Effects of HIV Infection on the Metabolic and Hormonal Status of Children with Severe Acute Malnutrition

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Introduction

Malnutrition is a major determinant of morbidity and mortality in the developing world and is the underlying cause of 3.5 million child deaths each year [1]. Poor nutrition increases greatly a child’s risk of dying from diarrhea, pneumonia, measles, and malaria and is associated with decreased adult height, lower educational achievement, lower socioeconomic status, and a possible increase in chronic diseases during adulthood [2,3]. Worldwide, malnutrition represents 35% of the burden of disease in children less than five years of age and 11% of disability-adjusted life years (DALYs) [1].
In sub-Saharan Africa, 30% of children with severe acute malnutrition (SAM) are infected with HIV, which increases mortality rates substantially; those with CD4 count $<$ 20% are at greatest risk [4,5]. However, the factors underlying the increased risk of mortality from HIV are poorly understood. Rates of pneumonia (68%), urinary tract infection (26%), and bacteremia (18%) are comparable in severely malnourished HIV-infected and HIV-negative children [6]. Furthermore, among those who survive, the rates of nutritional recovery are similar [7]. There is consequently a critical need to elucidate the pathophysiology of SAM in children with concurrent HIV infection.

In a previous study we used metabolomic profiling to characterize changes in various hormones, growth factors, cytokines, and metabolites during nutritional rehabilitation of severely malnourished Ugandan children [8]. Here we characterized differences in baseline metabolic and hormonal status between HIV-infected and HIV-negative children with SAM and compared their subsequent responses to nutritional therapy. We hypothesized that HIV infection would modify the hormonal and metabolic responses to malnutrition and nutrient therapy and that hormones and metabolites measured at baseline might be associated with mortality in HIV-infected children.

Methods

Study Cohort
The study was conducted at Mwanamugimu Nutrition Unit at Mulago Hospital, in Kampala, Uganda. Children ages six months to five years who met WHO criteria for SAM were eligible for enrollment. SAM was defined as having a weight-for-height $z$-score (W/H $z$) $\leq -3$, mid-upper arm circumference (MUAC) $\leq$ 11.5 cm, or bilateral pitting edema. Referrals came from the Mulago pediatric acute care unit (emergency department) and community clinics in and around Kampala.

Study Variables
A complete medical and diet history, sociodemographic profile, and physical exam including anthropometric data were obtained at time of enrollment. Lab studies included CBC and differential, blood smear, and CD4/CD8 counts (FACSCalibur, BD Biosci-
ences, USA). HIV status was assessed using an HIV rapid antibody test (Determine, Abbott, USA; STAT-Pak, Chembio Diagnostics, USA; Uni-Gold, Trinity Biotech, Ireland) for patients >18 months of age and HIV DNA PCR (AMPLICOR HIV-1 Monitor Test version 1.5, Roche, USA) for patients <18 months. Children whose mothers had a documented negative HIV test within the previous 30 days were presumed to be HIV negative. Children with known HIV infection did not have repeat HIV testing. Those with malaria were treated with anti-malarials. All patients received counseling from a trained HIV counselor at Mwanamugimu Nutrition Unit before delivering results; HIV-infected patients were referred for appropriate HIV-related care.

### Nutritional Interventions

Nutrition rehabilitation and management of medical complications were carried out according to WHO guidelines for inpatient treatment of SAM by medical house officers at Mwanamugimu Nutrition Unit [9]. Inpatient therapy was administered in two phases according to WHO guidelines: an initial stabilization phase during which acute medical conditions were managed; and a longer rehabilitation phase once clinical status improved. Patients were fed F75 mild-based liquid formula (75 kcal and 0.9 g protein/100 mL) during the first phase and F100 (100 kcal and 2.9 g protein/100 mL) during the rehabilitation phase. Micronutrient deficiencies were corrected with vitamin A, folic acid, zinc, and iron. All patients received empiric antibiotics [9]. Patients were followed from time of enrollment until death or discharge from the inpatient unit.

### Metabolomic Analysis

Blood samples (maximum 5 mL) were collected at time of enrollment (within 24 h of admission). A second blood sample was collected after 14 days of inpatient treatment or at time of discharge from the inpatient unit, whichever occurred first. Aprotinin (500 KIU/mL of blood; Sigma-Aldrich, USA) was added to prevent protein degradation. Blood samples were collected on ice and processed promptly; EDTA plasma was stored at −70 °C and shipped in bulk to the Duke University Stedman Nutrition Center for analysis. Detailed methods of the metabolic and hormonal analyses are described in the Supporting Information (Methods S1).

### Statistical Analysis

Sample size was based on commonly reported concentrations and variability of classical hormones (insulin, growth hormone, cortisol) in infants and children. We evaluated pre-treatment anthropometric variables and biomarkers using non-parametric Wilcoxon Rank-Sum and absolute change during treatment using Wilcoxon Signed-Rank based on variables of interest (HIV status, mortality). The association between HIV status and mortality was

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Table 1. Baseline Anthropometric and Hematologic Characteristics of HIV-infected and HIV-negative Patients.

| HIV-infected (n = 18) | HIV-negative (n = 56) | p-value |
|-----------------------|-----------------------|---------|
| **Male Sex**          |                       |         |
| Number (%)            |                       |         |
| 10/18 (55.6)          | 32/56 (57.1)          | 1.00    |
| Edema Present         |                       |         |
| 9/18 (50)             | 33/56 (58.9)          | 0.589   |
| Positive Malaria Smear|                       |         |
| 1/18 (5.6)            | 6/56 (10.7)           | 0.555   |
| Newly Diagnosed HIV infection | |         |
| 12/18 (66.7)          | -                     | -       |
| Current ARV treatment |                       |         |
| 4/6 (66.7)            | -                     | -       |
| Mortality             |                       |         |
| 6/18 (33.3)           | 3/56 (5.4)            | 0.0051  |

**Mean±SEM**

| HIV-infected         | HIV-negative         | p-value |
|----------------------|----------------------|---------|
| Age                  | 19.2±2.6             | 15.4±1.1| 0.315   |
| Days in Treatment    | 26.4±3.9             | 24.9±1.5| 0.850   |
| **W/H % (nonedematous)** |                       |         |
| 70.5±2.4             | 71.3±1.4             | 0.571   |
| **W/H Z-Score (nonedematous)** |                |         |
| −4.44±0.48           | −4.20±0.28           | 0.615   |
| **W/A Z-Score (nonedematous)** |             |         |
| −5.14±0.61           | −4.87±0.33           | 0.870   |
| **MUAC (nonedematous)** |                       |         |
| 9.6±0.5              | 9.9±0.2              | 0.599   |
| **L/A Z-score (all patients)** |                     |         |
| −3.17±0.40           | −2.90±0.20           | 0.492   |
| **H/C Z-score (all patients)** |                   |         |
| −0.72±0.32           | −1.25±0.21           | 0.246   |

**Hematology**

| HIV-infected         | HIV-negative         | p-value |
|----------------------|----------------------|---------|
| Abs CD4 Count        | 644±103              | 2734±253| <0.0001 |
| CD4%                 | 14.6±2.1             | 34.8±1.2| <0.0001 |
| Abs CD8 Count        | 1724±387             | 1610±120| 0.638   |
| CD8%                 | 37.3±2.6             | 213±1.0 | <0.0001 |
| CD4/CD8 Ratio        | 0.44±0.08            | 1.83±0.10| <0.0001 |
| WBC (10^3/µl)        | 8.5±1.2              | 13.2±1.0| 0.0082  |
| Hemoglobin (g/dL)    | 7.7±0.4              | 9.0±0.2 | 0.0130  |
| Platelets (10^3/µl)  | 267±37               | 371±24  | 0.0231  |

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assessed utilizing an odds ratio. We performed multivariable logistic and linear regression to evaluate associations between biomarkers levels, HIV status, and mortality. Analysis of leptin and total and high molecular weight (HMW) adiponectin excluded patients taking antiretroviral drugs (ARVs), given the known effects of these medications on these hormones [10–14]. Edematous children were excluded from analysis of anthropometric variables based on weight. All analyses were conducted using JMP Pro 9.0 (Cary, NC); a two-sided p-value, 0.05 was considered statistically significant for all tests.

Ethical considerations
The study protocol was approved by the institutional review boards at Duke University, Makerere University School of Public Health, and the Uganda National Council of Science and Technology. Sponsors of the study assisted with data interpretation but had no role in study design, data collection, or data analysis.

Written informed consent (in English and Luganda) to participate in the study and all its components was obtained from all guardians. Each patient received an insecticide-treated bed net at enrollment and transportation money to return home at the time of discharge as compensation for participation.

Results

Study Population
A total of 77 patients were referred to Mwanamugimu Inpatient Nutrition Unit and screened for study enrollment between

| Table 2. Baseline Metabolic Profile of HIV-infected and HIV-negative Patients. |
|---------------------------------------------------------------|
| **Fatty Acid Metabolites**                                    |
| NEFA (mmol/L)                                                |
| Mean±SEM HIV-infected (n = 16) 0.65±0.10 HIV-negative (n = 46) 0.54±0.06 0.285 |
| Total Ketones (μmol/L)                                       |
| 826±259 HIV-infected (n = 16) 424±95 HIV-negative (n = 46) 0.0387 |

| **Acylcarnitines**                                           |
| C2 (μmol/L)                                                 |
| Mean±SEM HIV-infected (n = 16) 22.3±3.5 HIV-negative (n = 46) 14.4±2.4 0.0103 |
| C3 (μmol/L)                                                 |
| Mean±SEM HIV-infected (n = 16) 0.47±0.07 HIV-negative (n = 46) 0.38±0.04 0.179 |
| C2/C3 Ratio                                                 |
| Mean±SEM HIV-infected (n = 16) 54.3±9.7 HIV-negative (n = 46) 44.7±5.7 0.195 |

| **Hormones**                                                 |
| Insulin (IU/ml)                                              |
| Mean±SEM HIV-infected (n = 16) 1.81±0.48 HIV-negative (n = 46) 2.45±0.45 0.321 |
| Growth Hormone (ng/ml)                                       |
| Mean±SEM HIV-infected (n = 16) 12.4±2.7 HIV-negative (n = 46) 110.0±1.3 0.380 |
| IGF-1 (ng/ml)                                                |
| Mean±SEM HIV-infected (n = 16) 13.3±4.4 HIV-negative (n = 46) 9.4±1.7 0.597 |
| Total Ghrelin (pg/ml)                                        |
| Mean±SEM HIV-infected (n = 16) 357.7±566 HIV-negative (n = 46) 4040±320 0.435 |
| GLP-1 (pg/ml)                                                |
| Mean±SEM HIV-infected (n = 16) 128.8±21.3 HIV-negative (n = 46) 96.0±12.8 0.106 |
| PYY (pg/ml)                                                  |
| Mean±SEM HIV-infected (n = 16) 1195±160 HIV-negative (n = 46) 1202±105 0.866 |
| Cortisol (μg/dl)                                             |
| Mean±SEM HIV-infected (n = 16) 54.0±3.2 HIV-negative (n = 46) 46.0±2.7 0.106 |

| **Adipocytokines**                                           |
| Leptin (pg/ml)                                               |
| Mean±SEM HIV-infected (n = 16) 69.8±26.6 HIV-negative (n = 46) 292±52 0.0163 |
| Total Adiponectin (ng/ml)                                    |
| Mean±SEM HIV-infected (n = 16) 8049±1081 HIV-negative (n = 46) 15268±1133 0.0017 |
| HMW Adiponectin (ng/ml)                                      |
| Mean±SEM HIV-infected (n = 16) 4409±757 HIV-negative (n = 46) 9356±761 0.0014 |

| **Amino Acids**                                              |
| Amino Acid Molar Sum (μmol/L)                                |
| Mean±SEM HIV-infected (n = 16) 1230±62 HIV-negative (n = 46) 1190±51 0.417 |

| **Inflammatory Cytokines**                                   |
| IL-2 (pg/ml)                                                 |
| Mean±SEM HIV-infected (n = 16) 7.7±2.7 HIV-negative (n = 46) 3.6±1.2 0.0158 |
| IL-6 (pg/ml)                                                 |
| Mean±SEM HIV-infected (n = 16) 96.3±58.4 HIV-negative (n = 46) 25.6±15.9 0.139 |
| IL-8 (pg/ml)                                                 |
| Mean±SEM HIV-infected (n = 16) 299.3±191.0 HIV-negative (n = 46) 75.2±25.9 0.060 |
| TNF-α (pg/ml)                                                |
| Mean±SEM HIV-infected (n = 16) 43.0±5.5 HIV-negative (n = 46) 37.4±9.5 0.0248 |

| **Other**                                                    |
| Glucose (mg/dl)                                              |
| Mean±SEM HIV-infected (n = 16) 77.1±7.9 HIV-negative (n = 46) 85.9±3.9 0.474 |
| Creatinine (mg/dl)                                           |
| Mean±SEM HIV-infected (n = 16) 0.30±0.04 HIV-negative (n = 46) 0.27±0.03 0.296 |
| Phosphorus (mg/dl)                                           |
| Mean±SEM HIV-infected (n = 16) 2.99±1.40 HIV-negative (n = 46) 3.28±1.02 0.390 |
| Albumin (g/dl)                                               |
| Mean±SEM HIV-infected (n = 16) 2.0±0.2 HIV-negative (n = 46) 2.0±0.1 0.847 |
| CRP (mg/L)                                                   |
| Mean±SEM HIV-infected (n = 16) 63.7±16.5 HIV-negative (n = 46) 26.8±5.3 0.0730 |
| Triglycerides (mg/dl)                                        |
| Mean±SEM HIV-infected (n = 16) 177.6±14.0 HIV-negative (n = 46) 122.9±12.2 0.0008 |

*Excludes patients on ARVs.
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December 2010 and March 2011. One patient refused to participate, another was deemed clinically unstable by the medical house officer for extra blood draws, and a third was transferred from the ward after only HIV status was assessed. Therefore, 75 patients had known HIV status and 74 had complete admission anthropometry. Analyses of hormones, metabolites, and cytokines were performed on blood samples from 62 patients at admission (16 HIV-infected including three on ARVs and 46 HIV-negative); 54 of these patients had repeat samples analyzed after 14 days of hospitalization and eight patients died before the second sample was obtained. Initial samples were insufficient in one additional patient (who died) and were not analyzed in 11 patients who left the ward prior to completing at least 14 days of treatment (including two HIV-infected patients, one of whom was on ARVs) (Figure 1).

The patient population was 57.3% male; mean age was 16.3 ± 1.0 months (mean ± SE). 56.8% (42/74) presented with edematous malnutrition. Non-edematous children had an initial W/H z-score of 2.42 ± 0.24 and MUAC of 9.8 ± 0.2 cm. Mean length-for-age (L/A) z-score was 2.97 ± 0.18; head circumference-for-age (HC/A) z-score was 1.15 ± 0.18. 9.5% (7/74) had malaria; one HIV-infected patient had concurrent malaria (Table 1).

As previously reported, overall mortality was 12.2% (9/74). Of those who successfully completed inpatient nutritional rehabilitation, mean length of stay was 25.2 days. During hospitalization, mean W/H z-score increased 1.00 ± 0.18 in non-edematous children. Among surviving patients, 80% (52/65) were followed until discharge and 20% (13/65) left the ward against medical advice before achieving nutritional stability.

Baseline Characteristics of HIV-infected and HIV-negative Patients

HIV prevalence in the study population was 24% (18/75); two-thirds (12/18) of these were newly diagnosed HIV infections. Four of the six previously diagnosed patients were being treated with ARVs upon admission (Table 1). Similar proportions of HIV-infected and HIV-negative patients presented with edematous malnutrition (p = 0.589). Non-edematous HIV-infected and HIV-negative patients presented with similar degrees of wasting (W/H z-score of 2.44 ± 0.24 vs. 2.40, p = 0.615). HIV-infected children had lower absolute CD4 counts (644 vs. 2734, p < 0.0001), WBC counts, hemoglobin, and platelets (Table 1).

HIV-infected patients had increased mortality rates: 33.3% for seropositive children compared with 5.4% for seronegative children (OR = 8.83, CI 1.93–40.43, p = 0.0051). Among those who survived, there were similar improvements in W/H z in (non-edematous) HIV-infected and HIV-negative patients after 14 days (0.85 vs. 0.43, p = 0.412).

### Table 3. Baseline Amino Acid Levels of HIV-infected and HIV-negative patients.

|                      | HIV-infected (n = 16) | HIV-negative (n = 46) | p-value |
|----------------------|-----------------------|----------------------|---------|
| Glycine (µmol/L)     | 235 ± 21.4            | 237 ± 12.6           | 0.866   |
| Alanine              | 153 ± 27.4            | 217 ± 16.2           | 0.0330  |
| Serine               | 99.2 ± 99.5           | 113 ± 5.6            | 0.464   |
| Proline              | 152 ± 14.4            | 153 ± 8.5            | 0.904   |
| Valine               | 100 ± 10.7            | 75.6 ± 6.3           | 0.0248  |
| Leucine/Isoleucine   | 82.0 ± 8.9            | 70.5 ± 5.2           | 0.237   |
| Methionine           | 16.1 ± 1.7            | 15.6 ± 1.0           | 0.742   |
| Histidine            | 69.1 ± 7.0            | 53.3 ± 4.1           | 0.250   |
| Phenylalanine        | 79.6 ± 7.7            | 43.0 ± 4.5           | 0.0067  |
| Tyrosine             | 30.1 ± 4.6            | 22.6 ± 2.7           | 0.207   |
| Aspartate            | 39.2 ± 4.8            | 36.5 ± 2.8           | 0.853   |
| Glutamate            | 111 ± 10.5            | 91.3 ± 6.2           | 0.435   |
| Ornithine            | 27.4 ± 3.5            | 26.2 ± 2.1           | 0.381   |
| Citrulline           | 8.9 ± 1.2             | 8.2 ± 0.7            | 0.421   |
| Arginine             | 28.3 ± 3.3            | 27.7 ± 1.9           | 0.323   |

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### Table 4. Multivariate regression assessing the effect of HIV status on leptin, total adiponectin, and HMW adiponectin when controlling for admission W/H z-score.

|                      | Beta (HIV status) | p-value | Adjusted R² |
|----------------------|-------------------|---------|-------------|
| Leptin               | 63.7 ± 44.4       | 0.1573  | 0.294       |
| Total Adiponectin    | 2752 ± 1051       | 0.0113  | 0.274       |
| HMW Adiponectin      | 1949 ± 720        | 0.0090  | 0.253       |

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Figure 2. Comparison of metabolic response to inpatient rehabilitation in 54 patients who completed treatment (10 HIV-infected and 44 HIV-negative children). Analysis of leptin and HMW adiponectin excluded those patients taking ARVs. Data are represented as the mean ± SEM.

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Table 5. Changes in Metabolic Profiles of HIV-infected (surviving) and HIV-negative patients.

|                               | HIV-infected (n = 10) | HIV-negative (n = 44) |
|-------------------------------|-----------------------|----------------------|
|                               | Mean±SEM              | p-value              |
|                               | Admission 14-day      | Mean±SEM              | p-value        |
| Fatty Acid Metabolites        |                       |                      |
| NEFA (mmol/L)                 | 0.72±0.16             | 0.24±0.06            | 0.0020         | 0.53±0.06 | 0.36±0.037 | 0.0723 |
| Total Ketones (µmol/L)        | 948±362               | 39±14                | 0.0098         | 431±99     | 179±56     | 0.0256 |
| Acylcarnitines                |                       |                      |
| C2 (µmol/L)                   | 25.2±5.2              | 7.2±0.8              | 0.0098         | 14.5±2.6   | 9.3±0.94   | 0.171  |
| C3 (µmol/L)                   | 0.50±0.11             | 0.65±0.11            | 0.275          | 0.36±0.03  | 0.67±0.06  | <0.0001 |
| C2/C3 Ratio                   | 60.6±14.7             | 13.4±2.0             | 0.0020         | 45.7±5.9   | 22.5±4.5   | <0.0001 |
| Even-Chain Acylcarnitine Molar Sum (µmol/L) | 27.1±5.5             | 8.2±0.9              | 0.0098         | 16±2.7     | 10.6±1     | 0.216  |
| Hormones                      |                       |                      |
| Insulin (mIU/ml)              | 2.18±0.70             | 4.43±1.42            | 0.084          | 2.52±0.47  | 3.4±0.53   | 0.108  |
| Growth Hormone (ng/ml)        | 13.3±4.3              | 5.2±1.2              | 0.027          | 11.2±1.4   | 10.1±1.7   | 0.147  |
| IGF-1 (ng/ml)                 | 213±5.8               | 316±8.1              | 0.250          | 8.8±1.6    | 27.6±3.6   | <0.0001 |
| Total Ghrelin (pg/ml)         | 2851±603              | 2439±570             | 0.084          | 4029±319   | 2692±303   | <0.0001 |
| GLP-1 (pg/ml)                 | 116.7±27.5            | 86.0±22.1            | 0.375          | 93.6±1.3   | 88.8±12.4  | 0.863  |
| PYY (pg/ml)                   | 916±118               | 893±112              | 1.0            | 1144±101   | 1011±78    | 0.100  |
| Cortisol (µg/dl)              | 56.6±3.4              | 41.1±6.7             | 0.0625         | 45.3±2.8   | 38.3±3     | 0.0123 |
| Adipocytokines*               | n = 7                 |                      |
| Leptin (pg/ml)                | 123.1±39.7            | 446±238              | 0.128          | 305±53.5   | 748±188    | 0.0011 |
| Total Adiponectin (ng/ml)     | 8383±1569             | 10154±1815           | 0.297          | 15115±1093 | 20792±1329 | <0.0001 |
| HMW Adiponectin (ng/ml)       | 5308±1259             | 5717±795             | 0.625          | 9350±748   | 14895±1245 | <0.0001 |
| Amino Acids                   |                       |                      |
| Amino Acid Molar Sum (µmol/L) | 1198±84.4             | 1850±145             | 0.005          | 1192±53.6  | 1944±80.5  | <0.0001 |
| Inflammatory Cytokines        |                       |                      |
| IL-2 (pg/ml)                  | 5.0±2.6               | 2.2±0.6              | 0.477          | 2.5±0.5    | 1.8±0.2    | 0.255  |
| IL-6 (pg/ml)                  | 49±32                 | 3.4±0.7              | 0.0371         | 9.9±1.5    | 7.8±2.8    | 0.0256 |
| IL-8 (pg/ml)                  | 344±300               | 29.7±3.4             | 0.0645         | 50.6±9.0   | 41.7±4.4   | 0.638  |
| TNF-α (pg/ml)                 | 38.3±7.1              | 34.7±5.5             | 0.922          | 37.5±10    | 32.5±4.5   | 1.0    |
| Other                         |                       |                      |
| Glucose (mg/dl)               | 72.1±9.3              | 75.4±3.7             | 0.625          | 84.2±3.8   | 77.0±2.2   | 0.0634 |
| Creatinine (mg/dl)            | 0.32±0.06             | 0.36±0.11            | 0.910          | 0.25±0.03  | 0.32±0.07  | 0.694  |
| Phosphorus (mg/dl)            | 3.2±0.4               | 4.2±0.2              | 0.086          | 3.4±0.2    | 4.6±0.1    | <0.0001 |
| Albumin (g/dl)                | 2.03±0.32             | 2.28±0.20            | 0.219          | 2.01±0.17  | 2.51±0.12  | <0.0001 |
| CRP (mg/L)                    | 70.7±21.1             | 14.2±7.2             | 0.0391         | 26.8±5.7   | 9.9±4.1    | 0.0003 |
| Triglycerides (mg/dl)         | 184±22.8              | 127±8.7              | 0.0391         | 124.3±12.7 | 127.7±11.2 | 0.416  |

*Excludes patients on ARVs.
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Effects of HIV Infection on Baseline Metabolic Profile

Both HIV-infected and HIV-negative patients presented in a severe catabolic state with exaggerated lipolysis, fatty acid oxidation, and hypoaminoacidemia (Table 2 and 3). Non-esterified fatty acids (NEFA), ketones, C2 acyl (acyetylcarcinine, and even-chained acylcarcinine molar sum were elevated in both groups at presentation, though ketones (p = 0.039), acetylcarcinine (p = 0.0103), and even-chained acylcarcinine molar sum (p = 0.0108) were higher in HIV-infected patients. Levels of albumin, amino acids, and C3 acyl [propionyl]carcinine, a byproduct of branched chain amino acid catabolism, were comparably low in both groups. Yet blood glucose levels were maintained in the normal range. Triglycerides were higher in HIV-infected patients (p<0.001). Moreover, a number of inflammatory markers, including CRP, IL-2, IL-6, IL-8, and TNF-α were higher in HIV-infected patients, with IL-2 (p = 0.016) and TNF-α (p = 0.025) reaching statistical significance (Table 2). Edematous patients had higher alanine amino transferase (ALT) and gamma glutamyl transpeptidase (GGT) levels and lower albumin and amino acid levels than non-edematous patients (data not shown); these metrics did not differ among the HIV-infected and HIV-negative groups. Edematous patients also had lower total and HMW adiponectin levels; however, edema did not modify the association between HIV status and hypoaldipoincinemia.
Table 6. Changes in Amino Acid Levels of HIV-infected (surviving) and HIV-negative patients.

| Amino Acid | HIV-infected (n = 10) | HIV-negative (n = 44) |
|------------|-----------------------|----------------------|
|            | Mean ± SEM            | p-value | Mean ± SEM | p-value |
|            | Admission 14-day       |         | Admission 14-day |         |
| Glycine    | 216 ± 16.6            | 313 ± 28.1 | 238 ± 13.7  | 305 ± 13.4 | <0.0001 |
| Alanine    | 150 ± 18.1            | 409 ± 57.7 | 218 ± 18.6  | 418 ± 27.5 | <0.0001 |
| Serine     | 98.8 ± 4.5            | 125 ± 14.6 | 113 ± 6.4   | 157 ± 6.9  | <0.0001 |
| Proline    | 155 ± 14.5            | 296 ± 42.6 | 152 ± 9.4   | 282 ± 20.3 | <0.0001 |
| Valine     | 102 ± 15.8            | 130 ± 21.3 | 76.8 ± 6.2  | 152 ± 10.2 | <0.0001 |
| Leucine/Isoleucine | 80.1 ± 15.0 | 112 ± 15.2 | 71.0 ± 5.2  | 132 ± 7.3  | <0.0001 |
| Methionine | 16.8 ± 2.8            | 19.7 ± 3.3 | 15.6 ± 1.0  | 24.5 ± 1.6  | <0.0001 |
| Histidine  | 64.5 ± 12.7           | 53.9 ± 5.7 | 52.8 ± 3.4  | 50.7 ± 2.7  | 0.954 |
| Phenylalanine | 78.4 ± 18.2 | 59.8 ± 5.3 | 43.3 ± 2.8  | 53.0 ± 2.5  | 0.0184 |
| Tyrosine   | 29.8 ± 7.2            | 45.8 ± 10.6 | 22.6 ± 2.7  | 52.1 ± 5.0  | <0.0001 |
| Aspartate  | 31.3 ± 6.3            | 50.4 ± 10.5 | 35.4 ± 2.7  | 51.0 ± 5.0  | <0.0001 |
| Glutamate  | 109 ± 12.7            | 135 ± 17.3 | 91.3 ± 4.7  | 152 ± 8.2  | <0.0001 |
| Ornithine  | 29.3 ± 3.1            | 40.2 ± 6.8 | 26.7 ± 2.3  | 47.9 ± 3.3  | <0.0001 |
| Citrulline | 8.9 ± 1.1             | 16.3 ± 14  | 8.3 ± 0.7   | 19.0 ± 1.6  | <0.0001 |
| Arginine   | 29.2 ± 2.4            | 44.5 ± 6.9 | 27.8 ± 2.2  | 49.7 ± 3.3  | <0.0001 |

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Insulin and IGF-1 levels were low in both HIV-infected and HIV-negative subjects, while growth hormone (GH), ghrelin, cortisol, GLP-1, and peptide YY (PYY) were high (compare levels to those in references [15–18]). Excluding analysis of three patients taking ARVs, which are known to affect adipose tissue function, the levels of leptin (p = 0.016), total adiponectin (p = 0.0017), and high molecular weight (HMW) adiponectin (p = 0.0014) were significantly lower in HIV-infected than in HIV-negative subjects (Table 2). Multivariate logistic regression controlling for the degree of wasting (as assessed by W/H z-score) established that HIV infection was associated with lower total adiponectin (p = 0.0014) than HIV-negative subjects (Table 4).

Predictors of Mortality During Inpatient Treatment

Non-edematous patients who died had more striking manifestations of wasting than those who survived, as reflected in lower W/H z-score (−6.28 vs. −3.98, p = 0.0244) and MUAC (7.8 vs. 10.1, p = 0.0019). In all patients, there was a greater degree of stunting than those who died (L/A z-score −4.03 vs. −2.92, p = 0.0454) (Table 7).

In addition to HIV infection, factors at baseline associated with subsequent mortality were hypoalbuminemia (p = 0.0002), low levels of HMW adiponectin (p = 0.0149), and high levels of PYY (p = 0.0087), IL2 (p = 0.0004), IL6 (p = 0.004), and TNF-α (p = 0.0203) (Table 7) [8]. Multivariate logistic regression analysis controlling for HIV status and admission W/H z showed that hypoalbuminemia at baseline remained a significant predictor of mortality (OR 0.906, CI 0.827–0.993, p = 0.035) while HMW adiponectin at baseline became insignificant. Mortality did not vary with other baseline measures including presence of edema, hemoglobin, glucose, creatinine, albumin, phosphorus, other hormones and growth factors, fatty acid metabolites, or amino acid or cytokine levels.
Discussion

Malnutrition remains a major cause of morbidity and mortality, with the greatest impact in low-income countries. HIV infection is detected in 30% of children with SAM and is associated with greatly increased mortality rates [4]. The role of HIV in the pathophysiology of malnutrition is poorly understood. Here we characterized differences in baseline metabolic and hormonal status between HIV-infected and HIV-negative children with SAM and compared their subsequent responses to current WHO recommended nutritional therapy. A major finding of this study is that HIV-infected children with SAM present with significant reductions in the adipocytokines leptin and adiponectin that are associated with mortality during inpatient hospitalization.

In our study the prevalence of HIV infection was 24%, with two-thirds of these representing new diagnoses. Mortality in HIV-infected children was very high (33.3%), similar to results from a previous meta-analysis [4]. HIV-infected and HIV-negative patients presented with similar degrees of wasting and edema, and among those who survived, achieved similar rates of growth and recovery. A previous study found HIV-infected patients to be more wasted at baseline; nevertheless that study, like ours, noted that seropositive and seronegative patients achieve similar rates of catch-up growth during nutritional treatment and that increased wasting at presentation is associated with mortality [7].

Both HIV-infected and HIV-negative children presented in a severe catabolic state characterized by elevated NEFA, total ketones, and even-numbered acylcarnitines (derived from fatty acid oxidation) and striking reductions in serum albumin and

Table 7. Baseline Characteristics and Metabolic Profiles associated with Mortality.

|                          | Died (n = 8) | Survived (n = 54) | p-value |
|--------------------------|-------------|-------------------|---------|
| **Anthropometry (nonedematous)** |             |                   |         |
| Admission W/H % | 61.1 ± 4.6  | 72.5 ± 1.0 | 0.0121  |
| Admission W/H Z-Score | −6.28 ± 1.05 | −3.98 ± 0.19 | 0.0244  |
| Admission W/A Z-Score | −7.01 ± 0.86 | −4.63 ± 0.26 | 0.0221  |
| Admission MUAC | 7.8 ± 0.3  | 10.1 ± 0.2 | 0.0019  |
| L/A z-score (all patients) | −4.03 ± 0.58 | −2.82 ± 0.18 | 0.0454  |
| **Fatty Acid Metabolites** |             |                   |         |
| NEFA (mmol/L) | 0.57 ± 0.15 | 0.56 ± 0.06 | 0.557   |
| Total Ketones (μmol/L) | 539 ± 276 | 526 ± 106 | 0.456   |
| **Acylcarnitines** |             |                   |         |
| C2 (μmol/L) | 16.3 ± 2.2 | 16.5 ± 2.3 | 0.139   |
| C3 (μmol/L) | 0.51 ± 0.09 | 0.38 ± 0.03 | 0.128   |
| C2/C3 Ratio | 38.5 ± 7.7 | 48.4 ± 5.5 | 0.858   |
| **Hormones** |             |                   |         |
| Insulin (μIU/ml) | 1.10 ± 0.97 | 2.46 ± 0.38 | 0.182   |
| Growth Hormone (ng/ml) | 10.5 ± 3.4 | 11.5 ± 1.3 | 0.442   |
| IGF-1 (ng/ml) | 5.6 ± 4.8 | 11.1 ± 1.8 | 0.118   |
| Total Ghrelin (pg/ml) | 4660 ± 771 | 3811 ± 297 | 0.361   |
| GLP-1 (pg/ml) | 149.1 ± 30.3 | 97.9 ± 11.7 | 0.080   |
| PYY (pg/ml) | 1866 ± 227 | 1101 ± 87 | 0.0089  |
| Cortisol (μg/dl) | 52.2 ± 6.2 | 47.4 ± 2.4 | 0.484   |
| **Adipocytokines** |             |                   |         |
| Leptin (μg/ml) | 7.1 ± 4.4 | 280 ± 47 | 0.0002   |
| Total Adiponectin (ng/ml) | 10403 ± 3360 | 14191 ± 1017 | 0.075   |
| HMW Adiponectin (ng/ml) | 4894 ± 1922 | 8795 ± 693 | 0.0184   |
| **Amino Acids** |             |                   |         |
| Amino Acid Molar Sum (μmol/L) | 1248 ± 70 | 1193 ± 46 | 0.319   |
| **Inflammatory Cytokines** |             |                   |         |
| IL-2 (pg/ml) | 16.2 ± 2.8 | 2.9 ± 1.1 | 0.0004  |
| IL-6 (pg/ml) | 223.6 ± 47.9 | 17.2 ± 18.4 | 0.166   |
| IL-8 (pg/ml) | 323 ± 147 | 105 ± 57 | 0.0042  |
| TNF-α (pg/ml) | 46.8 ± 20.2 | 37.6 ± 7.8 | 0.0203  |

*Excludes patients on ARVs.
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amino acids. At the same time, blood glucose levels were maintained in the normal range. Leptin, adiponectin, insulin, and IGF-1 levels were low while growth hormone, cortisol, and ghrelin levels were high. [9] This profile suggests a state in which fat catabolism and glucose production are prioritized above energy storage and growth [9,20–25]. At baseline, serum triglycerides, ketones, and even-chain acylcarnitines were higher and leptin and HMW adiponectin lower in HIV-infected patients than in HIV-negative patients. When controlling for W/H z-score, lower HMW adiponectin levels remained significantly associated with HIV infection, though leptin levels did not.

Nutritional treatment reversed the state of lipid mobilization and fatty acid oxidation and increased the levels of amino acids and C3 acyl (propionyl)carnitine. Insulin and IGF-1 rose while GH, cortisol, and ghrelin declined. HIV status did not modify the effect of treatment on most metabolites, hormones, growth factors, and cytokines. However, nutritional intervention increased HMW and total adiponectin levels in HIV-negative patients but not in HIV-infected patients; their levels remained significantly lower despite high calorie feeds. Leptin, on the other hand, increased in both HIV-infected and HIV-negative subjects.

Previous studies have linked decreased levels of leptin and adiponectin to HIV infection, particularly in the context of HIV-associated lipodystrophy, a syndrome characterized by fat redistribution, dyslipidemia, and metabolic syndrome. Many investigations implicate ARVs in the development of this syndrome, citing medication-induced adipose dysregulation and mitochondrial toxicity as potential mechanisms [10–14]. Additional studies, however, in untreated adults and mice have shown that HIV infection itself may be associated with adipose tissue dysfunction and decreased levels of adiponectin and leptin [26–28].

Adiponectin is produced by mature adipocytes; over-expression of adiponectin increases hepatic insulin sensitivity, while low levels of adiponectin are associated with insulin resistance and the metabolic syndrome [29]. Circulating leptin levels rise in proportion to white adipose tissue mass; higher levels are associated with obesity and lower levels with fasting and malnutrition [22]. The severe hypoadiponectinemia and hypo-leptinemia in our HIV-infected children suggest a state of insulin resistance associated with depletion of white adipose tissue reserves.

The pre-existing mass and function of white adipose tissue appear to play roles in the adaptation to, and recovery from, malnutrition because low levels of leptin and adiponectin at baseline were associated with subsequent mortality. Indeed, baseline hypo-leptinemia remained a strong predictor of mortality when controlling for HIV infection and W/H z-score in a multivariate analysis. While these findings do not prove that mortality is caused by hypo-adiponectinemia and/or hypo-leptinemia, there are potential mechanisms by which hypo-adiponectinemia and hypo-leptinemia might contribute to mortality risk. For example, a lack of pre-existing adipose tissue stores, suggested by hypo-leptinemia at presentation, may limit a child’s ability to sustain energy production for critical cardiorespiratory function during the initial phases of acute severe malnutrition. Moreover, leptin and adiponectin may mediate inflammatory processes that may modulate the response to infectious pathogens. Leptin activates NK cells, induces neutrophil chemotaxis, enhances secretion of pro-inflammatory cytokines, and induces activation and proliferation of T-cells, while adiponectin promotes production of numerous anti-inflammatory cytokines [30,31]. It is possible that the combined effects of HIV infection and malnutrition on adipose tissue and immune function may increase mortality risk.

There were several limitations to our study. Blood samples were not obtained after fasting, as this could not be justified in critically ill patients. Our small sample size prevented us from conducting potentially important analyses of subgroups including HIV-infected patients who died (n = 6) and those already taking ARVs. Additionally, 17.6% (n = 13) of the original patient population left the ward prior to completing nutritional rehabilitation.

Nevertheless, this study provides a comprehensive analysis of the effects of HIV on the pathophysiology and recovery from SAM in childhood. Our findings suggest a critical interplay between HIV infection and adipose tissue storage and function in the adaptation to malnutrition.

Mortality in malnutrition is predicted by low W/H z and low MUAC. However, these can be difficult or impossible to interpret in infants and children with nutritional edema. Currently, all patients with nutritional edema are categorized as having SAM and treated accordingly. Our finding that hypo-leptinemia predicts mortality in edematous as well as non-edematous subjects suggests that leptin assays might in the future be used to identify and target malnourished children at highest risk of death [8].

Finally, it should be noted that the optimal timing for initiating ARV treatment in HIV-infected children with SAM is currently unknown. In some cases, early initiation of therapy may increase the risk of clinical deterioration; in clinically stable children, however, it appears to improve outcomes [12,32–35]. Future studies should determine if the effects of ARVs on adipose tissue metabolism and function influence the clinical response to treatment of severely malnourished children.

Supporting Information
Methods S1 Detailed methods on the assays used for the metabolic and hormonal analyses.
(DOC)

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Author Contributions
Conceived and designed the experiments: AM SB MF. Performed the experiments: AM SB TK EK. Analyzed the data: AM SB MF CH JVP JDI. Contributed reagents/materials/analysis tools: J. Bain MM RS CN. Wrote the paper: AM SB MF. Facilitated site coordination: J. Bartlett.

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