# Altered methionine-sulfone levels are associated with impaired growth in HIV-exposed-uninfected children

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# Altered methionine-sulfone levels are associated with impaired growth in HIV-exposed-uninfected children

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**Objective:** To determine immune-metabolic dysregulation in children born to women living with HIV.

**Methods:** Longitudinal immune-metabolomic analyses of plasma of 32 pregnant women with HIV (WHIV) and 12 uninfected women and their children up to 1.5 years of age were performed.

**Results:** Using liquid chromatography-mass spectrometry and a multiplex bead assay, 280 metabolites (57 amino acids, 116 positive lipids, 107 signalling lipids) and 24 immune mediators (e.g. cytokines) were quantified. combinational antiretroviral therapy (cART) exposure was categorized as cART initiation preconception (long), cART initiation postconception up to 4 weeks before birth (medium) and cART initiation within 3 weeks of birth (short). Plasma metabolite profiles differed between HIV-exposed-uninfected (HEU)-children with long cART exposure compared to HIV-unexposed-children (HUU). Specifically, higher levels of methionine-sulfone, which is associated with oxidative stress, were detected in HEU-children with long cART exposure compared to HUU-children. High infant methionine-sulfone levels were reflected by high prenatal plasma levels in the mother. Increased methionine-sulfone levels in the children were associated with decreased growth, including both weight and length.

**Conclusion:** These findings based on longitudinal data demonstrate that dysregulation of metabolite networks associated with oxidative stress in children born to WHIV is associated with restricted infant growth.



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## Introduction

The successful roll-out of combinational antiretroviral therapy (cART) has greatly improved health of women with HIV (WHIV) and their children and reduced vertical transmission of HIV [1]. Since the introduction of cART, an increasing number of pregnant WHIV receive cART, including women that initiated cART preconception [2]. Consequently, the population of HIV-exposed-uninfected (HEU) children is steadily increasing [3]. Compared to HIV-unexposed-uninfected (HUU) children, HEU-children, despite being uninfected, experience reduced *in utero* and postnatal growth, altered cardiac functioning, impaired immunity and enhanced susceptibility to infections [4–6]. In addition, concerns have been raised regarding neurodevelopment in HEU-children [7]. HIV-associated maternal immune activation as well as adverse effects of cART have been suggested to underlie these clinical observations [8,9].

Maternal cART use in pregnancy has been linked to mitochondrial toxicity in HEU-children [9]. Previously, we reported elevated levels of reactive oxygen species (ROS)-catalysed lipid peroxidation products in cord blood in HEU compared with HUU-children living in Europe [10]. Emerging data indicate that nutritional status and the local environment, including the microbiome, affect host metabolism limiting translation of findings between different settings [11]. Comprehensive immune-metabolic studies of pregnant women and their children in high HIV prevalence regions are needed to take these factors into account. Furthermore, it is critical to determine whether changes persist after birth and are associated with clinical parameters, such as growth. Here, we report immune-metabolomic analyses of plasma of HEU and HUU-children from birth up to 1.5 years of age, paired with third trimester maternal blood samples, to assess dysregulation of the metabolism in the child and associated maternal metabolites.

## Methods

### Study design

The observational University of Zimbabwe Birth Cohort Study in Harare, Zimbabwe, provided the opportunity to analyse plasma samples of WHIV and uninfected women and their children (Table 1, Supplemental Digital Content, http://links.lww.com/QAD/C864) [12]. Ethical approval was obtained from the Joint Research Ethics Committee (JREC) of the University of Zimbabwe and Parirentatwa group of Hospitals (JREC/18/15), the Medical Research Council of Zimbabwe (MRCZ/A/1968), the Research Council of Zimbabwe (SC/9) and ethics committee of the Aerztekammer Hamburg. All study participants provided written informed consent before participation. At enrolment, all women answered a structured questionnaire for assessment of clinical and socio-demographic characteristics. Gestational age was calculated based on the last day of menstruation. The standard of care for all pregnant WHIV to prevent vertical transmission of HIV in Zimbabwe during the study period consisted of TENOLAM-E (tenofovir, lamivudine and efavirenz). Duration of cART use was categorized as long (initiation preconception), medium (initiation post conception up to 4 weeks before birth), and no or short (initiation <3 weeks before delivery). According to national guidelines exclusive breastfeeding is encouraged during the first 6 months of life, afterwards appropriate complementary foods are advised to be added to breastfeeding. Blood samples were collected at enrolment from mothers and infants at delivery and during follow-up in the neonatal period ('NN', within 14 days of birth), early infancy ('EI', ≤6 months) and late infancy ('LI', >6 months). HIV-1 infection was assessed using a 1.5 Roche Amplicor HIV-1 proviral DNA PCR kit (Roche Diagnostics Incorporation, Branchburg, New Jersey, USA) performed on dried blood spots within the first 10 days of life, and regular intervals up to 72 weeks or until cessation of breast feeding, whichever came first [12]. All children were confirmed to be HIV-1-negative.

### Metabolomic and soluble immune mediator profiling

Targeted metabolomics analyses for amines, signalling lipids and positive lipids (total of 280 metabolites), were performed using standard operating procedures described previously [13–15]. Samples for amines, signalling lipids and positive lipids were analysed using the ultra-high-performance liquid chromatography system coupled to mass spectrometers (SCIEX QTRAP 6500, 6500+, Triple TOF 6600, respectively). A multiparameter multiplex bead assay (Invitrogen) was used to measure plasma levels of 24 cytokines, chemokines and growth factors according to the manufacturer's instructions. The detailed procedures and target compound lists are provided in the Supplemental Methods, Supplemental Digital Content, http://links.lww.com/QAD/C865.

### Statistical analysis

Profiled biochemicals were assessed for quality and preprocessed as described in Supplemental Methods, Supplemental Digital Content, http://links.lww.com/QAD/C865. Sample size details on included category intersections are listed in Table 1, Supplemental Digital Content, http://links.lww.com/QAD/C864. Given the limited number of children with no or short cART exposure, data from children with no or short cART exposure were excluded from the main regression analysis and were instead shown as individual points for explorative interpretation in supplementary data. Biochemical dynamics for the medium and long exposure groups were compared against HUU-children using main time effects and exposure × time interaction effects in a univariate Linear Mixed Model [16]. The longitudinal dependence between measurements from the same infant was taken into account, as were potential confounders:

sex, birth weight centile, gestational age of the child and the mother's BMI upon study entry. Biochemicals for which at least one overall exposure group difference survived a resampling-based FDR multiple testing procedure were considered further [17]. We detected the lowest 20 raw *P*-values to be smaller than 0.009 and controlled below a false discovery rate (FDR) threshold of $q$-value $< 0.17$. This false positive control delivered robust inference when compared against other multiple testing procedures (Table 2, Supplemental Digital Content, http://links.lww.com/QAD/C864). To unravel temporal dynamics, statistical significance of exposure x time interaction effects in these shortlisted models was assessed using posthoc Wald tests, and relevant comparisons were made to the maternal sample using paired *t*-tests. Details are included in Supplemental Methods, Supplemental Digital Content, http://links.lww.com/QAD/C865. To interpret these findings, Pearson correlations were used to assess associations between neonatal metabolites and maternal cytokines, as well as metabolites and cytokines in late infancy. A coefficient of 0.5 was considered to indicate moderate to high relevance [18] and these correlations were statistically tested (H0: $r = 0$ vs. H1: $r \neq 0$) using the cor.test function from the *stats* package (version 4.0.2) in R. Further, a growth analysis based on a reference population of sex-specific WHO standardized *Z*-scores on weight-for-age (WAZ), height-for-age (HAZ) and head circumference-for-age (HCAZ) [19] was used to correlate metabolites to growth parameters and included a specific model for boys and girls.

## Results

### Patient demographics

In the study 44 women and their children were included. The maternal and child characteristics are shown in Table 3, Supplemental Digital Content, http://links.lww.com/QAD/C864. Duration of cART differed between WHIW and was associated with viral load, mid-upper arm circumference, systolic blood pressure, total protein (TP) and high-density lipoprotein (HDL). However, all WHIV had a $CD4^+$ T-cell count exceeding $300 \times 10^6$ cells/l, indicating women included were not severely immune suppressed. HEU-children and HUU-children furthermore had a similar distribution with regards to gestational age, birth weight, APGAR scores at 5 min, mode of delivery, sex and duration of breastfeeding. There were no differences in breastfeeding observed between the different cART exposure groups.

### Maternal HIV and combinational antiretroviral therapy exposure have long-term effects on metabolic networks in children

To determine the immune-metabolic perturbations associated with HIV and cART exposure in children and mothers, plasma samples from 32 WHIV and 12 uninfected mothers and their children were analysed, using targeted metabolomics approaches and a multiplex bead assay. In total 280 metabolites, including 57 amino acids, 116 positive lipids and 107 signalling lipids, as well as 24 immune mediators (e.g. cytokines), were assessed in a univariate Linear Mixed Model to identify long-term metabolic changes associated with HIV and cART exposure duration. Methionine-sulfone showed the strongest association with cART duration ($q$-value $<0.05$) (Table 1). An additional six metabolites, phosphatidylcholine (PC) (38:3), ceramides (Cer) (d18:1/23:0) and Cer(d18:1/22:0)), sphingomyelin (SM) (d18:1/16:1) and lyso-phosphatidylethanolamine (LPE) (20:3) were identified with a $q$-value of $< 0.15$ in association with cART duration (Table 1). We included the top 20 metabolites for further analysis, allowing us to observe metabolites from the same chemical class. Of note, the top 20 metabolites all had a raw unadjusted *P*-value $<0.05$. These metabolites included sulphur-containing amino acids, sphingolipids, prostaglandins and glycerophospholipids. Taken together, maternal cART duration during pregnancy was associated with an altered metabolic profile in HEU-children.

### Levels of metabolites indicating oxidative stress are associated with prenatal combinational antiretroviral therapy exposure in HIV-exposed-uninfected-children

Increased levels of methionine-sulfone were observed in HEU-children with long cART exposure compared to the HUU and HEU-children with medium cART exposure (Table 1 and Fig. 1). Methionine-sulfone is the result of increased *in utero* oxidation by ROS of methionine, and is associated with the loss of methionine antioxidant and anti-inflammatory activity [20]. In line, there was a trend towards higher methionine levels in its unoxidized form in HUU-children (Fig. 1, Figure 1, Supplemental Digital Content, http://links.lww.com/QAD/C864). HEU-children with medium cART exposure displayed less pronounced changes in methionine-sulfone levels during infancy compared to HEU-children with long cART exposure. Mothers of children with long ART exposure had lower viral loads and higher $CD4^+$ T-cell counts suggesting reduced HIV-associated disease compared to women initiating cART during pregnancy. The increased levels of methionine-sulfone detected in HEU-children with long cART exposure therefore are more likely associated with cART duration rather than the maternal HIV infection and immune consequences. This was supported by observations in HEU-children with no or short cART-exposure, which had similar lower levels of methionine-sulfone to children born to women who initiated cART during pregnancy (Figure 2, Supplemental Digital Content, http://links.lww.com/QAD/C864).

A trend towards increased cysteine levels, also a sulphur-containing amino acid, was observed in HEU-children

**Table 1. Top 20 metabolites with most significant overall group − group differences from linear mixed model.**

| Order | Metabolites | Medium-cART-HEU vs. HUU | Long-cART-HEU vs. HUU | Long-cART-HEU vs. Medium-cART-HEU |
|---|---|---|---|---|
| 1 | Methionine-sulfone | 0.019 | 0.000*** | 0.007 |
| 2 | PC(38:3) | 0.602 | 0.001** | 0.043 |
| 3 | Cer(d18:1/23:0) | 0.190 | 0.000* | 0.306 |
| 4 | SM(d18:1/16:1) | 0.012 | 0.001* | 0.430 |
| 5 | LPE(20:3) | 0.328 | 0.001* | 0.140 |
| 6 | Cer(d18:1/22:0) | 0.130 | 0.001* | 0.179 |
| 7 | SM(d18:1/23:1) | 0.024 | 0.001* | 0.632 |
| 8 | PC(38:4) | 0.142 | 0.003 | 0.624 |
| 9 | 11β-$PGE_2$ | 0.035 | 0.004 | 0.459 |
| 10 | LPI(16:1) | 0.116 | 0.006 | 0.600 |
| 11 | LPE(16:1) | 0.010 | 0.005 | 0.698 |
| 12 | Cysteine | 0.253 | 0.003 | 0.003 |
| 13 | CE(18:3) | 0.078 | 0.004 | 0.097 |
| 14 | TG(58:10) | 0.038 | 0.003 | 0.193 |
| 15 | PE(36:4) | 0.262 | 0.006 | 0.077 |
| 16 | *S*-Methylcysteine | 0.005 | 0.005 | 0.375 |
| 17 | SM(d18:1/24:2) | 0.105 | 0.008 | 0.381 |
| 18 | Cer(d18:1/24:0) | 0.231 | 0.004 | 0.242 |
| 19 | LPE (22:5) | 0.486 | 0.006 | 0.202 |
| 20 | DGLEA | 0.061 | 0.009 | 0.645 |

Columns contain raw *P*-values on respective comparison medium-cART-HEU vs. HUU, long-cART-HEU vs. HUU and long-cART-HEU vs. medium-cART-HEU groups. 11β-$PGE_2$, 11β-prostaglandin $E_2$; cART, combinational antiretroviral therapy; CE, cholesterol ester; Cer, ceramide; DGLEA, dihomo-γ-linolenic acid; HEU, HIV-exposed-uninfected; HUU, HIV-unexposed-uninfected; LPE, lysophosphatidylethanolamine; LPI, lysophosphatidylinositol; PC, phosphatidylcholines; PE, phosphatidylethanolamine; SM, sphingomyelin; TG, triglyceride. [a] indicates FDR-controlled statistical significance under resampling-based *q*-values: ***$q < 0.05$, **$q < 0.10$, *$q < 0.15$.

with long cART-exposure compared to HUU-children. Cysteine is upregulated to reduce oxidative stress [21]. The antioxidant S-methylcysteine, the product of posttranslational methylation of cysteine, showed a trend towards decreasing levels compared to cysteine, indicating that cysteine synthesis may be enhanced in HEU-children with long-cART exposure (Fig. 1, Figure 1, Supplemental Digital Content, http://links.lww.com/QAD/C864). Furthermore, a trend towards increased levels of 11β-$PGE_2$ (unadjusted $P = 0.004$), a $PGE_2$ isomer, were detected in HEU-children with long cART exposure compared to the HUU-children (Table 1, Figures 1 and 3, Supplemental Digital Content, http://links.lww.com/QAD/C864), however after correction for multiple testing this was not significant (*q*-value > 0.15). Prostaglandins are produced upon lipid oxidation induced by ROS or enzymatic catabolism of Arachidonic Acid and well known for their induction of labour [22,23]. In sum, metabolic changes revealing patterns indicative of increased oxidative stress, were observed in HEU-children whose mothers had initiated cART prior to conception.

## Glycerophospholipid levels are increased in HIV-exposed-uninfected-children with long combinational antiretroviral therapy-exposure

Lipid dysregulation is frequently observed in cART treated people living with HIV [24]. Several species of glycerophospholipids (PC and LPE) showed a trend towards increased levels in HEU-children with long cART exposure compared to HEU-children with medium cART exposure or HUU-children (Table 1). Glycerophospholipids are the most abundant complex lipids and function as building blocks of cellular membranes and signalling molecules [25]. PC(38:3) and LPE(20:3) were increased in HEU-children with long cART exposure compared to the HUU-children (Fig. 2) with 5 related glycerophospholipids identified in the top 20 of the overall model. Although not significant after correction for multiple testing additional analyses of glycerophospholipids at the specific time points indicated that glycerophospholipids (Figure 1, Supplemental Digital Content, http://links.lww.com/QAD/C864) were especially higher early in infancy in HEU-children with long cART exposure compared to the HUU-children, and levels attenuated afterwards.

## Remodelling of the sphingomyelin-ceramides metabolism in HIV-exposed-uninfected-children with long combinational antiretroviral therapy exposure

Ceramides, the precursors of all complex sphingolipids, are potent signalling molecules and furthermore function as building blocks of neuronal membranes [26]. HEU-children with long-cART exposure showed a trend towards an altered sphingolipid/ceramide profile compared to HUU-children. Specifically, two ceramides [Cer(d18:1/23:0) and Cer(d18:1/22:0); *q*-value < 0.12] and two sphingomyelins [SM(d18:1/16:1) and SM(d18:1/23:1); *q*-value < 0.13] displayed trends towards altered levels in HEU-children with long cART exposure compared to HUU-children (Table 1). The top 20 included an additional ceramide and sphingomyelin with differential levels observed between the groups. From birth, ceramides and sphingomyelins followed a similar

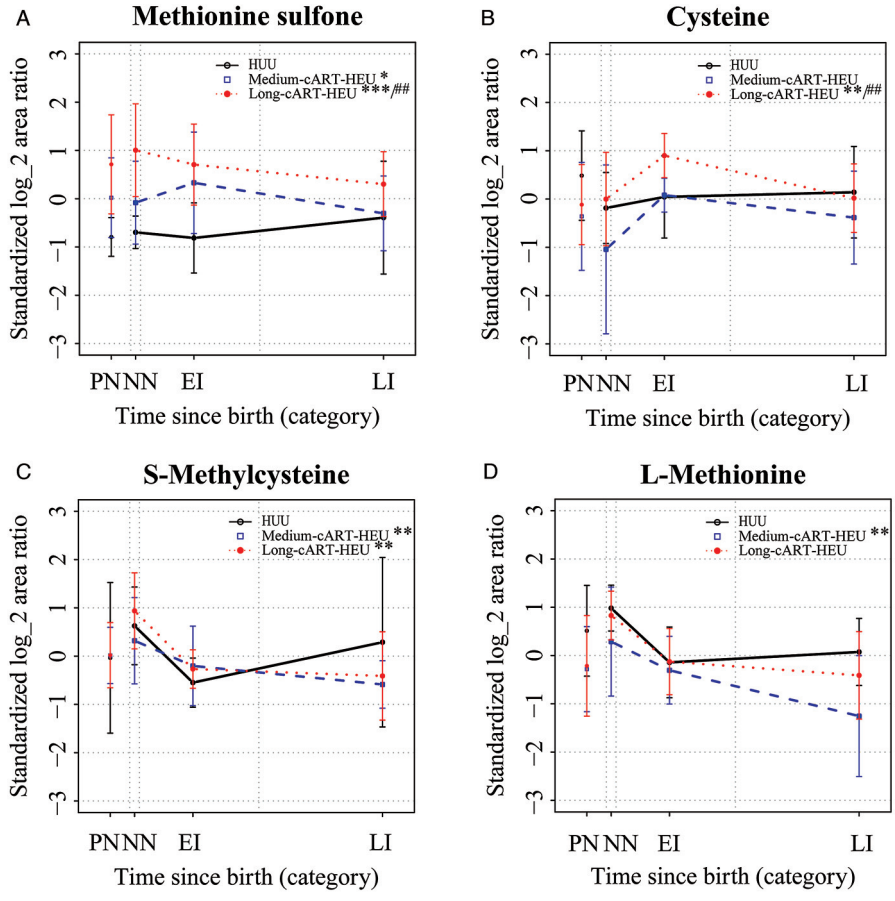


**Fig. 1. Longitudinal trajectories of sulphur containing amino acids in children.** Longitudinal trajectories of (a) methionine-sulfone and three related amino acids ((b) cysteine, hit 12; (c) *S*-methylcysteine: hit 16; (d) L-methionine: hit 75 from the main model) in children and their paired maternal prenatal (PN) samples. The points represent the means of the standardized, $\log_2$ area ratios per age category for the exposure groups (black circles represent HUU-children, blue squares represent children in medium-cART-HEU group and red circles represent children in long-cART-HEU group) (age categories: NN: neonate; EI: early infancy; LI: late infancy). The lines connecting the points are interpolations. The maternal prenatal ''PN'' exposure group means are included as disconnected points at the left of each figure. *represent the corresponding significance level of the raw *P*-value of group comparisons to HUU from the univariate linear mixed model in the infant cohort (***$P < 0.001$, **$P < 0.01$, *$P < 0.05$); #represent the corresponding significance level of the raw *P*-value of group comparisons to medium-cART-HEU group from the univariate linear mixed model in the infant cohort (##$P < 0.01$, #$P < 0.05$). The specific raw *P*-values are furthermore listed in Table 1.

trajectory, showing increased levels in early infancy in HEU-children with long cART exposure compared to HUU-children (Fig. 3, Figure 1, Supplemental Digital Content, http://links.lww.com/QAD/C864). Taken together, these findings suggest that the sphingolipid/ceramide metabolism in HEU-children with long cART exposure may be altered, although the difference did not reach significance after correction for multiple testing at single biochemicals.

## Infant metabolic dysregulation is associated with maternal metabolic dysregulation

Next, we investigated whether maternal methionine-sulfone levels predicted infant methionine-sulfone levels. In line with the data presented in Fig. 1, maternal methionine-sulfone levels reflected infant levels, as no significant differences were observed between maternal and infant methionine sulfone levels (*t*-test: maternal to infant HUU: $P = 0.406$; maternal to infant medium-HEU: $P = 0.972$; and maternal to infant long-HEU: $P = 0.261$, respectively), indicating that the maternal methionine-sulfone levels are an adequate predictor of altered levels in the child. These findings suggest that metabolic dysregulation in the mother associated with cART duration underlies metabolic changes in the infant, however this does not rule out that maternal immune activation in the mother may impact the child as well. To this end, we performed immune-metabolic correlation analyses between maternal immune mediators and infant metabolites at birth, using a correlation coefficient cut-off of ($r$) $\geq 0.5$ or $\leq -0.5$ [18]. Methionine-sulfone was not correlated with $CD4^+$ T-cell count, viral load, or

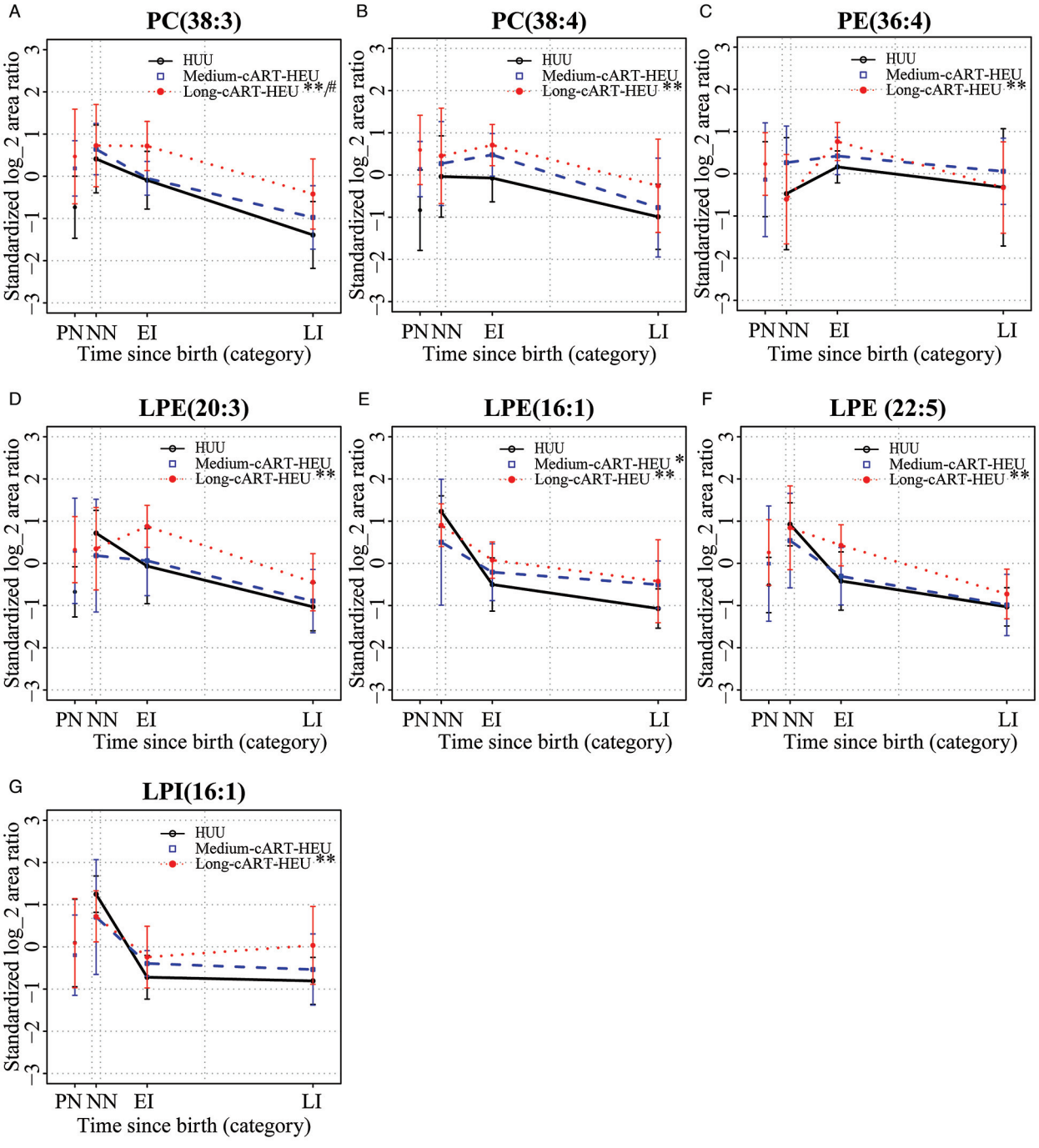


**Fig. 2. Longitudinal trajectories of glycerophospholipids in children.** Longitudinal trajectories of glycerophospholipids ((a–g) all within top 20 hits from the main model) in children and their paired maternal prenatal (PN) samples. The points represent the means of the standardized, $\log_2$ area ratios per age category for the exposure groups (black circles represent HUU-children, blue squares represent children in medium-cART-HEU group and red circles represent children in Long-cART-HEU group) (age categories NN: neonate; EI: early infancy; LI: late infancy). The lines connecting the points are interpolations. The maternal prenatal ''PN'' exposure group means are included as disconnected points at the left of each figure. *represent the corresponding significance level of the raw *P*-value of group comparisons to HUU from the univariate linear mixed model in the infant cohort (***$P < 0.001$, **$P < 0.01$, *$P < 0.05$); #represent the corresponding significance level of the raw *P*-value of group comparisons to medium-cART-HEU group from the univariate linear mixed model in the infant cohort (##$P < 0.01$, #$P < 0.05$). The specific raw *P*-values are furthermore listed in Table 1. LPE, lysophosphatidylethanolamine; LPI, lysophosphatidylinositol; PC, phosphatidylcholines; PE, phosphatidylethanolamine.

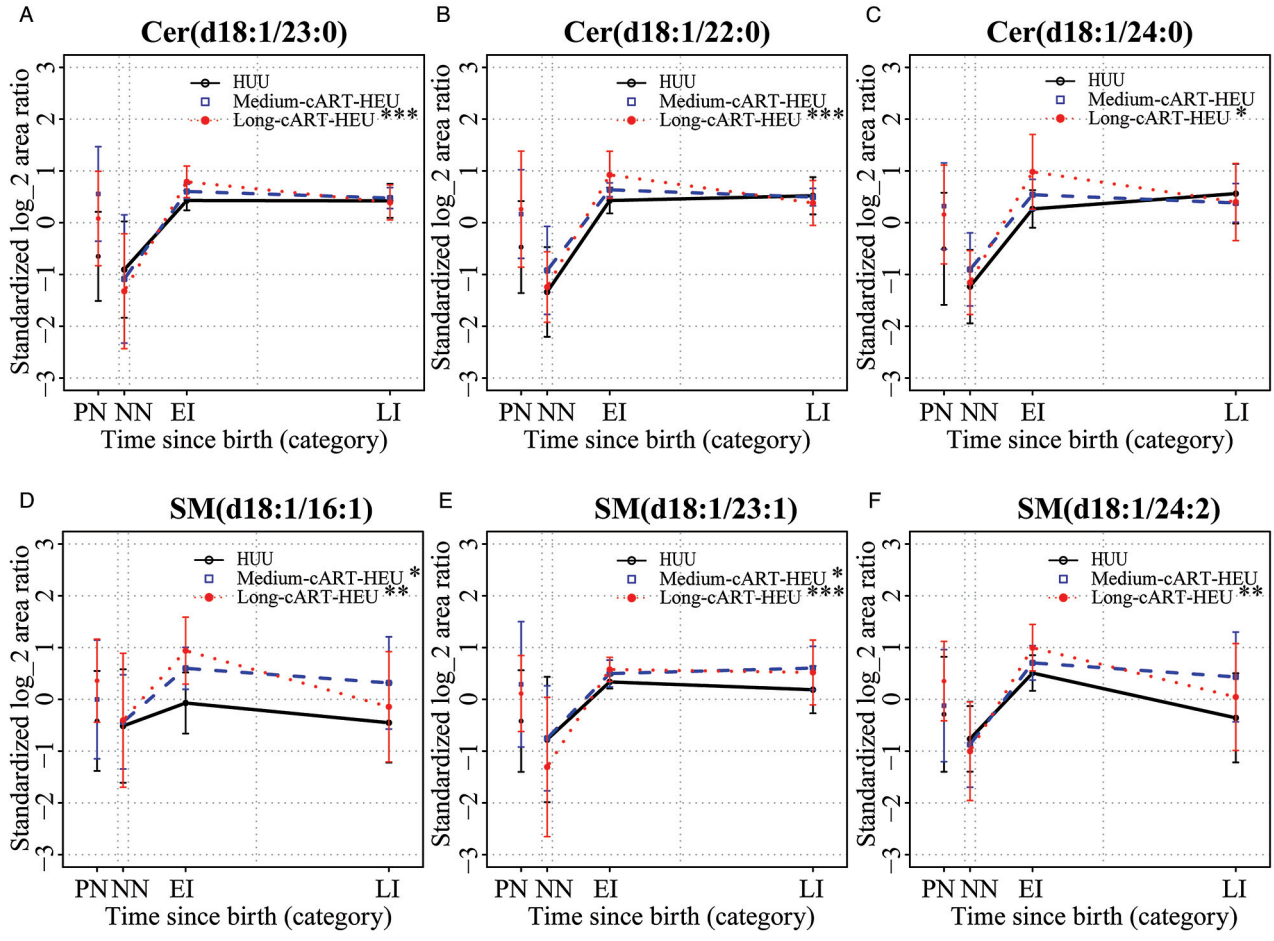


**Fig. 3. Longitudinal trajectories of sphingomyelins and ceramides in children.** Longitudinal trajectories of sphingolipids ((a–f) all within the top 20 hits from the main model) in the children and their paired maternal prenatal (PN) samples. The points represent the means of the standardized, $\log_2$ area ratios per age category for the exposure groups (black circles represent children in HUU group, blue open squares represent children in Medium-cART-HEU group and red circles represent children in long-cART-HEU group) (NN: neonate; EI: early infancy; LI: late infancy). The lines connecting the points are interpolations. The maternal prenatal ''PN'' exposure group means are included as disconnected points at the left-hand side of each figure. *represent the corresponding significance level of the raw *P*-value of group comparisons to HUU from the univariate linear mixed model in the infant cohort (***$P < 0.001$, **$P < 0.01$, *$P < 0.05$); #represent the corresponding significance level of the raw *P*-value of group comparisons to medium-cART-HEU group from the univariate linear mixed model in the infant cohort (##$P < 0.01$, #$P < 0.05$). The specific raw *P*-values are furthermore listed in Table 1. Cer, ceramides; SM, sphingomyelin.

maternal plasma cytokine levels (Figure 4, Supplemental Digital Content, http://links.lww.com/QAD/C864, Table 4, Supplemental Digital Content, http://links.lww.com/QAD/C864). Infant L-methionine levels were correlated to maternal $CD4^+$ T-cell count and infant cysteine with maternal viral load indicating that next to the association of maternal cART duration with methionine-sulfone in the infant, potential effects of maternal HIV on methionine metabolism cannot be excluded. Furthermore, infant ceramides and sphingomyelins were negatively associated with maternal cytokines, including interleukin (IL)-2 and IL-1β. To assess whether infant metabolic dysregulation after 6 months of age was associated with infant immune mediators, infant metabolites were correlated to infant immune parameters. However, few infant metabolites correlated to infant immune parameters, suggesting that metabolic dysregulation in children may not have long-term effects on soluble immune mediators. Specifically, no significant associations were observed between infant soluble immune mediators and methionine-sulfone levels (Figure 5, Supplemental Digital Content, http://links.lww.com/QAD/C864 and Table 5, Supplemental Digital Content, http://links.lww.com/QAD/C864). In sum, these findings suggest that methione-sulfone dysregulation in HEU-infants is associated with metabolic dysregulation in the mother rather than maternal immune dysregulation.

### Increased levels of methionine-sulfone are associated with decreased infant growth

Associations with maternal immune dysregulation and cART have been suggested to underlie decreased growth in HEU-children [6]. As methionine-sulfone dysregulation is a characteristic of oxidative stress and reduced energy production [20], we determined whether infant methionine-sulfone levels were associated with infant growth. The association between methionine-sulfone and height, weight and head circumference was investigated using a linear regression model, separately for boys and girls. Growth parameters were expressed as *Z*-scores according to WHO definitions [19]. Elevated infant methionine-sulfone levels negatively correlated to weight ($P = 0.015$) in boys, and reduced weight ($P = 0.036$) and length ($P = 0.020$) in girls (Fig. 4). There was a trend towards reduced head circumference in girls with higher methionine-sulfone levels. In sum, metabolic dysregulation with increased levels of methionine-sulfone in infants are associated with decreased growth.

## Discussion

Although the number of HEU-children is rapidly increasing concerns remain regarding the long-term consequences of maternal HIV and cART exposure. Here, we demonstrate based on longitudinal immune-metabolomics analyses of Zimbabwean mother–child pairs long-term disrupted metabolic functioning in HEU-children. In particular, children born to WHIV who had initiated cART prior to conception exhibited metabolic changes and increased levels of methionine-sulfone. Increased infant methionine-sulfone levels were associated with decreased growth. Methionine-sulfone levels in the child correlated to those in the mother and may offer a potential novel clinical correlate for risk of impaired growth in HEU-children.

cART during pregnancy dramatically improves health in women and children [8], however some studies have suggested that initiation prior to conception may increase the risk of adverse effects [27]. In this study methionine-sulfone was especially elevated in HEU-children born to women who had initiated cART prior to conception. This is in line with previous findings showing increased methionine-sulfone levels in people with HIV and receiving long-term cART compared to uninfected individuals [28]. Oxidized-methionine residues and their reduction by the Msr system impair the resilience of antioxidant defense, which is critical to main cellular homeostasis [29]. Several ARTs are known for affecting mitochondrial dysfunction through the inhibition of DNA pol-γ [30]. Tenofovir has been implicated in the depletion of the cellular antioxidant system and mitochondrial damage [31,32], indicating the potential contribution of tenofovir to changes in methionine-sulfone levels in mothers and infants observed in this study. Efavirenz, which was furthermore used in the maternal cART regimen in this study, is less well known for its mitochondrial toxicity. Recent studies however showed increased levels of metabolites associated with oxidative stress in people with HIV treated with efavirenz [33]. Although endogenous production of methionine-sulfone would fit the long-term higher levels in HEU-children, maternal methionine-sulfone transferred across the placenta or taken up by the infant intestine from breastmilk cannot be excluded. Studies in animals may suggest that this could also occur [34,35]. Furthermore, a trend towards elevated prostaglandin levels were observed in women who initiated cART prior to conception, and in their children. Prostaglandins are potent inducers of labour and the trend towards increased levels of 11-β-$PGE_2$ presented here may suggest their involvement in labour induction in WWH [23]. The unique comparison

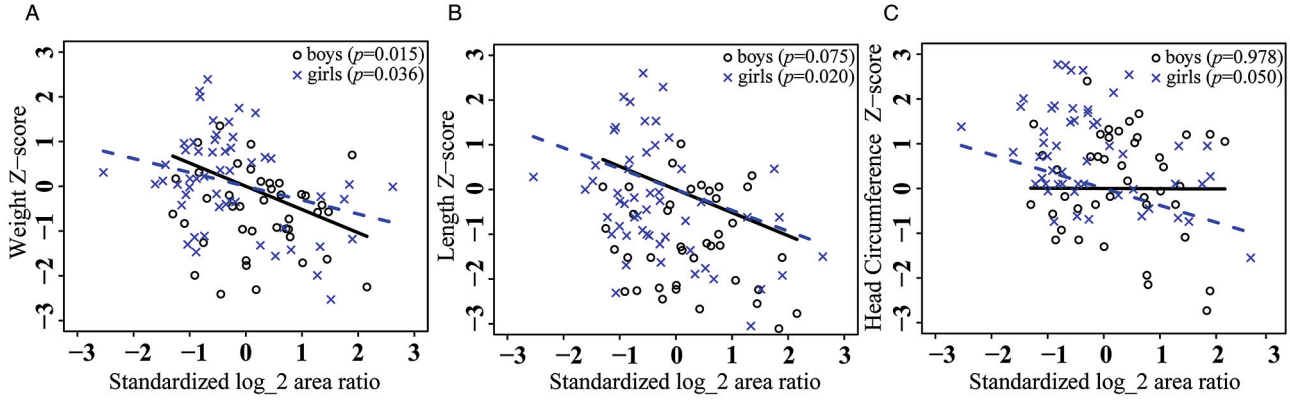


**Fig. 4. Correlations of methionine-sulfone with weight *Z*-score (a), length *Z*-score (b), and head circumference *Z*-score (c).** The points represent standardized $\log_2$ area ratios across all longitudinal infant samples for children in all groups included in the main model (black circles for boys; blue crosses for girls). Regression lines and associated mixed model *P*-values included for boys (black solid line) and girls (blue dashed line).

in this longitudinal cohort provided the opportunity to compare different cART exposure times as well as a small group of children with no or less than 3 weeks cART exposure. The latter showed more similar methionine-sulfone levels to HUU-children and HEU-children with medium cART exposure compared to HEU-children with long cART exposure. Furthermore, maternal $CD4^+$ T-cell count, viral load or maternal plasma cytokines, were not strongly associated with methionine-sulfone. Considering that long-term cART is critical for the mother's health further studies are needed to verify the results and identify potential pathways that can be targeted to reduce adverse effects of cART. Furthermore, alternative cART regimens are increasingly used. Longitudinal studies are needed to assess whether these alternative cART regimens have a reduced potential to perturb the maternal and infant metabolism. Taken together, increased methionine-sulfone levels indicative of oxidative stress are observed in HEU-children born to WHIV who initiated cART prior to conception.

Furthermore, we observed a trend towards altered sphingolipid and ceramide levels in children born to WHIV. Ceramides, the precursors of all complex sphingolipids, are potent signalling molecules and well known biomarkers for cardiovascular diseases [36]. Sphingolipid metabolism is furthermore critical for efficient neuronal functioning [37]. Ceramide intracellular levels are fine-tuned and alteration of the sphingomyelin-ceramide signalling profile are associated with the development of age-related, neurological and neuroinflammatory diseases [37–39]. Although under debate, studies suggest that HEU-children may be at risk of altered neurological development [7]. Our findings, although important to note not reaching biochemical-specific significance after correction for multiple testing, may suggest that sphingolipid metabolism could be altered in HEU-children and indicate that further studies are needed. Lipid metabolism was less affected than reported in previous studies in HEU-children and in people with HIV [40]. This may reflect the less lipid toxic profile of TENOLAM vs. protease inhibitors [10,28].

Decreased infant growth is an important clinical parameter of child health and associated with increased morbidity and mortality [41]. HEU-children are smaller for gestational age and have an increased risk for reduced postnatal growth [6,42]. Our data suggests that that increased oxidative stress may have consequences for infant growth. As maternal methionine-sulfone levels were similar to the children, maternal methionine-sulfone furthermore may provide a new biomarker for decreased growth during pregnancy in mothers and during follow-up in HEU-children. These observations require further studies to assess the validity of maternal and infant methionine-sulfone to predict postnatal growth. In conclusion, our findings demonstrate metabolic dysregulation indicating increased oxidative stress in WHIV and their uninfected children, which is associated with timing of cART initiation and decreased growth. Future studies are needed to uncover the mechanisms underlying long-term mitochondrial dysregulation in HEU-children and inform on interventions that can restore metabolic functioning in WHIV and children to promote healthy development.

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### Conflicts of interest

There are no conflicts of interest.

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