# Young CPHIV Have Low Proinsulin to C-Peptide Ratios That Inversely Correlate With T-Cell Exhaustion

971

Correspondence: wl1@iu.edu
akhaita@iu.edu

Wei Li[1], Emily Sims[2], Mussa Mwamzuka[3], Fatma Marshed[3], Aabid Ahmed[3], Alka Khaitan[1]

[1]Ryan White Center for Pediatric Infectious Diseases and Global Health, Indiana University School of Medicine, Indianapolis, IN, USA. [2]Center for Diabetes and Metabolic Diseases, Indiana University School of Medicine, Indianapolis, IN, USA. [3]Bomu Hospital, Mombasa, Kenya

## Introduction

People living with HIV have an increased risk of developing diabetes mellitus. HIV-associated diabetes, is clinically similar to Type 2 diabetes, which occurs due to increased peripheral insulin resistance combined with β cell dysfunction[1]. Children with perinatal HIV (CPHIV) have higher rates of insulin resistance compared with healthy children[2, 3], but whether β cell dysfunction contributes is unknown.

The ratio of immature (Pro-insulin) relative to mature (C-peptide) insulin products (PI:C) in circulation serves as a biomarker of pancreatic β cell stress[4] and is predictive of progression to type 1 or type 2 diabetes[5-8]. Adults living with HIV (ALHIV) did not have an elevated PI:C ratio. Instead, a lower PI:C (reduced β cell stress ) was observed in ART-naïve subjects with reduced CD4 counts compared to those on ART treatment or without HIV[9], suggesting immune dysregulation may preserve β cell function. In this study, we investigated PI:C ratios in CPHIV and their correlations with clinical, intestinal damage, and immune activation and exhaustion markers.

## Methods

We quantified plasma levels of proinsulin (TECO intact proinsulin ELISA) and C-peptide (TOSOH immunoassay) in 200 Kenyan children who were HIV unexposed (HIV-) and CPHIV who were treatment naïve (ART-) or virally suppressed on treatment (ART+) aged 0-5 years ("0-5y" n=28 ART-, 21 ART+, 36 HIV-) and 5-20 years ("5-20y" n=41 ART-, 28 ART+, 46 HIV-). We calculated PI:C and assessed its correlations with HIV viral load, CD4 count, CD4%, CD4:CD8, markers of systemic inflammation (CRP, IL-6), monocyte (sCD14, sCD163) and T cell (CD38+HLA-DR+) activation markers, and immune checkpoints (ICPs: PD-1, CD160, TIM3) on memory T cells. Mann-Whitney test, Kruskal–Wallis test, Chi-Square test, and Spearman's correlations were performed on GraphPad Prism 10.

### Table 1a: 0-5y Participant Characteristics

| | HIV- | ART- | ART+ | p |
|---|---|---|---|---|
| n | 36 | 28 | 21 | |
| Age (y) | 2.6 (1.3-3.6) | 2.1 (1.1-3.3) | 3.0 (2.1-3.5) | ns[a] |
| #Female | 20 (56%) | 15 (54%) | 10 (48%) | ns[b] |
| % CD4 | 33 (28-39) | 21 (14-32) | 32 (28-35) | 0.0002 [a] |
| CD4:CD8 | 1.9 (1.2-2.5) | 0.5 (0.2-1.1) | 1.4 (1.0-1.7) | <0.0001[a] |
| HIV log copies/ml | | 5.2 (4.3-5.8) | 2.0 (2.0-2.0) | <0.0001[c] |

### Table 1b: 5-20y Participant Characteristics

| | HIV- | ART- | ART+ | p |
|---|---|---|---|---|
| n | 46 | 41 | 28 | |
| Age (y) | 11.7 (9.4-15.7) | 10.7 (8.3-13.9) | 11.2 (8.2-13.1) | ns[a] |
| #Female | 19 (41%) | 23 (56%) | 14 (50%) | ns[b] |
| % CD4 | 38 (33-42) | 24 (14-28) | 33 (29-41) | <0.0001 [a] |
| CD4:CD8 | 1.8 (1.2-2.3) | 0.5 (0.3-0.8) | 1.0 (0.5-1.4) | <0.0001[a] |
| HIV log copies/ml | | 4.8 (4.3-5.2) | 2.0 (2.0-2.0) | <0.0001[c] |

Data are presented as median values with interquartile range or #number and percentage. (a) Kruskal-Wallis; (b) Chi-Square; (c) Mann-Whitney. ns: not significant.

## Results

### Figure 1. Proinsulin and C-peptide Ratios

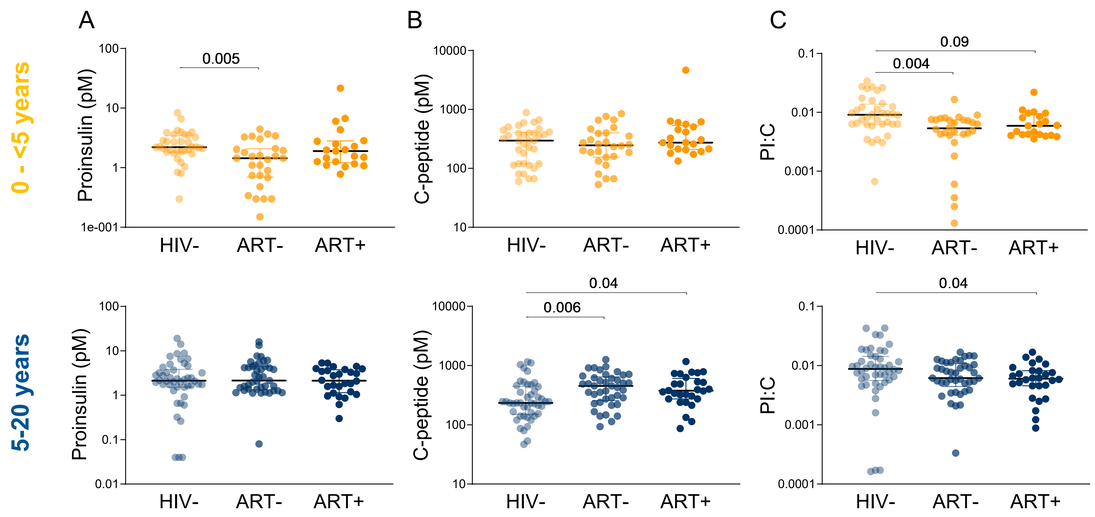



**Fig 1. Proinsulin, C-peptide and PI:C ratios in CPHIV.** Comparison of (A) Proinsulin, (B) C-peptide and (C) PI:C in CPHIV 0-5 years (orange) or 5-20 years (blue). Compared to HIV-, 0-5y ART- group had lower PI:C (C) and proinsulin levels (A), whereas ART+ trended toward lower PI:C (C). In 5-20y, ART+ had lower PI:C (C) and both ART- and ART+ had a higher C-peptide level (B) than HIV- children. P values less than 0.05 are shown.

### Figure 2. PI:C and HIV Disease Progression

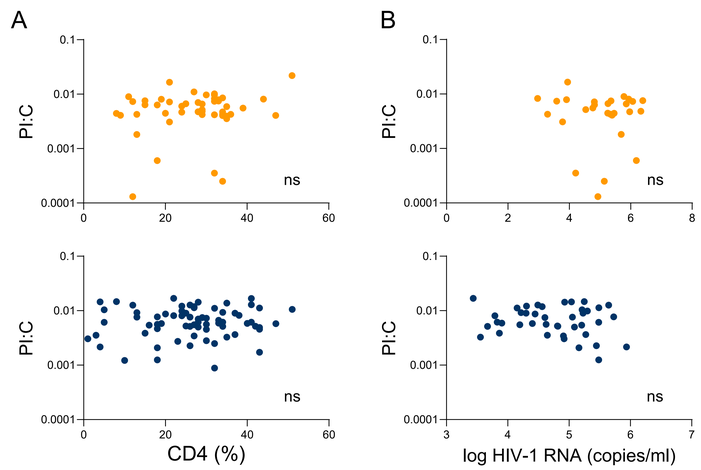



**Fig 2. Correlations between PI:C and (A) CD4% and (B) HIV viral load in CPHIV 0-5 years (orange) or 5-20 years (blue).** PI:C did not correlate with (A) CD4 T % in 0-5y old or 5-20y old CPHIV. PC:C also did not show correlations with (B) HIV viral load (log RNA copies/ml) in ART- CPHIV. ns: not significant.

### Figure 3. PI:C and Inflammation

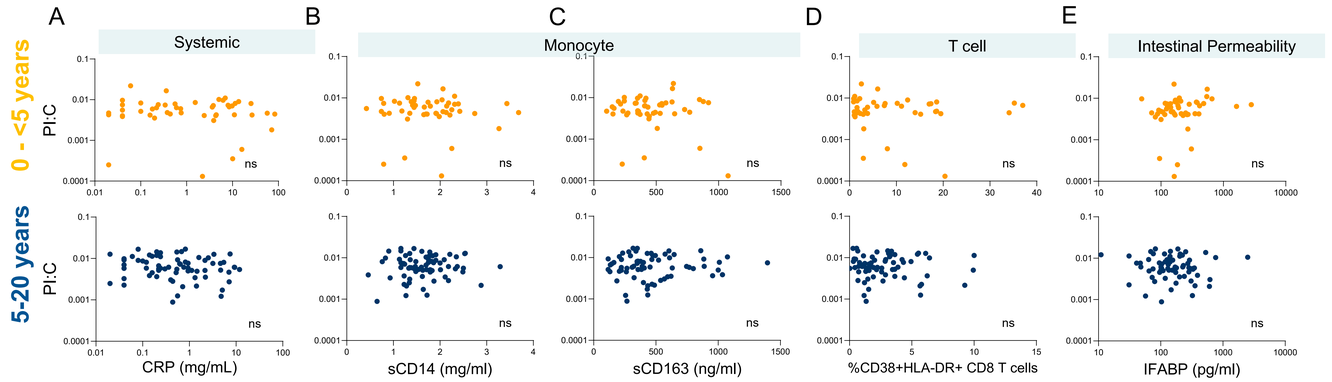



**Fig 3. Correlations between PI:C and (A) CRP, (B) sCD14, (C) sCD163, (D) CD38+HLA-DR+ CD8 T, (E) IFABP in CPHIV 0-5 years (orange) or 5-20 years (blue).** PI:C did not correlate with levels of (A) CRP, (B) sCD14, (C) sCD163, (D) the frequency of HLA-DR+CD38+ CD8 T cells in peripheral blood mononuclear cells, and (E) IFABP in 0-5y old, or 5-20y old CPHIV.

### Figure 4. PI:C and Immune Checkpoints

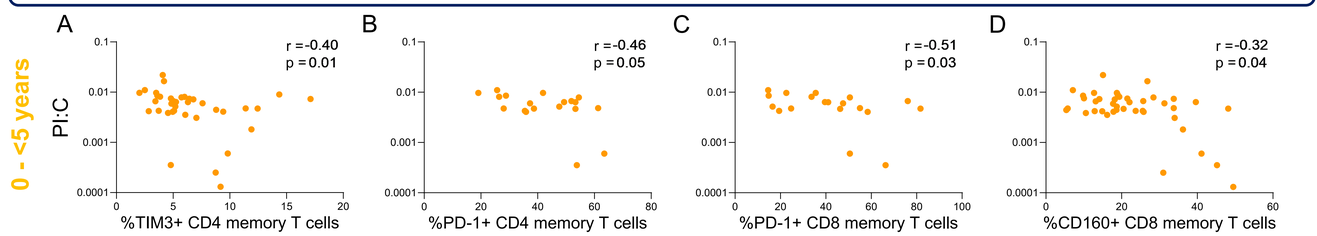



**Figure 4. Correlations between PI:C and (A) TIM3+ and (B) PD-1+ CD4m T cells, (C) PD-1+ and (D) CD160+ CD8m T cells in CPHIV 0-5 years.** PI:C negatively correlated with percentage of (A) TIM3+ and (B) PD-1+ memory CD4 T cells and (C) PD-1+ and (D) CD160+ memory CD8 T cells. There were no correlations between PI:C and ICPs in 5-20y CPHIV (data not shown).

## Conclusions

CPHIV had a lower PI:C compared with HIV-, stemming from lower proinsulin in younger and higher C-peptide in older CPHIV.

Clinical markers of HIV disease progression and inflammation were not associated with β cell stress in CPHIV.

PI:C had differential associations with ICPs on T cells in younger and older CPHIV.

- In 0-5y CPHIV, PI:C inversely correlated with PD-1 and TIM3 on memory CD4 and PD-1 and CD160 on memory CD8 T cells.
- In 5-20y CPHIV, PI:C was not significantly associated with ICPs.

Our data show no evidence of β cell dysfunction in CPHIV and suggest ICPs may play a protective role against β cell stress in young CPHIV.

## References

1. Chiasson JL, Rabasa-Lhoret R. Prevention of type 2 diabetes: insulin resistance and beta-cell function. Diabetes. 2004;53 Suppl 3:S34-38.
2. Dirajlal-Fargo S, Musiime V, Cook A, et al. Insulin Resistance and Markers of Inflammation in HIVinfected Ugandan Children in the CHAPAS-3 Trial. Pediatr Infect Dis J. 2017;36(8):761-767.
3. Dirajlal-Fargo S, Shan L, Sattar A, et al. Insulin resistance and intestinal integrity in children with and without HIV infection in Uganda. HIV Med. 2020;21(2):119-127.5.
4. Rodriguez-Calvo T, Chen YC, Verchere CB, et al. Altered beta-Cell Prohormone Processing and Secretion in Type 1 Diabetes. Diabetes. 2021.
5. Kahn SE, Halban PA. Release of incompletely processed proinsulin is the cause of the disproportionate proinsulinemia of NIDDM. Diabetes. 1997;46(11):1725-1732.
6. Kahn SE, Leonetti DL, Prigeon RL, Boyko EJ, Bergstom RW, Fujimoto WY. Proinsulin levels predict the development of non-insulin-dependent diabetes mellitus (NIDDM) in Japanese-American men. Diabet Med. 1996;13(9 Suppl 6):S63-66.
7. Sims EK, Bahnson HT, Nyalwidhe J, et al. Proinsulin Secretion Is a Persistent Feature of Type 1 Diabetes. Diabetes Care. 2019;42(2):258-264.
8. Sims EK, Chaudhry Z, Watkins R, et al. Elevations in the Fasting Serum Proinsulin-to-C-Peptide Ratio Precede the Onset of Type 1 Diabetes. Diabetes Care. 2016;39(9):1519-1526
9. Sims EK, Park G, Mather KJ, Mirmira RG, Liu Z, Gupta SK. Immune reconstitution in ART treated, but not untreated HIV infection, is associated with abnormal beta cell function. PLoS One. 2018;13(5):e0197080.

This research was funded by a grant from Wells Center for Pediatric Research, Clinical Translational Research to AK

INDIANA UNIVERSITY SCHOOL OF MEDICINE

Riley Children's Health
Indiana University Health