Anthropometry, body composition, early growth, and chronic disease risk factors among Zambian adolescents exposed or not to perinatal maternal HIV

Suzanne Filteau,[1] Lackson Kasonka,[2] Jonathan C Wells,[3] Grace Munthali,[4] Molly Chisenga,[2] Andrea M Rehman[1]

[1]Faculty of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine
[2]University Teaching Hospital - Women and Newborn
[3]Institute of Child Health, University College London
[4]National Institute for Scientific and Industrial Research, Lusaka, Zambia

Corresponding author: Suzanne Filteau, Faculty of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E7HT, UK

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Abstract

Early life exposures and growth patterns may affect long-term risk of chronic non-communicable diseases (NCDs). We followed up in adolescence two Zambian cohorts (N=322) recruited in infancy to investigate how two early exposures – maternal HIV exposure without HIV infection (HEU), and early growth profile – were associated with later anthropometry, body composition, blood lipids, haemoglobin (Hb) and HbA1c, blood pressure and grip strength. Although in analyses controlled for age and sex, HEU children were thinner, but not shorter, than HIV-unexposed, uninfected (HUU) children, with further control for sociodemographic factors, these differences were not significant. HEU children had higher HDL cholesterol than HUU children and marginally lower HbA1c but no other biochemical or clinical differences. We identified three early growth profiles – adequate growth, declining and malnourished – which tracked into adolescence when differences in anthropometry and body fat were still seen. In adolescence, the early malnourished group, compared with the adequate group, had lower blood triglycerides and higher HDL, lower grip strength (difference: -1.87 kg, 95% CI: -3.47, -0.27; P=0.02) and higher HbA1c (difference: 0.5%, 95% CI 0.2, 0.9, P=0.005). Lower grip strength and higher HbA1c suggest the early malnourished children could be at increased risk of NCDs in later life. Including early growth profile in analyses of HIV exposure reduced the associations between HIV and outcomes. The results suggest that perinatal HIV exposure may have no long-term effects unless accompanied by poor early growth. Reducing the risk of young child malnutrition may lessen children's risk of later NCDs.

Keywords: HIV, growth trajectory, body composition, chronic disease risk, adolescent, HbA1c, cohort

Introduction

The prevalence of chronic non-communicable diseases (NCDs) is rising globally, including in low- and middle-income countries.[1] Overweight and obesity, which are also increasing in prevalence, are important risk factors for NCDs, but there is evidence from Africa that some NCDs, notably diabetes, may frequently occur in the absence of overweight and at younger ages than seen in high-income countries.[2] Environmental factors, both concurrent and earlier in life, are likely to be important contributors to the different phenotypes of NCDs in Africa compared to those in high-income countries.

Severe infectious diseases such as malaria, HIV and tuberculosis are common in Africa. Malaria both earlier in life and concurrently among Ugandan children aged about 10 years was associated with altered blood lipid profiles.[3] HIV infection in adults can increase risk of some NCDs[4; 5; 6] and people who start antiretroviral therapy (ART) with advanced disease, as indicated by a low CD4 count, may be at especially high risk.[7] Advanced HIV can also lead to weight loss and we have shown that prior malnutrition due mainly to HIV or tuberculosis was associated with later increased risk of low insulin production among Tanzanian adults. [8]

Although being HIV-infected seems to increase risk of NCDs, there is limited information as to whether there are similar risks of perinatal exposure to maternal HIV in children who do not themselves become infected, that is, who are HIV-exposed, uninfected (HEU). For these children some effects of HIV exposure may be indirect, rather than direct causes of the virus. Figure 1 shows a conceptual framework linking exposure to maternal HIV, sociodemographic variables, and early growth pattern to the outcome of NCD risk. Children of HIV-infected mothers may be born at lower birth weight than HIV-unexposed, uninfected (HUU) children [9; 10] which may increase their NCD risk.[11] The lower birth weight could result from virus-induced factors such as inflammation[12] but also socioeconomic factors such as HIV-infected parents being less able to work with consequences for household food security and nutrition.[13] Demographic factors may also interact with HIV and socioeconomic factors over time; for example, HIV-infected parents may die, leaving their children as single or double orphans with consequent economic and other risks for nutrition and health. The long-term NCD risk appears to occur with malnutrition in childhood as well as prenatally, as evidenced by studies showing that exposure to famine during childhood was associated with increased adult diabetes in the Netherlands[14] and China.[15]

Although catch-up growth before age 2 years after being born low birth weight appears to carry little risk, later fast growth may increase NCD risk.[16; 17] Fast childhood weight gain is becoming increasingly common in countries undergoing the nutrition transition,[18] although it may be less common in children of the lowest socioeconomic status. While overt NCDs typically emerge in adult life, many studies have linked markers of fetal undernutrition with markers of poorer cardiometabolic health during childhood, such as raised blood pressure, insulin resistance and dyslipidaemia that may track into adulthood.[19; 20]

We have studied two cohorts of Zambian children whom we recruited for prior studies related to nutrition and HIV in order to investigate long-term effects of perinatal HIV exposure or early linear and ponderal growth trajectory on anthropometry, body composition and biomarkers for NCDs in early adolescence.

Methods

Design

This follow-up study, with field work conducted 2018-2019, was an analysis of children from previously followed up cohorts. Our primary exposures were perinatal exposure to maternal HIV and growth trajectory profiles in early life.

Participants

There were two separate cohorts of children: one previously recruited for a randomised controlled nutrition trial and the other for an observational cohort study. Both studies were conducted by the same research team and recruited from the same catchment area of Lusaka, Zambia. Most of these children had been previously followed up in 2014.[21]

For the Breastfeeding and Postpartum Health (BFPH) longitudinal cohort study,[9] HIV-infected and uninfected mothers of the children were recruited when pregnant and children were born between 2001 and 2004. Detailed information on maternal and infant health, infant feeding, and infant growth was collected until age 16 weeks. HIV status of all mothers was known through antenatal testing at the local government clinic. Children's HIV status was not assessed in the original BFPH study. At the time of the study the only antiretroviral therapy (ART) regimen available for prevention of mother-to-child transmission (PMTCT) in the area was perinatal nevirapine to both mother and infant. ART was not available for the women

themselves. Recruited HIV-infected women were slightly older and less likely to be primiparous than HIV-uninfected women and infants of the HIV-infected women were born at about 100 g lower weight.[9]

For the Chilenje Infant Growth, Nutrition and Infection Study (CIGNIS) randomised controlled trial comparing two locally made complementary foods, children born between 2005 and 2007 were recruited at age 6 months and participated until they were 18 months.[22] At the time of the study perinatal nevirapine was the local regimen for PMTCT. ART was available only for adults with CD4 count < 200 cells/□L until towards the end of the study when the cut-off was changed to < 350 cells/□L; only 5 of the CIGNIS children's mothers were on any ART. Agreement to HIV-testing of children by antibodies at 18 months, the only test available locally throughout most of the trial, was an inclusion criterion of the study. Children who died or defaulted before 18 months were not tested for HIV. Knowledge of maternal HIV status was not required, although antenatal HIV status from routine government health services was known for most of the women. HIV-infected mothers were older than HIV-uninfected mothers, were of lower education and more likely to be in the lowest tercile of an asset index. HIV-infected mothers were less likely to initiate breastfeeding and stopped earlier compared to HIV-uninfected mothers [23]. There were no differences in linear growth, the trial primary outcome, between the diet treatment groups.[22]

HIV status and exposure

Children of HIV-uninfected mothers who had themselves never been tested were considered HIV-unexposed, uninfected (HUU). Children of HIV-infected mothers whose own HIV status was missing, were included as HIV-exposed, uninfected (HEU) since we expected that, by adolescence, they would have begun to show symptoms on clinical examination if they were perinatally HIV-infected. Children whose mother's HIV status was unknown were coded as unknown HIV exposure. Only seven children were HIV-infected at the recent follow-up; they contributed to the determination of early growth trajectories but were not included in analyses of adolescent outcomes since it is already established that HIV-infected children often grow poorly. Furthermore, because they were few in number we lacked statistical power to include them as a comparison group. We therefore compared HUU against HEU.

Ethics

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human participants were approved by the University of Zambia Biomedical Research Ethics Committee and the London School of Hygiene and Tropical Medicine ethics committee. Written consent was obtained from carers for participants under 18 years old and from the participants themselves if over 18 years. Written assent was also obtained from all under-age children. If reason for medical intervention was found on clinical examination, children were referred to the local government services on the same site as the clinic.

Assessments at study visits

Children and parents or guardians were invited to the University Teaching Hospital (UTH) research clinic for a single visit. Questionnaires were used to collect demographic, socioeconomic and morbidity history data and children were given a clinical examination. We asked girls if they had started menstruating and we examined boys for Tanner stage.

Anthropometry data - weight, height, mid-upper arm circumference (MUAC), waist and hip circumferences, triceps, subscapular and suprailiac skinfolds – was collected by research nurses trained and experienced in anthropometry. The child's right side was used for MUAC and triceps skinfold. Blood pressure was measured using an automatic Omron IP20 sphygmomanometer. Grip strength for both hands was measured using a Takei GRIP-D dynamometer**.** The measurements, excluding body composition, were taken twice and analyses used the mean for most variables but the maximum for grip strength.

Venous blood samples were collected for triglycerides and total, HDL and LDL cholesterol. Blood lipids were measured using enzymatic assay kits from Pointe Scientific (Canton, MI, USA). Finger-prick blood samples were used for measurement of haemoglobin (Hb) and haemoglobin A1c (HbA1c) using handheld instruments from Hemocue (Angelhom, Sweden); due to problems with equipment or supplies, some children did not have these measurements. HbA1c was the only feasible method for assessing diabetes risk in the study because it was not possible to have children come to the clinic fasting or to conduct glucose tolerance tests. Mild anemia was defined as Hb ≥80 g/L and <120 g/L and severe anemia as Hb<80 g/L. It is unclear what level of HbA1c should be considered high in this population since preliminary analysis using a cut-off of >5.43% which is the 90th percentile for American children aged

10-14 years[24] found an unlikely percentage (82%) of the cohort with high HbA1c, possibly due to differences in genetic ancestry.[25] We chose to use a common adult cut-off for diabetes, HbA1c ≥6.5%, for internal comparisons, while recognising the limited ability to predict diabetes risk.

Body composition was measured using three independent methods: bioelectrical impedance (BIA) using Tanita BC418 instrumentation (Tokyo, Japan), air displacement plethysmography (ADP) using a BodPod (Life Measurement, Concord, CA, USA), and deuterium (D_2O) dilution according to standard methods.[26] For BIA and ADP, fat mass (FM) and fat-free mass (FFM) were obtained using in-built manufacturers' equations. For D_2O dilution, a baseline saliva sample was collected from participants at least 2 hours after their last meal. Each participant then received an oral dose (0.1 g/kg body weight) of D_2O (99.8% atom excess, Cambridge Isotope Laboratories, USA). Two end-point saliva samples were collected at 3 and 4 hours after D_2O dose ingestion; if they agreed within 3 mg/kg, indicating equilibration by 3 hours, the average was used; if not, the 4-hour sample was reanalysed as the duplicate. Saliva samples were stored in plastic saliva vials at −20 °C until analysis for D_2O abundance using a Fourier transform infrared spectrometer (Agilent Technologies, Malaysia, model 4500s). The enrichment was calculated by subtracting the value of the baseline sample from the value of the post dose sample. The calculated D_2O enrichment was then used in the calculation of body composition, using published values for FFM hydration to convert body water to FFM.[27] FM was calculated by difference of weight and FFM. For all body composition measures, we used in analyses fat mass index (FMI) and fat-free mass index (FFMI) which were calculated by dividing kgs fat or fat-free mass by height-squared, analogous to body mass index (BMI).

Data management and statistical analyses

Data was collected using the RedCap system and imported into Stata 16.1 for analysis. Height-for-age and BMI-for-age Z-scores were calculated using the World Health Organization (WHO) standards in the Stata zanthro command. Principal component analysis (PCA) was used to create a socioeconomic (SES) score from questionnaire data on family assets - car, bicycle, radio, television, refrigerator, mobile phone, livestock and poultry. The SES score was divided into terciles of low, middle and high socio-economic status. The PCA for SES was based on a larger group (n=514) of families from a wider cross-section of

Lusaka neighbourhoods since we were conducting a related study in parallel.[28] We collected data on both maternal and paternal education and occupation but present only maternal since a large proportion of paternal data was missing.

Preliminary analyses comparing FMI from the three measures of body composition (BIA, ADP and deuterium dilution) found that correlation coefficients among the measures ranged from 0.83 to 0.92, all with P<0.001. FMI was slightly lower for ADP (and consequently FFMI slightly higher since they sum to BMI) but the differences were small; FMI was 3.98 kg/m^2 (SD 3.22) by ADP, 4.39 kg/m^2 (SD 2.67) by BIA, and 4.47 kg/m^2 (SD 2.91) by D_2O. This suggested that the three body composition methods, though having different underlying assumptions and potential sources of error, were in general agreement. Moreover, as demonstrated previously,[29] an aggregate value for body composition obtained from several methods is more accurate than values from individual methods, as the error associated with each technique tends to cancel out. Therefore, we used as the FMI and FFMI outcomes in analyses the arithmetic means of results from the three methods.[29] Although there was a small amount of missing data for body composition by each method alone (1 for D_2O, 2 for ADP, 19 for BIA), the combined FMI and FFMI outcomes had no missing data.

We conducted two sets of analyses of the same outcome variables with different exposure variables. The first exposure was perinatal exposure to maternal HIV for which we compared HEU children with HUU children. The second exposure was early growth trajectory profile. To identify these we used latent class structural equation models, the *gsem* command, and weight-for-age (WAZ) and length-for-age (LAZ) Z scores from three time points: birth (only weight available in CIGNIS), infancy (4 months in BFPH, 6 months in CIGNIS), and early childhood (~3 years in BFPH, 18 months in CIGNIS). All data available, including from children who died or were lost to follow-up, was included in the determination of growth trajectory profile. To determine the optimal number of profiles which fitted the data best, we used the Bayesian information criteria (BIC, lower values are better), entropy values (values closer to 1 are better), predicted posterior probabilities (considered the certainty that a given participant was a member in their particular allocated profile), and ensured sample sizes in each group were a minimum of 62 individuals (5% of the sample size available to determine latent class grouping).

Outcome variables compared between our exposure groups, were anthropometry, FMI, FFMI, blood lipids, blood pressure, Hb, HbA1c, and grip strength. The primary analyses, using linear regression to generate mean differences and 95% confidence intervals (CI), were controlled for age and sex. We then conducted multivariable analyses adjusting for factors which either differed among HIV exposure groups in order to separate HIV exposure itself from sociodemographic factors associated with exposure, or were associated with loss to follow-up since original recruitment in order to control for survivor or other follow-up bias. Pubertal stage was not included as a covariate in analyses since it was collinear with age. To analyse all children's records, including the 26 who were missing HIV exposure information, the 87 missing HbA1c, and the 146 missing Hb plus a few other variables with small amounts of missing data, we used multiple imputation with chained equations to generate 10 multiple imputation datasets including exposures and all outcomes in the imputation model. We used overall Wald tests to determine the association between exposures and outcomes with a significance level of 0.05 and no correction for multiple testing.

In exploratory analysis and without formally fitting mediation models, we wished to investigate whether there was any evidence that early growth trajectory mediated the relationship between the HIV exposure and outcomes. Therefore, for outcomes associated with HIV exposure in age- and sex-controlled analyses, we added early growth profile to the regression models to determine whether associations with HIV exposure were modified by inclusion of early growth profile. We chose to include outcomes where the relationship between HIV exposure and outcome had $P<0.05$ in the age- and sex-controlled analyses, rather than those in the multivariable analyses, because the multiple associations between maternal HIV, early growth trajectory and sociodemographic variables meant that inclusion of the latter would be over-controlling.

Sample size

The sample size was dictated by the number in the cohorts who were available at follow-up (n=322). This number of children provided 90% power to detect outcome differences of effect size 0.36 between the two groups of HIV exposure and of 0.44 between pair-wise comparisons of the three early growth trajectory profiles. In order to have adequate statistical power when fitting structural equation models, the sample size of 322 individuals meets recommendations of at least 20 participants per explanatory variable. [30]

Results

Figure 2 shows that at this follow-up 322 HIV-uninfected children and adolescents were available from the original BFPH (n=48) and CIGNIS (n=274) studies. Seven HIV-infected children were recruited but not analysed for their follow-up data. Although dropout was high from both studies, especially from BFPH for whom their mothers were recruited 17-20 years ago, BFPH participants studied in 2019 did not differ from the those in the original cohort who were not followed up in terms of sex ratio, birth weight, HIV exposure or sociodemographic variables (Supplementary Table 1). The CIGNIS children followed up did not differ from those not followed up in sex ratio, birth weight or HIV exposure but were of higher socioeconomic status and their mothers were more likely to have been married and employed.

Associations of outcomes with HIV exposure

HIV status and exposure were known for all participants except 26 from the CIGNIS study. The proportion of participants HIV-exposed was similar within each cohort over time. All groups were evenly divided between boys and girls (Table 1). The HIV-unknown group was slightly younger and boys were at earlier pubertal stage than the other groups, likely because there were no BFPH children in this group. Socioeconomic tercile and maternal occupation did not differ among groups but fewer mothers of HEU children were married and they tended to have less education than mothers of HUU or HIV-unknown children.

Table 2 shows marginal mean differences between HIV exposure groups and anthropometry, body composition and grip strength using the imputed dataset; crude means without imputation are shown in Supplementary Table 2. Almost all differences were negative, i.e. the HEU children were smaller, and a few outcomes (hip circumference, triceps and subscapular skinfolds, FMI) were different at $P<0.05$ from HUU children in analyses controlled for age and sex. However, when further controlled for factors associated with HIV exposure or loss from the initial cohort (children of more educated or married mothers or in the higher socioeconomic terciles tended to be larger), there were no significant associations of outcomes with HIV exposure. Girls did not differ from boys in height but had higher BMI and indicators of body fat (data not shown).

Table 3 shows marginal mean differences in biochemical data according to child HIV exposure. Few differences were seen except that, in both age- and sex-controlled and fully

controlled analyses, HEU children had higher blood HDL, and in the fully adjusted analysis HEU children had borderline lower HbA1c than HUU children. Based on the imputed data, mild anemia (Hb between 80-120 g/L) was present in 27% of HUU and 28% of HEU children and severe anemia (Hb<80 g/L) was present in 5% of HUU and 2% of HEU children (chi^2 P=0.001). HbA1c was ≥6.5% for 53% of HUU and 47% of HEU children.

Associations of outcomes with latent class growth profiles.

Three latent class profiles were determined from analysis of the early growth data; we call these adequate growth, declining, and malnourished (Figure 3). The adequate growth group (454 children from the combined original data sets) gained considerably in WAZ and somewhat in LAZ during early life, the decliners (683 children) had fairly stable WAZ but declining LAZ, and the malnourished group (104 children) had mean WAZ and LAZ <-2 at all time points. More CIGNIS than BFPH children were in the declining (60% vs 46%) or malnourished (9.5% vs 6.3%) groups. Unsurprisingly, children who died during the original studies were more likely to be in the declining class (67% vs 54% for those who completed the original study and 59% for those lost to follow-up) or the malnourished class (21% vs 7.7% among completers and 9.7% among those lost). More children of HIV-infected mothers than of HIV-uninfected or HIV-unknown mothers (12% vs 7% and 9%) were in the malnourished group. Child HIV status was mostly unknown during the ages of data in the latent variable analysis but we know few children overall were HIV-infected themselves. Child sex was not associated with latent class.

Figure 4 shows that early growth trajectories tracked into later childhood. The malnourished group were significantly smaller than the adequate growth group for all anthropometric measures as well as FMI and FFMI. The declining group was smaller than the adequate growth group in HAZ, BMIZ, MUAC, hip and waist circumferences but not in skinfolds, FMI or FFMI.

Clinical outcomes according to early growth trajectories are in Table 4. The declining group had lower systolic blood pressure than the adequate growth group but no other differences. The malnourished group had lower grip strength and blood triglycerides than the improving group and higher HbA1c and HDL cholesterol. The prevalence of moderate and severe anemia did not differ by early growth profile. There was a trend (chi^2 P=0.08) towards

differences in the proportion of HbA1c ≥6.5% with 34% of the adequate growth group, 40% of the declining group, and 58% of the malnourished group having high HbA1c.

Combined HIV and early growth profiles

To explore evidence that early growth profile mediated the relationship between HIV exposure and outcomes, we selected outcomes associated, P<0.05, with HIV exposure in age- and sex-controlled analyses (Tables 2 and 3): BMI, hip circumference, triceps and subscapular skinfolds, FMI, HDL, and, because of its clinical importance and borderline associations, HbA1c. For all outcomes the coefficients for HIV exposure became closer to zero and the significance decreased (Supplementary Table 3). Coefficients for early growth classes changed little from those in age- and sex-controlled analyses shown in Figure 4 and Table 4 (data not shown).

Discussion

Similarly to when we studied the cohorts in 2014,[21] we found that by adolescence HEU children had lower markers of adiposity than HUU children when values were adjusted only for age and sex. However, with further adjustment for sociodemographic factors, of which maternal education appeared to have the most important associations, these differences were no longer significant, suggesting that sociodemographic factors interacting with HIV exposure were important. Hb, HbA1c, blood pressure and most blood lipids also did not differ between HEU and HUU groups. HDL cholesterol was higher in the HEU group. We wondered whether this was related to these children being thinner than the HUU children in this generally non-overweight population and found this was supported by exploratory analyses: when we included as covariables our indicators of adiposity (three skinfold thicknesses and FMI) the associations of HIV exposure with HDL were decreased and became non-significant (data not shown).

Perinatal HIV exposure may be associated with decreased *in utero* growth, as indicated by lower birth weight,[9; 10] and the present study showed it was also associated in some children with having a malnourished early postnatal growth profile. Different early growth trajectories according to HIV exposure could result from catch-up growth or from ongoing environmental factors which may differ from those HUU children experience, for example lower socioeconomic status, increased exposure to infections in the household, and increased risk of orphanhood. Exposure to parental opportunistic infections was likely common when the

BFPH and CIGNIS children were young since ART was not generally available for their parents. Different early growth trajectories between HEU and HUU or in households with different sociodemographic characteristics could have influenced anthropometry, body composition and risk factors for chronic disease later in life. These early growth patterns tracked into adolescence with the groups remaining different in height and markers of both lean mass and adiposity. We used the improving group as the reference in analyses but in general they exhibited low risk markers for NCDs, supporting previous studies which showed that early rapid growth is not a risk for NCDs among children in low- or middle-income countries.[16; 17] The children with the malnourished trajectory in early life had in early adolescence lower grip strength, higher HbA1c, lower blood triglycerides and higher HDL cholesterol. Low grip strength is associated with adverse health outcomes in many adult populations[31; 32] and higher HbA1c suggests an increased risk of diabetes, though we recognise the limitations of the cut-off we used for high HbA1c. Poor post-natal growth has been linked with increased diabetes risk in high-income settings for men born early in the 20th century.[33] Poor post-natal growth in the absence of specific diseases is currently rare in high-income countries but remains common in low- and middle-income countries. Our results differ from most studies of long-term health after childhood malnutrition which identify cases once they come to the clinic, whereas we were able to look at the critical infant period when children are still in the process of becoming malnourished.

Analysis of both HIV exposure and latent growth class together, reduced the associations between HIV exposure and outcomes which were significantly associated in the age- and sex-controlled analyses. This provides weak evidence that early growth mediates the relationship between maternal HIV exposure and outcomes. Further work is needed to determine the mechanisms whereby maternal HIV and sociodemographic variables led to poorer early growth and its consequences. We considered whether changes between initial recruitment in infancy and current follow-up in adolescence in the sociodemographic covariables investigated – mother's education and marital status and SES – influenced our findings. SES was determined differently in the two original studies and at follow-up so comparisons were not possible. Maternal education increased somewhat since recruitment so that the percentage with primary only decreased from 26% to 14%. More women at follow-up, 18%, were widowed or divorced than in the original studies, 4%; however, marital status was not associated with most outcomes. Furthermore, since we do not know at what stage these changes occurred, we were unable to control for them in analyses.

The study had several strengths including access to a well-characterised longitudinal cohort of children followed either from prenatal or infant life, detailed anthropometry, body composition measured in three ways, and analysis using multiple imputation to account for missing data. An important limitation is the large loss to follow-up from both cohorts since their original recruitment up to 20 years before; however, we used a missing at random analysis and controlled in the multivariable regressions for recruitment variables which differed between participants who were followed up or not. Although we had detailed early growth data from both cohorts, the exact ages at which these were measured differed between cohorts and we had no birth lengths for CIGNIS children; both these factors could have affected the allocation of individuals to latent class resulting in the potential for misclassification bias. HbA1c levels were unexpectedly high in the cohort which made it difficult to determine a cut-off which would be associated with current or later diabetes. We considered whether there may have been technical problems with the instrument but we think this unlikely since a) the instrument was calibrated according to the manufacturer's recommendations with both daily and weekly calibration, b) HbA1c values were not grossly elevated, and c) the study was done in parallel with a related study in a slightly younger population and we did not find such high prevalence of HbA1c ≥6.5% in those children.[28]

In conclusion, our results are encouraging by suggesting no serious long-term adverse effects on anthropometry, body composition or risk factors for NCDs among most HEU children from a relatively middle-class urban Zambian population. However, parental HIV in some families may be associated with sociodemographic factors which lead to poor growth which tracks into adolescence and is associated with some NCD risk factors: low grip strength and high HbA1c. Our work is innovative in that it captured growth trajectory profiles over time when children were in the process of becoming malnourished, not just at a point when they were brought for care of severe malnutrition. More rapid growth during infancy and early childhood was not associated with increased NCD risk. Our results suggest social support for improved nutrition and better health care, not necessarily just for HIV-affected families although for them it could be delivered within ART support programmes, would benefit the long-term health of children in Zambia and other African settings.

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Conflicts of interest

None

Authorship

AMR and SF designed and conducted statistical analyses. LK was study principal investigator and supervised the overall project. MC led the clinical work. GM was the technical expert and lead for body composition analyses. JCW advised on body composition analyses and data interpretation. SF drafted the manuscript and all authors contributed to it and approved the final version.

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Table 1: Characteristics of children according to HIV exposure

	HIV-unexposed	HIV-exposed, uninfected	HIV-unknown exposure	P[1]
N	208	88	26	
Sex, n (%) male	103 (50%)	40 (45%)	14 (54%)	0.70
Age (years), mean (SD)	12.8 (1.5)	13.3 (2.0)	11.8 (1.0)	<0.001
Girls started menstruating, n (%)[2]	55/104 (53%)	27/48 (56%)	4/12 (42%)	0.66
Boys' Tanner stage, n (%)[2]	3/102 (3%)	0/40 (0%)	0/14 (0%)	0.05
1	37/102 (36%)	10/40 (25%)	9/14 (64%)	
2	31/102 (30%)	10/40 (25%)	4/14 (29%)	
3	27/102 (26%)	14/40 (35%)	1/14 (7%)	
4	4/102 (4%)	6/40 (15%)	0/14 (0%)	
5				
Mother's marital status n (%)				
Married/cohabiting	153 (74%)	49 (56%)	22 (85%)	0.004
Single	19 (9%)	11 (13%)	1 (4%)	
Widowed	18 (9%)	14 (16%)	0	
Divorced	14 (7%)	9 (10%)	2 (8%)	
Unknown	4 (2%)	5 (6%)	1 (4%)	
Mother's education, n (%)				
Primary or less	24 (12%)	16 (18%)	5 (19%)	0.006
Some secondary	40 (19%)	32 (36%)	3 (12%)	
Completed secondary	53 (25%)	17 (19%)	7 (27%)	
College, university	88 (42%)	18 (20%)	10 (38%)	
Unknown	3 (1%)	5 (6%)	1 (4%)	
Mother's occupation, n (%)				
Employed	154 (74%)	54 (61%)	17 (65%)	0.36
Housewife	36 (17%)	21 (24%)	5 (19%)	

Unemployed/student	6 (3%)	6 (7%)	2 (8%)	
Other/unknown	12 (6%)	7 (8%)	2 (8%)	
Socioeconomic tertiles,[2] n (%)	90 (43%)	51 (58%)	12 (46%)	0.11
Low	26 (13%)	11 (13%)	3 (12%)	
Medium	92 (44%)	26 (30%)	11 (42%)	
High				

[1] P values are from ANOVA for continuous variables and chi-square for categorical variables

[2] Menstruation missing for 1 HIV-unexposed girl and Tanner stage for 1 HIV-unexposed boy

[3] Calculated using principal components analysis (PCA) from a list of assets terciles included another group of children followed at the same time for a different study which is why terciles are imbalanced.

Table 2. Association of HIV exposure and status with anthropometry, body composition and grip strength using data from multiple imputation [1,2]

Outcome		Imputed marginal means (95% CI)[4]	Coefficient adjusted for age and sex (95% CI)	P	Multivariable coefficient (95% CI)[5]	P
Height (cm)		152.3 (151.1, 153.5)	Ref		Ref	
HUU		152.3 (150.7, 154.0)	-0.01 (-1.71, 1.69)	0.99	0.84 (-0.90, 2.59)	0.34
	HEU					
Height-for-age Z		-0.27 (-0.39, -0.14)	Ref		Ref	
HUU		-0.28 (-0.45, -0.11)	-0.01 (-0.23, 0.20)	0.89	0.09 (-0.14, 0.32)	0.44
	HEU					
Weight (kg)		45.7 (44.0, 47.4)	Ref		Ref	
HUU		43.5 (41.5, 45.5)	-2.18 (-4.59, 0.22)	0.08	-0.60 (-3.02, 1.81)	0.62
	HEU					
Body mass index (kg/m^2)		19.5 (18.9, 20.1)	Ref		Ref	
HUU		18.6 (17.9, 19.3)	-0.91 (-1.80, -0.02)	0.05	-0.41 (-1.31, 0.49)	0.37
	HEU					
Body mass index-for-age Z		0.04 (-0.14, 0.23)	Ref		Ref	
HUU		-0.22 (-0.46, 0.03)	-0.26 (-0.57, 0.05)	0.10	-0.07 (-0.38, 0.25)	0.67
	HEU					
MUAC (cm)		23.7 (23.2, 24.3)	Ref		Ref	
HUU		23.0 (22.4, 23.6)	-0.8 (-1.6, 0.0)	0.06	-0.3 (-1.1, 0.6)	0.56

	HEU					
Hip circumference (cm)		83.5 (82.0, 85.1)	Ref		Ref	
HUU		81.2 (79.4, 83.0)	-2.3 (-4.5, -0.1)	0.04	-0.9 (-3.1, 1.4)	0.45
	HEU					
Waist circumference (cm)		65.8 (64.6, 67.1)	Ref		Ref	
HUU		64.3 (62.8, 65.9)	-1.5 (-3.4, 0.5)	0.13	-0.5 (-2.5, 1.4)	0.59
	HEU					
Triceps skinfold (mm)		13.0 (12.1, 13.8)	Ref		Ref	
HUU		11.4 (10.3, 12.5)	-1.5 (-2.9, -0.2)	0.03	-0.8 (-2.2, 0.6)	0.26
	HEU					
Subscapular skinfold (mm)		11.0 (10.2, 11.8)	Ref		Ref	
HUU		9.5 (8.7, 10.4)	-1.4 (-2.6, -0.3)	0.02	-0.8 (-1.9, 0.4)	0.19
	HEU					
Suprailiac skinfold (mm)		10.2 (9.3, 11.1)	Ref		Ref	
HUU		9.0 (7.9, 10.1)	-1.2 (-2.5, 0.2)	0.10	-0.5 (-1.9, 0.9)	0.50
	HEU					
Fat mass index (kg/m^2)[3]		4.5 (4.1, 4.9)	Ref		Ref	
HUU		3.8 (3.4, 4.3)	-0.62 (-1.20, -0.03)	0.04	-0.37 (-0.97, 0.22)	0.22
	HEU					
Fat-free mass index (kg/m^2) [3]		14.9 (14.7, 15.2)	Ref		Ref	
HUU		14.7 (14.4, 15.1)	-0.22 (-0.66, 0.23)	0.34	0.07 (-0.40, 0.55)	0.76
	HEU					

Grip strength (kg)		21.9 (21.1, 22.6)	Ref		Ref	
HUU		22.0 (21.0, 23.0)	0.2 (-1.0, 1.3)	0.27	0.5 (-0.8, 1.7)	0.47
	HEU					

[1] HEU, HIV-exposed, uninfected; HUU, HIV-unexposed; MUAC, mid-upper arm circumference

[2] There were 227 children in the HUU group and 95 in the HEU group. Numbers in analyses differed by imputation dataset.

[3] Mean body composition combining results from bioelectrical impedance, air displacement plethysmography and deuterium dilution methods. Indices are fat or fat-free mass in kg divided by height in meters-squared.

[4] Marginal means controlling for age and sex; marginal means from multivariable differed from these only modestly and are not shown.

[5] Multivariable coefficients represent the difference from the HUU group, adjusted for age, sex, maternal education, maternal marital status and socioeconomic tercile.

Table 3. Association of HIV exposure and infection with clinical variables using data from multiple imputation [1,2]

Outcome		Imputed marginal means (95% CI)[3]	Difference adjusted for age and sex (95% CI)	P	Multivariable coefficient (95% CI)[4]	P
Hemoglobin (g/L)		106 (102, 109)	Ref		Ref	
HUU		103 (98, 108)	-2.6 (-8.7, 3.5)	0.40	-2.4 (-8.9, 4.1)	0.47
	HEU					
HbA1c (%)		5.9 (5.7, 6.0)	Ref		Ref	
HUU		5.7 (5.5, 5.8)	-0.2 (-0.5, 0.1)	0.12	-0.3 (-0.5, 0.0)	0.05
	HEU					
Systolic blood pressure (mmHg)		105 (103, 106)	Ref		Ref	
HUU		106 (103, 108)	1.0 (-1.7, 3.7)	0.46	1.1 (-1.6, 3.8)	0.42
	HEU					
Diastolic blood pressure (mmHg)		66 (65, 66)	Ref		Ref	
HUU		66 (64, 67)	0.2 (-1.6, 2.1)	0.80	0.0 (-1.8, 1.8)	0.98
	HEU					
Blood lipids						

Triglycerides (mmol/L)		0.84 (0.78, 0.90)	Ref		Ref	
HUU		0.79 (0.71, 0.88)	-0.05 (-0.16, 0.06)	0.40	-0.02 (-0.13, 0.09)	0.74
	HEU					
Cholesterol (mmol/L)		3.83 (3.71, 3.94)	Ref		Ref	
HUU		3.87 (3.72, 4.02)	0.04 (-0.15, 0.23)	0.69	0.11 (-0.09, 0.30)	0.26
	HEU					
HDL cholesterol (mmol/L)		1.09 (1.05, 1.13)	Ref		Ref	
HUU		1.16 (1.11, 1.22)	0.07 (0.00, 0.14)	0.04	0.08 (0.01, 0.15)	0.03
	HEU					
LDL cholesterol (mmol/L)		2.35 (2.26, 2.44)	Ref		Ref	
HUU		2.34 (2.22, 2.47)	-0.01 (-0.16, 0.15)	0.94	0.05 (-0.11, 0.22)	0.51
	HEU					

[1] HEU, HIV-exposed, uninfected; HUU, HIV-unexposed; HbAc1, haemoglobin A1c; HDL, high density lipoprotein; LDL, low density lipoprotein

[2] There were 227 children in the HUU group and 95 in the HEU group. Numbers in analyses differed by imputation dataset.

[3] Marginal means controlling for age and sex; marginal means from multivariable differed from these only modestly and are not shown.

[4] Multivariable coefficients represent the difference from the HUU group, adjusted for age, sex, maternal education, maternal marital status and socioeconomic tercile.

Table 4. Association of early growth latent variable classes with clinical variables using data from multiple imputation [1,2]

Outcome		Imputed marginal means (95% CI)[4]	Difference adjusted for age and sex (95% CI)	P[3]	Multivariable coefficient (95% CI)[5]	P[3]
Grip strength (kg) growth	adequate	22.6 (21.7, 23.5)	Ref		Ref	
	declining	21.7 (20.9, 22.5)	-0.84 (-1.91, 0.23)	0.12	-0.71 (-1.79, 0.38)	0.20
	malnourished	20.4 (19.0, 21.7)	-2.20 (-3.72, -0.69)	0.005	-1.87 (-3.47, -0.27)	0.02
	overall P		P=0.02		P=0.07	
Hemoglobin (g/L) growth	adequate	104 (100, 108)	Ref		Ref	
	declining	107 (103, 111)	2.8 (-3.0, 8.6)	0.35	3.2 (-2.8, 9.1)	0.29
	malnourished	99 (88, 109)	-5.3 (-16.4, 5.7)	0.34	-5.2 (-16.3, 5.9)	0.36
	overall P		P=0.30		P=0.26	
HbA1c (%) growth	adequate	5.7 (5.5, 5.8)	Ref		Ref	
	declining	5.9 (5.6, 6.1)	0.2 (-0.1, 0.5)	0.13	0.2 (-0.1, 0.5)	0.17
	malnourished	6.2 (5.9, 6.6)	0.6 (0.2, 0.9)	0.001	0.5 (0.2, 0.9)	0.005

	malnourished		P=0.004		P=0.02	
	overall P					
Systolic blood pressure (mmHg)						
	adequate growth	107 (105, 108)	Ref		Ref	
	declining	104 (102, 105)	-2.9 (-5.3, -0.5)	0.02	-2.8 (-5.2, -0.5)	0.02
	malnourished	106 (102, 110)	-1.2 (-5.5, 3.2)	0.60	-1.8 (-6.5, 2.9)	0.46
	overall P		P=0.05		P=0.07	
Diastolic blood pressure (mmHg)						
	adequate growth	66 (65, 67)	Ref		Ref	
	declining	65 (64, 67)	-0.3 (-2.0, 1.4)	0.73	-0.4 (-2.1, 1.3)	0.62
	malnourished	65 (63, 68)	-0.6 (-3.4, 2.3)	0.69	-1.3 (-4.3, 1.6)	0.38
	overall P		P=0.90		P=0.67	
<u>Blood lipids</u>						
Triglycerides (mmol/L)	adequate growth	0.88 (0.79, 0.97)	Ref		Ref	
		0.82 (0.76, 0.88)	-0.06 (-0.17, 0.05)	0.26	-0.04 (-0.15, 0.07)	0.45

	declining	0.63 (0.55, 0.72)	-0.25 (-0.38, -0.12)	<0.001	-0.22 (-0.37, -0.08)	0.003
	malnourished		P<0.001		P=0.003	
	overall P					
Cholesterol (mmol/L) growth	adequate	3.84 (3.69, 3.99)	Ref		Ref	
		3.84 (3.72, 3.96)	0.00 (-0.19, 0.20)	0.98	0.06 (-0.14, 0.26)	0.59
	declining	3.81 (3.47, 4.15)	-0.03 (-0.40, 0.34)	0.88	0.04 (-0.34 0.42)	0.83
	malnourished		P=0.98		P=0.86	
	overall P					
HDL cholesterol (mmol/L)						
	adequate growth	1.08 (1.03, 1.13)	Ref		Ref	
	declining	1.11 (1.07, 1.15)	0.03 (-0.03, 0.09)	0.38	0.03 (-0.03, 0.10)	0.35
	malnourished	1.29 (1.15, 1.43)	0.21 (0.07, 0.36)	0.005	0.23 (0.08, 0.38)	0.003
	overall P		P=0.02		P=0.01	
LDL cholesterol (mmol/L)						
	adequate growth	2.39 (2.27, 2.51)	Ref		Ref	

declining	2.34 (2.24, 2.44)	-0.05 (-0.21, 0.11)	0.55	-0.01 (-0.17, 0.15)	0.94
malnourished	2.19 (1.91, 2.47)	-0.20 (-0.50, 0.10)	0.20	-0.14 (-0.45, 0.17)	0.38
overall P		P=0.43		P=0.64	

[1] HEU, HIV-exposed, uninfected; HUU, HIV-unexposed; HbAc1, haemoglobin A1c; HDL, high density lipoprotein; LDL, low density lipoprotein

[2] There were 112 children in the adequate growth class, 184 in the declining class, and 26 in the malnourished class.

[3] P values within columns of coefficients are for the overall association with latent growth profile while those in columns are for comparisons with the adequate growth group

[4] Marginal means controlling for age and sex; marginal means from multivariable differed from these only modestly and are not shown.

[5] Multivariable coefficients represent the difference from the HUU group, adjusted for age, sex, maternal education, maternal marital status and socioeconomic tercile.

Supplementary Table 1: Characteristics of children from the original BFPH and CIGNIS studies according to whether they were or were not available in the 2018-2019 follow-up

	BFPH followed up	BFPH not followed up	P[1]	CIGNIS followed up	CIGNIS not followed up	P[1]
N	49	380		280	531	
Sex, [2] n (%) male	25 (51%)	171 (47%)	0.57	140 (50%)	245 (46%)	0.30
Birth weight[2] (kg), mean (SD)	3.08 (0.47)	2.98 (0.43)	0.93	3.09 (0.50)	3.02 (0.48)	0.96
HIV exposure, n (%)	26 (53%)	192 (51%)	0.73	182 (65%)	375 (71%)	0.47
unexposed	23 (47%)	188 (49%)		72 (26%)	109 (21%)	
Exposed	0 (0%)	0 (0%)		26 (9%)	47 (9%)	
unknown						
Mother's marital status n (%)						
Married	30 (61%)	278 (73%)	0.18	227 (81%)	380 (72%)	0.03
Single or cohabiting	18 (37%)	93 (24%)		40 (14%)	126 (24%)	
Widowed/divorced/other	1 (2%)	9 (2%)		13 (5%)	25 (5%)	
Mother's education, n (%)						
Primary or less	7 (14%)	62 (16%)	0.92	81 (29%)	188 (35%)	0.15
Secondary	29 (59%)	215 (57%)		110 (39%)	199 (37%)	
College, university	13 (27%)	102 (27%)		89 (32%)	144 (27%)	
Mother's occupation, n (%)						
Employed	12 (24%)	97 (25%)	0.94	103 (37%)	136 (26%)	0.02

Housewife	37 (76%)	282 (74%)		139 (50%)	283 (53%)	
Unemployed/student/unknown	0 (0%)	1 (0.3%)		38 (14%)	102 (21%)	
Socioeconomic tertiles,[3] n (%)						
Low	8 (16%)	90 (24%)	0.27	73 (26%)	199 (37%)	0.004
Medium	37 (76%)	242 (64%)		107 (38%)	163 (31%)	
High	4 (8%)	47 (12%)		100 (34%)	169 (32%)	

[1] P values are from t test for continuous variables and chi-square for categorical variables

[2] In BFPH women were recruited during pregnancy so infant data is missing for those lost to follow-up before delivery; therefore, for the not-followed group, sample size is 366 for infant age and sex. In CIGNIS birth weights were from infant clinic cards which were missing for 1 child followed up and 12 not followed up.

[3] Socioeconomic was determined by housing location in BFPH and using principal components analysis (PCA) from a list of assets in CIGNIS.

Supplementary Table 2: Anthropometric, body composition, clinical and biochemical outcomes of children according to HIV exposure; original data with no multiple imputation

	HIV-unexposed	HIV-exposed, uninfected	HIV-unknown exposure	P[2]
N[1]	208	88	26	
Height (cm)	152.1 (SD 9.2)	154.2 (SD 10.7)	147.1 (SD 8.1)	0.004
Height-for-age Z	-0.27 (SD 0.98)	-0.29 (SD 0.84)	-0.20 (0.97)	0.92
Weight (kg)	45.6 (SD 13.3)	45.5 (SD 12.1)	39.1 (SD 9.6)	0.05
Body mass index (kg/m^2)	19.5 (SD 4.6)	19.0 (SD 3.9)	17.9 (SD 2.8)	0.16
Body mass index-for-age Z	0.04 (SD 1.42)	-0.20 (SD 1.25)	-0.11 (SD 1.19)	0.37
Mid-upper arm circumference (cm)	23.7 (SD 4.3)	23.5 (SD 3.5)	22.0 (SD 3.2)	0.13
Hip circumference (cm)	83.4 (SD 12.2)	82.3 (SD 10.6)	78.3 (SD 9.0)	0.11
Waist circumference (cm)	65.9 (SD 9.6)	65.2 (SD 8.3)	62.3 (SD 5.7)	0.16
Triceps skinfold (mm)	12.9 (SD 6.8)	11,9 (SD 6.1)	11.1 (SD 5.7)	0.23
Subscapular skinfold (mm)	11.0 (SD 6.3)	9.9 (SD 4.8)	9.2 (SD 3.5)	0.17
Suprailiac skinfold (mm)	10.3 (SD 6.6)	9.4 (SD 6.0)	8.3 (SD 4.0)	0.24
Fat mass index (kg/m^2)[3]	4.8 (SD 3.0)	4.0 (SD 2.4)	3.6 (SD 1.8)	0.19
Fat-free mass index (kg/m^2) [3]	14.9 (SD 2.1)	15.0 (SD 2.1)	14.2 (SD 1.5)	0.22
Grip strength (kg)	21.6 (SD 5.5)	22.9 (SD 6.4)	20.1 (SD 4.7)	0.06
Hemoglobin (g/L)	106 (SD 19)	103 (SD 18)	103 (SD 16)	0.67
Hemoglobin A1c (%)	5.9 (SD 1.1)	5.7 (SD 0.8)	5.6 (SD 0.5)	0.25
Systolic blood pressure (mmHg)	104 (SD 10)	106 (SD 12)	105 (SD 8)	0.44
Diastolic blood pressure (mmHg)	65 (SD 7)	66 (SD 8)	64 (SD 6)	0.47
Blood lipids				

Triglycerides (mmol/L)	0.84 (SD 0.47)	0.78 (SD 0.37)	0.88 (SD 0.48)	0.48
Cholesterol (mmol/L)	3.8 (SD 0.9)	3.9 (SD 0.7)	4.0 (SD 0.8)	0.46
HDL cholesterol (mmol/L)	1.1 (SD 0.3)	1.2 (SD 0.3)	1.1 (SD 0.3)	0.09
LDL cholesterol (mmol/L)	2.3 (SD 0.7)	2.3 (SD 0.6)	2.6 (SD 0.7)	0.26

[1] For most outcomes, sample sizes are 208 HIV-unexposed (HUU), 88 HIV-exposed (HEU), 26 HIV-exposure unknown. Missing data were 3 HUU suprailiac skinfolds, 1 HUU and 1 HEU grip strength, 57 HUU, 22 HEU and 8 HIV-unknown HbA1c, 93 HUU, 41 HEU and 12 HIV-unknown for haemoglobin.

[2] P values are from linear regression

[3] Mean body composition combining results from bioelectrical impedance, air displacement plethysmography and deuterium dilution methods.

Supplementary Table 3. Modification of associations between HIV exposure and outcomes by inclusion of latent growth class in analyses[1,2]

	HEU versus HUU		HEU versus HUU including early growth class	
	Coefficient (95% confidence interval)	**P**	**Coefficient (95% confidence interval)**	**P**
Body mass index (kg/m^2)	-0.91 (-1.80, -0.02)	0.05	-0.76 (-1.64, 0.13)	0.09
Hip circumference (cm)	-2.3 (-4.5, -0.1)	0.04	-1.9 (-4.0, 0.3)	0.09
Triceps skinfold (mm)	-1.5 (-2.9, -0.2)	0.03	-1.4 (-2.8, 0.0)	0.05
Subscapular skinfold (mm)	-1.4 (-2.6, -0.3)	0.02	-1.3 (-2.4, -0.1)	0.03
Fat mass index (kg/m^2)	-0.62 (-1.20, -0.03)	0.04	-0.52 (-1.10, 0.05)	0.08
HbA1c (%)	-0.2 (-0.5, 0.1)	0.12	-0.2 (-0.5, 0.0)	0.08
HDL cholesterol (mmol/L)	0.07 (0.00, 0.14)	0.04	0.06 (-0.01, 0.13)	0.08

[1]HEU, HIV-exposed, uninfected; HUU, HIV-unexposed, uninfected

[2]Coefficients and P values are from linear regressions comparing HEU with HUU children, controlling for age and sex, without or with inclusion of early latent growth class (adequate growth, declining, malnourished). Values without latent growth class are the same as those in Tables 2 and 3.

Figure 1. Conceptual framework linking HIV exposure and early growth trajectory to later risk factors for non-communicable diseases

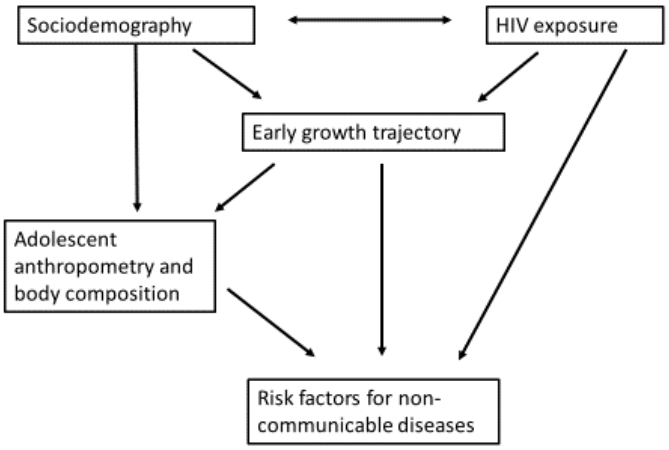

Figure 2. Flow chart of study participants[1]

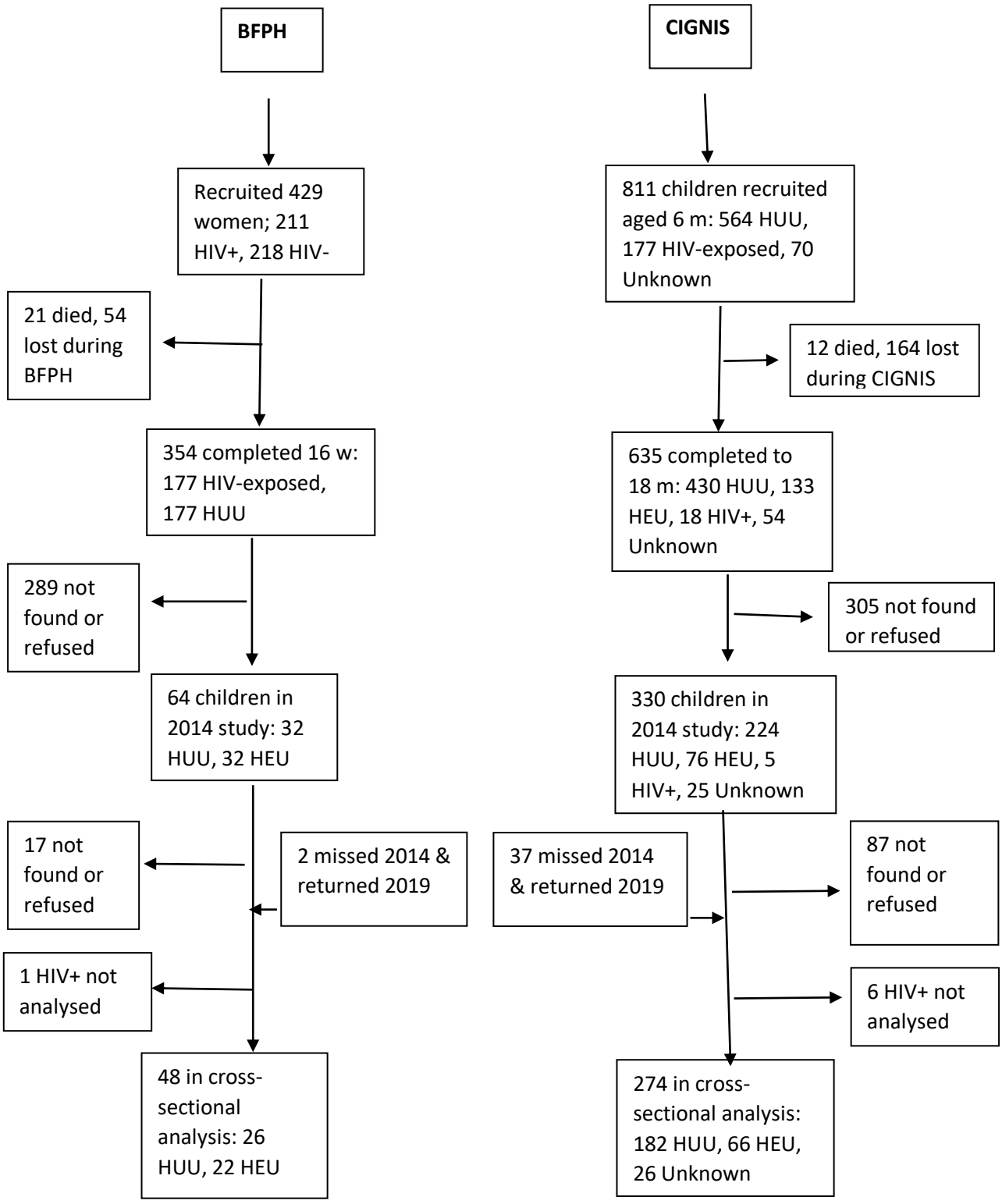

Figure 3. Early growth trajectory profiles determined by latent class analysis

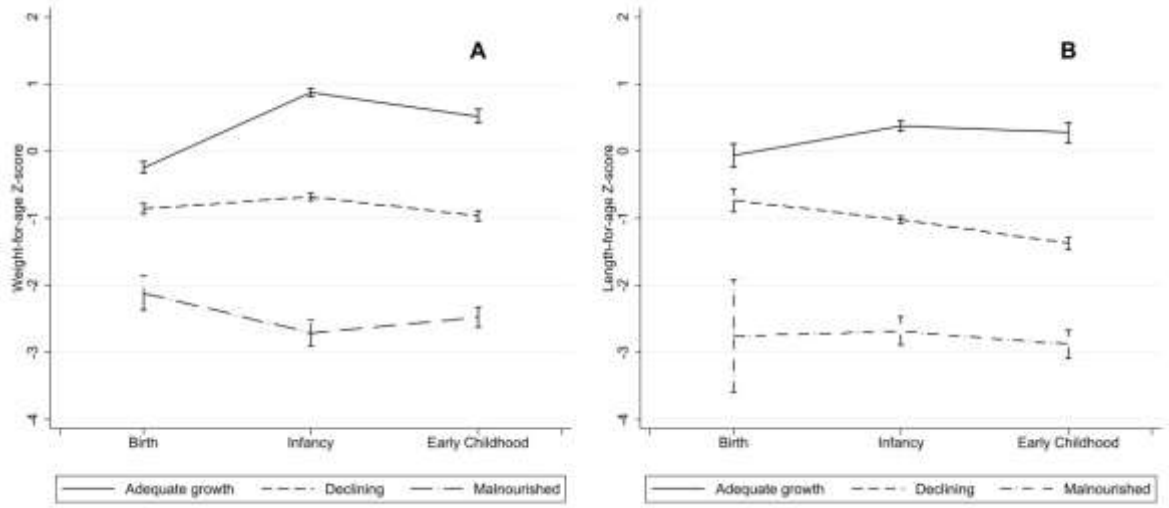

Figure 4. Anthropometry at follow-up according to early growth trajectory profile