

**Inflammation, cytomegalovirus and the growth hormone axis in HIV-exposed  
uninfected Zimbabwean infants**

Ceri Evans MRCPCH,<sup>1,2</sup> Bernard Chasekwa MSc,<sup>1</sup> Sandra Rukobo DMLT,<sup>1</sup> Margaret Goyha  
DMLT,<sup>1</sup> Kuda Mutasa MPH,<sup>1</sup> Robert Ntozini MPH,<sup>1</sup> Jean H. Humphrey ScD,<sup>1,3</sup> Andrew J.  
Prendergast DPhil<sup>1,2,3</sup>

<sup>1</sup>Zvitambo Institute for Maternal and Child Health Research, Harare, Zimbabwe

<sup>2</sup>Blizard Institute, Queen Mary University of London, London, UK

<sup>3</sup>Department of International Health, Johns Hopkins Bloomberg School of Public  
Health, Baltimore, MD, USA

**Corresponding author**

Dr. Ceri Evans MRCPCH, Blizard Institute, Queen Mary University of London, 4 Newark  
Street, London E1 2AT, UK. Tel +44 207 882 2269, fax +44 207 882 2195,  
ceri.evans@qmul.ac.uk.

**Conflicts of Interest and Source of Funding**

None of the authors declare any conflicts of interest.

This work was funded by a grant from Barts Charity, awarded to CE (MGU0300), and by the Wellcome Trust awarded to AJP (093768/Z/10/Z and 108065/Z/15/Z). CE was funded by the Wellcome Trust (210807/Z/18/Z) and the National Institute for Health Research. AJP was funded by the Wellcome Trust (093768/Z/10/Z and 108065/Z/15/Z). The ZVITAMBO trial was supported by the Canadian International Development Agency (CIDA) (R/C Project 690/M3688), United States Agency for International Development (USAID) (cooperative agreement number HRN-A-00-97-00015-00 between Johns Hopkins University and the Office of Health and Nutrition – USAID) and a grant from the Bill and Melinda Gates Foundation, Seattle WA. Additional funding was received from the SARA Project, which is operated by the Academy for Educational Development, Washington DC and is funded by USAID’s Bureau for Africa, Office of Sustainable Development under the terms of Contract AOT-C-00-99-00237-00, the Rockefeller Foundation (NY, NY) and BASF (Ludwigshafen, Germany).

### **Previous presentations**

This work was presented at the Conference on Retroviruses and Opportunistic Infections (CROI), Boston, MA, 2018.

### **ACKNOWLEDGEMENTS**

CE and AJP designed the analysis. CE, SR, MG and KM carried out the laboratory assays. CE, BC and RN carried out the statistical analyses. CE wrote the first draft of the manuscript. All authors critically reviewed and revised the manuscript. JHH designed and recruited to the original trial and RN was a co-investigator on the original trial.

**Key words:** HIV-exposed uninfected; childhood growth; growth hormone axis; cytomegalovirus; inflammation; Africa.

## ABSTRACT

**Objectives** Despite avoiding HIV infection, HIV-exposed uninfected (HEU) infants have poorer clinical outcomes than HIV-unexposed infants, including impaired growth. The growth hormone (GH) axis is an important regulator of infant growth through hepatic synthesis of insulin-like growth-factor-1 (IGF-1), and may be disrupted by chronic inflammation and acute infections, including cytomegalovirus (CMV). We tested the hypothesis that these factors lead to disruption of the GH axis in HEU infants, which might contribute to their impaired growth.

**Design:** Sub-study of 343 infants from the ZVITAMBO trial in Harare, Zimbabwe.

**Methods:** IGF-1, growth parameters, C-reactive protein (CRP) and CMV viremia were evaluated in 243 HEU infants and 100 HIV-unexposed infants. Univariable linear and logistic regression models were used to determine associations between IGF-1 and growth parameters, CRP and CMV.

**Results:** Mean 6-week IGF-1 was significantly lower in HEU compared to HIV-unexposed infants (29.6 vs. 32.6ng/mL;  $P=0.01$ ), and associated with subsequent linear and ponderal growth through 6 months of age. CRP was inversely correlated with IGF-1 in all infants regardless of HIV exposure status ( $\beta=-0.84$ ;  $P=0.03$ ). CMV viral loads were inversely correlated with IGF-1 in HEU ( $\beta=-1.16$ ;  $P=0.008$ ) but not HIV-unexposed ( $\beta=0.21$ ;  $P=0.83$ ) infants.

**Conclusions:** Overall, we found evidence for greater disruption of the GH axis in HEU compared to HIV-unexposed infants as early as 6 weeks of age, suggesting a role for reduced IGF-1 in mediating growth impairment in HEU infants. Inflammation and co-infections may be drivers of growth impairment in HEU infants by disrupting the growth hormone axis.

## **INTRODUCTION**

HIV-exposed uninfected (HEU) infants have poorer growth than HIV-unexposed infants<sup>1-3</sup>. Impaired linear growth is associated with increased mortality, reduced neurodevelopment and lower human capital. Although the pathogenesis of growth failure remains unclear, systemic inflammation and co-infections such as cytomegalovirus (CMV) may contribute.<sup>4,5</sup> The growth hormone (GH) axis regulates infant growth through hepatic synthesis of insulin-like growth-factor-1 (IGF-1),<sup>6</sup> which is disrupted by chronic inflammation<sup>7</sup> and acute infections,<sup>8</sup> including CMV.<sup>9</sup> We recently showed that Zimbabwean HEU infants have elevated inflammatory markers, a high prevalence of CMV infection in early life, and higher CMV viral loads compared to HIV-unexposed infants.<sup>10</sup> Here, we tested the hypothesis that these factors suppress the GH axis and contribute to impaired growth in HEU infants.

## **METHODS**

The ZVITAMBO trial was undertaken prior to maternal or infant ART availability (1997-2001).<sup>11</sup> Briefly, 14110 mother-infant pairs were recruited at birth and followed for 1-2 years. Exclusion criteria included birthweight <1500g, plans to leave Harare, multiple pregnancy, and life-threatening medical conditions. Mothers underwent longitudinal HIV testing.<sup>11</sup> Longitudinal blood samples from children were cryopreserved; the last available sample was HIV-tested by DNA PCR (Roche Amplicor, v1.5) or ELISA (GeneScreen; Sanofi Diagnostics Pasteur) depending on age. HEU infants in this study were defined as infants born to HIV-positive mothers who remained HIV-negative through 6-months of age.

## **Infant selection**

We selected all HEU infants in whom mother and infant survived through 6-months, with available infant anthropometry and IGF-1 measurement from 6-weeks of age. A random selection of 100 HIV-unexposed infants meeting these criteria provided comparative data.

## **Anthropometry**

Weight, length and head circumference were measured at birth, 6wks, 3mo and 6mo using previously described methods,<sup>12</sup> and converted to Z-scores using WHO standards.<sup>13</sup>

## **Laboratory assays**

Plasma IGF-1 and C-reactive protein (CRP) were measured by ELISA (R&D Systems, MN). Viral nucleic acid was extracted from plasma using the QIAamp DSP Virus Spin Kit (Qiagen, Hilden), and CMV was detected by quantitative PCR using the Abbott RealTime CMV Amplification Reagent Kit (Abbott Laboratories, IL) on the Abbott m2000rt platform.<sup>10</sup>

## **Analyses**

We used unpaired *t*-tests and Mann-Whitney tests to compare continuous variables, and Fisher's exact tests for categorical variables. Univariable linear and logistic regression was used to determine associations between IGF-1 and growth. Where there was a possible interaction with HIV exposure ( $P < 0.2$ ), results were stratified by maternal HIV status. We used univariable linear and logistic regression to determine associations between IGF-1 and CRP and CMV, and to determine the modifying effect of HIV exposure. Where there was a possible interaction with HIV exposure ( $P < 0.2$ ), analyses were stratified, and multivariable linear regression was used to adjust for maternal HIV disease severity. Analyses were undertaken using Prism v6.0 (Graphpad, CA) and STATA v15.1 (StataCorp, TX).

## Ethical approvals

Mothers provided written informed consent. The trial and this sub-study were approved by the Medical Research Council of Zimbabwe, Johns Hopkins Bloomberg School of Public Health Committee on Human Research, and Montreal General Hospital Ethics Committee.

## RESULTS

243 HEU and 100 HIV-unexposed infants were included. Baseline characteristics are shown in Supplementary Table 1, <http://links.lww.com/QAD/B799>. HEU and HIV-unexposed infants were similar at baseline, but HEU infants were born to mothers with lower mid-upper arm circumference (25.6 vs. 26.2cm;  $P=0.04$ ) and haemoglobin (113 vs. 121g/L;  $P=0.005$ ), and there were differences in breastfeeding.<sup>10</sup> Although there were no statistically significant differences in growth between HEU and HIV-unexposed infants in this sub-study (Supplementary Table 1, <http://links.lww.com/QAD/B799>), analysis of the whole ZVITAMBO cohort (N=14,110) demonstrated significantly poorer growth amongst HEU compared to HIV-unexposed infants.<sup>2</sup>

### IGF-1 and growth

Mean IGF-1 at 6-weeks was lower in HEU infants (29.5ng/mL (SD 10.2) than HIV-unexposed infants (32.6ng/mL (10.4);  $P=0.014$ ; Figure 1A). Table 1 shows associations between IGF-1 and growth from univariable linear regression analyses. Six-week IGF-1 was associated with length-for-age Z-score (LAZ) between 6wks-6mo (Figure 1B). The effect of IGF-1 on LAZ was not modified by infant HIV exposure status (all time-points  $P>0.2$ ). IGF-1 was positively associated with weight-for-age Z-score (WAZ) at all time-points, and not modified by HIV exposure ( $P >0.2$ ). IGF-1 was positively associated with weight-for-length Z-score (WLZ) at 6wks; there was some evidence that this association was modified by HIV

exposure at 3 months and 6 months of age. We therefore stratified results by HIV exposure status, and found associations between IGF-1 and WLZ in HIV-unexposed but not HEU infants at 3 and 6mo. IGF-1 was not significantly associated with head circumference-for-age Z-scores (HCZ) between birth and 3mo. However, there was some evidence that the association between IGF-1 and HCZ was modified by HIV exposure at 6mo; when results were stratified, there was an association in HIV-unexposed infants ( $\beta=2.39 \times 10^{-2}$ ;  $P=0.04$ ) but not HEU infants ( $\beta=0.28 \times 10^{-2}$ ;  $P=0.72$ ).

Taken together, IGF-1 at 6wks was lower in HEU compared to HIV-unexposed infants. IGF-1 was associated with future linear growth in HEU infants and with both linear and ponderal growth in HIV-unexposed infants.

### **Inflammation and IGF-1**

We hypothesised that a pro-inflammatory state is associated with lower circulating IGF-1. CRP was measured in 324/343 (94%) infants. On univariable linear regression, there was an inverse relationship between log CRP and IGF-1 at 6 weeks of age ( $\beta=-0.84$ ;  $P=0.03$ ; Figure 1C), which was not modified by HIV exposure status ( $P=0.97$ ). CRP was not directly associated with anthropometric measures (data not shown).

### **CMV DNAemia and IGF-1**

We hypothesised that CMV affects growth by disrupting the GH axis. Plasma CMV was measured in 331/343 (97%) infants at 6 weeks of age. 81.4% and 74.0% of HEU and HIV-unexposed infants had CMV DNAemia. We found no difference in mean (SD) IGF-1 between infants with (30.1 (10.1) ng/mL) or without (31.5 (11.4) ng/mL) CMV DNAemia ( $P=0.30$ ), regardless of HIV exposure (interaction  $P=0.25$ ). However, among infants with CMV DNAemia, there was an inverse relationship between log CMV viral load and IGF-1

( $\beta=-1.04$ ;  $P=0.01$ ), which was modified by HIV exposure ( $P=0.16$ ): when disaggregated by HIV status, the relationship between log CMV viral load and IGF-1 was significant in HEU ( $\beta=-1.16$ ;  $P=0.008$ ; Figure 1D) but not HIV-unexposed ( $\beta=0.21$ ;  $P=0.83$ ) infants. The relationship in HEU infants was not confounded by maternal HIV disease severity: the association between log CMV viral load and IGF-1 in HEU infants persisted after adjusting for maternal CD4 count and HIV viral load ( $\beta=-1.25$ ;  $P=0.02$ ). The relationship was also not mediated through CRP (after adjusting for CRP,  $\beta=-1.00$ ;  $P=0.016$ ).

Taken together, detection of CMV was not associated with IGF-1 levels, but among those with CMV DNAemia, CMV viral load was inversely associated with IGF-1 in HEU, but not HIV-unexposed infants; this finding was not explained by maternal HIV disease severity or the inflammatory milieu in HEU infants.

### **Effect of trial arm**

Because this sub-study was nested in a placebo-controlled trial of maternal and/or infant vitamin A, data were also analysed with adjustment for trial arm, with no meaningful effects on findings (Supplementary Table 2, <http://links.lww.com/QAD/B800>).

## **DISCUSSION**

Delineating the mechanisms underlying growth failure in HEU infants is necessary to design interventions to promote healthy growth and development in this expanding population.<sup>3</sup> We have previously hypothesised that drivers of growth impairment include inflammation, viral co-infections, and maternal HIV disease.<sup>4</sup> The current study has four key findings. First, HEU infants had modest reductions in IGF-1 compared to HIV-unexposed infants at 6wks of age; second, IGF-1 concentrations at 6wks were associated with subsequent linear growth through 6 months amongst HEU children; third, IGF-1 was inversely related to CRP, suggesting that



systemic inflammation may reduce IGF-1 levels;<sup>6</sup> and, fourth, CMV viral load was inversely related to IGF-1 in HEU infants but not in HIV-unexposed infants. Collectively, these findings suggest independent effects of early-life CMV acquisition and systemic inflammation in disruption of the GH axis in HEU infants.

GH is an important regulator of infant growth through hepatic synthesis of IGF-1.<sup>6</sup> Here we show that IGF-1 is approximately 10% lower in HEU compared to HIV-unexposed infants as early as 6-weeks. Six-week IGF-1 was associated with length and weight at most subsequent time points, suggesting that lower concentrations of IGF-1 could plausibly contribute to growth impairment in HEU infants. There was evidence that HIV exposure modified the association between IGF-1 and WLZ and HCZ, such that the effect was more pronounced in HIV-unexposed infants. This may indicate a greater, or different, range of drivers of growth failure in HEU compared to HIV-unexposed infants,<sup>4</sup> and should be investigated further.

In a previous study among HIV-unexposed infants from this cohort, we reported that stunting is characterized by chronic inflammation, which appears to disrupt the GH axis,<sup>7</sup> as in other inflammatory diseases.<sup>6</sup> We also reported raised inflammatory markers among HEU infants from as early as 6-weeks of age.<sup>10</sup> Here we extend these findings by showing that the inverse relationship between IGF-1 and CRP is not modified by HIV-exposure status, meaning inflammation in HEU infants likely contributes to growth impairment through reduced IGF-1 as in HIV-unexposed infants. Whether raised inflammatory markers persist in the PMTCT era remains unknown and warrants investigation.

The ZVITAMBO trial reported increased frequency and severity of common childhood infections in HEU infants<sup>4</sup> and a failure to effectively control CMV infection.<sup>10</sup> We have also shown in the same cohort that acute clinical infections disrupt the GH axis.<sup>8</sup> CMV has previously been associated with impaired growth among HIV-exposed and HIV-unexposed

children in Zambia.<sup>14</sup> Here we hypothesised that CMV viraemia contributes to disruption of the GH axis. The presence of CMV DNAemia at 6 weeks had no association with IGF-1; however, among HEU (but not HIV-unexposed) infants with CMV viraemia, there was an inverse relationship between CMV viral load and IGF-1 concentrations. This is consistent with our previous finding that HEU infants have impaired handling of infections,<sup>10,15</sup> and suggests this may have consequences for growth. Importantly, the relationship between CMV viral load and IGF-1 was not explained by severity of maternal HIV, or by elevated inflammatory markers in those infants with higher CMV viral loads. Collectively, these findings indicate two distinct potential mechanisms underlying growth failure in HEU infants: chronic inflammation and impaired control of viral infections.

This study has strengths and limitations. HIV-exposed infants were known to be HIV-uninfected to at least 6 months of age, meaning the group was not contaminated by infants becoming postnatally HIV-infected through breastfeeding. The trial took place before ART availability, meaning positive or negative effects of ART on growth were eliminated. We included all available HEU infants in this analysis; however, it is possible that the study was underpowered to identify true modifying effects of HIV exposure. Measuring CRP only may not have fully captured the inflammatory milieu, including CMV-related inflammation. We were unable to delineate causal relationships, but our findings warrant further mechanistic exploration.

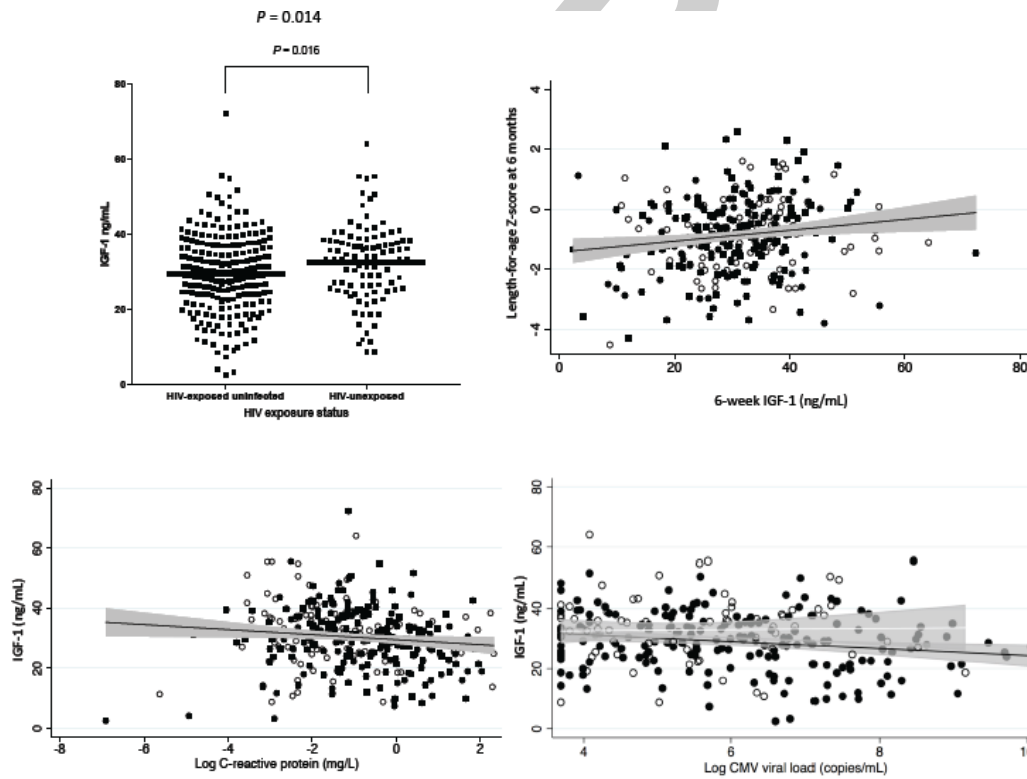
In summary, we find evidence for greater disruption of the GH axis in HEU compared to HIV-unexposed infants by 6 weeks of age, which suggests a potential role for reduced IGF-1 in mediating growth impairment in HEU infants. The previously reported pro-inflammatory state of HEU infants likely represents one pathway underlying growth impairment, but we also found an independent association between greater CMV viraemia and reduced IGF-1, suggesting that both chronic inflammation and poor handling of infections might contribute

to stunting in HEU infants. These pathways provide an opportunity to evaluate targeted interventions for HEU infants, to promote healthy growth and development.

## References

1. 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. *AIDS*. 2016;30(15):2323-2328.
2. Omoni AO, Ntozini R, Evans C, et al. Child Growth According to Maternal and Child HIV Status in Zimbabwe. *Pediatr Infect Dis J*. 2017;36(9):869-876.
3. Evans C, Jones CE, Prendergast AJ. HIV-exposed, uninfected infants: new global challenges in the era of paediatric HIV elimination. *Lancet Infect Dis*. 2016;16(6):e92-e107.
4. Evans C, Humphrey JH, Ntozini R, Prendergast AJ. HIV-Exposed Uninfected Infants in Zimbabwe: Insights into Health Outcomes in the Pre-Antiretroviral Therapy Era. *Front Immunol*. 2016;7:190.
5. Filteau S, Rowland-Jones S. Cytomegalovirus Infection May Contribute to the Reduced Immune Function, Growth, Development, and Health of HIV-Exposed, Uninfected African Children. *Front Immunol*. 2016;7:257.
6. Walters TD, Griffiths AM. Mechanisms of growth impairment in pediatric Crohn's disease. *Nat Rev Gastroenterol Hepatol*. 2009;6(9):513-523.
7. Prendergast A, Rukobo, S, Chasekwa, B, Mutasa, K, Ntozini, R, Mbuya, MN, Jones, A, Moulton, LH, Stoltzfus, RJ, Humphrey, JH. Stunting is characterized by chronic inflammation in Zimbabwean infants. *PLoS One*. 2014;9(2):e86928.
8. Jones AD, Rukobo S, Chasekwa B, et al. Acute illness is associated with suppression of the growth hormone axis in Zimbabwean infants. *Am J Trop Med Hyg*. 2015;92(2):463-470.
9. Baricević I, Nedić O, Nikolić JA, Nedeljković J. The insulin-like growth factor system in the circulation of patients with viral infections. *Clin Chem Lab Med*. 2004;42(10):1127-1131.
10. Evans C, Chasekwa B, Rukobo S, et al. CMV acquisition and inflammation in HIV-exposed uninfected Zimbabwean infants. *J Infect Dis*. 2016.
11. Humphrey JH, Iliff PJ, Marinda ET, et al. Effects of a single large dose of vitamin A, given during the postpartum period to HIV-positive women and their infants, on child HIV infection, HIV-free survival, and mortality. *J Infect Dis*. 2006;193(6):860-871.
12. Gibson RS. *Principles of nutritional assessment*. Oxford University Press; 1990.
13. WHO. WHO child growth standards: methods and development. [http://www.who.int/childgrowth/publications/technical\\_report\\_pub/en/index.html](http://www.who.int/childgrowth/publications/technical_report_pub/en/index.html). Published 2006. Accessed 15th September 2015, 2015.
14. Gompels UA, Larke N, Sanz-Ramos M, et al. Human cytomegalovirus infant infection adversely affects growth and development in maternally HIV-exposed and unexposed infants in Zambia. *Clin Infect Dis*. 2012;54(3):434-442.
15. Koyanagi A, Humphrey JH, Ntozini R, et al. Morbidity among human immunodeficiency virus-exposed but uninfected, human immunodeficiency virus-infected, and human immunodeficiency virus-unexposed infants in Zimbabwe before availability of highly active antiretroviral therapy. *Pediatr Infect Dis J*. 2011;30(1):45-51.

**Figure 1. IGF-1, growth, inflammation and cytomegalovirus.** (A) IGF-1 concentration at 6 weeks of age in HIV-exposed uninfected versus HIV-unexposed children. Horizontal lines at mean. (B) Association between IGF-1 concentration and length-for-age Z-score at 6 months of age amongst HIV-exposed uninfected (black circles) and HIV-unexposed (white circles) children. Line shows regression with 95% confidence intervals amongst all children. (C) Association between C-reactive protein and IGF-1 concentrations at 6 weeks of age amongst HIV-exposed uninfected (black circles) and HIV-unexposed (white circles) children. Line shows regression with 95% confidence intervals amongst all children. (D) Association between CMV viral load and IGF-1 concentration in CMV-viremic HIV-exposed uninfected (black circles) and HIV-unexposed (white circles) children. HIV exposure status modified the effect of CMV viral load on IGF-1, so two regression lines are shown. The black line shows regression with 95% confidence intervals for HIV-exposed uninfected children. The white line shows regression with 95% confidence intervals for HIV-unexposed children. IGF-1: insulin-like growth factor-1; CMV cytomegalovirus.



**Table 1. Associations between insulin-like growth factor-1 at 6 weeks of age and infant growth**

	Age at anthropometry measurement			
	Birth	6 wk	3 mo	6 mo
<b>Length-for-age Z score</b>				
6wk IGF-1 (ng/mL) x HIV exposure (interaction)	$P = 0.39$	$P = 0.83$	$P = 0.84$	$P = 0.46$
Regression* $\beta_{IGF-1}$	$0.49 \times 10^{-2}, P = 0.45$	$1.45 \times 10^{-2}, P = 0.03$	$1.39 \times 10^{-2}, P = 0.06$	$1.82 \times 10^{-2}, P = 0.007$
<b>Weight-for-age Z score</b>				
6wk IGF-1 (ng/mL) x HIV exposure (interaction)	$P = 0.82$	$P = 0.72$	$P = 0.21$	$P = 0.49$
Regression $\beta_{IGF-1}$	$1.22 \times 10^{-2}, P = 0.04$	$2.53 \times 10^{-2}, P < 0.001$	$2.36 \times 10^{-2}, P < 0.001$	$2.28 \times 10^{-2}, P = 0.001$
<b>Weight-for-length Z score</b>				
6wk IGF-1 (ng/mL) x HIV exposure (interaction)	$P = 0.51$	$P = 0.79$	$P = 0.14\#$	$P = 0.16\#$
Regression $\beta_{IGF-1}$ (all infants)	$0.23 \times 10^{-2}, P = 0.80$	$1.67 \times 10^{-2}, P = 0.02$	-	-
HEU regression $\beta_{IGF-1}$	-	-	$0.49 \times 10^{-2}, P = 0.55$	$0.87 \times 10^{-2}, P = 0.29$
HIV-unexposed regression $\beta_{IGF-1}$	-	-	$2.63 \times 10^{-2}, P = 0.03$	$2.95 \times 10^{-2}, P = 0.03$
<b>Head circumference-for-age Z score</b>				
6wk IGF-1 (ng/mL) x HIV exposure (interaction)	$P = 0.57$	$P = 0.25$	$P = 0.38$	$P = 0.13\#$
Regression $\beta_{IGF-1}$ (all infants)	$0.81 \times 10^{-2}, P = 0.18$	$0.95 \times 10^{-2}, P = 0.16$	$1.01 \times 10^{-2}, P = 0.08$	-
HEU regression $\beta_{IGF-1}$	-	-	-	$0.28 \times 10^{-2}, P = 0.72$
HIV-unexposed regression $\beta_{IGF-1}$	-	-	-	$2.39 \times 10^{-2}, P = 0.04$

All associations estimated by univariable linear regression.

HEU: HIV-exposed uninfected; IGF-1: insulin-like growth factor-1; # $P < 0.2$  (significance level for interaction).

\*The  $\beta$  co-efficient shows the change in Z-score for each anthropometry variable per unit rise in IGF-1 (measured in ng/mL).