 2021 Aug 12];77(13):7383. Available from: /pmc/articles/PMC164778/

55. Campion EW, Luzuriaga K, Mofenson LM. Challenges in the Elimination of Pediatric HIV-1 Infection. New Engl J Med Downloaded from nejm.org. 2016;374:761–70.

56. Rainwater-Lovett K, Luzuriaga K, Persaud D. Very early combination antiretroviral therapy in infants: prospects for cure. Curr Opin HIV AIDS [Internet]. 2015 Jan 12 [cited 2021 Aug 12];10(1):4. Available from: /pmc/articles/PMC4351817/

57. Persaud D, Gay H, Ziemniak C, Chen YH, Piatak MJ, Chun T-W, et al. Absence of Detectable HIV-1 Viremia after Treatment Cessation in an Infant. http://dx.doi.org/101056/NEJMoa1302976 [Internet]. 2013 Nov 6 [cited 2021 Aug 12];369(19):1828–35. Available from: https://www.nejm.org/doi/full/10.1056/nejmoa1302976

58. Perelson AS, Essunger P, Cao Y, Vesanen M, Hurley A, Saksela K, Markowitz M HD. Decay characteristics of HIV-1-infected compartments during combination therapy. Nature [Internet]. 1997 May 8 [cited 2021 Aug 12];387(6629):188–91. Available from: https://pubmed.ncbi.nlm.nih.gov/9144290/

59. Siliciano JD, Kajdas J, Finzi D, Quinn TC, Chadwick K, Margolick JB, Kovacs C, Gange SJ SR. Long-term follow-up studies confirm the stability of the latent reservoir for HIV-1 in resting CD4+ T cells. Nat Med [Internet]. 2003 Jun 1 [cited 2021 Aug 12];9(6):727–8. Available from: https://pubmed.ncbi.nlm.nih.gov/12754504/

60. Havlir DV, Strain MC, Clerici M, Ignacio C, Trabattoni D, Ferrante P WJ. Productive infection maintains a dynamic steady state of residual viremia in human immunodeficiency virus type 1-infected persons treated with suppressive antiretroviral therapy for five years. J Virol [Internet]. 2003 Oct 15 [cited 2021 Aug 12];77(20):11212–9. Available from: https://pubmed.ncbi.nlm.nih.gov/14512569/

61. Persaud D, Palumbo PE, Ziemniak C, Hughes MD, Alvero CG, Luzuriaga K, et al. Dynamics of the resting CD4+ T-cell latent HIV reservoir in infants initiating HAART less than 6 months of age. AIDS [Internet]. 2012 Jul 31 [cited 2021 Aug 12];26(12):1483. Available from: /pmc/articles/PMC3495308/

62. Chun T-W, Engel D, Berrey MM, Shea T, Corey L, Fauci AS. Early establishment of a pool of latently infected, resting CD4+ T cells during primary HIV-1 infection. Proc Natl Acad Sci U S A [Internet]. 1998 Jul 21 [cited 2021 Aug 12];95(15):8869. Available from: /pmc/articles/PMC21169/

63. Matthew C. Strain, Susan J. Little, Eric S. Daar, Diane V. Havlir, Huldrych F. Günthard, Ruby Y. Lam, Otto A. Daly, Juin Nguyen, Caroline C. Ignacio, Celsa A. Spina, Douglas D. Richman JKW. Effect of treatment, during primary infection, on establishment and clearance of cellular reservoirs of HIV-1. J Infect Dis [Internet]. 2005 May 1 [cited 2021 Aug 12];191(9):1410–8. Available from: https://pubmed.ncbi.nlm.nih.gov/15809898/

64. Stevenson M. HIV-1 pathogenesis. 2003 [cited 2021 Aug 17]; Available from: http://www.nature.com/naturemedicine

65. Fletcher C V., Staskus K, Wietgrefe SW, Rothenberger M, Reilly C, Chipman JG, et al. Persistent HIV-1 replication is associated with lower antiretroviral drug concentrations in lymphatic tissues. Proc Natl Acad Sci U S A [Internet]. 2014 Feb 11 [cited 2021 Aug 12];111(6):2307. Available from: /pmc/articles/PMC3926074/

66. van Marle G, Church DL, Nunweiler KD, Cannon K, Wainberg MA, Gill MJ. Higher levels of Zidovudine resistant HIV in the colon compared to blood and other gastrointestinal compartments in HIV infection. Retrovirology [Internet]. 2010 Sep 13 [cited 2022 Jan 21];7:74. Available from: /pmc/articles/PMC2949729/

67. Chung, Doo Ryeon, Dennis L. Kasper, Ronald J. Panzo, Tanuja Chtinis, M. J. Grusby MHS and AOT. CD4+ T cells mediate abscess formation in intra-abdominal sepsis by an IL-17-dependent mechanism. J Immunol [Internet]. 2003 Feb 15 [cited 2021 Aug 19];170(4):1958–63. Available from: https://pubmed.ncbi.nlm.nih.gov/12574364/

68. Kao CY, Chen Y, Thai P, Wachi S, Huang F, Kim C, Harper RW WR. IL-17 markedly up-regulates beta-defensin-2 expression in human airway epithelium via JAK and NF-kappaB signaling pathways. J Immunol [Internet]. 2004 Sep 1 [cited 2021 Aug 19];173(5):3482–91. Available from: https://pubmed.ncbi.nlm.nih.gov/15322213/

69. Liang SC, Tan XY, Luxenberg DP, Karim R, Dunussi-Joannopoulos K, Collins M F LA. Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. J Exp Med [Internet]. 2006 [cited 2021 Aug 19];203(10):2271–9. Available from: https://pubmed.ncbi.nlm.nih.gov/16982811/

70. Kolls JK LA. Interleukin-17 family members and inflammation. Immunity [Internet]. 2004 Oct [cited 2021 Aug 19];21(4):467–76. Available from: https://pubmed.ncbi.nlm.nih.gov/15485625/

71. Raffatellu M, Santos RL, Verhoeven DE, George MD, Wilson RP, Winter SE, et al. Simian immunodeficiency virus–induced mucosal interleukin-17 deficiency promotes Salmonella dissemination from the gut. Nat Med [Internet]. 2008 Apr [cited 2021 Aug 19];14(4):421. Available from: /pmc/articles/PMC2901863/

72. C H, S D, P K, R D, RM D, CH H. Human immunodeficiency virus infection of enterocytes and mononuclear cells in human jejunal mucosa. Gastroenterology [Internet]. 1991 [cited 2021 Aug 19];100(6):1521–7. Available from: https://pubmed.ncbi.nlm.nih.gov/2019358/

73. Epple HJ, Allers K, Tröger H, Kühl A, Erben U, Fromm M, et al. Acute HIV infection induces mucosal infiltration with CD4+ and CD8+ T cells, epithelial apoptosis, and a mucosal barrier defect. Gastroenterology. 2010;139(4).

74. Nazli A, Chan O, Dobson-Belaire WN, Ouellet M, Tremblay MJ, Gray-Owen SD, Arsenault AL KC. Exposure to HIV-1 directly impairs mucosal epithelial barrier integrity allowing microbial translocation. PLoS Pathog [Internet]. 2010 [cited 2021 Aug 19];6(4):1–20. Available from: https://pubmed.ncbi.nlm.nih.gov/20386714/

75. Asmuth, David M SMH and CAW. Physiological effects of HIV infection on human intestinal epithelial cells: an in vitro model for HIV enteropathy. AIDS [Internet]. 1994 [cited 2021 Aug 19];8(2):205–11. Available from: https://pubmed.ncbi.nlm.nih.gov/7913816/

76. Bettelli E, Korn T, Oukka M, Kuchroo VK. Induction and effector functions of TH17 cells. Nature [Internet]. 2008 Jun 19 [cited 2021 Aug 19];453(7198):1051. Available from: /pmc/articles/PMC6280661/

77. Brenchley JM, Price DA, Schacker TW, Asher TE, Silvestri G, Rao S, et al. Microbial

translocation is a cause of systemic immune activation in chronic HIV infection. 2006 [cited 2021 Aug 18]; Available from: http://www.nature.com/naturemedicine

78. Ziegler TR, Luo M, Estívariz CF, Moore DA 3rd, Sitaraman SV, Hao L, Bazargan N, Klapproth JM, Tian J, Galloway JR, Leader LM, Jones DP GA. Detectable serum flagellin and lipopolysaccharide and upregulated anti-flagellin and lipopolysaccharide immunoglobulins in human short bowel syndrome. Am J Physiol Regul Integr Comp Physiol [Internet]. 2008 Feb [cited 2021 Aug 18];294(2). Available from: https://pubmed.ncbi.nlm.nih.gov/18003793/

79. TB C, KM D, ES L, AY Z, Y Y, JN W. Recognition of peptidoglycan from the microbiota by Nod1 enhances systemic innate immunity. Nat Med [Internet]. 2010 Feb [cited 2021 Aug 18];16(2):228–31. Available from: https://pubmed.ncbi.nlm.nih.gov/20081863/

80. Jiang W, Lederman MM, Hunt P, Sieg SF, Haley K, Rodriguez B, et al. Plasma Levels of Bacterial DNA Correlate with Immune Activation and the Magnitude of Immune Restoration in Persons with Antiretroviral-Treated HIV Infection. J Infect Dis [Internet]. 2009 Apr 15 [cited 2021 Aug 18];199(8):1177. Available from: /pmc/articles/PMC2728622/

81. Haruta I, Hashimoto E, Shibata N, Kato Y, Kobayashi M SK. Lipoteichoic acid may affect the pathogenesis of PBC-like bile duct damage and might be involved in systemic multifocal epithelial inflammations in chronic colitis-harboring TCRalpha-/-xAIM-/- mice. Autoimmunity [Internet]. 2007 Aug [cited 2021 Aug 18];40(5):372–9. Available from: https://pubmed.ncbi.nlm.nih.gov/17612899/

82. Francés R, Zapater P, González-Navajas JM, Muñoz C, Caño R, Moreu R, Pascual S, Bellot P, Pérez-Mateo M SJ. Bacterial DNA in patients with cirrhosis and noninfected ascites mimics the soluble immune response established in patients with spontaneous bacterial peritonitis. Hepatology [Internet]. 2008 Mar [cited 2021 Aug 18];47(3):978–85. Available from: https://pubmed.ncbi.nlm.nih.gov/18306221/

83. Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nat Publ Gr. 2010;11.

84. Santos-Oliveira JR, Regis EG, Leal CR, Cunha RV, Bozza PT D-CA. Evidence that lipopolisaccharide may contribute to the cytokine storm and cellular activation in patients with visceral leishmaniasis. PLoS Negl Trop Dis [Internet]. 2011 Jul [cited 2021 Aug 19];5(7). Available from: https://pubmed.ncbi.nlm.nih.gov/21765960/

85. Barbara da Silva, William Singer, Ignatius W. Fong CAO. In vivo cytokine and neuroendocrine responses to endotoxin in human immunodeficiency virus-infected subjects. J Infect Dis [Internet]. 1999 [cited 2021 Aug 19];180(1):106–15. Available from: https://pubmed.ncbi.nlm.nih.gov/10353868/

86. Gordon SN, Cervasi B, Odorizzi P, Silverman R, Aberra F, Ginsberg G, et al. Disruption of Intestinal CD4+ T Cell Homeostasis Is a Key Marker of Systemic CD4+ T Cell Activation in HIV-Infected Individuals. J Immunol [Internet]. 2010 Nov 1 [cited 2021 Nov 18];185(9):5169. Available from: /pmc/articles/PMC3155848/

87. Klatt NR, Estes JD, Sun X, Ortiz AM, Barber JS, Harris LD, et al. Loss of mucosal CD103 DCs and IL-17 and IL-22 lymphocytes is associated with mucosal damage in SIV infection. Mucosal Immunol. 2012 Nov;5(6):646–57.

88. Cassol E, Malfeld S, Mahasha P, Van Der Merwe S, Cassol S, Seebregts C, et al. Persistent microbial translocation and immune activation in HIV-1-infected South Africans receiving combination antiretroviral therapy. J Infect Dis [Internet]. 2010 Sep 1 [cited 2021 Nov 16];202(5):723–33. Available from: https://pubmed.ncbi.nlm.nih.gov/20629534/

89. Sandler NG, Wand H, Roque A, Law M, Nason MC, Nixon DE, Pedersen C, Ruxrungtham K,

Lewin SR, Emery S, Neaton JD, Brenchley JM, Deeks SG, Sereti I DD. Plasma levels of soluble CD14 independently predict mortality in HIV infection. J Infect Dis [Internet]. 2011 Mar 15 [cited 2021 Aug 18];203(6):780–90. Available from: https://pubmed.ncbi.nlm.nih.gov/21252259/

90. Deeks SG, Tracy R, Douek DC. Systemic effects of inflammation on health during chronic HIV infection. Immunity [Internet]. 2013 Oct 17 [cited 2021 Nov 16];39(4):633–45. Available from: https://pubmed.ncbi.nlm.nih.gov/24138880/

91. Kristoff J, Haret-Richter G, Ma D, Ribeiro RM, Xu C, Cornell E, et al. Early microbial translocation blockade reduces SIV-mediated inflammation and viral replication. J Clin Invest [Internet]. 2014 Jun 2 [cited 2021 Aug 11];124(6):2802–6. Available from: http://www.jci.org

92. Kawakami K, Scheidereit C RR. Identification and purification of a human immunoglobulin-enhancer-binding protein (NF-kappa B) that activates transcription from a human immunodeficiency virus type 1 promoter in vitro. Proc Natl Acad Sci U S A [Internet]. 1988 [cited 2021 Aug 17];85(13):4700–4. Available from: https://pubmed.ncbi.nlm.nih.gov/3133660/

93. Reeves RK, Rajakumar PA, Evans TI, Connole M, Gillis J, Wong FE, et al. Gut inflammation and indoleamine deoxygenase inhibit IL-17 production and promote cytotoxic potential in NKp44+ mucosal NK cells during SIV infection. Blood [Internet]. 2011 Sep 22 [cited 2021 Nov 16];118(12):3321. Available from: /pmc/articles/PMC3179400/

94. Sandler NG, Douek DC. Microbial translocation in HIV infection: Causes, consequences and treatment opportunities [Internet]. Vol. 10, Nature Reviews Microbiology. 2012 [cited 2020 Feb 14]. p. 655–66. Available from: http://www.nature.com/articles/nrmicro2848

95. Favre D, Mold J, Hunt PW, Kanwar B, Loke P, Seu L, et al. Tryptophan Catabolism by Indoleamine 2,3-Dioxygenase 1 Alters the Balance of TH17 to Regulatory T Cells in HIV Disease. Sci Transl Med [Internet]. 2010 [cited 2021 Aug 18];2(32):32ra36. Available from: /pmc/articles/PMC3034445/

96. Brenchley JM, Price DA, Douek DC. HIV disease: fallout from a mucosal catastrophe? Nat Immunol [Internet]. 2006 [cited 2021 Aug 18];7(3). Available from: http://www.nature.com/natureimmunology

97. Fox ES, Thomas P BS. Clearance of gut-derived endotoxins by the liver. Release and modification of 3H, 14C-lipopolysaccharide by isolated rat Kupffer cells. Gastroenterology [Internet]. 1989 [cited 2021 Jul 27];96(2 Pt 1):456–61. Available from: https://pubmed.ncbi.nlm.nih.gov/2642878/

98. Mathison J, Ulevitch R. The clearance, tissue distribution, and cellular localization of intravenously injected lipopolysaccharide in rabbits. undefined. 1979;

99. Zlydaszyk JC MR. Fate of 51Cr-labeled lipopolysaccharide in tissue culture cells and livers of normal mice. Infect Immun [Internet]. 1976 [cited 2021 Jul 27];14(1):100–5. Available from: https://pubmed.ncbi.nlm.nih.gov/780270/

100. Stabinski L, Reynolds SJ, Ocama P, Laeyendecker O, Boaz I, Ndyanabo A, et al. High Prevalence of Liver Fibrosis Associated with HIV Infection: A Cross-Sectional Study in Rural Rakai, Uganda. Antivir Ther [Internet]. 2011 [cited 2021 Jul 27];16(3):405. Available from: /pmc/articles/PMC3142695/

101. Vispo E, Morello J, Rodriguez-Novoa S S V. Noncirrhotic portal hypertension in HIV infection. Curr Opin Infect Dis [Internet]. 2011 Feb [cited 2021 Jul 27];24(1):12–8. Available from: https://pubmed.ncbi.nlm.nih.gov/21157331/

102. Papagno L, Spina CA, Marchant A, Salio M, Rufer N, Little S, et al. Immune Activation and

CD8+ T-Cell Differentiation towards Senescence in HIV-1 Infection. PLoS Biol [Internet]. 2004 [cited 2021 Aug 17];2(2). Available from: /pmc/articles/PMC340937/

103. Douek DC, Brenchley JM, Betts MR, Ambrozak DR, Hill BJ, Okamoto Y, et al. HIV preferentially infects HIV-specific CD4+ T cells. Nat 2002 4176884 [Internet]. 2002 May 2 [cited 2021 Aug 17];417(6884):95–8. Available from: https://www.nature.com/articles/417095a

104. Betts MR, Ambrozak DR, Douek DC, Bonhoeffer S, Brenchley JM, Casazza JP, et al. Analysis of Total Human Immunodeficiency Virus (HIV)-Specific CD4+ and CD8+ T-Cell Responses: Relationship to Viral Load in Untreated HIV Infection. J Virol [Internet]. 2001 Dec 15 [cited 2021 Aug 18];75(24):11983. Available from: /pmc/articles/PMC116093/

105. Alter G, Suscovich TJ, Teigen N, Meier A, Streeck H, Brander C, et al. Single-stranded RNA derived from HIV-1 serves as a potent activator of NK cells. J Immunol [Internet]. 2007 Jun 15 [cited 2021 Nov 18];178(12):7658–66. Available from: https://pubmed.ncbi.nlm.nih.gov/17548602/

106. Beignon AS, McKenna K, Skoberne M, Manches O, DaSilva I, Kavanagh DG, et al. Endocytosis of HIV-1 activates plasmacytoid dendritic cells via Toll-like receptor–viral RNA interactions. J Clin Invest [Internet]. 2005 Nov [cited 2021 Nov 18];115(11):3265. Available from: /pmc/articles/PMC1253628/

107. Doitsh G, Cavrois M, Lassen KG, Zepeda O, Yang Z, Santiago ML, et al. Abortive HIV infection mediates CD4 T cell depletion and inflammation in human lymphoid tissue. Cell [Internet]. 2010 Nov 24 [cited 2021 Nov 17];143(5):789–801. Available from: https://pubmed.ncbi.nlm.nih.gov/21111238/

108. Decrion AZ, Dichamp I, Varin A, Herbein G. HIV and Inflammation. Curr HIV Res. 2005;3:243–59.

109. Merrill JE, Koyanagi Y, Chen IS. Interleukin-1 and tumor necrosis factor alpha can be induced from mononuclear phagocytes by human immunodeficiency virus type 1 binding to the CD4 receptor. J Virol [Internet]. 1989 Oct [cited 2021 Aug 17];63(10):4404–8. Available from: https://pubmed.ncbi.nlm.nih.gov/2789293/

110. Patke CL, Shearer WT. gp120- and TNF-α-induced modulation of human B cell function: Proliferation, cyclic AMP generation, Ig production, and B-cell receptor expression. J Allergy Clin Immunol. 2000;105(5):975–82.

111. Wang JK, Kiyokawa E, Verdin E TD. The Nef protein of HIV-1 associates with rafts and primes T cells for activation. Proc Natl Acad Sci U S A [Internet]. 2000 Jan 4 [cited 2021 Aug 17];97(1):394–9. Available from: https://pubmed.ncbi.nlm.nih.gov/10618429/

112. Simmons A, Aluvihare V MA. Nef triggers a transcriptional program in T cells imitating single-signal T cell activation and inducing HIV virulence mediators. Immunity [Internet]. 2001 [cited 2021 Aug 17];14(6):763–77. Available from: https://pubmed.ncbi.nlm.nih.gov/11420046/

113. Swingler S, Mann A, Jacqué J, Brichacek B, Sasseville VG, Williams K, Lackner AA, Janoff EN, Wang R, Fisher D SM. HIV-1 Nef mediates lymphocyte chemotaxis and activation by infected macrophages. Nat Med [Internet]. 1999 Sep [cited 2021 Aug 17];5(9):997–1003. Available from: https://pubmed.ncbi.nlm.nih.gov/10470075/

114. Smed-Sörensen A, Loré K, Vasudevan J, Louder MK, Andersson J, Mascola JR, Spetz AL KR. Differential susceptibility to human immunodeficiency virus type 1 infection of myeloid and plasmacytoid dendritic cells. J Virol [Internet]. 2005 Jul [cited 2021 Aug 18];79(14):8861–9. Available from: https://pubmed.ncbi.nlm.nih.gov/15994779/

115. Smith MZ, Bastidas S, Karrer U, Oxenius A. Impact of antigen specificity on CD4+ T cell activation in chronic HIV-1 infection. BMC Infect Dis [Internet]. 2013 Feb 25 [cited 2021 Aug

19];13(1):100. Available from: /pmc/articles/PMC3598342/

116. Doisne JM, Urrutia A, Lacabaratz-Porret C, Goujard C, Meyer L, Chaix ML, Sinet M VA. CD8+ T cells specific for EBV, cytomegalovirus, and influenza virus are activated during primary HIV infection. J Immunol [Internet]. 2004 Aug 15 [cited 2021 Aug 19];173(4):2410–8. Available from: https://pubmed.ncbi.nlm.nih.gov/15294954/

117. Naeger DM, Martin JN, Sinclair E, Hunt PW, Bangsberg DR, Hecht F, Hsue P, McCune JM DS. Cytomegalovirus-specific T cells persist at very high levels during long-term antiretroviral treatment of HIV disease. PLoS One [Internet]. 2010 Jan 29 [cited 2021 Aug 19];5(1). Available from: https://pubmed.ncbi.nlm.nih.gov/20126452/

118. Wittkop L, Bitard J, Lazaro E, Neau D, Bonnet F, Mercie P, et al. Effect of Cytomegalovirus-Induced Immune Response, Self Antigen-Induced Immune Response, and Microbial Translocation on Chronic Immune Activation in Successfully Treated HIV Type 1-Infected Patients: The ANRS CO3 Aquitaine Cohort Downloaded from. J Infect Dis [Internet]. 2011 [cited 2021 Aug 19];207:207. Available from: http://jid.oxfordjournals.org/

119. Mehandru S, Poles MA, Tenner-Racz K, Jean-Pierre P, Manuelli V, Lopez P, et al. Lack of Mucosal Immune Reconstitution during Prolonged Treatment of Acute and Early HIV-1 Infection. PLoS Med [Internet]. 2006 Dec 5 [cited 2021 Aug 12];3(12):e484. Available from: /pmc/articles/PMC1762085/

120. Mehandru S, Poles MA, Tenner-Racz K, Horowitz A, Hurley A, Hogan C, et al. Primary HIV-1 infection is associated with preferential depletion of CD4+ T lymphocytes from effector sites in the gastrointestinal tract. J Exp Med [Internet]. 2004 Sep 20 [cited 2020 Feb 14];200(6):761–70. Available from: https://rupress.org/jem/article/200/6/761/40151/Primary-HIV1-Infection-Is-Associated-with

121. Guadalupe M, Sankaran S, George MD, Reay E, Verhoeven D, Shacklett BL, et al. Viral Suppression and Immune Restoration in the Gastrointestinal Mucosa of Human Immunodeficiency Virus Type 1-Infected Patients Initiating Therapy during Primary or Chronic Infection. J Virol [Internet]. 2006 [cited 2021 Aug 13];80(16):8236. Available from: /pmc/articles/PMC1563811/

122. Mavigner M, Cazabat M, Dubois M, L'Faqihi F-E, Requena M, Pasquier C, et al. Altered CD4+ T cell homing to the gut impairs mucosal immune reconstitution in treated HIV-infected individuals. J Clin Invest [Internet]. 2012 Jan 3 [cited 2021 Aug 13];122(1):62. Available from: /pmc/articles/PMC3248296/

123. Sankaran S, George MD, Reay E, Guadalupe M, Flamm J, Prindiville T, et al. Rapid Onset of Intestinal Epithelial Barrier Dysfunction in Primary Human Immunodeficiency Virus Infection Is Driven by an Imbalance between Immune Response and Mucosal Repair and Regeneration. J Virol. 2008 Jan 1;82(1):538–45.

124. Hunt PW, Sinclair E, Rodriguez B, Shive C, Clagett B, Funderburg N, Robinson J, Huang Y, Epling L, Martin JN, Deeks SG, Meinert CL, Van Natta ML, Jabs DA LM. Gut epithelial barrier dysfunction and innate immune activation predict mortality in treated HIV infection. J Infect Dis [Internet]. 2014 Oct 15 [cited 2021 Aug 18];210(8):1228–38. Available from: https://pubmed.ncbi.nlm.nih.gov/24755434/

125. Kelesidis T, Kendall MA, Yang OO, Hodis HN, Currier JS. Biomarkers of Microbial Translocation and Macrophage Activation: Association With Progression of Subclinical Atherosclerosis in HIV-1 Infection. J Infect Dis [Internet]. 2012 Nov 15 [cited 2021 Aug 18];206(10):1558. Available from: /pmc/articles/PMC3475633/

126. Ancuta P, Kamat A, Kunstman KJ, Kim EY, Autissier P, Wurcel A, et al. Microbial Translocation Is Associated with Increased Monocyte Activation and Dementia in AIDS Patients. PLoS One

[Internet]. 2008 Jun 25 [cited 2021 Nov 16];3(6). Available from: /pmc/articles/PMC2424175/

127. Gumusoglu SB, Stevens HE. Maternal Inflammation and Neurodevelopmental Programming: A Review of Preclinical Outcomes and Implications for Translational Psychiatry. Biol Psychiatry [Internet]. 2019 Jan 15 [cited 2022 Jan 21];85(2):107–21. Available from: https://pubmed.ncbi.nlm.nih.gov/30318336/

128. Hagberg H, Mallard C. Effect of inflammation on central nervous system development and vulnerability. Curr Opin Neurol. 2005;18:117–23.

129. Croonenberghs J, Bosmans E, Deboutte D, Kenis G, Maes M. Activation of the inflammatory response system in autism. Neuropsychobiology [Internet]. 2002 [cited 2021 Nov 22];45(1):1–6. Available from: https://pubmed.ncbi.nlm.nih.gov/11803234/

130. Malek-Ahmadi P. Cytokines and etiopathogenesis of pervasive developmental disorders. Med Hypotheses. 2001 Mar 1;56(3):321–4.

131. Patterson PH. Maternal infection: window on neuroimmune interactions in fetal brain development and mental illness. Curr Opin Neurobiol [Internet]. 2002 Feb 1 [cited 2021 Nov 23];12(1):115–8. Available from: https://pubmed.ncbi.nlm.nih.gov/11861174/

132. Gilmore JH, Jarskog LF, Vadlamudi S, Lauder JM. Prenatal Infection and Risk for Schizophrenia: IL-1β, IL-6, and TNFα Inhibit Cortical Neuron Dendrite Development. Neuropsychopharmacol 2004 297 [Internet]. 2004 Apr 7 [cited 2021 Nov 23];29(7):1221–9. Available from: https://www.nature.com/articles/1300446

133. Patterson PH. Maternal infection and immune involvement in autism. Trends Mol Med. 2011 Jul 1;17(7):389–94.

134. Jonakait GM. The effects of maternal inflammation on neuronal development: possible mechanisms. [cited 2021 Aug 30]; Available from: www.elsevier.com/locate/ijdevneu

135. Irving WL, James DK, Stephenson T, Laing P, Jameson C, Oxford JS, Chakraverty P, Brown DW, Boon AC ZM. Influenza virus infection in the second and third trimesters of pregnancy: a clinical and seroepidemiological study. BJOG [Internet]. 2000 [cited 2021 Sep 9];107(10):1282–9. Available from: https://pubmed.ncbi.nlm.nih.gov/11028582/

136. Shi L, Tu N PP. Maternal influenza infection is likely to alter fetal brain development indirectly: the virus is not detected in the fetus. Int J Dev Neurosci [Internet]. 2005 [cited 2021 Sep 9];23(2–3):299–305. Available from: https://pubmed.ncbi.nlm.nih.gov/15749254/

137. Short SJ, Lubach GR, Karasin AI, Olsen CW, Styner M, Knickmeyer RC, Gilmore JH CC. Maternal influenza infection during pregnancy impacts postnatal brain development in the rhesus monkey. Biol Psychiatry [Internet]. 2010 May 15 [cited 2021 Sep 9];67(10):965–73. Available from: https://pubmed.ncbi.nlm.nih.gov/20079486/

138. Urakubo A, Jarskog LF, Lieberman JA, Gilmore JH. Prenatal exposure to maternal infection alters cytokine expression in the placenta, amniotic fluid, and fetal brain. Schizophr Res [Internet]. 2001 [cited 2021 Aug 25];47:27–36. Available from: www.elsevier.com/locate/schres

139. Bell MJ, Hallenbeck JM, Gallo V. Determining the Fetal Inflammatory Response in an Experimental Model of Intrauterine Inflammation in Rats. Pediatr Res 2004 564 [Internet]. 2004 Oct [cited 2021 Nov 27];56(4):541–6. Available from: https://www.nature.com/articles/pr2004512

140. Gilmore JH, Jarskog LF, Vadlamudi S. Maternal poly I:C exposure during pregnancy regulates TNFa, BDNF, and NGF expression in neonatal brain and the maternal-fetal unit of the rat. [cited 2021 Aug 25]; Available from: www.elsevier.com/locate/jneuroim

141. Meyer U, Nyffeler M, Engler A, Urwyler A, Schedlowski M, Knuesel I, et al. The Time of Prenatal Immune Challenge Determines the Specificity of Inflammation-Mediated Brain and Behavioral Pathology. J Neurosci [Internet]. 2006 [cited 2021 Aug 30];26(18):4752. Available from: /pmc/articles/PMC6674174/

142. Dahlgren J, Samuelsson A-M, Jansson T, Holmäng A. Interleukin-6 in the maternal circulation reaches the rat fetus in mid-gestation. Pediatr Res [Internet]. 2006 Aug [cited 2021 Aug 25];60(2):147–51. Available from: https://pubmed.ncbi.nlm.nih.gov/16864694/

143. Ponzio NM, Servatius R, Beck K, Marzouk A, Kreider T. Cytokine Levels during Pregnancy Influence Immunological Profiles and Neurobehavioral Patterns of the Offspring. Ann NY Acad Sci. 2007;1107:118–28.

144. Zaretsky M V., Alexander JM, Byrd W, Bawdon RE. Transfer of inflammatory cytokines across the placenta. Obstet Gynecol [Internet]. 2004 Mar [cited 2021 Aug 25];103(3):546–50. Available from: https://pubmed.ncbi.nlm.nih.gov/14990420/

145. Letterio J, Geiser A, Kulkarni A, Roche N, Sporn M, Roberts A. Maternal rescue of transforming growth factor-beta 1 null mice. Science (80-) [Internet]. 1994 Jun 24 [cited 2021 Aug 30];264(5167):1936–8. Available from: https://science.sciencemag.org/content/264/5167/1936

146. Calhoun DA, Gersting JA, Lunøe M, Du Y CR. Transfer of recombinant human granulocyte colony stimulating factor (rhG-CSF) from the maternal to the fetal circulation is not dependent upon a functional G-CSF-receptor. Placenta [Internet]. 2001 [cited 2021 Aug 30];22(6):609–12. Available from: https://pubmed.ncbi.nlm.nih.gov/11440552/

147. Aaltonen R, Heikkinen T, Hakala K, Laine K, Alanen A. Transfer of proinflammatory cytokines across term placenta. Obstet Gynecol [Internet]. 2005 [cited 2021 Nov 27];106(4):802–7. Available from: https://pubmed.ncbi.nlm.nih.gov/16199639/

148. Pasca AM, Penn AA. The placenta: The lost neuroendocrine organ. Neoreviews. 2010;11(2).

149. Hsiao EY, Patterson PH. Activation of the maternal immune system induces endocrine changes in the placenta via IL-6. Brain Behav Immun [Internet]. 2011 May [cited 2022 Feb 2];25(4):604–15. Available from: https://pubmed.ncbi.nlm.nih.gov/21195166/

150. Holmlund U, Cebers G, Dahlfors AR, Sandstedt B, Bremme K, EkstrÖm ES, et al. Expression and regulation of the pattern recognition receptors Toll-like receptor-2 and Toll-like receptor-4 in the human placenta. Immunology [Internet]. 2002 [cited 2021 Aug 25];107(1):145. Available from: /pmc/articles/PMC1782774/

151. Schaefer TM, Fahey J V., Wright JA, Wira CR. Innate Immunity in the Human Female Reproductive Tract: Antiviral Response of Uterine Epithelial Cells to the TLR3 Agonist Poly(I:C). J Immunol [Internet]. 2005 Jan 15 [cited 2021 Aug 30];174(2):992–1002. Available from: https://www.jimmunol.org/content/174/2/992

152. Schaefer TM, Desouza K, Fahey JV, Beagley KW WC. Toll-like receptor (TLR) expression and TLR-mediated cytokine/chemokine production by human uterine epithelial cells. Immunology [Internet]. 2004 Jul [cited 2021 Aug 30];112(3):428–36. Available from: https://pubmed.ncbi.nlm.nih.gov/15196211/

153. Abrahams VM MG. Toll-like receptors and their role in the trophoblast. Placenta [Internet]. 2005 [cited 2021 Aug 30];26(7):540–7. Available from: https://pubmed.ncbi.nlm.nih.gov/15993703/

154. Reuben JM, Turpin JA, Lee B-N, Doyle M, Gonik B, Jacobson R, et al. Induction of Inflammatory Cytokines in Placental Monocytes of Gravidae Infected with the Human Immunodeficiency Virus Type 1. J Interf CYTOKINE Res. 1996;16:963–71.

155. Lee B-N, Ordonez N, Popek EJ, Lu J-G, Helfgott A, Eriksen N, et al. Inflammatory Cytokine Expression Is Correlated with the Level of Human Immunodeficiency Virus (HIV) Transcripts in HIV-Infected Placental Trophoblastic Cells. J Virol [Internet]. 1997 [cited 2021 Sep 6];71(5):3628–35. Available from: https://journals.asm.org/journal/jvi

156. Faye A, Pornprasert S, Mary J-Y, Dolcini G, Derrien M, Barré-Sinoussi F, et al. Characterization of the main placental cytokine profiles from HIV-1-infected pregnant women treated with anti-retroviral drugs in France. Clin Exp Immunol [Internet]. 2007 Sep [cited 2021 Sep 11];149(3):430. Available from: /pmc/articles/PMC2219329/

157. Thellin O, Coumans B, Zorzi W, Igout A HE. Tolerance to the foeto-placental "graft": ten ways to support a child for nine months. Curr Opin Immunol [Internet]. 2000 [cited 2021 Sep 9];12(6):731–7. Available from: https://pubmed.ncbi.nlm.nih.gov/11102780/

158. Sykes L, MacIntyre DA, Yap XJ, Teoh TG BP. The Th1:th2 dichotomy of pregnancy and preterm labour. Mediators Inflamm [Internet]. 2012 [cited 2021 Sep 9];2012. Available from: https://pubmed.ncbi.nlm.nih.gov/22719180/

159. AL M, DH M. Immunology at the maternal-fetal interface: lessons for T cell tolerance and suppression. Annu Rev Immunol [Internet]. 2000 [cited 2021 Sep 9];18:367–91. Available from: https://pubmed.ncbi.nlm.nih.gov/10837063/

160. Alijotas-Reig J, Esteve-Valverde E, Ferrer-Oliveras R, Llurba E GJ. Tumor Necrosis Factor-Alpha and Pregnancy: Focus on Biologics. An Updated and Comprehensive Review. Clin Rev Allergy Immunol [Internet]. 2017 Aug 1 [cited 2021 Sep 9];53(1):40–53. Available from: https://pubmed.ncbi.nlm.nih.gov/28054230/

161. Chaouat G, Menu E, Clark DA, Dy M, Minkowski M, Wegmann TG. IL-10 prevents naturally occurring fetal loss in the CBA x DBA/2 mating combination, and local defect in IL-10 production in this abortion-prone combination is corrected by in vivo injection of IFN-tau. J Reprod Fertil. 1990;89(2):447–58.

162. Hill JA, Polgar K, Anderson DJ. T-Helper 1-Type Immunity to Trophoblast in Women With Recurrent Spontaneous Abortion. [cited 2021 Sep 11]; Available from: http://jama.jamanetwork.com/

163. Richardson K, Weinberg A. Dynamics of Regulatory T-Cells during Pregnancy: Effect of HIV Infection and Correlations with Other Immune Parameters. PLoS One [Internet]. 2011 Nov 29 [cited 2021 Sep 11];6(11):28172. Available from: /pmc/articles/PMC3226670/

164. Finocchario-Kessler S, Goggin K, Staggs V, Wanyenze RK, Beyeza-Kashesya J, Mindry D, et al. High report of miscarriage among women living with HIV who want to conceive in Uganda 11 Medical and Health Sciences 1117 Public Health and Health Services. BMC Res Notes [Internet]. 2018 Oct 22 [cited 2022 Jan 22];11(1):1–5. Available from: https://bmcresnotes.biomedcentral.com/articles/10.1186/s13104-018-3857-9

165. Mikyas Y, Aziz N, Harawa ' N, Gorre M, Dillon M, Boyer PJ, et al. Immunologic activation during pregnancy: serial measurement of lymphocyte phenotype and serum activation molecules in HIV-infected and uninfected women. J Reprod Immunol. 1997;33:157–70.

166. Romano MF, Buffolano W, Bisogni R, Russo R, Liuzzi R, Bunders M, et al. Increased CD154 expression in uninfected infants born to HIV-positive mothers exposed to antiretroviral prophylaxis. Viral Immunol [Internet]. 2006 [cited 2021 Nov 22];19(3):363–72. Available from: https://www.researchgate.net/publication/6807493_Increased_CD154_Expression_in_Uninfected_Infants_Born_to_HIV-Positive_Mothers_Exposed_to_Antiretroviral_Prophylaxis

167. Ono E, dos Santos AMN, de Menezes Succi RC, Machado DM, de Angelis DSA, Salomão R, et al. Imbalance of naive and memory T lymphocytes with sustained high cellular activation

during the first year of life from uninfected children born to HIV-1-infected mothers on HAART. Brazilian J Med Biol Res [Internet]. [cited 2021 Nov 22];41(8):700–8. Available from: https://www.academia.edu/3358659/Imbalance_of_naive_and_memory_T_lymphocytes_with_sustained_high_cellular_activation_during_the_first_year_of_life_from_uninfected_children_born_to_HIV1_infected_mothers_on_HAART

168. Rich KC, Siegel JN, Jennings C, Rydman RJ, Landay AL. Function and phenotype of immature CD4+ lymphocytes in healthy infants and early lymphocyte activation in uninfected infants of human immunodeficiency virus-infected mothers. Clin Diagn Lab Immunol [Internet]. 1997 [cited 2021 Nov 22];4(3):358. Available from: /pmc/articles/PMC170532/?report=abstract

169. M Saresella, F Colombo, S Fossati, N Sala, D Bricalli, M L Villa, P Ferrante, L Dally, A Vigano MC. T-lymphocyte maturation abnormalities in uninfected newborns and children with vertical exposure to HIV - PubMed. 2000 Dec 1 [cited 2021 Sep 8]; Available from: https://pubmed.ncbi.nlm.nih.gov/11090071/

170. R Cheynier , P Langlade-Demoyen, M R Marescot, S Blanche, G Blondin, S Wain-Hobson, C Griscelli, E Vilmer FP. Cytotoxic T lymphocyte responses in the peripheral blood of children born to human immunodeficiency virus-1-infected mothers. European Journal of Immunology, 22(9), 2211–2217 | 10.1002/eji.1830220905 [Internet]. [cited 2021 Sep 8]. Available from: https://sci-hub.se/10.1002/eji.1830220905

171. Velilla PA, Montoya CJ, Hoyos A, Moreno ME, Chougnet C, Rugeles MT. Effect of intrauterine HIV-1 exposure on the frequency and function of uninfected newborns' dendritic cells. Clin Immunol. 2008 Mar 1;126(3):243–50.

172. Abu-Raya B, Smolen KK, Willems F, Kollmann TR, Marchant A. Transfer of Maternal Antimicrobial Immunity to HIV-Exposed Uninfected Newborns. Front Immunol [Internet]. 2016 Aug 31 [cited 2021 Sep 6];7(AUG):1. Available from: /pmc/articles/PMC5005931/

173. A DM, C C, L M. Occurrence of human immunodeficiency virus type 1 (HIV-1)-specific cytolytic T cell activity in apparently uninfected children born to HIV-1-infected mothers. J Infect Dis [Internet]. 1994 [cited 2021 Sep 8];170(5):1296–9. Available from: https://pubmed.ncbi.nlm.nih.gov/7963731/

174. Bunders MJ, van Hamme JL, Jansen MH, Boer K, Kootstra NA, Kuijpers TW. Fetal exposure to HIV-1 alters chemokine receptor expression by CD4+T cells and increases susceptibility to HIV-1. Sci Reports 2014 41 [Internet]. 2014 Oct 24 [cited 2021 Sep 7];4(1):1–8. Available from: https://www.nature.com/articles/srep06690

175. Reikie BA, Adams RCM, Leligdowicz A, Ho K, Naidoo S, Rusk CE, et al. Altered innate immune development in HIV-exposed uninfected infants. J Acquir Immune Defic Syndr [Internet]. 2014 Jul 1 [cited 2021 Sep 7];66(3):245. Available from: /pmc/articles/PMC4146715/

176. Borges-Almeida E, Mbpm Milanez H, Marluce M, Vilela S, Cunha FG, Abramczuk BM, et al. The impact of maternal HIV infection on cord blood lymphocyte subsets and cytokine profile in exposed non-infected newborns. BMC Infect Dis [Internet]. 2011 [cited 2021 Sep 7];11:38. Available from: http://www.biomedcentral.com/1471-2334/11/38

177. Hygino J, Lima PG, Filho RGS, Silva AAL, Saramago CSM, Andrade RM, et al. Altered immunological reactivity in HIV-1-exposed uninfected neonates. 2008;

178. Kennedy RH, Silver R. Neuroimmune Signaling: Cytokines and the CNS. Neurosci 21st Century [Internet]. 2015 [cited 2021 Sep 4];1–41. Available from: https://link.springer.com/referenceworkentry/10.1007/978-1-4614-6434-1_174-1

179. Banks WA, Erickson MA. The blood-brain barrier and immune function and dysfunction. Neurobiol Dis [Internet]. 2010 Jan [cited 2021 Nov 13];37(1):26–32. Available from:

https://pubmed.ncbi.nlm.nih.gov/19664708/

180. Gutierrez EG, Banks WA, Kastin AJ. Murine tumor necrosis factor alpha is transported from blood to brain in the mouse. J Neuroimmunol [Internet]. 1993 [cited 2021 Nov 13];47(2):169–76. Available from: https://pubmed.ncbi.nlm.nih.gov/8370768/

181. Banks WA, Kastin AJ, Gutierrez EG. Interleukin-1 alpha in blood has direct access to cortical brain cells. Neurosci Lett [Internet]. 1993 Nov 26 [cited 2021 Nov 13];163(1):41–4. Available from: https://pubmed.ncbi.nlm.nih.gov/8295729/

182. Banks WA, Farr SA, Morley JE. Entry of blood-borne cytokines into the central nervous system: effects on cognitive processes. Neuroimmunomodulation [Internet]. 2002 [cited 2021 Nov 13];10(6):319–27. Available from: https://pubmed.ncbi.nlm.nih.gov/12907838/

183. Pan W, Xiang S, Tu H, Kastin AJ. Cytokines Interact with the Blood-Brain Barrier. Blood-Brain Barriers From Ontog to Artif Interfaces [Internet]. 2007 May 31 [cited 2021 Nov 14];1:247–64. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/9783527611225.ch11

184. Wuchert F, Ott D, Murgott J, Rafalzik S, Hitzel N, Roth J, et al. Rat area postrema microglial cells act as sensors for the toll-like receptor-4 agonist lipopolysaccharide. J Neuroimmunol [Internet]. 2008 Nov 15 [cited 2021 Nov 13];204(1–2):66–74. Available from: https://pubmed.ncbi.nlm.nih.gov/18786731/

185. Pan W, Kastin AJ. Cytokine Transport Across the Injured Blood-Spinal Cord Barrier. Curr Pharm Des [Internet]. 2008 Jun 24 [cited 2021 Nov 13];14(16):1620. Available from: /pmc/articles/PMC3927781/

186. Pan W, Zhang L, Liao J, Csernus B, Kastin AJ. Selective increase in TNF alpha permeation across the blood-spinal cord barrier after SCI. J Neuroimmunol [Internet]. 2003 Jan [cited 2021 Nov 13];134(1–2):111–7. Available from: https://pubmed.ncbi.nlm.nih.gov/12507778/

187. Saunders NR, Knott GW, Dziegielewska KM. Barriers in the Immature Brain. Cell Mol Neurobiol 2000 201 [Internet]. 2000 [cited 2021 Nov 14];20(1):29–40. Available from: https://link.springer.com/article/10.1023/A:1006991809927

188. Saunders NR, Habgood MD, Dziegielewska KM. Barrier mechanisms in the brain, II. Immature brain. Clin Exp Pharmacol Physiol [Internet]. 1999 [cited 2021 Nov 14];26(2):85–91. Available from: https://pubmed.ncbi.nlm.nih.gov/10065326/

189. Barichello T, Fagundes GD, Generoso JS, Paula Moreira A, Costa CS, Zanatta JR, et al. Brain-blood barrier breakdown and pro-inflammatory mediators in neonate rats submitted meningitis by Streptococcus pneumoniae. Brain Res [Internet]. 2012 Aug 30 [cited 2021 Nov 14];1471:162–8. Available from: https://pubmed.ncbi.nlm.nih.gov/22796596/

190. Meyer U, Murray P, Urwyler A, Yee B, Schedlowski M, Feldon J. Adult behavioral and pharmacological dysfunctions following disruption of the fetal brain balance between pro-inflammatory and IL-10-mediated anti-inflammatory signaling. Mol Psychiatry [Internet]. 2008 Feb [cited 2021 Aug 31];13(2):208–21. Available from: https://pubmed.ncbi.nlm.nih.gov/17579604/

191. Silver RM, Lohner WS, Daynes RA, Mitchell MD BD. Lipopolysaccharide-induced fetal death: the role of tumor-necrosis factor alpha. Biol Reprod [Internet]. 1994 [cited 2021 Aug 31];50(5):1108–12. Available from: https://pubmed.ncbi.nlm.nih.gov/8025168/

192. Li XY, Zhang C, Wang H, Ji YL, Wang SF, Zhao L, Chen X XD. Tumor necrosis factor alpha partially contributes to lipopolysaccharide-induced intra-uterine fetal growth restriction and skeletal development retardation in mice. Toxicol Lett [Internet]. 2006 May 5 [cited 2021 Aug 31];163(1):20–9. Available from: https://pubmed.ncbi.nlm.nih.gov/16263228/

193. Smith SE, Li J, Garbett K, Mirnics K PP. Maternal immune activation alters fetal brain development through interleukin-6. J Neurosci [Internet]. 2007 Oct 3 [cited 2021 Aug 31];27(40):10695–702. Available from: https://pubmed.ncbi.nlm.nih.gov/17913903/

194. Meyer U, Feldon J, Yee BK. A review of the fetal brain cytokine imbalance hypothesis of schizophrenia. Schizophr Bull [Internet]. 2009 Sep [cited 2022 Jan 24];35(5):959–72. Available from: https://pubmed.ncbi.nlm.nih.gov/18408229/

195. Microglial Physiology: Unique Stimuli, Specialized Responses. Annual Review of Immunology, 27(1), 119–145 | 10.1146/annurev.immunol.021908.132528 [Internet]. [cited 2021 Sep 2]. Available from: https://sci-hub.se/10.1146/annurev.immunol.021908.132528

196. Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. Nat Rev Immunol [Internet]. 2008 Dec [cited 2021 Sep 3];8(12):958. Available from: /pmc/articles/PMC2724991/

197. UK H. Microglia as a source and target of cytokines. Glia [Internet]. 2002 Nov 1 [cited 2021 Sep 2];40(2):140–55. Available from: https://pubmed.ncbi.nlm.nih.gov/12379902/

198. Garden GA, Möller T. Microglia Biology in Health and Disease. 2006;

199. Chan WY, Kohsaka S, Rezaie P. The origin and cell lineage of microglia-New concepts. 2006;

200. Rezaie P, Male D. Colonisation of the Developing Human Brain and Spinal Cord by Microglia: A Review. Microsc Res Tech. 1999;45:359–82.

201. Nimmerjahn A, Kirchhoff F HF. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science [Internet]. 2005 May 27 [cited 2021 Sep 2];308(5726):1314–8. Available from: https://pubmed.ncbi.nlm.nih.gov/15831717/

202. WJ S, MB G, GW K. Functional plasticity of microglia: a review. Glia [Internet]. 1988 [cited 2021 Sep 2];1(5):301–7. Available from: https://pubmed.ncbi.nlm.nih.gov/2976393/

203. Bilbo SD, Schwarz JM. Early-Life Programming of Later-Life Brain and Behavior: A Critical Role for the Immune System. Front Behav Neurosci [Internet]. 2009 Aug 24 [cited 2021 Sep 2];3(AUG). Available from: /pmc/articles/PMC2737431/

204. Davalos D, Grutzendler J, Yang G, Kim JV, Zuo Y, Jung S, Littman DR, Dustin ML GW. ATP mediates rapid microglial response to local brain injury in vivo. Nat Neurosci [Internet]. 2005 Jun [cited 2021 Sep 2];8(6):752–8. Available from: https://pubmed.ncbi.nlm.nih.gov/15895084/

205. Borrell J, Vela JM, Arévalo-Martin A, Molina-Holgado E GC. Prenatal immune challenge disrupts sensorimotor gating in adult rats. Implications for the etiopathogenesis of schizophrenia. Neuropsychopharmacology [Internet]. 2002 [cited 2021 Sep 7];26(2):204–15. Available from: https://pubmed.ncbi.nlm.nih.gov/11790516/

206. Ratnayake U, Quinn TA, Castillo-Melendez M, Dickinson H WD. Behaviour and hippocampus-specific changes in spiny mouse neonates after treatment of the mother with the viral-mimetic Poly I:C at mid-pregnancy. Brain Behav Immun [Internet]. 2012 Nov [cited 2021 Sep 7];26(8):1288–99. Available from: https://pubmed.ncbi.nlm.nih.gov/22960545/

207. Graciarena M, Depino AM PF. Prenatal inflammation impairs adult neurogenesis and memory related behavior through persistent hippocampal TGFβ1 downregulation. Brain Behav Immun [Internet]. 2010 Nov [cited 2021 Sep 7];24(8):1301–9. Available from: https://pubmed.ncbi.nlm.nih.gov/20600816/

208. Juckel G, Manitz MP, Brüne M, Friebe A, Heneka MT WR. Microglial activation in a neuroinflammational animal model of schizophrenia--a pilot study. Schizophr Res [Internet]. 2011 Sep [cited 2021 Sep 7];131(1–3):96–100. Available from:

https://pubmed.ncbi.nlm.nih.gov/21752601/

209. Giovanoli S, Engler H, Engler A, Richetto J, Voget M, Willi R, Winter C, Riva MA, Mortensen PB, Feldon J, Schedlowski M MU. Stress in puberty unmasks latent neuropathological consequences of prenatal immune activation in mice. Science [Internet]. 2013 Mar 1 [cited 2021 Sep 7];339(6123):1100–2. Available from: https://pubmed.ncbi.nlm.nih.gov/23449593/

210. Kim SU, De Vellis J. Microglia in Health and Disease.

211. Cai Z, Lin S, Fan LW, Pang Y RP. Minocycline alleviates hypoxic-ischemic injury to developing oligodendrocytes in the neonatal rat brain. Neuroscience [Internet]. 2006 [cited 2021 Sep 4];137(2):425–35. Available from: https://pubmed.ncbi.nlm.nih.gov/16289838/

212. Chechneva O, Dinkel K, Cavaliere F, Martinez-Sanchez M RK. Anti-inflammatory treatment in oxygen-glucose-deprived hippocampal slice cultures is neuroprotective and associated with reduced cell proliferation and intact neurogenesis. Neurobiol Dis [Internet]. 2006 Aug [cited 2021 Sep 4];23(2):247–59. Available from: https://pubmed.ncbi.nlm.nih.gov/16733089/

213. Hagberg H, Gressens P, Mallard C. Inflammation during Fetal and Neonatal Life: Implications for Neurologic and Neuropsychiatric Disease in Children and Adults.

214. Perry VH, Cunningham C, Boche D. Atypical inflammation in the central nervous system in prion disease.

215. Cunningham C, Wilcockson DC, Campion S, Lunnon K PV. Central and systemic endotoxin challenges exacerbate the local inflammatory response and increase neuronal death during chronic neurodegeneration. J Neurosci [Internet]. 2005 Oct 5 [cited 2021 Sep 7];25(40):9275–84. Available from: https://pubmed.ncbi.nlm.nih.gov/16207887/

216. Godbout JP, Chen J, Abraham J, Richwine AF, Berg BM, Kelley KW JR. Exaggerated neuroinflammation and sickness behavior in aged mice following activation of the peripheral innate immune system. FASEB J [Internet]. 2005 Aug [cited 2021 Sep 7];19(10):1329–31. Available from: https://pubmed.ncbi.nlm.nih.gov/15919760/

217. Pitossi F, Rey A Del, Kabiersch A, Besedovsky H. Induction of Cytokine Transcripts in the Central Nervous System and Pituitary Following Peripheral Administration of Endotoxin to Mice. J Neurosci Res. 1997;48:287–98.

218. FO M, A S, A M, M L. Macrophage activation and polarization. Front Biosci [Internet]. 2008 [cited 2021 Sep 16];13(2):453–61. Available from: https://pubmed.ncbi.nlm.nih.gov/17981560/

219. Pace JL, Russell SW, LeBlanc PA, Murasko DM. Comparative effects of various classes of mouse interferons on macrophage activation for tumor cell killing. J Immunol. 1985;134(2):977–97781.

220. Ullian EM, Harris BT, Wu A, Chan JR BB. Schwann cells and astrocytes induce synapse formation by spinal motor neurons in culture. Mol Cell Neurosci [Internet]. 2004 Feb [cited 2021 Sep 16];25(2):241–51. Available from: https://pubmed.ncbi.nlm.nih.gov/15019941/

221. Kéry R, Chen APF, Kirschen GW. Genetic targeting of astrocytes to combat neurodegenerative disease. Neural Regen Res [Internet]. 2020 Feb 1 [cited 2021 Sep 16];15(2):199. Available from: /pmc/articles/PMC6905329/

222. Henn A, Kirner S, Leist M. TLR2 Hypersensitivity of Astrocytes as Functional Consequence of Previous Inflammatory Episodes. . J Immunol J Immunol Univ Michigan [Internet]. 2015 [cited 2021 Sep 16];186:3237–47. Available from: http://www.jimmunol.org/content/186/5/3237http://www.aai.org/ji/copyright.htmlhttp://jimmunol.org/cgi/alerts/etochttp://www.jimmunol.org/

223. JS M. Early adrenalectomy stimulates subsequent growth and development of the rat brain. Exp Neurol [Internet]. 1983 [cited 2021 Sep 15];82(2):432–46. Available from: https://pubmed.ncbi.nlm.nih.gov/6628629/

224. Matthews SG. Antenatal Glucocorticoids and Programming of the Developing CNS. Pediatr Res 2000 473 [Internet]. 2000 [cited 2021 Sep 15];47(3):291–300. Available from: https://www.nature.com/articles/pr200051

225. Antonow-Schlorke I, Schwab M, Li C, Nathanielsz PW. Glucocorticoid exposure at the dose used clinically alters cytoskeletal proteins and presynaptic terminals in the fetal baboon brain. J Physiol [Internet]. 2003 Feb 15 [cited 2021 Sep 15];547(Pt 1):117. Available from: /pmc/articles/PMC2342613/

226. Ratnayake U, Quinn T, Walker DW, Dickinson H. Cytokines and the neurodevelopmental basis of mental illness. Front Neurosci [Internet]. 2013 [cited 2021 Sep 16];7(7 OCT). Available from: /pmc/articles/PMC3797953/

227. Zhang J-M, An J. Cytokines, Inflammation and Pain. Int Anesthesiol Clin [Internet]. 2007 Mar [cited 2021 Sep 14];45(2):27. Available from: /pmc/articles/PMC2785020/

228. Goehler LE, Gaykema RPA, Hansen MK, Anderson K, Maier SF, Watkins LR. Vagal immune-to-brain communication: a visceral chemosensory pathway. Auton Neurosci Basic Clin [Internet]. 2000 [cited 2021 Sep 15];85:49–59. Available from: www.elsevier.com/locate/autneu

229. Pavlov VA, Tracey KJ. The cholinergic anti-infammatory pathway. Brain Behav Immun [Internet]. 2005 [cited 2021 Sep 15];19:493–9. Available from: www.elsevier.com/locate/ybrbi

230. Gallowitsch-Puerta M, Pavlov VA. Neuro-immune interactions via the cholinergic anti-inflammatory pathway. Life Sci [Internet]. 2007 May 30 [cited 2021 Sep 15];80(24–25):2325. Available from: /pmc/articles/PMC2921074/

231. Johnston GR, Webster NR. Cytokines and the immunomodulatory function of the vagus nerve. [cited 2021 Sep 15]; Available from: http://bja.oxfordjournals.org/

232. Borovikova L V., Ivanova S, Zhang M, Yang H, Botchkina GI, Watkins LR, et al. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. Nat 2000 4056785 [Internet]. 2000 May 25 [cited 2021 Sep 14];405(6785):458–62. Available from: https://www.nature.com/articles/35013070

233. Tracey KJ. The inflammatory reflex. 2002 [cited 2021 Sep 15]; Available from: www.nature.com/nature

234. Plata-Salamán CR, Ffrench-Mullen JMH. Interleukin-2 modulates calcium currents in dissociated hippocampal CA1 neurons. undefined. 1993;4(5):579–81.

235. Han&h U-K, Seto D, Quirion R. Modulation of Hippocampal Acetylcholine Release: A Potent Central Action of Interleukin-2. 1993;

236. Awatsuji H, Furukawa Y, Nakajima M, Furukawa S, Hayashi K. Interleukin-2 as a neurotrophic factor for supporting the survival of neurons cultured from various regions of fetal rat brain. J Neurosci Res [Internet]. 1993 [cited 2021 Nov 14];35(3):305–11. Available from: https://pubmed.ncbi.nlm.nih.gov/8350391/

237. Araujo D, Lapchak P, Chabot J, Nair N, Quirion R. Characterization and possible role of growth factor and lymphokine receptors in the regulation of cholinergic function in the mammalian brain. undefined. 1989;

238. Nemni R, Iannaccone S, Quattrini A, Smirne S, Sessa M, Lodi M, et al. Effect of chronic treatment with recombinant interleukin-2 on the central nervous system of adult and old

mice. Brahl Res. 1992;591:248–52.

239. Schwarcz R, Pellicciari R. Manipulation of brain kynurenines: glial targets, neuronal effects, and clinical opportunities. J Pharmacol Exp Ther [Internet]. 2002 Oct [cited 2021 Nov 15];303(1):1–10. Available from: https://pubmed.ncbi.nlm.nih.gov/12235226/

240. Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. Nat Rev Neurosci [Internet]. 2008 Jan [cited 2021 Nov 15];9(1):46–56. Available from: https://pubmed.ncbi.nlm.nih.gov/18073775/

241. Borland LM, Michael AC. Voltammetric study of the control of striatal dopamine release by glutamate. J Neurochem [Internet]. 2004 Oct 1 [cited 2021 Nov 15];91(1):220–9. Available from: https://onlinelibrary.wiley.com/doi/full/10.1111/j.1471-4159.2004.02708.x

242. Mcnally L, Bhagwagar Z, Hannestad J. Inflammation, Glutamate, and Glia in Depression: A Literature Review To Receive Credit for This Activity to be eligible for credit. 2010;

243. Hardingham GE, Fukunaga Y, Bading H. Extrasynaptic NMDARs oppose synaptic NMDARs by triggering CREB shut-off and cell death pathways. Nat Neurosci 2002 55 [Internet]. 2002 Apr 15 [cited 2021 Nov 15];5(5):405–14. Available from: https://www.nature.com/articles/nn835

244. Rios C, Santamaria A. Quinolinic acid is a potent lipid peroxidant in rat brain homogenates. Neurochem Res 1991 1610 [Internet]. 1991 Oct [cited 2021 Nov 15];16(10):1139–43. Available from: https://link.springer.com/article/10.1007/BF00966592

245. Müller N, Schwarz MJ. The immune-mediated alteration of serotonin and glutamate: towards an integrated view of depression. Mol Psychiatry [Internet]. 2007 Nov [cited 2021 Nov 15];12(11):988–1000. Available from: https://pubmed.ncbi.nlm.nih.gov/17457312/

246. Matute C, Domercq M, Sánchez-Gómez M-V. Glutamate-Mediated Glial Injury: Mechanisms and Clinical Importance. [cited 2021 Nov 15]; Available from: www.interscience.

247. Miller AH, Maletic V, Raison CL. Inflammation and Its Discontents: The Role of Cytokines in the Pathophysiology of Major Depression. Biol Psychiatry [Internet]. 2009 May 1 [cited 2021 Nov 15];65(9):732. Available from: /sites/ppmc/articles/PMC2680424/

248. Ida T, Hara M, Nakamura Y, Kozaki S, Tsunoda S, Ihara H. Cytokine-induced enhancement of calcium-dependent glutamate release from astrocytes mediated by nitric oxide. 2007 [cited 2021 Nov 15]; Available from: www.sciencedirect.com

249. Cusick SE, Georgieff MK. The Role of Nutrition in Brain Development: The Golden Opportunity of the "First 1000 Days." J Pediatr [Internet]. 2016 Aug 1 [cited 2022 Jun 14];175:16. Available from: /pmc/articles/PMC4981537/

250. Rowland I, Gibson G, Heinken A, Scott K, Swann J, Thiele I, et al. Gut microbiota functions: metabolism of nutrients and other food components. Eur J Nutr [Internet]. 2018 Feb 1 [cited 2023 Jan 18];57(1). Available from: https://pubmed.ncbi.nlm.nih.gov/28393285/

251. Liu J, Tan Y, Cheng H, Zhang D, Feng W, Peng C. Functions of Gut Microbiota Metabolites, Current Status and Future Perspectives. Aging Dis [Internet]. 2022 Jul 16 [cited 2023 Jan 18];13(4):1106–26. Available from: https://pubmed.ncbi.nlm.nih.gov/35855347/

252. Fang H, Kang J, Zhang D. Microbial production of vitamin B12: a review and future perspectives. Microb Cell Fact [Internet]. 2017 Jan 30 [cited 2023 Jan 18];16(1). Available from: https://pubmed.ncbi.nlm.nih.gov/28137297/

253. Vujkovic-Cvijin I, Dunham RM, Iwai S, Maher MC, Albright RG, Broadhurst MJ, et al. Dysbiosis of the gut microbiota is associated with HIV disease progression and tryptophan catabolism. Sci Transl Med [Internet]. 2013 Jul 10 [cited 2021 Sep 17];5(193):193ra91. Available from:

/pmc/articles/PMC4094294/

254. Dillon SM, Lee EJ, Kotter C V., Austin GL, Dong Z, Hecht DK, et al. An altered intestinal mucosal microbiome in HIV-1 infection is associated with mucosal and systemic immune activation and endotoxemia. Mucosal Immunol. 2014 Jan 8;7(4):983–94.

255. Yeshanew AG, Gizaw AK, Tebeje BM. Effect of Highly Active Antiretroviral Therapy on T-Cell Sub-population Profile in Human Immunodeficiency Virus-1 Infected Patients. http://www.sciencepublishinggroup.com [Internet]. 2015 Nov 26 [cited 2021 Nov 11];3(6):57. Available from: https://article.sciencepublishinggroup.com/html/10.11648.j.iji.20150306.11.html

256. Mutlu EA, Keshavarzian A, Losurdo J, Swanson G, Siewe B, Forsyth C, et al. A Compositional Look at the Human Gastrointestinal Microbiome and Immune Activation Parameters in HIV Infected Subjects. PLoS Pathog [Internet]. 2014 [cited 2021 Nov 10];10(2). Available from: /pmc/articles/PMC3930561/

257. McHardy IH, Li X, Tong M, Ruegger P, Jacobs J, Borneman J, et al. HIV Infection is associated with compositional and functional shifts in the rectal mucosal microbiota. Microbiome [Internet]. 2013 Oct 12 [cited 2021 Sep 17];1(1). Available from: https://pubmed.ncbi.nlm.nih.gov/24451087/

258. Vázquez-Castellanos JF, Serrano-Villar S, Latorre A, Artacho A, Ferrús ML, Madrid N, et al. Altered metabolism of gut microbiota contributes to chronic immune activation in HIV-infected individuals. Mucosal Immunol [Internet]. 2014 [cited 2021 Sep 17]; Available from: www.nature.com/mi

259. Dinh DM, Volpe GE, Duffalo C, Bhalchandra S, Tai AK, Kane A V., et al. Intestinal Microbiota, microbial translocation, and systemic inflammation in chronic HIV infection. In: Journal of Infectious Diseases. Oxford University Press; 2015. p. 19–27.

260. Sevenoaks T, Wedderburn CJ, Donald KA, Barnett W, Zar HJ, Stein DJ, et al. Association of maternal and infant inflammation with neurodevelopment in HIV-exposed uninfected children in a South African birth cohort. Brain Behav Immun [Internet]. 2021 Jan 1 [cited 2021 Nov 19];91:65. Available from: /pmc/articles/PMC7772888/

261. Lozupone CA, Rhodes ME, Neff CP, Fontenot AP, Campbell TB, Palmer BE. Gut Microbes HIV-induced alteration in gut microbiota. 2016 [cited 2020 Feb 13]; Available from: http://www.tandfonline.com/action/journalInformation?journalCode=kgmi20

262. Mazmanian SK, Round JL, Kasper DL. A microbial symbiosis factor prevents intestinal inflammatory disease. Nature [Internet]. 2008 May 29 [cited 2021 Nov 11];453(7195):620–5. Available from: https://pubmed.ncbi.nlm.nih.gov/18509436/

263. Pinto-Cardoso S, Lozupone C, Briceño O, Alva-Hernández S, Téllez N, Adriana A, et al. Fecal Bacterial Communities in treated HIV infected individuals on two antiretroviral regimens. Sci Rep [Internet]. 2017 Mar 6 [cited 2021 Oct 7];7. Available from: /pmc/articles/PMC5338340/

264. Jost T, Lacroix C, Braegger CP, Rochat F. Vertical mother-neonate transfer of maternal gut bacteria via breast-feeding. 2014;

265. Asnicar F, Manara S, Zolfo M, Truong DT, Scholz M, Armanini F, et al. Studying Vertical Microbiome Transmission from Mothers to Infants by Strain-Level Metagenomic Profiling. mSystems [Internet]. 2017 Feb 28 [cited 2023 Aug 19];2(1). Available from: https://pubmed.ncbi.nlm.nih.gov/28144631/

266. Collado MC, Rautava S, Aakko J, Isolauri E, Salminen S. Human gut colonisation may be initiated in utero by distinct microbial communities in the placenta and amniotic fluid. Sci Rep [Internet]. 2016 Mar 22 [cited 2021 Nov 11];6. Available from:

https://pubmed.ncbi.nlm.nih.gov/27001291/

267. Jiménez E, Marín ML, Martín R, Odriozola JM, Olivares M, Xaus J, et al. Is meconium from healthy newborns actually sterile? 2008 [cited 2020 Feb 5]; Available from: www.elsevier.com/locate/resmic

268. Rautava S, Collado MC, Salminen S, Isolauri E. Probiotics modulate host-microbe interaction in the placenta and fetal gut: A randomized, double-blind, placebo-controlled trial. Neonatology. 2012 Sep;102(3):178–84.

269. Ardissone AN, De La Cruz DM, Davis-Richardson AG, Rechcigl KT, Li N, Drew JC, et al. Meconium microbiome analysis identifies bacteria correlated with premature birth. PLoS One. 2014 Mar 10;9(3).

270. Satokari R, Grönroos T, Laitinen K, Salminen S, Isolauri E. Bifidobacterium and Lactobacillus DNA in the human placenta. Lett Appl Microbiol. 2009 Jan;48(1):8–12.

271. Steel JH, Malatos S, Kennea N, Edwards AD, Miles L, Duggan P, et al. Bacteria and inflammatory cells in fetal membranes do not always cause preterm labor. Pediatr Res. 2005 Mar;57(3):404–11.

272. Jiménez E, Fernández L, Marín ML, Martín R, Odriozola JM, Nueno-Palop C, et al. Isolation of commensal bacteria from umbilical cord blood of healthy neonates born by cesarean section. Curr Microbiol. 2005 Oct;51(4):270–4.

273. Nowak P, Troseid M, Avershina E, Barqasho B, Neogi U, Holm K, et al. Gut microbiota diversity predicts immune status in HIV-1 infection. AIDS [Internet]. 2015 Nov [cited 2020 Feb 13];29(18):2409–18. Available from: https://insights.ovid.com/crossref?an=00002030-201511280-00004

274. Bender JM, Li F, Martelly S, Byrt E, Rouzier V, Leo M, et al. Maternal HIV infection influences the microbiome of HIV-uninfected infants. Sci Transl Med. 2016 Jul 27;8(349).

275. Zevin AS, McKinnon L, Burgener A, Klatt NR. Microbial translocation and microbiome dysbiosis in HIV-associated immune activation. Vol. 11, Current Opinion in HIV and AIDS. Lippincott Williams and Wilkins; 2016. p. 182–90.

276. Moffett JR, Ross B, Arun P, Madhavarao CN, Namboodiri AMA. N-Acetylaspartate in the CNS: From neurodiagnostics to neurobiology. Vol. 81, Progress in Neurobiology. Prog Neurobiol; 2007. p. 89–131.

277. Goldstein FB. Biosynthesis of N-acetyl-l-aspartic acid. BBA - Biochim Biophys Acta. 1959;33(2):583–4.

278. Clark JF, Doepke A, Filosa JA, Wardle RL, Lu A, Meeker TJ, et al. N-Acetylaspartate as a reservoir for glutamate. Med Hypotheses. 2006;67(3):506–12.

279. D'Adamo AF, Gidez LI, Yatsu FM. Acetyl transport mechanisms. Involvement of N-Acetyl aspartic acid in de novo fatty acid biosynthesis in the developing rat brain. Exp Brain Res. 1968 May;5(4):267–73.

280. D'Adamo AF, Yatsu FM. ACETATE METABOLISM IN THE NERVOUS SYSTEM. N-ACETYL-l-ASPARTIC ACID AND THE BIOSYNTHESIS OF BRAIN LIPIDS. J Neurochem [Internet]. 1966 Oct 1 [cited 2020 Feb 24];13(10):961–5. Available from: http://doi.wiley.com/10.1111/j.1471-4159.1966.tb10292.x

281. Blüml S, Moreno-Torres A, Shic F, Nguy C-H, Ross BD. Tricarboxylic acid cycle of glia in the *in vivo* human brain. NMR Biomed [Internet]. 2002 Feb 1 [cited 2020 Feb 24];15(1):1–5. Available from: http://doi.wiley.com/10.1002/nbm.725

282. Barker P. Magnetic resonance spectroscopy of the human brain. Mol Chem Neuropathol [Internet]. 1996 Jan [cited 2020 Feb 24];27(1):42–4. Available from: http://link.springer.com/10.1007/BF02815033

283. Curtis DR, Johnston GA. Amino acid transmitters in the mammalian central nervous system. Vol. 69, Ergebnisse der Physiologie, biologischen Chemie und experimentellen Pharmakologie. Ergeb Physiol; 1974. p. 97–188.

284. Erecińska M, Silver IA. Metabolism and role of glutamate in mammalian brain. Vol. 35, Progress in Neurobiology. Prog Neurobiol; 1990. p. 245–96.

285. McEntee WJ, Crook TH. Glutamate: its role in learning, memory, and the aging brain. Vol. 111, Psychopharmacology. Springer-Verlag; 1993. p. 391–401.

286. Coyle JT. The glutamatergic dysfunction hypothesis for schizophrenia. Vol. 3, Harvard Review of Psychiatry. Lippincott Williams and Wilkins; 1996. p. 241–53.

287. Ueda Y, Doi T, Tokumaru J, Yokoyama H, Nakajima A, Mitsuyama Y, et al. Collapse of extracellular glutamate regulation during epileptogenesis: Down-regulation and functional failure of glutamate transporter function in rats with chronic seizures induced by kainic acid. J Neurochem. 2001;76(3):892–900.

288. Schousboe A. Role of astrocytes in the maintenance and modulation of glutamatergic and GABAergic neurotransmission. Neurochem Res. 2003 Feb 1;28(2):347–52.

289. Schousboe A, Waagepetersen HS. Role of astrocytes in glutamate homeostasis: Implications for excitotoxicity. Neurotox Res. 2005;8(3–4):221–5.

290. Y D, M Y. Compartmentation of brain glutamate metabolism in neurons and glia. J Nutr. 2000;130(4S Suppl).

291. Madhavarao CN, Arun P, Moffett JR, Szucs S, Surendran S, Matalon R, et al. Defective N-acetylaspartate catabolism reduces brain acetate levels and myelin lipid synthesis in Canavan's disease. Proc Natl Acad Sci U S A. 2005 Apr 5;102(14):5221–6.

292. Moffett JR, Tieman SB, Weinberger DR, Coyle JT, Namboodiri AMA, editors. N-Acetylaspartate: A unique neuronal molecule in the central nervous system [Internet]. Boston, MA: Springer US; 2006 [cited 2020 Feb 24]. (Advances in Experimental Medicine and Biology; vol. 576). Available from: http://link.springer.com/10.1007/0-387-30172-0

293. Manev H, Favaron M, Guidotti A, Costa E. Delayed increase of Ca2+ influx elicited by glutamate: role in neuronal death. undefined. 1989;

294. Wallimann T, Wyss M, Brdiczka D, Nicolay K, Eppenberger HM. Intracellular compartmentation, structure and function of creatine kinase isoenzymes in tissues with high and fluctuating energy demands: The "phosphocreatine circuit" for cellular energy homeostasis. Vol. 281, Biochemical Journal. Biochem J; 1992. p. 21–40.

295. Wallimann T, Tokarska-Schlattner M, Schlattner U. The creatine kinase system and pleiotropic effects of creatine. Vol. 40, Amino acids. Springer; 2011. p. 1271–96.

296. Braissant O, Henry H, Loup M, Eilers B, Bachmann C. Endogenous synthesis and transport of creatine in the rat brain: An in situ hybridization study. Mol Brain Res. 2001 Jan 31;86(1–2):193–201.

297. Braissant O, Henry H, Villard AM, Speer O, Wallimann T, Bachmann C. Creatine synthesis and transport during rat embryogenesis: Spatiotemporal expression of AGAT, GAMT and CT1. BMC Dev Biol. 2005 May 26;5:9.

298. Rackayova V, Cudalbu C, Pouwels PJW, Braissant O. Creatine in the central nervous system:

From magnetic resonance spectroscopy to creatine deficiencies. Anal Biochem. 2017 Jul 15;529:144–57.

299. Braissant O, Béard E, Torrent C, Henry H. Dissociation of AGAT, GAMT and SLC6A8 in CNS: relevance to creatine deficiency syndromes. Neurobiol Dis [Internet]. 2010 Feb [cited 2023 Aug 25];37(2):423–33. Available from: https://pubmed.ncbi.nlm.nih.gov/19879361/

300. Foster JA, McVey Neufeld KA. Gut-brain axis: how the microbiome influences anxiety and depression. Trends Neurosci [Internet]. 2013 May [cited 2021 Nov 11];36(5):305–12. Available from: https://pubmed.ncbi.nlm.nih.gov/23384445/

301. Sanders LM, Zeisel SH. Choline: Dietary requirements and role in brain development. Vol. 42, Nutrition Today. Nutr Today; 2007. p. 181–6.

302. Zeisel SH. Choline: Critical Role During Fetal Development and Dietary Requirements in Adults. Annu Rev Nutr. 2006 Aug;26(1):229–50.

303. Gibellini F, Smith TK. The Kennedy pathway-De novo synthesis of phosphatidylethanolamine and phosphatidylcholine. IUBMB Life [Internet]. 2010 Jun 1 [cited 2020 Feb 26];62(6):n/a-n/a. Available from: http://doi.wiley.com/10.1002/iub.337

304. Blusztajn JK, Wurtman RJ. Choline and cholinergic neurons. undefined. 1983;221(4611):614–20.

305. Zeisel SH, Da Costa KA. Choline: an essential nutrient for public health. Nutr Rev [Internet]. 2009 Nov [cited 2022 Feb 4];67(11):615–23. Available from: https://pubmed.ncbi.nlm.nih.gov/19906248/

306. Zeisell SH. BIOCHEMISTRY, PHYSIOLOGY, AND PHARMACOLOGY. Ann Rev Nutr 1981 [Internet]. [cited 2021 Nov 8];1(9):121. Available from: www.annualreviews.org

307. Bernheim F, Bernheim MLC. THE CHOLINE OXIDASE OF LIVER. undefined. 1937 Dec 31;121(1):55–60.

308. Haubrich DR, Gerber NH. Choline dehydrogenase: Assay, properties and inhibitors. Biochem Pharmacol. 1981 Nov 1;30(21):2993–3000.

309. Davison JM, Mellott TJ, Kovacheva VP BJ. Gestational choline supply regulates methylation of histone H3, expression of histone methyltransferases G9a (Kmt1c) and Suv39h1 (Kmt1a), and DNA methylation of their genes in rat fetal liver and brain. J Biol Chem [Internet]. 2009 Jan 23 [cited 2021 Oct 18];284(4):1982–9. Available from: https://pubmed.ncbi.nlm.nih.gov/19001366/

310. Hirata F, Viveros OH, Diliberto EJ, Axelrod J. Identification and properties of two methyltransferases in conversion of phosphatidylethanolamine to phosphatidylcholine. Proc Natl Acad Sci U S A. 1978;75(4):1718–21.

311. Blusztajn JK, Zeisel SH, Wurtman RJ. Synthesis of lecithin (phosphatidylcholine) from phosphatidylethanolamine in bovine brain. Brain Res [Internet]. 1979 Dec 28 [cited 2022 Jan 11];179(2):319–27. Available from: https://pubmed.ncbi.nlm.nih.gov/509240/

312. Sastry BVR, Statham CN, Axelrod J, Hirata F. Evidence for two methyltransferase involved in the conversion of phosphatidylethanolamine to phosphatidylcholine in the rat liver. Arch Biochem Biophys [Internet]. 1981 Oct 15 [cited 2022 Jan 11];211(2):762–73. Available from: https://pubmed.ncbi.nlm.nih.gov/7305396/

313. Bekdash RA. Choline, the brain and neurodegeneration: Insights from epigenetics. Front Biosci - Landmark. 2018 Jan 1;23(6):1113–43.

314. Babb SM, Ke Y, Lange N, Kaufman MJ, Renshaw PF, Cohen BM. Oral choline increases choline

metabolites in human brain. Psychiatry Res - Neuroimaging. 2004 Jan 15;130(1):1–9.

315. Stoll AL, Renshaw PF, De Micheli E, Wurtman R, Pillay SS, Cohen BM. Choline Ingestion Increases the Resonance of Choline-Containing Compounds in Human Brain: An In Vivo Proton Magnetic Resonance Study. 1995;

316. Bekdash RA. Choline and the Brain: An Epigenetic Perspective. Adv Neurobiol. 2016;12.

317. Wu BTF, Dyer RA, King DJ, Richardson KJ, Innis SM. Early Second Trimester Maternal Plasma Choline and Betaine Are Related to Measures of Early Cognitive Development in Term Infants. PLoS One [Internet]. 2012 Aug 20 [cited 2021 Oct 27];7(8):e43448. Available from: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0043448

318. Sweiry JH, Yudilevich DL. Characterization of choline transport at maternal and fetal interfaces of the perfused guinea-pig placenta. J Physiol [Internet]. 1985 Sep 1 [cited 2021 Nov 2];366(1):251. Available from: /pmc/articles/PMC1193030/?report=abstract

319. Chao CK, Pomfret EA, Zeisel SH. Uptake of choline by rat mammary-gland epithelial cells. Biochem J [Internet]. 1988 Aug 15 [cited 2021 Oct 14];254(1):33–8. Available from: /biochemj/article/254/1/33/24012/Uptake-of-choline-by-rat-mammary-gland-epithelial

320. McMahon KE, Farrell PM. Measurement of free choline concentrations in maternal and neonatal blood by micropyrolysis gas chromatography. Clin Chim Acta [Internet]. 1985 Jun 30 [cited 2021 Nov 2];149(1):1–12. Available from: https://pubmed.ncbi.nlm.nih.gov/4028430/

321. Zeisel SH, Mar MH, Zhou Z da CK. Pregnancy and lactation are associated with diminished concentrations of choline and its metabolites in rat liver. J Nutr [Internet]. 1995 [cited 2021 Nov 2];125(12):3049–54. Available from: https://pubmed.ncbi.nlm.nih.gov/7500183/

322. Holmes-McNary MQ, Cheng WL, Mar MH, Fussell S ZS. Choline and choline esters in human and rat milk and in infant formulas. Am J Clin Nutr [Internet]. 1996 [cited 2021 Nov 2];64(4):572–6. Available from: https://pubmed.ncbi.nlm.nih.gov/8839502/

323. Romano KA, Vivas EI, Amador-Noguez D, Rey FE. Intestinal Microbiota Composition Modulates Choline Bioavailability from Diet and Accumulation of the Proatherogenic Metabolite Trimethylamine-N-Oxide. MBio [Internet]. 2015 Mar 17 [cited 2021 Oct 27];6(2). Available from: /pmc/articles/PMC4453578/

324. Baker J, Chaykin S. The biosynthesis of trimethylamine-N-oxide. BBA - Biochim Biophys Acta. 1960 Jul 15;41(3):548–50.

325. Shih DM, Wang Z, Lee R, Meng Y, Che N, Charugundla S, et al. Flavin containing monooxygenase 3 exerts broad effects on glucose and lipid metabolism and atherosclerosis. J Lipid Res [Internet]. 2015 Jan 1 [cited 2021 Oct 27];56(1):22. Available from: /pmc/articles/PMC4274068/

326. Canyelles M, Tondo M, Cedó L, Farràs M, Escolà-Gil JC, Blanco-Vaca F. Trimethylamine N-Oxide: A Link among Diet, Gut Microbiota, Gene Regulation of Liver and Intestine Cholesterol Homeostasis and HDL Function. Int J Mol Sci [Internet]. 2018 [cited 2021 Oct 27];19(10). Available from: /pmc/articles/PMC6214130/

327. Best JG, Stagg CJ, Dennis A. Other Significant Metabolites. Myo-Inositol, GABA, Glutamine, and Lactate. In: Magnetic Resonance Spectroscopy: Tools for Neuroscience Research and Emerging Clinical Applications. Elsevier Inc.; 2013. p. 122–38.

328. Uldry M, Ibberson M, Horisberger JD, Chatton JY, Riederer BM, Thorens B. Identification of a mammalian H+-myo-inositol symporter expressed predominantly in the brain. EMBO J. 2001;20(16):4467–77.

329. Hauser G, Finelli VN. The Biosynthesis of Free and Phosphatide Myo-inositol from Glucose by

Mammalian Tissue Slices. J Biol Chem [Internet]. 1963 Oct 1 [cited 2023 Aug 30];238(10):3224–8. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0021925818486504

330. Blüml S, Wisnowski JL, Nelson MD, Paquette L, Gilles FH, Kinney HC, et al. Metabolic Maturation of the Human Brain From Birth Through Adolescence: Insights From In Vivo Magnetic Resonance Spectroscopy. Cereb Cortex. 2013;23(12):2944–55.

331. Cortey A, Jarvik JG, Lenkinski RE, Grossman RI, Frank L, Delivoria-Papadopoulos M. Proton MR Spectroscopy of Brain Abnormalities in Neonates Born to HIV-Positive Mothers.

332. Holmes MJ, Robertson FC, Little F, Randall SR, Cotton MF, Van Der Kouwe AJW, et al. Longitudinal increases of brain metabolite levels in 5-10 year old children. Harezlak J, editor. PLoS One [Internet]. 2017 Jul 1 [cited 2020 Feb 22];12(7):e0180973. Available from: https://dx.plos.org/10.1371/journal.pone.0180973

333. Graham AS, Holmes MJ, Little F, Dobbels E, Cotton MF, Laughton B, et al. MRS suggests multi-regional inflammation and white matter axonal damage at 11 years following perinatal HIV infection. NeuroImage Clin [Internet]. 2020 Jan 1 [cited 2022 Jan 11];28. Available from: https://pubmed.ncbi.nlm.nih.gov/33395994/

334. Ernst T, Jiang CS, Nakama H, Buchthal S, Chang L. Lower brain glutamate is associated with cognitive deficits in HIV patients: a new mechanism for HIV-associated neurocognitive disorder. J Magn Reson Imaging [Internet]. 2010 Nov [cited 2022 Jan 17];32(5):1045–53. Available from: https://pubmed.ncbi.nlm.nih.gov/21031507/

335. Le Roux SM, Donald KA, Brittain K, Phillips TK, Zerbe A, Nguyen KK, et al. Neurodevelopment of breastfed HIV-exposed uninfected and HIV-unexposed children in South Africa. Aids. 2018;32(13):1781–91.

336. Wedderburn CJ, Yeung S, Rehman AM, Stadler JAM, Nhapi RT, Barnett W, et al. Neurodevelopment of HIV-exposed uninfected children in South Africa: outcomes from an observational birth cohort study. Lancet Child Adolesc Heal [Internet]. 2019;3(11):803–13. Available from: http://dx.doi.org/10.1016/S2352-4642(19)30250-0

337. Chandna J, Mrcpch CE, Chasekwa B, Kandawasvika G, Msw NT, Mba BM, et al. EARLY CHILD DEVELOPMENT OF HIV-EXPOSED UNINFECTED CHILDREN IN RURAL ZIMBABWE. 2019;784.

338. McHenry MS, Oyungu E, Yang Z, Ombitsa AR, Cherop C VR. Neurodevelopmental outcomes in young children born to HIV-positive mothers in rural Yunnan, China. Pediatr Int [Internet]. 2018 Jul 1 [cited 2021 Aug 11];60(7):618–25. Available from: https://pubmed.ncbi.nlm.nih.gov/29663621/

339. Alcock KJ, Abubakar A, Newton CR, Holding P. The effects of prenatal HIV exposure on language functioning in Kenyan children: establishing an evaluative framework. BMC Res Notes. 2016;

340. Springer PE, Slogrove AL, Laughton B, Bettinger JA, Saun-Ders HH, Molteno CD, et al. Neurodevelopmental Outcome of HIV- exposed but uninfected infants in the Mother and Infants Health Study, Cape Town, South Africa.

341. Chaudhury S, Williams PL, Mayondi GK, Leidner J, Holding P, Tepper V, et al. Neurodevelopment of HIV-exposed and HIV-unexposed uninfected children at 24 months. Pediatrics. 2017;140(4).

342. Jahanshad N, Couture MC, Prasitsuebsai W, Nir TM, Aurpibul L, Thompson PM, et al. Brain Imaging and Neurodevelopment in HIV-uninfected Thai Children Born to HIV-infected Mothers. Pediatr Infect Dis J. 2015 Sep 26;34(9):e211–6.

343. Akay C, Cooper M, Odeleye A, Jensen BK, White MG, Vassoler F, et al. Antiretroviral drugs induce oxidative stress and neuronal damage in the central nervous system. J Neurovirol [Internet]. 2014 Feb [cited 2021 Nov 22];20(1):39–53. Available from: https://pubmed.ncbi.nlm.nih.gov/24420448/

344. Brown LAM, Jin J, Ferrell D, Sadic E, Obregon D, Smith AJ, et al. Efavirenz promotes β-secretase expression and increased Aβ1-40,42 via oxidative stress and reduced microglial phagocytosis: Implications for HIV Associated Neurocognitive Disorders (HAND). PLoS One. 2014 Apr 23;9(4).

345. Etherton MR, Lyons JL, Ard KL. HIV-associated Neurocognitive Disorders and Antiretroviral Therapy: Current Concepts and Controversies. Curr Infect Dis Reports 2015 176 [Internet]. 2015 Apr 28 [cited 2021 Nov 22];17(6):1–6. Available from: https://link.springer.com/article/10.1007/s11908-015-0485-6

346. Ma Q, Vaida F, Wong J, Sanders CA, Kao Y ting, Croteau D, et al. Long-term efavirenz use is associated with worse neurocognitive functioning in HIV-infected patients. J NeuroVirology 2015 222 [Internet]. 2015 Sep 25 [cited 2021 Nov 22];22(2):170–8. Available from: https://link.springer.com/article/10.1007/s13365-015-0382-7

347. Robertson K, Liner J, Meeker RB. Antiretroviral neurotoxicity. J NeuroVirology 2012 185 [Internet]. 2012 Jul 19 [cited 2021 Nov 22];18(5):388–99. Available from: https://link.springer.com/article/10.1007/s13365-012-0120-3

348. Azari N, Soleimani F, Vameghi R, Sajedi F, Shahshahani S, Karimi H, et al. A Psychometric Study of the Bayley Scales of Infant and Toddler Development in Persian Language Children. Iran J Child Neurol [Internet]. 2017 Dec 1 [cited 2022 Jan 20];11(1):50. Available from: /pmc/articles/PMC5329760/

349. Williams PL, Marino M, Malee K, Brogly S, Hughes MD, Mofenson LM. Neurodevelopment and In Utero Antiretroviral Exposure of HIV-Exposed Uninfected Infants. Pediatrics [Internet]. 2010 [cited 2021 Nov 20];125:250–60. Available from: www.pediatrics.org/cgi/doi/10.1542/peds.2009-1112

350. Alimenti A, Forbes JC, Oberlander TF, Money DM, Grunau RE, Papsdorf MP, et al. A Prospective Controlled Study of Neurodevelopment in HIV-Uninfected Children Exposed to Combination Antiretroviral Drugs in Pregnancy. Pediatrics [Internet]. 2007 Nov 1 [cited 2021 Nov 22];120(Supplement_3):S159–60. Available from: /pediatrics/article/120/Supplement_3/S159/69772/A-Prospective-Controlled-Study-of-Neurodevelopment

351. Sirois PA, Huo Y, Williams PL, Malee K, Garvie PA, Kammerer B, et al. Safety of Perinatal Exposure to Antiretroviral Medications: Developmental Outcomes in Infants. Pediatr Infect Dis J [Internet]. 2013 Jun [cited 2021 Nov 20];32(6):648. Available from: /pmc/articles/PMC3723344/

352. Rice ML, Zeldow B, Siberry GK, Purswani M, Malee K, Hoffman HJ, et al. Evaluation of Risk for Late Language Emergence after In Utero Antiretroviral Drug Exposure in HIV-exposed Uninfected Infants. Pediatr Infect Dis J [Internet]. 2013 [cited 2021 Nov 20];32(10):e406. Available from: /pmc/articles/PMC3785009/

353. Nozyce ML, Huo Y, Williams PL, Kapetanovic S, Hazra R, Nichols S, et al. Safety of in utero and neonatal antiretroviral exposure: Cognitive and academic outcomes in HIV-exposed, Uninfected Children 5-13 Years of Age. Pediatr Infect Dis J. 2014;33(11):1128–33.

354. Culnane M, Fowler MG, Lee SS, McSherry G, Brady M, O'Donnell K, et al. Lack of long-term effects of in utero exposure to zidovudine among uninfected children born to HIV-infected women. J Am Med Assoc [Internet]. 1999 Jan 13 [cited 2021 Nov 20];281(2):151–7. Available

from: https://jhu.pure.elsevier.com/en/publications/lack-of-long-term-effects-of-in-utero-exposure-to-zidovudine-amon-3

355. Ramaphosa MC, Matsoso MP. South Africa's National Strategic Plan for HIV, TB and STIs 2017-2022. 2017;

356. Jacobson SW, Chiodo LM, Sokol RJ, Jacobson JL. Validity of maternal report of prenatal alcohol, cocaine, and smoking in relation to neurobehavioral outcome. Pediatrics [Internet]. 2002 [cited 2022 Oct 13];109(5):815–25. Available from: https://pubmed.ncbi.nlm.nih.gov/11986441/

357. Jacobson SW, Stanton ME, Molteno CD, Burden MJ, Fuller DS, Hoyme HE, et al. Impaired Eyeblink Conditioning in Children With Fetal Alcohol Syndrome. Alcohol Clin Exp Res [Internet]. 2008 Feb 1 [cited 2022 Oct 13];32(2):365–72. Available from: https://onlinelibrary.wiley.com/doi/full/10.1111/j.1530-0277.2007.00585.x

358. Mollica RF, Caspi-Yavin Y, Bollini P, Truong T, Tor S LJ. The Harvard Trauma Questionnaire. Validating a cross-cultural instrument for measuring torture, trauma, and posttraumatic stress disorder in Indochinese refugees - PubMed [Internet]. [cited 2022 Oct 13]. Available from: https://pubmed.ncbi.nlm.nih.gov/1737972/

359. Halepota AA, Wasif SA. Harvard Trauma Questionnaire Urdu Translation: The only cross-culturally validated screening instrument for the assessment of trauma and torture and their sequelae.

360. Cerniglia L, Cimino S, Ballarotto G, Monniello G. Parental Loss During Childhood and Outcomes on Adolescents' Psychological Profiles: A Longitudinal Study. Curr Psychol 2014 334 [Internet]. 2014 May 14 [cited 2022 Jan 20];33(4):545–56. Available from: https://link.springer.com/article/10.1007/s12144-014-9228-3

361. Flory JD, Bierer LM, Yehuda R. Maternal Exposure to the Holocaust and Health Complaints in Offspring Offspring. Dis Markers [Internet]. 2011 [cited 2022 Jan 20];30:133–9. Available from: https://academicworks.cuny.edu

362. Yehuda R, Daskalakis NP, Lehrner A, Desarnaud F, Bader HN, Makotkine I, et al. Influences of maternal and paternal PTSD on epigenetic regulation of the glucocorticoid receptor gene in Holocaust survivor offspring. Am J Psychiatry [Internet]. 2014 [cited 2022 Jan 20];171(8):872. Available from: /pmc/articles/PMC4127390/

363. D'Andrea W, Ford J, Stolbach B, Spinazzola J, van der Kolk BA. Understanding interpersonal trauma in children: why we need a developmentally appropriate trauma diagnosis. Am J Orthopsychiatry [Internet]. 2012 Apr [cited 2022 Jan 20];82(2):187–200. Available from: https://pubmed.ncbi.nlm.nih.gov/22506521/

364. Ammaniti M, Tambelli R, Odorisio F. Exploring Maternal Representations During Pregnancy in Normal and At-Risk Samples: The Use of the Interview of Maternal Representations During Pregnancy. Infant Ment Health J [Internet]. 2013 Jan 1 [cited 2022 Jan 20];34(1):1–10. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/imhj.21357

365. van Ee E, Kleber RJ, Mooren TTM. War trauma lingers on: Associations between maternal posttraumatic stress disorder, parent-child interaction, and child development. Infant Ment Health J [Internet]. 2012 Sep [cited 2022 Jan 20];33(5):459–68. Available from: https://pubmed.ncbi.nlm.nih.gov/28520264/

366. Diego MA, Field T, Jones NA, Hernandez-Reif M. Withdrawn and intrusive maternal interaction style and infant frontal EEG asymmetry shifts in infants of depressed and non-depressed mothers. Infant Behav Dev [Internet]. 2006 Apr [cited 2022 Jan 22];29(2):220–9. Available from: https://pubmed.ncbi.nlm.nih.gov/17138277/

367. Dubowitz L, Ricci D, Mercuri E. The Dubowitz neurological examination of the full-term newborn. Ment Retard Dev Disabil Res Rev [Internet]. 2005 Feb 1 [cited 2022 Oct 13];11(1):52–60. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/mrdd.20048

368. van der Kouwe AJW, Benner T, Salat DH, Fischl B. Brain morphometry with multiecho MPRAGE. Neuroimage. 2008 Apr 1;40(2):559–69.

369. Klose U. In vivo proton spectroscopy in presence of eddy currents. undefined. 1990;14(1):26–30.

370. Provencher SW. Automatic quantitation of localized in vivo 1H spectra with LCModel. NMR Biomed. 2001;14(4):260–4.

371. Kreis R, Ernst T, Ross BD. Absolute Quantitation of Water and Metabolites in the Human Brain. II. Metabolite Concentrations. J Magn Reson Ser B [Internet]. 1993 Aug [cited 2020 Feb 27];102(1):9–19. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1064186683710563

372. Ernst T, Kreis R, Ross BD. Absolute Quantitation of Water and Metabolites in the Human Brain. I. Compartments and Water. J Magn Reson Ser B. 1993 Aug 1;102(1):1–8.

373. Adhikari PMR, Chowta MN, Ramapuram JT, Rao S, Udupa K, Acharya SD. Prevalence of Vitamin B12 and folic acid deficiency in HIV-positive patients and its association with neuropsychiatric symptoms and immunological response. Indian J Sex Transm Dis AIDS [Internet]. 2016 Jul 1 [cited 2021 Nov 3];37(2):178. Available from: /pmc/articles/PMC5111305/

374. Beach RS, Morgan R, Wilkie F, Mantero-Atienza E, Blaney N, Shor-Posner G, Lu Y, Eisdorfer C BM, Y L, C E, MK B. Plasma vitamin B12 level as a potential cofactor in studies of human immunodeficiency virus type 1-related cognitive changes. Arch Neurol [Internet]. 1992 [cited 2021 Nov 3];49(5):501–6. Available from: https://pubmed.ncbi.nlm.nih.gov/1580812/

375. Beach RS, Morgan R, Wilkie F, Mantero-Atienza E, Blaney N, Shor-Posner G, Lu Y, Eisdorfer C BM. Vitamin B12 in early human immunodeficiency virus-1 infection. Arch Intern Med [Internet]. 1991 May 1 [cited 2021 Nov 3];151(5):1019b – 1020. Available from: https://pubmed.ncbi.nlm.nih.gov/2025127/

376. Beach RS, Mantero-Atienza E, Shor-Posner G, Javier JJ, Szapocznik J, Morgan R, Sauberlich HE, Cornwell PE, Eisdorfer C BM. Specific nutrient abnormalities in asymptomatic HIV-1 infection. AIDS [Internet]. 1992 [cited 2021 Nov 3];6(7):701–8. Available from: https://pubmed.ncbi.nlm.nih.gov/1503689/

377. Rath S, Heidrich B, Pieper DH, Vital M. Uncovering the trimethylamine-producing bacteria of the human gut microbiota. Microbiome [Internet]. 2017 [cited 2021 Oct 27];5(1). Available from: /pmc/articles/PMC5433236/

378. Jensen HH, Batres-Marquez SP, Carriquiry A, Schalinske KL. Choline in the diets of the US population: NHANES, 2003–2004. FASEB J [Internet]. 2007 Jan 1 [cited 2021 Oct 13];21(6):LB46–LB46. Available from: https://onlinelibrary.wiley.com/doi/full/10.1096/fasebj.21.6.LB46-c

379. Boeke CE, Gillman MW, Hughes MD, Rifas-Shiman SL, Villamor E, Oken E. Choline Intake During Pregnancy and Child Cognition at Age 7 Years. Am J Epidemiol [Internet]. 2013 Jun 15 [cited 2021 Oct 13];177(12):1338. Available from: /pmc/articles/PMC3676149/

380. Shaw GM, Carmichael SL, Yang W, Selvin S SD. Periconceptional dietary intake of choline and betaine and neural tube defects in offspring. Am J Epidemiol [Internet]. 2004 Jul 15 [cited 2021 Oct 13];160(2):102–9. Available from: https://pubmed.ncbi.nlm.nih.gov/15234930/

381. Shaw GM, Finnell RH, Blom HJ, Carmichael SL, Vollset SE, Yang W, et al. Choline and risk of neural tube defects in a folate-fortified population. Epidemiology [Internet]. 2009 [cited 2023 Aug 21];20(5):714–9. Available from: https://pubmed.ncbi.nlm.nih.gov/19593156/

382. Carmichael SL, Yang W, Shaw GM. Periconceptional nutrient intakes and risks of neural tube defects in California. Birth Defects Res A Clin Mol Teratol [Internet]. 2010 Aug [cited 2023 Aug 21];88(8):670–8. Available from: https://pubmed.ncbi.nlm.nih.gov/20740594/

383. Chandler AL, Hobbs CA, Mosley BS, Berry RJ, Canfield MA, Qi YP, et al. Neural tube defects and maternal intake of micronutrients related to one-carbon metabolism or antioxidant activity. Birth Defects Res A Clin Mol Teratol [Internet]. 2012 Nov [cited 2023 Aug 21];94(11):864–74. Available from: https://pubmed.ncbi.nlm.nih.gov/22933447/

384. Mills JL, Fan R, Brody LC, Liu A, Ueland PM, Wang Y, et al. Maternal choline concentrations during pregnancy and choline-related genetic variants as risk factors for neural tube defects. Am J Clin Nutr [Internet]. 2014 Oct 1 [cited 2023 Aug 21];100(4):1069–74. Available from: https://pubmed.ncbi.nlm.nih.gov/25240073/

385. Skurnick JH, Bogden JD, Baker H, Kemp FW, Sheffet A, Quattrone G LD. Micronutrient profiles in HIV-1-infected heterosexual adults. J Acquir Immune Defic Syndr Hum Retrovirol [Internet]. 1996 [cited 2021 Oct 19];12(1):75–83. Available from: https://pubmed.ncbi.nlm.nih.gov/8624765/

386. Gongvatana A, Harezlak J, Buchthal S, Daar E, Schifitto G, Campbell T, et al. Progressive Cerebral Injury in the Setting of Chronic HIV Infection and Antiretroviral Therapy. J Neurovirol [Internet]. 2013 Jun [cited 2021 Oct 20];19(3):209. Available from: /pmc/articles/PMC3740160/

387. Cornford EM, Cornford ME. Nutrient transport and the blood-brain barrier in developing animals - PubMed. [cited 2021 Nov 8]; Available from: https://pubmed.ncbi.nlm.nih.gov/2872083/

388. Garner SC, Mar MH, Zeisel SH. Choline Distribution and Metabolism in Pregnant Rats and Fetuses are Influenced by the Choline Content of the Maternal Diet. J Nutr [Internet]. 1995 Nov 1 [cited 2021 Nov 8];125(11):2851–8. Available from: https://academic.oup.com/jn/article/125/11/2851/4730554

389. Geula C, Nagykery N, Nicholas A, Wu C-K. Cholinergic Neuronal and Axonal Abnormalities Are Present Early in Aging and in Alzheimer Disease. J Neuropathol Exp Neurol [Internet]. 2008 Apr 1 [cited 2021 Oct 18];67(4):309–18. Available from: https://academic.oup.com/jnen/article/67/4/309/2916971

390. Zeisel SH, Blusztajn JK. CHOLINE AND HUMAN NUTRITION. Rev Nutr [Internet]. 1994 [cited 2021 Oct 19];14:269–96. Available from: www.annualreviews.org

391. Amenta F, Parnetti L, Gallai V WA. Treatment of cognitive dysfunction associated with Alzheimer's disease with cholinergic precursors. Ineffective treatments or inappropriate approaches? Mech Ageing Dev [Internet]. 2001 [cited 2021 Oct 19];122(16):2025–40. Available from: https://pubmed.ncbi.nlm.nih.gov/11589920/

392. Meck WH, Williams CL, Cermak JM BJ. Developmental periods of choline sensitivity provide an ontogenetic mechanism for regulating memory capacity and age-related dementia. Front Integr Neurosci [Internet]. 2008 May 3 [cited 2021 Oct 19];1(MAY). Available from: https://pubmed.ncbi.nlm.nih.gov/18958235/

393. Morell P, Quarles RH. Characteristic Composition of Myelin. 1999 [cited 2022 Jan 11]; Available from: https://www.ncbi.nlm.nih.gov/books/NBK28221/

394. N S, LA P. Tissue levels of S-adenosylmethionine and S-adenosylhomocysteine in rats fed

methyl-deficient, amino acid-defined diets for one to five weeks. Carcinogenesis [Internet]. 1983 Aug [cited 2021 Oct 19];4(8):1051–7. Available from: https://pubmed.ncbi.nlm.nih.gov/6872150/

395. R H, GW G. DNA methylation and mutation. Mutat Res [Internet]. 1993 [cited 2021 Oct 19];285(1):61–7. Available from: https://pubmed.ncbi.nlm.nih.gov/7678134/

396. Suzuki MM, Bird A. DNA methylation landscapes: provocative insights from epigenomics. Nat Rev Genet [Internet]. 2008 Jun [cited 2021 Nov 8];9(6):465–76. Available from: https://pubmed.ncbi.nlm.nih.gov/18463664/

397. A J. Beyond Watson and Crick: DNA methylation and molecular enzymology of DNA methyltransferases. Chembiochem [Internet]. 2002 Apr 1 [cited 2021 Oct 19];3(4):274–93. Available from: https://europepmc.org/article/MED/11933228

398. MD N, CN C, SH Z. Gene expression profiling of choline-deprived neural precursor cells isolated from mouse brain. Brain Res Mol Brain Res [Internet]. 2005 Apr 4 [cited 2021 Oct 14];134(2):309–22. Available from: https://pubmed.ncbi.nlm.nih.gov/15836926/

399. Mehedint MG, Craciunescu CN, Zeisel SH. Maternal dietary choline deficiency alters angiogenesis in fetal mouse hippocampus. Proc Natl Acad Sci U S A [Internet]. 2010 Jul 20 [cited 2021 Oct 14];107(29):12834. Available from: /pmc/articles/PMC2919920/

400. Weisberg IS, Jacques PF, Selhub J, Bostom AG, Chen Z, Curtis Ellison R, et al. The 1298A-->C polymorphism in methylenetetrahydrofolate reductase (MTHFR): in vitro expression and association with homocysteine. Atherosclerosis [Internet]. 2001 [cited 2021 Nov 9];156(2):409–15. Available from: https://pubmed.ncbi.nlm.nih.gov/11395038/

401. Olthof MR, Van Vliet T, Verhoef P, Zock PL, Katan MB. Effect of homocysteine-lowering nutrients on blood lipids: results from four randomised, placebo-controlled studies in healthy humans. PLoS Med [Internet]. 2005 [cited 2021 Nov 9];2(5):0446–56. Available from: https://pubmed.ncbi.nlm.nih.gov/15916468/

402. Jacques PF, Bostom AG, Wilson PWF, Rich S, Rosenberg IH, Selhub J. Determinants of plasma total homocysteine concentration in the Framingham Offspring cohort. Am J Clin Nutr [Internet]. 2001 [cited 2021 Nov 9];73(3):613–21. Available from: https://pubmed.ncbi.nlm.nih.gov/11237940/

403. Shelnutt KP, Kauwell GPA, Chapman CM, Gregory JF, Maneval DR, Browdy AA, et al. Folate Status Response to Controlled Folate Intake Is Affected by the Methylenetetrahydrofolate Reductase 677C→T Polymorphism in Young Women. J Nutr [Internet]. 2003 Dec 1 [cited 2021 Nov 9];133(12):4107–11. Available from: https://academic.oup.com/jn/article/133/12/4107/4687453

404. S S, A B, J S, PF J, IH R, RB D, et al. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. N Engl J Med [Internet]. 2002 Feb 14 [cited 2021 Oct 19];346(7):476–83. Available from: https://pubmed.ncbi.nlm.nih.gov/11844848/

405. Elias MF, Sullivan LM, D'agostino RB, Elias PK, Jacques PF, Selhub J, et al. Homocysteine and Cognitive Performance in the Framingham Offspring Study: Age Is Important.

406. Hobbs CA, Cleves MA, Melnyk S, Zhao W JS. Congenital heart defects and abnormal maternal biomarkers of methionine and homocysteine metabolism. Am J Clin Nutr [Internet]. 2005 [cited 2021 Oct 19];81(1):147–53. Available from: https://pubmed.ncbi.nlm.nih.gov/15640474/

407. Nelen WLDM, Blom HJ, Steegers EAP, Den Heijer M, Thomas CMG, Eskes TKAB. Homocysteine and folate levels as risk factors for recurrent early pregnancy loss. Obstet Gynecol. 2000 Apr 1;95(4):519–24.

408. Zeisel SH. The supply of choline is important for fetal progenitor cells. Semin Cell Dev Biol [Internet]. 2011 [cited 2021 Nov 9];22(6):624–8. Available from: https://pubmed.ncbi.nlm.nih.gov/21693194/

409. Varela-Moreiras G, Selhub J, daCosta KA, Zeisel SH. Effect of chronic choline deficiency in rats on liver folate content and distribution. J Nutr Biochem. 1992 Oct 1;3(10):519–22.

410. Robertson KR, Stern RA, Hall CD, Perkins DO, Wilkins JW, Gortner DT, Donovan MK, Messenheimer JA, Whaley R ED. Vitamin B12 deficiency and nervous system disease in HIV infection. Arch Neurol [Internet]. 1993 [cited 2021 Nov 3];50(8):807–11. Available from: https://pubmed.ncbi.nlm.nih.gov/8352665/

411. SA R, M H, C C, W L, AV H. Serum vitamin B12 and transcobalamin levels in early HIV disease. Am J Hematol [Internet]. 1994 [cited 2021 Nov 3];47(3):167–71. Available from: https://pubmed.ncbi.nlm.nih.gov/7942779/

412. Paltiel O, Falutz J, Veilleux M, Rosenblatt DS, Gordon K. Clinical correlates of subnormal vitamin B12 levels in patients infected with the human immunodeficiency virus. Am J Hematol [Internet]. 1995 Aug 1 [cited 2021 Nov 3];49(4):318–22. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/ajh.2830490410

413. Irlam JH, Siegfried N, Visser ME, Rollins NC. A clinical review of micronutrients in HIV infection. J Int Assoc Physicians AIDS Care (Chic) [Internet]. 2002 Apr [cited 2021 Nov 3];1(2):63–75. Available from: https://pubmed.ncbi.nlm.nih.gov/12942678/

414. Fadilah A, Musson R, Ong MT, Desurkar A V, Mordekar SR. Vitamin B12 deficiency in infants secondary to maternal deficiency: A case series of seven infants. Eur J Paediatr Neurol [Internet]. 2017 [cited 2021 Nov 12];21:e3. Available from: http://dx.doi.org/10.1016/j.ejpn.2017.04.1335

415. White M, Feucht UD, Duffley E, Molokoane F, Durandt C, Cassol E, et al. Does in utero HIV exposure and the early nutritional environment influence infant development and immune outcomes? Findings from a pilot study in Pretoria, South Africa. Pilot feasibility Stud [Internet]. 2020 Dec 1 [cited 2021 Nov 12];6(1). Available from: https://pubmed.ncbi.nlm.nih.gov/33308322/

416. Boudes P, Zittoun J, Sobel A. Folate, vitamin B12, and HIV infection. Lancet [Internet]. 1990 Jun 9 [cited 2021 Nov 4];335(8702):1401–2. Available from: http://www.thelancet.com/article/014067369091279J/fulltext

417. Fuchs D, Hausen A, Reibnegger G, Werner ER, Dierich MP WH. Neopterin as a marker for activated cell-mediated immunity: application in HIV infection. Immunol Today [Internet]. 1988 [cited 2021 Nov 4];9(5):150–5. Available from: https://pubmed.ncbi.nlm.nih.gov/3076770/

418. Smith I, Howells DW, Kendall B, Levinsky R HK. Folate deficiency and demyelination in AIDS. Lancet (London, England) [Internet]. 1987 Jul 25 [cited 2021 Nov 4];2(8552):215. Available from: https://pubmed.ncbi.nlm.nih.gov/2885665/

419. Jacob RA, Jenden DJ, Allman-Farinelli MA, Swendseid ME. Folate nutriture alters choline status of women and men fed low choline diets. J Nutr [Internet]. 1999 [cited 2021 Nov 4];129(3):712–7. Available from: https://pubmed.ncbi.nlm.nih.gov/10082779/

420. Allen LH. Causes of vitamin B12 and folate deficiency. Food Nutr Bull [Internet]. 2008 [cited 2021 Nov 13];29(2 Suppl). Available from: https://pubmed.ncbi.nlm.nih.gov/18709879/

421. Hui DY. Intestinal phospholipid and lysophospholipid metabolism in cardiometabolic disease. Curr Opin Lipidol [Internet]. 2016 Oct 1 [cited 2021 Oct 27];27(5):507. Available from: /pmc/articles/PMC5125253/

422. Cho CE, Taesuwan S, Malysheva O V, Bender E, Tulchinsky NF, Yan J, et al. Trimethylamine-N-oxide (TMAO) response to animal source foods varies among healthy young men and is influenced by their gut microbiota composition: A randomized controlled trial. Mol Nutr Food Res [Internet]. 2017 [cited 2021 Oct 27];1(12):1600324. Available from: www.mnf-journal.com

423. Ling Z, Jin C, Xie T, Cheng Y, Li L, Wu N. Alterations in the Fecal Microbiota of Patients with HIV-1 Infection: An Observational Study in A Chinese Population. Sci Rep. 2016 Aug 1;6(1):30673.

424. Romano KA, Martinez-del Campo A, Kasahara K, Chittim CL, Vivas EI, Amador-Noguez D, et al. Metabolic, Epigenetic, and Transgenerational Effects of Gut Bacterial Choline Consumption. Cell Host Microbe [Internet]. 2017 Sep 13 [cited 2021 Nov 10];22(3):279. Available from: /pmc/articles/PMC5599363/

425. Ross BM, Moszczynska A, Blusztajn JK, Sherwin A, Lozano A, Kish SJ. Phospholipid biosynthetic enzymes in human brain. Lipids 1997 324 [Internet]. 1997 Apr [cited 2022 Jan 13];32(4):351–8. Available from: https://link.springer.com/article/10.1007/s11745-997-0044-x

426. Marshall DL, De Micheli E, Bogdanov MB, Wurtman RJ. Effects of ethanolamine (Etn) administration on Etn and choline (Ch) levels in plasma, brain extracellular fluid (ECF) and brain tissue, and on brain phospholipid levels in rats: an in vivo study. Neurosci Res Commun. 18(2).

427. Wurtman RJ, Cansev M, Ulus IH. Choline and Its Products Acetylcholine and Phosphatidylcholine. Handb Neurochem Mol Neurobiol [Internet]. 2009 [cited 2021 Nov 8];443–501. Available from: https://link.springer.com/referenceworkentry/10.1007/978-0-387-30378-9_18

428. Shytle RD, Mori T, Townsend K, Vendrame M, Sun N, Zeng J, et al. Cholinergic modulation of microglial activation by alpha 7 nicotinic receptors. J Neurochem [Internet]. 2004 Apr [cited 2022 Jan 13];89(2):337–43. Available from: https://pubmed.ncbi.nlm.nih.gov/15056277/

429. Ances BM, Ortega M, Vaida F, Heaps J, Paul R. Independent effects of HIV, aging, and HAART on brain volumetric measures. J Acquir Immune Defic Syndr [Internet]. 2012 Apr 15 [cited 2022 Oct 31];59(5):469–77. Available from: https://pubmed.ncbi.nlm.nih.gov/22269799/

430. Nowroozalizadeh S, Månsson F, Da Silva Z, Repits J, Dabo B, Pereira C, et al. Microbial Translocation Correlates with the Severity of Both HIV-1 and HIV-2 Infections. undefined. 2010 Apr 15;201(8):1150–4.

431. Piconi S, Trabattoni D, Gori A, Parisotto S, Magni C, Meraviglia P, et al. Immune activation, apoptosis, and Treg activity are associated with persistently reduced CD4+ T-cell counts during antiretroviral therapy. AIDS [Internet]. 2010 Aug 24 [cited 2022 Jan 19];24(13):1991–2000. Available from: https://pubmed.ncbi.nlm.nih.gov/20651586/

432. Lim A, Amini A, D'Orsogna LJ, Rajasuriar R, Kramski M, Lewin SR, et al. Antibody and B-cell responses may control circulating lipopolysaccharide in patients with HIV infection. AIDS [Internet]. 2011 Jul 17 [cited 2022 Jan 19];25(11):1379–83. Available from: https://pubmed.ncbi.nlm.nih.gov/21572302/

433. Fernandez S, Tanaskovic S, Helbig K, Rajasuriar R, Kramski M, Murray JM, et al. CD4+ T-cell deficiency in HIV patients responding to antiretroviral therapy is associated with increased expression of interferon-stimulated genes in CD4+ T cells. J Infect Dis [Internet]. 2011 Dec 15 [cited 2022 Jan 19];204(12):1927–35. Available from: https://pubmed.ncbi.nlm.nih.gov/22006994/

434. Marchetti G, Cozzi-Lepri A, Merlini E, Bellistrì GM, Castagna A, Galli M, et al. Microbial

translocation predicts disease progression of HIV-infected antiretroviral-naive patients with high CD4+ cell count. AIDS [Internet]. 2011 Jul 17 [cited 2022 Jan 19];25(11):1385–94. Available from: https://pubmed.ncbi.nlm.nih.gov/21505312/

435. d'Ettorre G, Paiardini M, Zaffiri L, Andreotti M, Ceccarelli G, Rizza C, et al. HIV Persistence in the Gut Mucosa of HIV-Infected Subjects Undergoing Antiretroviral Therapy Correlates with Immune Activation and Increased Levels of LPS. Curr HIV Res [Internet]. 2011 Jun 6 [cited 2021 Nov 30];9(3):148–53. Available from: https://www.researchgate.net/publication/50986175_HIV_Persistence_in_the_Gut_Mucosa_of_HIV-Infected_Subjects_Undergoing_Antiretroviral_Therapy_Correlates_with_Immune_Activation_and_Increased_Levels_of_LPS

436. Hofer U, Speck RF. Disturbance of the gut-associated lymphoid tissue is associated with disease progression in chronic HIV infection. Semin Immunopathol [Internet]. 2009 Jul [cited 2022 Jan 19];31(2):257–66. Available from: https://pubmed.ncbi.nlm.nih.gov/19484240/

437. Connolly NC, Riddler SA, Rinaldo CR. Proinflammatory cytokines in HIV disease-a review and rationale for new therapeutic approaches. AIDS Rev [Internet]. 2005 Jul 1 [cited 2021 Nov 18];7(3):168–80. Available from: https://europepmc.org/article/MED/16302465

438. Wu Y, Lu YC, Jacobs M, Pradhan S, Kapse K, Zhao L, et al. Association of Prenatal Maternal Psychological Distress with Fetal Brain Growth, Metabolism, and Cortical Maturation. JAMA Netw Open. 2020 Jan 29;3(1).

439. Poland RE, Cloak C, Lutchmansingh PJ, McCracken JT, Chang L, Ernst T. Brain N-acetyl aspartate concentrations measured by 1H MRS are reduced in adult male rats subjected to perinatal stress: Preliminary observations and hypothetical implications for neurodevelopmental disorders. J Psychiatr Res. 1999 Jan 2;33(1):41–51.

440. Gapp K, Corcoba A, van Steenwyk G, Mansuy IM, Duarte JMN. Brain metabolic alterations in mice subjected to postnatal traumatic stress and in their offspring. J Cereb Blood Flow Metab. 2017 Jul 1;37(7):2423–32.

441. Macrì S, Ceci C, Canese R, Laviola G. Prenatal stress and peripubertal stimulation of the endocannabinoid system differentially regulate emotional responses and brain metabolism in mice. PLoS One. 2012;7(7).

442. Grace SL, Evindar A, Stewart DE. The effect of postpartum depression on child cognitive development and behavior: a review and critical analysis of the literature. Arch Womens Ment Health [Internet]. 2003 Nov [cited 2022 Jan 22];6(4):263–74. Available from: https://pubmed.ncbi.nlm.nih.gov/14628179/

443. Cimino S, Cerniglia L, Paciello M. Mothers with depression, anxiety or eating disorders: outcomes on their children and the role of paternal psychological profiles. Child Psychiatry Hum Dev [Internet]. 2015 Apr 1 [cited 2022 Jan 22];46(2):228–36. Available from: https://pubmed.ncbi.nlm.nih.gov/24687513/

444. Goodman RD, Miller MD, West-Olatunji CA. Traumatic stress, socioeconomic status, and academic achievement among primary school students. Psychol Trauma Theory, Res Pract Policy. 2012 May;4(3):252–9.

445. De Simone R, Ajmone-Cat MA, Carnevale D, Minghetti L. Activation of alpha7 nicotinic acetylcholine receptor by nicotine selectively up-regulates cyclooxygenase-2 and prostaglandin E2 in rat microglial cultures. J Neuroinflammation [Internet]. 2005 Jan 25 [cited 2022 Jan 13];2(1). Available from: https://pubmed.ncbi.nlm.nih.gov/15670336/

6. Appendix Supplement

A.1 Linear regression analysis models for the basal ganglia

$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_4$

where Y = NAA/Cr+PCr, X1 = HIV status (HUU vs HEU (pre-conception + post-conception)), X2 = GA (weeks) at the MRI scan, X3 = Head circumference (cm) at the MRI scan, X4 = Maternal Harvard Trauma Scores

$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_4 + \beta_5X_5$

where Y = Glu/Cr+CPr, X1 = HIV status (HUU vs HEU (pre-conception + post-conception)), X2 = FWHM, X3 = SNR, X4 = Length (cm) at the MRI scan, X5 = Maternal monthly income

$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_4$

where Y = GPC+PCh/Cr+PCr, X1 = HIV status (HUU vs HEU (pre-conception + post-conception)), X2 = SNR, X3 = Weight (g) at the MRI scan, X4 = Length (cm) at the MRI scan

$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_4 + \beta_5X_5$

where Y = Ins/Cr+PCr, X1 = HIV status (HUU vs HEU (pre-conception + post-conception)), X2 = Weight (g) at the MRI scan, X3 = Length (cm) at the MRI scan, X4 = Head circumference (cm) at the MRI scan, X5 = GA (weeks) at the MRI scan

Linear regression analysis models were similar where X1 = HIV group (HUU vs HEU (pre-conception) or HEU (post-conception))

A.2 Linear regression analysis models for the midfrontal gray matter

$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3$

where Y = NAA/Cr+PCr, X1 = HIV status (HUU vs HEU (pre-conception + post-conception)), X2 = FWHM, X3 = Head circumference (cm) at the MRI scan

$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_4$

where Y = Glu/Cr+CPr, X1 = HIV status (HUU vs HEU (pre-conception + post-conception)), X2 = FWHM, X3 = SNR, X4 = Maternal education

$Y = \beta_0 + \beta_1X_1 + \beta_2X_2$

where Y = GPC+PCh/Cr+PCr, X1 = HIV status (HUU vs HEU (pre-conception + post-conception)), X2 = FWHM

$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3$

where Y =I ns/Cr+PCr, X1 = HIV status (HUU vs HEU (pre-conception + post-conception)), X2 = GA (weeks) at the MRI scan, X3 = Length (cm) at the MRI scan

Linear regression analysis models were similar where X1 =H IV group (HUU vs HEU (pre-conception) or HEU (post-conception))

A.3 Interaction term using linear regression model

Interaction between status and maternal Harvard trauma scores

$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_4 + \beta_5X_4{:}X1$

where Y = NAA/Cr+PCr, X1 = HIV status (HUU vs HEU (pre-conception + post-conception)), X2 = GA (weeks) at the MRI scan, X3 = Head circumference (cm) at the MRI scan, X4 = Maternal Harvard Trauma Scores

Interaction between duration of ART in HEU neonates and maternal $CD4^+$ T cell count

$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_3{:}X1$

where Y = NAA/Cr+PCr, X1 = ART duration (HEU (pre-conception) vs HEU (post-conception)), X2 = GA (weeks) at the MRI scan, X3 = Maternal $CD4^+$ T cell count