RESEARCH ARTICLE

REVISED Systemic inflammation is negatively associated with early post discharge growth following acute illness among severely malnourished children - a pilot study [version 2; peer review: 2 approved]

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Abstract

Background: Rapid growth should occur among children with severe malnutrition (SM) with medical and nutritional management. Systemic inflammation (SI) is associated with death among children with SM and is negatively associated with linear growth. However, the relationship between SI and weight gain during therapeutic feeding following acute illness is unknown. We hypothesised that growth post-hospital discharge is associated with SI among children with SM.
Methods: We conducted secondary analysis of data from HIV-uninfected children with SM (n=98) who survived and were not readmitted to hospital during one year of follow-up. We examined the relationship between changes in absolute deficits in weight and mid-upper-arm circumference (MUAC) from enrolment at stabilisation to 60 days and one year later, and untargeted plasma proteome, targeted cytokines/chemokines, leptin, and soluble CD14 using multivariate regularized linear regression.
Results: The mean change in absolute deficit in weight and MUAC was -0.50kg (standard deviation; SD±0.69) and -1.20cm (SD±0.89), respectively, from enrolment to 60 days later. During the same period, mean weight and MUAC gain was 3.3g/kg/day (SD±2.4) and 0.22mm/day (SD±0.2), respectively. Enrolment interleukins; IL17-alpha and IL-2, and serum amyloid P were negatively associated with weight and MUAC gain during 60 days. Lipopolysaccharide binding protein and complement component 2 were negatively associated with weight gain only. Leptin was positively associated with weight gain. Soluble

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CD14, beta-2 microglobulin, and macrophage inflammatory protein 1 beta were negatively associated with MUAC gain only. Glutathione peroxidase 3 was positively associated with weight and MUAC gain during one year.
Conclusions: Early post-hospital discharge weight and MUAC gain were rapid and comparable to children with uncomplicated SM treated in the community. Higher concentrations of SI markers were associated with less weight and MUAC gain, suggesting inflammation negatively impacts recovery from wasting. This finding warrants further research on reducing inflammation on growth among children with SM.

Keywords
severe malnutrition, child growth, weight, mid-upper arm circumference, anthropometric deficit, inflammation, cytokines, proteome

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REVISED **Amendments from Version 1**

In the introduction, we have included additional citations on linear growth and post-hospital discharge persistence of markers of systemic inflammation among sepsis survivors in high income settings. We have also conducted further analysis with growth deficits (weight, MUAC, and height) at 1 year and presented these results in the main text and discussed the findings. An interesting and strong association between Glutathione peroxidase 3 and one-year weight and MUAC deficit changes emerged. We believe that this analysis of inflammation on longer-term impact on growth is a valuable contribution to the field where the data is quite limited. These changes have been reflected in updated figures and tables.

Any further responses from the reviewers can be found at the end of the article

Introduction

In 2018, approximately 50.5 million children under five years old globally were wasted, of which 16 million were severely wasted[1,2]. Wasting is associated with elevated mortality, mainly due to susceptibility to infectious diseases[3–5]. Current guidelines recommend that children with severe wasting or oedematous malnutrition who are acutely ill (complicated severe malnutrition; CSM) are initially medically treated and nutritionally stabilised as inpatients. Once stabilised, they are treated with high protein and energy feeds in the form of ready-to-use therapeutic foods (RUTF) to achieve catch-up weight gain as outpatients[6,7].

Severely malnourished children admitted to hospital with acute illness may suffer relapse, readmission, or death after discharge from hospital[8–13] and are at risk of impaired neurocognitive development[14–16]. Children may return to household settings of poverty, social disadvantage, environmental contamination, and inadequate access to healthcare[17–25]. Enhanced prevention of recurrent illnesses over longer periods following hospitalisation and improved dietary quality have been suggested as opportunities to improve growth[10,11].

RUTF was designed to fulfil 100% of the nutritional needs of children recovering from SM and may theoretically enable weight gain of up to 20 g/kg/day[26]. Weight gain velocity is usually high at the start of the therapeutic feeding, then decreases and later plateaus[27–34]. Weight gain may be affected by comorbidities such as HIV or other chronic infections but may also be related to intestinal or systemic inflammation (SI), leading to reduced appetite, nutrient malabsorption, and metabolic changes[25,35,36].

SI is demonstrable at the time of hospital discharge in children with SM[37]. However, it is not known how long inflammation persists or what its effects are on weight gain. In high income settings, survivors of sepsis have elevated markers of SI for up to one year and retain an increased mortality risk[38–42]. After sepsis, systemic levels of C-reactive protein (CRP) and soluble programmed death ligand 1 (a marker of immunosuppression) are elevated for up to one year in patients while interleukin 6 (IL6) and IL10 persists for several months in human and experimental models[38–40]. SI, characterised by elevated cytokines including IL1β and IL6, is known to suppress linear growth indirectly through the growth hormone/insulin growth factor 1 (GH/IGF1) axis, and directly through effects on long bone growth plate chondrocytes[25,43]. In Nepal, birth size was inversely associated with low-grade, chronic inflammation during pregnancy as indicated by serum Alpha 1 acid Glycoprotein[44,45]. Further, head size at birth among Nepalese[46] and attained z scores for height (HAZ) and weight-for-age (WAZ) at 6–8 years of age[47] were associated with a wide array of plasma proteins, including S100 calprotectin subunits, assessed at 6–8 years. In community settings, SI is elevated in 17–34% children in LMIC[48] (CRP >5mg/L or α1-antichymotrypsin >0.6g/L) and is associated with reduced linear growth[49–53]. Besides linear growth, SI may affect gain in adipose and muscle through promoting a persistent catabolic state and dysregulation of the usual hormonal and metabolic processes of these tissues[54–56].

Both nutrient scarcity and acute illness are associated with a catabolic state[55] with negative effects on the body's storage organs, mainly adipose and muscle. During refeeding of children with SM, significant systemic metabolic shifts are observed that relate to the muscle, liver, and the adipose tissue among others[57,58]. We therefore hypothesised that among children with SM treated in hospital for an acute illness, weight gain is associated with SI. The objective of this study was to investigate the relationship between plasma proteomic and cytokine profiles and weight gain among HIV negative children with SM in the first 60 days of post-hospital discharge following medical stabilisation.

Methods

Ethics approval and consent to participate

The trial was approved by the Kenya National Ethical Review Committee (SSC 1562) and the Oxford Tropical Research Ethics Committee (OXTREC reference 18–09). Secondary analyses were approved by the Scientific and Ethical Review Unit (SERU 2782). The trial was registered at clinicaltrials.gov (NCT00934492, 8th July 2009). Informed consent for data and sample collection, storage, and future research had been obtained from mothers or guardians of study participants during recruitment to the trial.

Study design and patient recruitment

This was a secondary analysis of data from a nested case control study[37] within a clinical trial (NCT00934492) that tested the efficacy of daily co-trimoxazole prophylaxis in reducing post-discharge mortality among HIV-uninfected children aged 2–59 months hospitalised with CSM in two urban (Mombasa and Nairobi) and two rural (Kilifi and Malindi) hospitals in Kenya[8]. Children were included in the trial if they had mid-upper-arm circumference (MUAC) <11.5cm if aged ≥6 months and <11.0cm if aged 2–5 months or had oedematous malnutrition; and had a negative HIV rapid-antibody test; and had completed the stabilisation phase of treatment as defined in WHO guidelines. Children were enrolled just prior to discharge from hospital. Samples were collected from study participants prior to initiation of the investigational product: co-trimoxazole or

placebo at discharge and constitute enrolment samples. Discharge was according to WHO guidelines, based on clinical recovery rather than achieving an anthropometric threshold. At hospital discharge, nutritional counselling was given to caregivers, along with RUTF dosed as per WHO and Kenyan guidelines, and families were actively referred to community-based management of acute malnutrition (CMAM) centres located either at the hospital or in community facilities to continue therapeutic feeding. Children were actively followed up for 12 months, monthly in the first six months, and at months eight, 10 and 12. Study participants were traced at home if they defaulted and loss to follow-up was minimal (≤5%). The trial intervention had no overall effect on reducing mortality or hospital readmission.

Participants selected for this study had served as controls in a previous case control study[37]. Briefly, the case to control ratio in the case control study was 1:1 and there were 121 cases (deaths) that were analysed that had sufficient samples from among 147 deaths that had occurred within the first 60 days of enrolment into the trial. Control children (n=120) had been randomly selected without replacement amongst 1119 children who survived and were not readmitted to hospital during 12 months of trial follow up using the '*sample*' command in STATA (version 15.1, TX, USA). For this study, 12 children who were oedematous at enrolment and another 10 children that lacked anthropometry data at month 2 to month 6 were excluded from the analysis. We therefore analysed data for 98 children in which plasma proteomic and cytokine measurements had been done on enrolment samples.

Data sources and measurements

During enrolment and at follow-up, child and caregiver demographic characteristics, immunisation status, clinical examination, admission diagnoses, chronic conditions, and anthropometry (weight, height or length, MUAC) were collected[8]. Weight was measured with the use of an electronic scale (Seca 825), length or height with the use of an infantometer (Seca 416) or stadiometer (Seca 215), and MUAC with the use of insertion tape (TALC)[8]. The WHO (2006) growth references were used to calculate Z scores.

Proteomics and cytokines measurement in plasma

Untargeted plasma proteomics were measured by liquid chromatography tandem mass spectrometry and targeted cytokines, chemokines, leptin and soluble CD14 (sCD14) by Luminex and ELISA as previously described[37].

Bioinformatics and statistical analysis

The primary and secondary outcomes were the change in absolute deficits in weight (DWAD) and MUAC (DMAD), respectively, from enrolment to 60 days. We further analysed the change in absolute weight, MUAC and height deficits from enrolment to 1 year. Absolute deficit was defined as the median value for age according to WHO growth charts minus the child's measured value. It was calculated as the difference between the measured weight, MUAC, or height and the median age- and sex-specific value obtained from the WHO 2006 growth standards[59–61]. Absolute deficit was used rather than Z scores for weight-for-age (WAZ) or weight-for-height (WHZ) because changes in standard deviation across age or length makes them less appropriate for measuring changes over time among children of different ages[60]. Exposure variables were the plasma proteome, leptin, sCD14 and a panel of targeted cytokines that are markers of inflammation and immune activation. Regression models were adjusted for age, sex, randomisation and site, whilst regression to the mean was addressed by including enrolment anthropometric values in the regression models. We hypothesised that proteins measured at baseline would have their strongest effect on early growth (within 60 days) than at later time points. We conducted the analysis in the R statistical software version 3.6.2[62] and performed a multivariate regularized linear regression analysis using an elastic net (EN) model implemented using the "glmnet" package. This package fits a generalized linear model via penalized maximum likelihood. EN is a penalized regression approach and integrates two regularized approaches, ridge regression and LASSO (Least Absolute Shrinkage and Selection Operator), wherein the contribution of each of these models to the final EN model is controlled by the α parameter[63,64]. The EN penalty is controlled by α and bridges the gap between LASSO (α=1, the default) and ridge (α=0). The tuning parameter lambda (λ) that controls the overall strength of the penalty was determined using five-fold cross validation. The strong penalization imposed by LASSO draws non-predictive coefficients to zero, thereby eliminating proteins from the models, whereas ridge regression addresses potential multi-collinearity problems in high-dimensional data[63,64]. Variables such as age, sex, randomisation arm and site were treated as prior confounders and were not subjected to penalization by imposing a penalty factor of 0. All other variables had a penalty factor of 1 and were subjected to penalization. We used the 'caret' package in R to automatically select the best tuning parameters alpha and lambda by testing a range of possible alpha and lambda values. The best alpha and lambda values are those values that minimize the cross-validation error. EN model generation was performed separately for each growth outcome: change in Weight Absolute Deficit (DWAD) (primary outcome) and change in MUAC Absolute Deficit (DMAD) (secondary outcome), with protein profiles, cytokines, and enrolment anthropometric variables as predictors. As a further analysis, EN models were generated for DWAD, DMAD and Height Absolute Deficit (DHAD) for changes during one year. The subset of variables assigned non-zero coefficients were considered optimal and were retained in each of the final multivariable models. Finally, bootstrapping was used to evaluate the robustness of selected proteins at 1000 iterations using the 'BootValidation' package in R on the elastic net model with the optimized regularization value (α=0.5) and analytes selected by the model for more than 60%[65] of times were considered as important protein features.

Results

Characteristics of study participants

Study participants' characteristics are shown in Table 1. At enrolment, 89% of the children were over six months of age[66]. Children were also severely stunted at enrolment and this was unchanged after 60 days despite large MUAC and weight gains

Table 1. Characteristics of study participants.

Characteristic	Enrolment (N=98)	60 Days (N=98)	$P._{adj}$
Demographics			
Median age (mo.) [IQR]	13 [9–16]	–	–
Girls (n) %	47 (48)	–	–
Born prematurely (%)	14 (14)	–	–
Born underweight n (%)	23 (23)	–	–
Recruitment hospital			
Kilifi County Hospital n (%)	5 (5)	–	–
Coast General Hospital n (%)	51 (51)	–	–
Malindi Subcounty Hospital n (%)	20 (20)	–	–
Mbagathi County Hospital n (%)	24 (24)	–	–
Randomized to co-trimoxazole n (%)	50(50)		-
Anthropometry			
Weight (kg), mean ±SD	5.8±1.3	6.8±1.3	0.015
MUAC (cm), mean ±SD	10.6±1.0	11.9±1.1	<0.001
Height (cm), mean ±SD	66.8±7.3	68.8±6.9	<0.001
Weight absolute deficit (kg), mean ±SD	-3.2±1.1	-2.8±1.2	0.16
MUAC absolute deficit (cm), mean ±SD)	-3.8±0.9	-2.6±1.0	0.001
Height absolute deficit (cm) mean ±SD	-6.6±4.4	-7.2±4.3	0.012
WAZ, mean ±SD	-3.90±1.0	-3.07±1.2	0.011
WHZ, mean ±SD	-3.14±1.2	-1.85±1.4	0.016
HAZ, mean ±SD	-2.87±1.7	-3.02±1.5	0.001
Full blood count			
Haemoglobin g/dl mean ±SD	9.95±2.0	10.4±2.3	<0.001
WBC count (x10^3/L) – median (IQR)	9.9 (6.3–12.7)	9.5 (6.6–12.1)	<0.001
Lymphocyte count (x10^3/L) – median (IQR)	5.0 (2.9–6.7)	4.9 (3.0–7.2)	0.003
Neutrophil count (x10^3/L) – median (IQR)	2.95 (1.9–4.7)	2.7 (2.1–4.3)	<0.001
Platelet count (x10^3/L) – median (IQR)	475 (280–579)	407 (233–529)	<0.001

mo. = months, n = number of study participants, SD = standard deviation, IQR = interquartile range, P.adj = P value adjusted for age, sex, randomisation arm, and the site of enrolment, MUAC = mid-upper-arm circumference, WAZ = weight for age z score, WHZ = weight for height z score, HAZ = height for age z score, WBC = white blood cell.

with nutritional rehabilitation (all P.adj<0.01). Haemoglobin, total white blood cell count, and lymphocyte count increased, while neutrophil and platelet counts decreased between enrolment and 60 days (P.adj<0.01) (Table 1).

Children have higher growth rates during the first two months post-discharge

Overall, mean weight gain for 60 days was 3.3g (SD: ±2.4) per kilogram per day and 3.2kg (SD: ±0.27) during one year from enrolment. The mean MUAC and length/height gains for 60 days were 0.22mm (SD: ±0.2) per day and 0.34mm (SD: ±0.25) per day, respectively (Table 2). The mean one-year gains in MUAC and height were 2.82cm (SD: ±1.34) and 11.15cm (SD: ±3.82), respectively. Changes in weight and MUAC from enrolment to 60 days were larger than during the later bimonthly time periods up to one year (p<0.01) (Table 2). Differences in height between enrolment to 60 days were not significantly different from bimonthly changes up to 6 months (both

Table 2. Bimonthly anthropometric growth indices of children during the first 180 days post-hospital discharge. **p values* refer to paired t tests between changes during 0-60 days, and changes during 61-120 days, 121-180 days or average bimonthly changes between days 181 and 365. Enr.=Enrolment, Δ=change, DWAD=change in absolute deficits in weight, DMAD= change in absolute deficits in MUAC, DHAD=change in absolute deficits in height.

Changes in anthropometry								
Characteristic	**Enr.–60 days**	**61–120 days**	***p value****	**121–180 days**	***p value****	**Bimonthly 181–365 days**	***p value****	**Overall growth (Enr.–356 days)**
Δ Weight (kg), mean ±SD	1.08±0.70	0.58±0.50	<0.001	0.40±0.44	<0.001	0.39±0.27	<0.001	3.23±0.27
Δ MUAC (cm), mean ±SD	1.33±0.89	0.51±0.65	<0.001	0.27±0.58	<0.001	0.24±0.29	<0.001	2.82±1.34
Δ Height (cm), mean ±SD	2.07±1.55	2.23±1.30	0.33	1.97±1.19	0.55	1.60±0.63	0.001	11.15±3.82
DWAD (kg), mean ±SD	-0.50±0.69	-0.10±0.48	<0.001	-0.05±0.43	<0.001	-0.02±0.28	<0.001	-0.51±1.23
DMAD (cm), mean ±SD	-1.20±0.89	-0.39±0.66	<0.001	-0.18±0.58	<0.001	-0.13±0.29	<0.001	-2.15±1.23
DHAD (cm), mean ±SD	-0.53±1.40	-0.08±1.23	0.03	-0.18±1.12	0.08	-0.29±0.59	0.37	-1.58±2.89

p>0.1) but was higher than in bimonthly periods after 6 months (Table 2).

The mean change in absolute deficits in weight (DWAD) and MUAC (DMAD) from enrolment to 60 days were -0.5kg (SD: ±0.69) and -1.20cm (SD: ±0.89), respectively, and these were higher when compared to later time periods (P<0.001). There was a significant difference in the change in height deficit (DHAD) between the first 60 days and 61–120 days (P=0.03) but not at 121–180 days or the later bimonthly average change after 180 days (P>0.05).

Inflammatory cytokines and proteins are negatively associated with change in growth deficit at two months

Change in weight absolute deficit (DWAD). In the multivariate elastic net (EN) regularized regression model adjusted for confounders, inflammatory cytokines interleukin 17 alpha (IL17a) and interleukin 2 (IL2), complement component 2 (C2), lipopolysaccharide binding protein (LBP), amyloid P component, serum (APCS or SAP), among others were negatively associated with DWAD in the first 60 days (Figure 1a). Further, our analysis showed that the adipokine leptin was positively associated with DWAD (Figure 1a).

Change in MUAC absolute deficit (DMAD). Inflammatory cytokines IL17a, IL2, and Macrophage inflammatory protein 1-beta (MIP1B) were negatively associated with DMAD in the first 60 days (Figure 1b). Angiotensinogen (AGT), the precursor of all angiotensin peptides; soluble CD14 (sCD14), a co-receptor for the detection of bacterial lipopolysaccharide (LPS); beta-2 microglobulin (β2M), a component of MHC class I molecules which are present on all nucleated cells; and SAP, were negatively associated with DMAD (Figure 1b). Only IL17a, IL2, and SAP were associated with both DWAD and DMAD (Figure 1c) even though these two anthropometric measurements were significantly correlated as shown in Figure 1d. Both models were significantly associated to their respective growth outcome, accounting for just over half of the variability in growth (DWAD r^2=0.51 and DMAD r^2=0.57, Table 3).

Bootstrap analysis. After 1000 bootstrap iterations, only IL17a was identified in >60% of the DWAD model repetitions (Figure 1e) indicating that this was the most robust feature associated with weight gain. Using similar iterations during bootstrap validation for the DMAD model, no features were extracted at the 60% threshold and the most frequently selected features were IL17a (55%), B2M (55%), AGT (49%), SAP (48%), and sCD14 (48%) as shown (Figure 1f).

Complement factors and Glutathione Peroxidase 3 are associated with one-year change in growth deficits

DWAD at one-year follow-up. Complement proteins; C2, factor D (CFD), C1r Subcomponent Like (C1RL), factor B (CFB), and component 8 (C8G), as well as AGT, CRP, and cytokines; MIP1a and IL1b were negatively associated with DWAD from enrolment to one year (Figure 2a). Glutathione Peroxidase 3 (GPX3), a selenium-dependent antioxidant enzyme that scavenge hydrogen peroxide in the presence of reduced glutathione was positively associated with one-year DWAD.

DMAD at one-year follow-up. Complement proteins; factor I (CFI), factor H Related 2 (CFHR2), CFD, and C2, as well as Attractin (ATRN), β2M, CRP, Zinc-alpha-2-glycoprotein (AZGP1 or ZAG), Vitamin K-dependent protein S (PROS1), and cytokines; IP10 and TNFα were negatively associated with one-year DMAD (Figure 2b). GPX3 and Pregnancy zone protein (PZP Alpha-2-Macroglobulin Like) was positively associated with one-year DWAD (Figure 2b).

DHAD at one-year follow-up. Complement factor B (CFB), β2M, MIP1a, and Haemoglobin Subunit Gamma 1 (HBG1) among others were negatively associated with one-year DHAD.

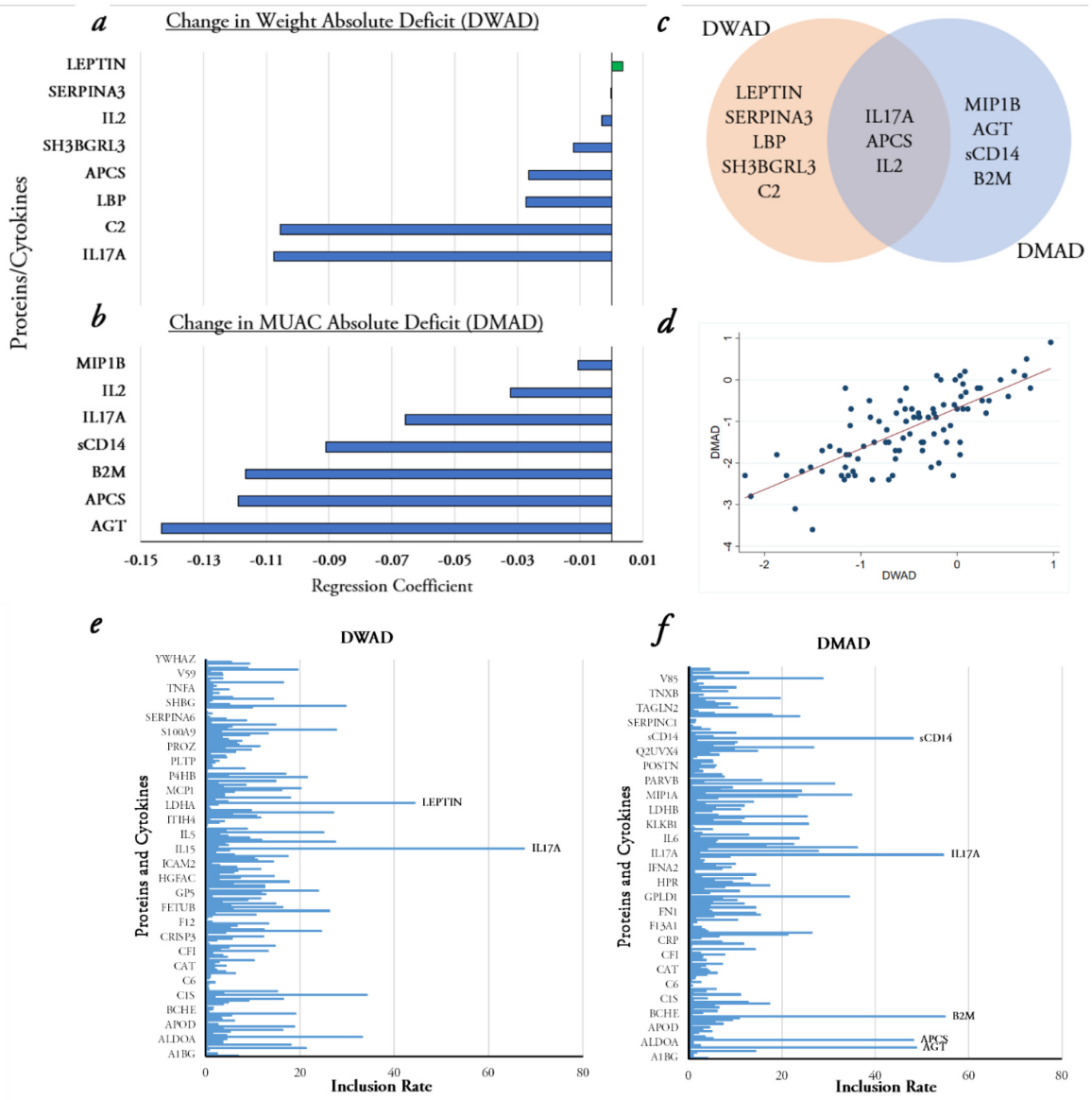

Figure 1. Multivariate analysis of plasma proteome and cytokines associated change in growth deficit at 60 days. Untargeted liquid chromatography tandem mass spectrometry plasma proteins, and targeted cytokines/chemokines, Leptin, and sCD14 associated with DWAD (***a***) and DMAD (***b***) in multivariate elastic net (EN) regularized linear regression models at two months. Log normalised protein values were used in the analysis and regression models were adjusted for age, randomisation arm, sex, respective enrolment growth deficits, and site. (***c***) A Venn diagram showing overlap of the proteins and cytokines associated with DWAD and DMAD. (***d***) A scatter plot showing that DWAD and DMAD are significantly correlated (P<0.001, R^2 =0.74). (***e*** and ***f***) Bar plots showing feature importance as depicted by the feature inclusion rate after 1000 bootstrap iterations during bootstrap validation for DWAD and DMAD, respectively. DWAD = change in weight absolute deficit, DMAD = change in MUAC absolute deficit, MUAC = mid-upper-arm circumference.

Bootstrap analysis (enrolment one–year). Using 1000 bootstrap iterations, GPX3 and MIP1a were identified in >60% of the one-year DWAD model repetitions (Figure 2d). GPX3 was also selected at >60% in the bootstrap validation for the DMAD model. Other frequently selected proteins included IP10 (60%) and PZP (59%). MIP1a was selected at >60% in the bootstrap validation of DHAD while HBG1 was selected at 58% of the iterations.

All the models were significantly associated to their respective growth outcome, accounting for over half of the variability in growth (Table 4).

Discussion

We investigated the relationship between inflammatory cytokines and plasma proteomic profiles and change in anthropometric deficits during the early post-hospital discharge period as

Table 3. Elastic Net regression model optimal alpha parameters and performance of proteins associated with change in growth deficits within 60 days.

EN Variable		Optimal alpha	r	[95% CI]	P value
DWAD	Exposure protein variables	0.5	0.51	0.34 – 0.64	<0.0001
DMAD	Exposure protein variables	0.5	0.57	0.41 – 0.69	<0.0001

Footnote: Optimal alpha parameter and correlation coefficients for the EN model enumerating the correlation between DWAD and DMAD at two months and exposure protein variables (untargeted plasma proteome, and targeted cytokine/chemokines, leptin, and sCD14) extracted by the multivariate regularized models.

EN = elastic net, DWAD = change in absolute deficits in weight, DMAD = change in absolute deficits in mid-upper-arm circumference, CI = confidence interval.

this is the period most likely to be related to biological factors measured at discharge and when catch up in weight deficit is at its greatest[8]. We also investigated associations between discharge proteomic profiles and inflammation status and change in growth deficits over one-year follow-up since little is known about extended influences of inflammation and longer-term growth in acutely ill undernourished populations. The mean weight gain rate of 3.3 g/kg/day observed was comparable to that reported for uncomplicated SM treated with a similar diet in the community[31,67–69]. However, there were significant reductions in absolute deficits of weight and MUAC. Although markers of SI were negatively associated with growth in the early post-hospital discharge period, substantial growth did occur in the presence of markers of inflammation. It is likely that the large metabolic shifts observed during refeeding with energy dense therapeutic feeds favours tissue accretion even in the presence of SI. It is notable that despite absolute increases in height, there was no significant reduction in absolute deficit of height, indicating that catch-up growth mainly occurs in the adipose and muscle and not long bones. No comparable data on inflammatory markers are published from children with uncomplicated SM. However, lack of an acute illness means it is plausible that there is less systemic inflammation (SI). Overall, our results indicate that growth is influenced by inflammation status.

Inflammatory cytokines IL17a, IL2, and MIP1B and inflammatory proteins sCD14, LBP, SAP, and β2M were negatively associated with weight gain and MUAC in the early post-discharge period. IL17a is produced by T-helper 17 (Th17) cells that play a role in host defence against extracellular pathogens through recruitment of neutrophils and macrophages to infected tissues[70–72]. IL17a[73] is involved in tissue inflammation by release of other pro-inflammatory cytokines and inducing neutrophil chemotaxis[74] and is implicated in obesity and adipogenesis[75]. In mouse models, IL17a has been proposed to play a role in weight gain in response to a high-fat diet[76]. In humans, increased expression of IL17a has been reported in inflammatory bowel disease[77–79]. sCD14 is secreted by monocytes and macrophages commonly in response to LPS translocation[80], while LBP is a plasma protein that binds to the lipid A moiety of bacterial LPS[81]. β2M is released by activated T and B lymphocytes and plasma β2M has been described as a predictive biomarker for many vascular inflammatory diseases[82,83]. SAP is an acute phase protein and belongs to the pentraxins family of proteins that also includes C-reactive protein, which exhibit calcium-dependent binding to several different molecules and pathogens[84]. Children included in this study were judged by trained clinicians as clinically stabilised following an acute illness and induction of these inflammatory cytokines and proteins likely results from induction by microbial molecules[85,86]. Several of these cytokines are elevated in patients with inflammatory bowel disease[77,87–89] and may reflect the presence of environmental enteric dysfunction, which is common in low- and middle-income countries, that is associated with linear growth failure[51,90,91].

Complement proteins were negatively associated with one-year gains in weight, MUAC and height. The complement system is comprised of plasma proteins and is part of the innate defence against common pathogens that enhances the ability of antibodies and phagocytic cells to opsonize and lyse microbes and damaged cells and promote inflammation[92]. The complement system is activated via three different pathways: the classic pathway, the alternative pathway, and the lectin pathway and the adipose tissue is the site of production of some complement components[93]. The activation of the alternative pathway is composed of complement C3, factors B and D which are mainly produced by the adipose tissue[94–96]. Previous studies have shown that levels of serum complement proteins are highly correlated with body weight and changes in body weight[97,98]. Malnutrition has been associated with low complement levels[99], although studied populations may have had concurrent infections or conditions which might affect complement levels. The negative association between complement and one-year growth implies that inflammation at discharge likely impacts longer-term growth in this population.

The negative relationship between growth at one year and inflammation as depicted by CRP, Attractin (ATRN; expressed on activated T cells and released extracellularly) and cytokines, mirrored findings from the early post discharge growth which was the period with the highest growth rate. We also found that Zinc-alpha-2-glycoprotein (ZAG) was negatively associated with gain in MUAC. ZAG also known as lipid-mobilizing factor stimulates lipolysis and is involved in depletion of fatty acids from adipose tissues[100–102].

Our results show that leptin was positively associated with early post-discharge growth. Leptin levels increase with accretion of adipose tissue mass and therefore leptin levels are related to body weight[103]. Leptin was first recognized for its prominent action on the hypothalamus to control food intake, energy expenditure and, hence, body weight[104–106]. It is also involved in immune homeostasis by differentially regulating T cells, enhancing Th1 and suppressing Th2 cytokine production, and reversing starvation-induced immunosuppression[107–109]. Among Ugandan children

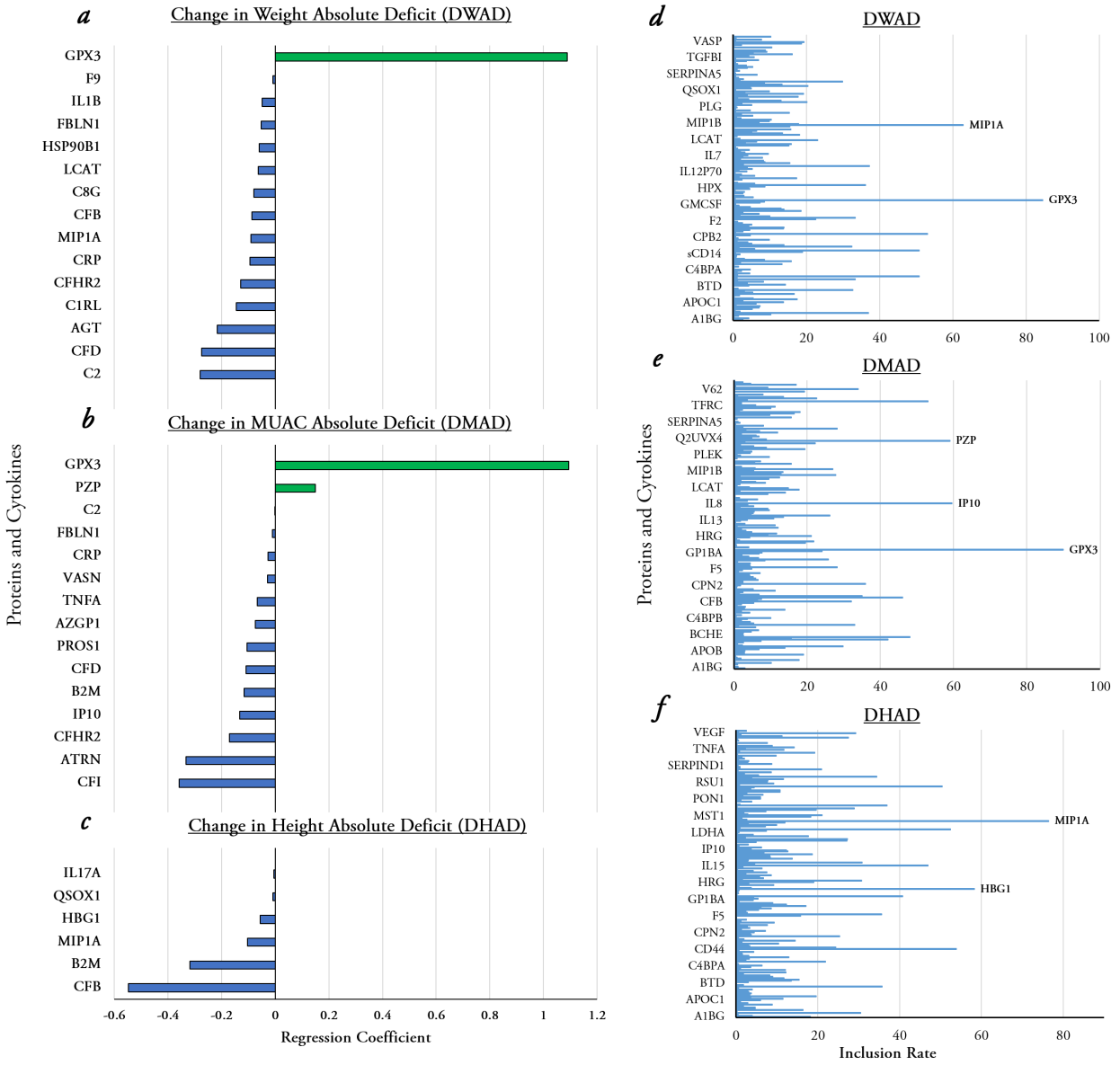

Figure 2. Multivariate analysis of plasma proteome and cytokines associated change in growth deficits from enrolment to one-year. Untargeted liquid chromatography tandem mass spectrometry plasma proteins, and targeted cytokines/chemokines, Leptin, and sCD14 associated with DWAD (***a***), DMAD (***b***) and DHAD (***c***) in multivariate elastic net (EN) regularized linear regression models at 1 year. (***d***, ***e***, and ***f***) Bar plots showing feature importance as depicted by the feature inclusion rate after 1000 bootstrap iterations during bootstrap validation for DWAD, DMAD, and DHAD respectively. Log normalised protein values were used in the analysis and regression models were adjusted for age, randomisation arm, sex, respective enrolment growth deficits, and site.

hospitalised with SM, nutritional stabilisation and weight gain was associated with significant increases in leptin levels[57]. However, it is worth noting that leptin levels which were measured at hospital discharge may still be low among children with SM.

One-year gains in weight and MUAC were positively associated with GPX3 at enrolment. GPX3 is a selenium-dependent enzyme playing a critical role in detoxifying reactive oxidative species including organic and lipid hydroperoxides as well as hydrogen peroxide[110–112]. Selenium via selenoproteins plays a crucial role in cellular redox balance, immunity, and metabolism and GPX3 is a marker of differentiated adipocytes[113,114]. In animal models, adipose GPX3 expression was suppressed by TNFα, LPS-induced SI, and by hypoxia, but was stimulated by the antioxidant N-acetyl cysteine[114]. Recent findings implicate

Table 4. Elastic Net regression model optimal alpha parameters and performance of proteins associated with change in growth deficits at one-year.

EN Variable		Optimal alpha	r	[95% CI]	P value
DWAD	Exposure protein variables	0.5	0.73	0.62 – 0.82	<0.0001
DMAD	Exposure protein variables	0.5	0.72	0.59 – 0.81	<0.0001
DHAD	Exposure protein variables	0.5	0.58	0.42 – 0.71	<0.0001

Footnote: Optimal alpha parameter and correlation coefficients for the EN model enumerating the correlation between DWAD, DMAD, and DHAD at one-year and exposure protein variables (untargeted plasma proteome, and targeted cytokine/chemokines, leptin, and sCD14) extracted by the multivariate regularized models.

GPX3 as a regulator of insulin receptor expression and insulin sensitivity in adipose tissue[115]. GPX3 has been identified as one of the strongest genes associated with traits of insulin sensitivity, adipogenesis, and Type 2 Diabetes[116]. GPX3 also belongs to a cluster of adipokines which is closely related to insulin sensitivity, hyperglycemia, and lipid metabolism[117]. Taken together, our findings imply that oxidative stress and increased SI during the acute phase are pathways that contribute to long-term post-discharge growth failure among acutely ill undernourished children.

Proinflammatory signalling in the adipocyte is required for proper adipose tissue remodelling and expansion[118] and recent studies suggest that low grade inflammation may play a positive role in weight gain in both children[119] and adults[120]. In a population-based longitudinal study in the Brazilian Amazon among children ≤10 years, low-grade inflammation (CRP <1 mg/L at baseline) was predictive of annual gain in BMI-for-age during follow-up[119]. During refeeding, severely malnourished children may adopt an obesogenic metabolic phenotype, where tissue accretion occurs in the presence of inflammation and reflecting restoration of adipose tissue lost due to malnutrition[121]. However, inflammation increases energy expenditure and in animal studies focusing on increasing production, SI is attributed to inefficient nutrient utilization efficiency which translated to low gain in weight, implying that in that context, persistent inflammation negatively affects growth[122–125] However, although we previously found CRP to be associated with mortality[37], other biomarkers of inflammation were negatively associated with growth in the present study.

Future work should investigate effects over a longer time period post-discharge and compare with community participants/children without acute illness to establish community norms and examine interventions to reduce inflammation. The strengths of this study included robust and standardised data collection within a clinical trial, follow-up for one year and very low loss to follow-up. The limitations include the relatively small sample size, the lack of serial measurements of inflammatory markers and body composition, the inability to control for other factors that may influence cytokine levels, and that children with oedema were excluded from the analysis. This study was carried out at four sites in Kenya only. Every child was tested for HIV, enabling us to exclude its effect. Important nutritional factors, hormones and growth factors, and metabolites which would have contributed further to the understanding of the relationship between SI and growth were not determined. Molecules such as LPS that would explain elevated SI were not determined and were beyond the scope of this study. The untargeted proteomics and targeted Luminex and ELISA approaches used in this study provided a broad array of protein molecules from which to identify molecules associated with early post-hospital discharge growth.

Conclusions

Among children with SM, early post-hospital discharge catch-up growth in weight and MUAC is rapid. Higher concentrations of markers of SI were associated with less early weight and MUAC gain, suggesting inflammation negatively impacts recovery from wasting. Our results indicate that growth is influenced by inflammation status and warrants further research on the role of inflammation on growth among children with SM.

Data availability

Underlying data

Specific variables such as personal identifiers and the longitude and latitude co-ordinates of study participants were removed to enhance participant anonymisation and can be accessed following application to our Data Governance Committee at dgc@kemri-wellcome.org. The replication data and analysis scripts for this manuscript are available from the Harvard Dataverse.

Harvard Dataverse: Replication data for: Systemic Inflammation is Negatively Associated with Early Post Discharge Growth following Acute Illness among Severely Malnourished Children-a Pilot Study. https://doi.org/10.7910/DVN/5DKLVI[66].

This project contains the following underlying data:

- (a) Njunge_CTX_15092020.dta
- (b) Njunge_CTX_15092020.csv

  Both (a) and (b) files contain similar information. The files contain anthropometric measurements at the time of hospital discharge and during follow up months 1, 2, 3,

4, 5, 6, 8, 10, and 12. A full blood count at enrolment, 2, 6, and 12 months. The two files were generated using STATA/IC (version 15.1; StataCorp, College Station, TX, USA)

- Njunge_Inflammation_Codebook.pdf: It contains a list of all the variables in the two datasets and their description.

Extended data

Harvard Dataverse: Replication data for: Systemic Inflammation is Negatively Associated with Early Post Discharge Growth following Acute Illness among Severely Malnourished Children- a Pilot Study. https://doi.org/10.7910/DVN/5DKLVI[66].

This project contains the following extended data:

- Njunge_EN_Glmnet_bootValidation.R: This analysis script uses Njunge_CTX_15092020.csv to perform multivariate regularized linear regression analysis using an elastic net (EN) model implemented using the "glmnet" package and fits a generalized linear model via penalized maximum likelihood. It generates an EN model separately for each growth outcome (change in Weight Absolute Deficit(DWAD) (primary outcome) and change in MUAC Absolute Deficit (DMAD) (secondary outcome), with protein profiles, cytokines, and enrolment anthropometric variables as predictors. The subset of variables assigned non-zero coefficients are retained in each of the final multi-variable models. It then performs Bootstrapping to evaluate the robustness of selected proteins at 1000 iterations using the 'BootValidation' package. The analysis was performed using R Studio (R version 3.6.2 (2019-12-12))
- Njunge_Stata_Do.do: This analysis script uses Njunge_CTX_15092020.dta to generate the summary participants characteristics at enrolment and at 2 months and calculate the changes in anthropometry
- NjungeJM_Inflammation_Readme.txt: It contains description of the related study, file contents, data license and usage instructions.

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References

1. UNICEF/WHO/World Bank: **Levels and trends in child malnutrition**. 2017.
Reference Source
2. Report, GN: **The Global Nutrition Report**. 2018.
Reference Source
3. Black RE, Victora CG, Walker SP, *et al.*: **Maternal and child undernutrition and overweight in low-income and middle-income countries.** *Lancet.* 2013; **382**(9890): 427–451.
PubMed Abstract | Publisher Full Text
4. Bejon P, Mohammed S, Mwangi I, *et al.*: **Fraction of all hospital admissions and deaths attributable to malnutrition among children in rural Kenya.** *Am J Clin Nutr.* 2008; **88**(6): 1626–1631.
PubMed Abstract | Publisher Full Text | Free Full Text
5. Liu L, *et al.*: **Global, regional, and national causes of under-5 mortality in 2000–15: an updated systematic analysis with implications for the Sustainable Development Goals.** *Lancet.* 2016; **388**(10063): 3027–3035.
PubMed Abstract | Publisher Full Text | Free Full Text
6. WHO: **WHO Guidelines Approved by the Guidelines Review Committee.** (World Health Organization, Geneva, 2013.
Reference Source
7. WHO: **Pocket Book of Hospital Care for Children: Guidelines for the Management of Common Childhood Illnesses.** Guidelines for the management of common illnesses, 2013; 412.
Reference Source
8. Berkley JA, Ngari M, Thitiri J, *et al.*: **Daily co-trimoxazole prophylaxis to prevent mortality in children with complicated severe acute malnutrition: a multicentre, double-blind, randomised placebo-controlled trial.** *Lancet Glob Health.* 2016; **4**(7): e464–473.
PubMed Abstract | Publisher Full Text | Free Full Text
9. Moisi JC, Gatakaa H, Berkley JA, *et al.*: **Excess child mortality after discharge from hospital in Kilifi, Kenya: a retrospective cohort analysis.** *Bull World Health Organ.* 2011; **89**(10): 725–732, 732A.
PubMed Abstract | Publisher Full Text | Free Full Text
10. Ngari MM, Iversen PO, Thitiri J, *et al.*: **Linear growth following complicated severe malnutrition: 1-year follow-up cohort of Kenyan children.** *Arch Dis Child.* 2019; **104**(3): 229–235.
PubMed Abstract | Publisher Full Text | Free Full Text
11. Ngari MM, Mwalekwa L, Timbwa M, *et al.*: **Changes in susceptibility to life-threatening infections after treatment for complicated severe malnutrition in Kenya.** *Am J Clin Nutr.* 2018; **107**(4): 626–634.
PubMed Abstract | Publisher Full Text | Free Full Text
12. Stobaugh HC, Mayberry A, McGrath M, *et al.*: **Relapse after severe acute malnutrition: A systematic literature review and secondary data analysis.** *Matern Child Nutr.* 2019; **15**(2): e12702.
PubMed Abstract | Publisher Full Text | Free Full Text
13. Nemetchek B, English L, Kissoon N, *et al.*: **Paediatric postdischarge mortality in developing countries: a systematic review.** *BMJ open.* 2018; **8**(12): e023445.
PubMed Abstract | Publisher Full Text | Free Full Text
14. Kar BR, Rao SL, Chandramouli BA: **Cognitive development in children with chronic protein energy malnutrition.** *Behav Brain Funct.* 2008; **4**: 31–31.
PubMed Abstract | Publisher Full Text | Free Full Text
15. Prado EL, Dewey KG: **Nutrition and brain development in early life.** *Nutr Rev.* 2014; **72**(4): 267–284.
PubMed Abstract | Publisher Full Text
16. Gladstone M, Mallewa M, Jalloh JJ, *et al.*: **Assessment of Neurodisability and Malnutrition in Children in Africa.** *Semin Pediatr Neurol.* 2014; **21**(1): 50–57.
PubMed Abstract | Publisher Full Text
17. Millward DJ: **Nutrition, infection and stunting: the roles of deficiencies of individual nutrients and foods, and of inflammation, as determinants of reduced linear growth of children.** *Nutr Res Rev.* 2017; **30**(1): 50–72.
PubMed Abstract | Publisher Full Text
18. 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.
PubMed Abstract | Publisher Full Text | Free Full Text
19. Mata LJ, Kronmal RA, García B, *et al.*: **Breast-feeding, weaning and the diarrhoeal syndrome in a Guatemalan Indian village.** *Ciba Found Symp.*

1976; (42): 311–338.
PubMed Abstract | Publisher Full Text

20. Tanner JM: **Growth as a measure of the nutritional and hygienic status of a population.** *Horm Res.* 1992; **38**(Suppl 1): 106–115.
PubMed Abstract | Publisher Full Text

21. Prendergast AJ, Humphrey JH: **The stunting syndrome in developing countries.** *Paediatr Int Child Health.* 2014; **34**(4): 250–265.
PubMed Abstract | Publisher Full Text | Free Full Text

22. Guerrant RL, Oriá RB, Moore SR, *et al.*: **Malnutrition as an enteric infectious disease with long-term effects on child development.** *Nutr Rev.* 2008; **66**(9): 487–505.
PubMed Abstract | Publisher Full Text | Free Full Text

23. Checkley W, Buckley Gillian, Gilman Robert H, *et al.*: **Multi-country analysis of the effects of diarrhoea on childhood stunting.** *Int J Epidemiol.* 2008; **37**(4): 816–830.
PubMed Abstract | Publisher Full Text | Free Full Text

24. Checkley W, Epstein LD, Gilman RH, *et al.*: **Effects of Cryptosporidium parvum Infection in Peruvian Children: Growth Faltering and Subsequent Catch-up Growth.** *Am J Epidemiol.* 1998; **148**(5): 497–506.
PubMed Abstract | Publisher Full Text

25. DeBoer MD, Scharf RJ, Leite AM, *et al.*: **Systemic inflammation, growth factors, and linear growth in the setting of infection and malnutrition.** *Nutrition.* 2017; **33**: 248–253.
PubMed Abstract | Publisher Full Text | Free Full Text

26. Diop el HI, Dossou NI, Ndour MM, *et al.*: **Comparison of the efficacy of a solid ready-to-use food and a liquid, milk-based diet for the rehabilitation of severely malnourished children: a randomized trial.** *Am J Clin Nutr.* 2003; **78**(2): 302–307.
PubMed Abstract | Publisher Full Text

27. Sandige H, Ndekha MJ, Briend A, *et al.*: **Home-Based Treatment of Malnourished Malawian Children with Locally Produced or Imported Ready-to-Use Food.** *J Pediatr Gastroenterol Nutr.* 2004; **39**(2): 141–146.
PubMed Abstract | Publisher Full Text

28. Yebyo HG, Kendall C, Nigusse D, *et al.*: **Outpatient therapeutic feeding program outcomes and determinants in treatment of severe acute malnutrition in tigray, northern ethiopia: a retrospective cohort study.** *PLoS One.* 2013; **8**(6): e65840.
PubMed Abstract | Publisher Full Text | Free Full Text

29. Kangas ST, Salpéteur C, Nikièma V, *et al.*: **Impact of reduced dose of ready-to-use therapeutic foods in children with uncomplicated severe acute malnutrition: A randomised non-inferiority trial in Burkina Faso.** *PLoS Med.* 2019; **16**(8): e1002887.
PubMed Abstract | Publisher Full Text | Free Full Text

30. Linneman Z, Matilsky D, Ndekha M, *et al.*: **A large-scale operational study of home-based therapy with ready-to-use therapeutic food in childhood malnutrition in Malawi.** *Matern Child Nutr.* 2007; **3**(3): 206–215.
PubMed Abstract | Publisher Full Text | Free Full Text

31. Aguayo VM, Badgaiyan N, Qadir SS, *et al.*: **Community management of acute malnutrition (CMAM) programme in Pakistan effectively treats children with uncomplicated severe wasting.** *Matern Child Nutr.* 2018; **14 Suppl 4**(Suppl 4): e12623.
PubMed Abstract | Publisher Full Text | Free Full Text

32. James PT, Van den Briel N, Rozet A, *et al.*: **Low-dose RUTF protocol and improved service delivery lead to good programme outcomes in the treatment of uncomplicated SAM: a programme report from Myanmar.** *Matern Child Nutr.* 2015; **11**(4): 859–869.
PubMed Abstract | Publisher Full Text | Free Full Text

33. Burza S, Mahajan R, Marino E, *et al.*: **Community-based management of severe acute malnutrition in India: new evidence from Bihar.** *Am J Clin Nutr.* 2015; **101**(4): 847–859.
PubMed Abstract | Publisher Full Text | Free Full Text

34. Das JK, Salam RA, Saeed M, *et al.*: **Effectiveness of Interventions for Managing Acute Malnutrition in Children under Five Years of Age in Low-Income and Middle-Income Countries: A Systematic Review and Meta-Analysis.** *Nutrients.* 2020; **12**(1): 116.
PubMed Abstract | Publisher Full Text | Free Full Text

35. Jones KD, Thitiri J, Ngari M, *et al.*: **Childhood malnutrition: toward an understanding of infections, inflammation, and antimicrobials.** *Food Nutr Bull.* 2014; **35**(2 Suppl): S64–70.
PubMed Abstract | Publisher Full Text | Free Full Text

36. Attia S, Versloot CJ, Voskuijl W, *et al.*: **Mortality in children with complicated severe acute malnutrition is related to intestinal and systemic inflammation: an observational cohort study.** *Am J Clin Nutr.* 2016; **104**(5): 1441–1449.
PubMed Abstract | Publisher Full Text | Free Full Text

37. Njunge JM, Gwela A, Kibinge NK, *et al.*: **Biomarkers of post-discharge mortality among children with complicated severe acute malnutrition.** *Sci Rep.* 2019; **9**(1): 5981.
PubMed Abstract | Publisher Full Text | Free Full Text

38. Yende S, D'Angelo G, Kellum JA, *et al.*: **Inflammatory markers at hospital discharge predict subsequent mortality after pneumonia and sepsis.** *Am J Respir Crit Care Med.* 2008; **177**(11): 1242–1247.
PubMed Abstract | Publisher Full Text | Free Full Text

39. Kaynar AM, Yende S, Zhu L, *et al.*: **Effects of intra-abdominal sepsis on atherosclerosis in mice.** *Crit Care.* 2014; **18**(5): 469.
PubMed Abstract | Publisher Full Text | Free Full Text

40. Yende S, Kellum JA, Talisa VB, *et al.*: **Long-term Host Immune Response Trajectories Among Hospitalized Patients With Sepsis.** *JAMA Netw Open.* 2019; **2**(8): e198686.
PubMed Abstract | Publisher Full Text | Free Full Text

41. Rahmel T, Schmitz S, Nowak H, *et al.*: **Long-term mortality and outcome in hospital survivors of septic shock, sepsis, and severe infections: The importance of aftercare.** *PLoS One.* 2020; **15**(2): e0228952.
PubMed Abstract | Publisher Full Text | Free Full Text

42. Póvoa P, Garvik OS, Vinholt PJ, *et al.*: **C-reactive protein and albumin kinetics after antibiotic therapy in community-acquired bloodstream infection.** *Int J Infect Dis.* 2020; **95**: 50–58.
PubMed Abstract | Publisher Full Text

43. Sederquist B, Fernandez-Vojvodich P, Zaman F, *et al.*: **Recent research on the growth plate: Impact of inflammatory cytokines on longitudinal bone growth.** *J Mol Endocrinol.* 2014; **53**(1): T35–44.
PubMed Abstract | Publisher Full Text

44. Sauder MW, Lee SE, Schulze KJ, *et al.*: **Inflammation throughout pregnancy and fetal growth restriction in rural Nepal.** *Epidemiol Infect.* 2019; **147**: e258.
PubMed Abstract | Publisher Full Text | Free Full Text

45. Hindle LJ, Gitau R, Filteau SM, *et al.*: **Effect of multiple micronutrient supplementation during pregnancy on inflammatory markers in Nepalese women.** *Am J Clin Nutr.* 2006; **84**(5): 1086–1092.
PubMed Abstract | Publisher Full Text

46. Lee SE, West Jr KP, Cole RN, *et al.*: **Novel Plasma Proteins in Nepalese School-aged Children are Associated with a Small Head Size at Birth.** *Sci Rep.* 2018; **8**(1): 6390.
PubMed Abstract | Publisher Full Text | Free Full Text

47. Lee SE, Stewart CP, Schulze KJ, *et al.*: **The Plasma Proteome Is Associated with Anthropometric Status of Undernourished Nepalese School-Aged Children.** *J Nutr.* 2017; **147**(3): 304–313.
PubMed Abstract | Publisher Full Text | Free Full Text

48. Muriuki JM, Mentzer AJ, Webb EL, *et al.*: **Estimating the burden of iron deficiency among African children.** *BMC Med.* 2020; **18**(1): 31.
PubMed Abstract | Publisher Full Text | Free Full Text

49. Rogawski ET, Liu J, Platts-Mills JA, *et al.*: **Use of quantitative molecular diagnostic methods to investigate the effect of enteropathogen infections on linear growth in children in low-resource settings: longitudinal analysis of results from the MAL-ED cohort study.** *Lancet Glob Health.* 2018; **6**(12): e1319–e1328.
PubMed Abstract | Publisher Full Text | Free Full Text

50. Prendergast AJ, Rukobo S, Chasekwa B, *et al.*: **Stunting Is Characterized by Chronic Inflammation in Zimbabwean Infants.** *PLoS One.* 2014; **9**(2): e86928.
PubMed Abstract | Publisher Full Text | Free Full Text

51. Campbell DI, Elia M, Lunn PG: **Growth faltering in rural Gambian infants is associated with impaired small intestinal barrier function, leading to endotoxemia and systemic inflammation.** *J Nutr.* 2003; **133**(5): 1332–1338.
PubMed Abstract | Publisher Full Text

52. Urlacher SS, *et al.*: **Tradeoffs between immune function and childhood growth among Amazonian forager-horticulturalists.** *Proc Natl Acad Sci U S A.* 2018; **115**(17): E3914–E3921.
PubMed Abstract | Publisher Full Text | Free Full Text

53. Jones KDJ, Hünten-Kirsch B, Laving AMR, *et al.*: **Mesalazine in the initial management of severely acutely malnourished children with environmental enteric dysfunction: a pilot randomized controlled trial.** *BMC Med.* 2014; **12**: 133.
PubMed Abstract | Publisher Full Text | Free Full Text

54. Costamagna D, Costelli P, Sampaolesi M, *et al.*: **Role of Inflammation in Muscle Homeostasis and Myogenesis.** *Mediators Inflamm.* 2015; **2015**: 805172.
PubMed Abstract | Publisher Full Text | Free Full Text

55. Sharma K, Mogensen KM, Robinson MK: **Pathophysiology of Critical Illness and Role of Nutrition.** *Nutrition in Clinical Practice.* 2019; **34**(1): 12–22.
PubMed Abstract | Publisher Full Text

56. Argiles JM, Lopez-Soriano FJ, Busquets S: **Counteracting inflammation: a promising therapy in cachexia.** *Crit Rev Oncog.* 2012; **17**(3): 253–262.
PubMed Abstract | Publisher Full Text

57. Bartz S, Mody A, Hornik C, *et al.*: **Severe acute malnutrition in childhood: hormonal and metabolic status at presentation, response to treatment, and predictors of mortality.** *J Clin Endocrinol Metab.* 2014; **99**(6): 2128–2137.
PubMed Abstract | Publisher Full Text | Free Full Text

58. Di Giovanni V, Bourdon C, Wang DX, *et al.*: **Metabolomic Changes in Serum of Children with Different Clinical Diagnoses of Malnutrition.** *J Nutr.* 2016; **146**(12): 2436–2444.
PubMed Abstract | Publisher Full Text | Free Full Text

59. Leroy JL, Ruel M, Habicht J-P, *et al.*: **Linear Growth Deficit Continues to Accumulate beyond the First 1000 Days in Low- and Middle-Income Countries: Global Evidence from 51 National Surveys.** *J Nutr.* 2014; **144**(9): 1460–1466.
PubMed Abstract | Publisher Full Text

60. Leroy JL, Ruel M, Habicht JP, *et al.*: **Using height-for-age differences (HAD)**

instead of height-for-age z-scores (HAZ) for the meaningful measurement of population-level catch-up in linear growth in children less than 5 years of age. *BMC Pediatr.* 2015; **15**: 145–145.
PubMed Abstract | Publisher Full Text | Free Full Text

61. WHO Multicentre Growth Reference Study Group: **WHO Child Growth Standards based on length/height, weight and age.** *Acta Paediatr Suppl.* 2006; **450**: 76–85.
PubMed Abstract | Publisher Full Text

62. R Core Team: **R: A language and environment for statistical computing.** R Foundation for Statistical Computing. 2017.
Reference Source

63. Friedman J, Hastie T, Tibshirani R: **Regularization Paths for Generalized Linear Models via Coordinate Descent.** *J Stat Softw.* 2010; **33**(1): 1–22.
PubMed Abstract | Free Full Text

64. Zou H, Hastie T: **Regularization and Variable Selection via the Elastic Net.** *J R Stat Soc Series B Stat Methodol.* 2005; **67**(2): 301–320.
Publisher Full Text

65. Abram SV, Helwig NE, Moodie CA, *et al.*: **Bootstrap Enhanced Penalized Regression for Variable Selection with Neuroimaging Data.** *Front Neurosci.* 2016; **10**: 344–344.
PubMed Abstract | Publisher Full Text | Free Full Text

66. Njunge JM, Gonzales GB, Ngari MM, *et al.*: **Replication Data for: Systemic Inflammation is Negatively Associated with Early Post Discharge Growth following Acute Illness among Severely Malnourished Children- a Pilot Study**. Harvard Dataverse, V1, UNF:6:QLujfK2zSCp6JTUtQTXP/Q== [fileUNF]. 2020.
http://www.doi.org/10.7910/DVN/5DKLVI

67. Isanaka S, Langendorf C, Berthé F, *et al.*: **Routine Amoxicillin for Uncomplicated Severe Acute Malnutrition in Children.** *N Engl J Med.* 2016; **374**(5): 444–453.
PubMed Abstract | Publisher Full Text

68. Trehan I, Goldbach HS, LaGrone LN, *et al.*: **Antibiotics as Part of the Management of Severe Acute Malnutrition.** *N Engl J Med.* 2016; **368**(5): 425–435.
PubMed Abstract | Publisher Full Text | Free Full Text

69. Ciliberto MA, Sandige H, Ndekha MJ, *et al.*: **Comparison of home-based therapy with ready-to-use therapeutic food with standard therapy in the treatment of malnourished Malawian children: a controlled, clinical effectiveness trial.** *Am J Clin Nutr.* 2005; **81**(4): 864–870.
PubMed Abstract | Publisher Full Text

70. Tesmer LA, Lundy SK, Sarkar S, *et al.*: **Th17 cells in human disease.** *Immunol Rev.* 2008; **223**: 87–113.
PubMed Abstract | Publisher Full Text | Free Full Text

71. Harrington LE, Hatton RD, Mangan PR, *et al.*: **Interleukin 17-producing CD4$^+$ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages.** *Nat Immunol.* 2005; **6**(11): 1123–1132.
PubMed Abstract | Publisher Full Text

72. Park H, Li Z, Yang XO, *et al.*: **A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17.** *Nat Immunol.* 2005; **6**(11): 1133–1141.
PubMed Abstract | Publisher Full Text | Free Full Text

73. Basso AS, Cheroutre H, Mucida D: **More stories on Th17 cells.** *Cell Res.* 2009; **19**(4): 399–411.
PubMed Abstract | Publisher Full Text | Free Full Text

74. Onishi RM, Gaffen SL: **Interleukin-17 and its target genes: mechanisms of interleukin-17 function in disease.** *Immunology.* 2010; **129**(3): 311–321.
PubMed Abstract | Publisher Full Text | Free Full Text

75. Ahmed M, Gaffen SL: **IL-17 in obesity and adipogenesis.** *Cytokine & growth factor reviews.* 2010; **21**: 449–453.
PubMed Abstract | Publisher Full Text | Free Full Text

76. Madhur MS, Funt SA, Li L, *et al.*: **Role of interleukin 17 in inflammation, atherosclerosis, and vascular function in apolipoprotein e-deficient mice.** *Arterioscler Thromb Vasc Biol.* 2011; **31**(7): 1565–1572.
PubMed Abstract | Publisher Full Text | Free Full Text

77. Fujino S, Andoh A, Bamba S, *et al.*: **Increased expression of interleukin 17 in inflammatory bowel disease.** *Gut.* 2003; **52**(1): 65–70.
PubMed Abstract | Publisher Full Text | Free Full Text

78. Sakuraba A, Sato T, Kamada N, *et al.*: **Th1/Th17 immune response is induced by mesenteric lymph node dendritic cells in Crohn's disease** *Gastroenterology.* 2009; **137**(5): 1736–1745.
PubMed Abstract | Publisher Full Text

79. Monteleone I, Pallone F, Monteleone G: **Th17-related cytokines: new players in the control of chronic intestinal inflammation.** *BMC Med.* 2011; **9**: 122.
PubMed Abstract | Publisher Full Text | Free Full Text

80. Kitchens RL, Thompson PA: **Modulatory effects of sCD14 and LBP on LPS-host cell interactions.** *J Endotoxin Res.* 2005; **11**(4): 225–229.
PubMed Abstract | Publisher Full Text

81. Schumann R, Leong SR, Flaggs GW, *et al.*: **Structure and function of lipopolysaccharide binding protein.** *Science.* 1990; **249**(4975): 1429–1431.
PubMed Abstract | Publisher Full Text

82. Argyropoulos CP, Chen SS, Ng YH, *et al.*: **Rediscovering Beta-2 Microglobulin As a Biomarker across the Spectrum of Kidney Diseases.** *Front Med (Lausanne).* 2017; **4**: 73.
PubMed Abstract | Publisher Full Text | Free Full Text

83. Hu Fy, Wu J, Tang Q, *et al.*: **Serum β2-Microglobulin Is Closely Associated With the Recurrence Risk and 3-Month Outcome of Acute Ischemic Stroke.** *Front Neurol.* 2020; **10**: 1334.
PubMed Abstract | Publisher Full Text | Free Full Text

84. Pilling D, Gomer RH: **The Development of Serum Amyloid P as a Possible Therapeutic.** *Front Immunol.* 2018; **9**: 2328–2328.
PubMed Abstract | Publisher Full Text | Free Full Text

85. Kolli D, Velayutham TS, Casola A: **Host-Viral Interactions: Role of Pattern Recognition Receptors (PRRs) in Human Pneumovirus Infections.** *Pathogens.* 2013; **2**(2): 232–263.
PubMed Abstract | Publisher Full Text | Free Full Text

86. Kawai T, Akira S: **Toll-like receptors and their crosstalk with other innate receptors in infection and immunity.** *Immunity.* 2011; **34**(5): 637–650.
PubMed Abstract | Publisher Full Text

87. Kader HA, Tchernev VT, Satyaraj E, *et al.*: **Protein microarray analysis of disease activity in pediatric inflammatory bowel disease demonstrates elevated serum PLGF, IL-7, TGF-beta1, and IL-12p40 levels in Crohn's disease and ulcerative colitis patients in remission versus active disease.** *Am J Gastroenterol.* 2005; **100**(2): 414–423.
PubMed Abstract | Publisher Full Text | Free Full Text

88. Ebrahimpour S, Shahbazi M, Khalili A, *et al.*: **Elevated levels of IL-2 and IL-21 produced by CD4+ T cells in inflammatory bowel disease.** *J Biol Regul Homeost Agents.* 2017; **31**(2): 279–287.
PubMed Abstract

89. Pastor Rojo O, Román ALS, Arbizu EA, *et al.*: **Serum lipopolysaccharide-binding protein in endotoxemic patients with inflammatory bowel disease.** *Inflamm Bowel Dis.* 2007; **13**(3): 269–277.
PubMed Abstract | Publisher Full Text

90. Lin A, Arnold BF, Afreen S, *et al.*: **Household environmental conditions are associated with enteropathy and impaired growth in rural Bangladesh.** *Am J Trop Med Hyg.* 2013; **89**(1): 130–137.
PubMed Abstract | Publisher Full Text | Free Full Text

91. Weisz AJ, Manary MJ, Stephenson K, *et al.*: **Abnormal gut integrity is associated with reduced linear growth in rural Malawian children.** *J Pediatr Gastroenterol Nutr.* 2012; **55**(6): 747–750.
PubMed Abstract | Publisher Full Text

92. Dunkelberger JR, Song W-C: **Complement and its role in innate and adaptive immune responses.** *Cell Res.* 2010; **20**(1): 34–50.
PubMed Abstract | Publisher Full Text

93. Vlaicu SI, Tatomir A, Boodhoo D, *et al.*: **The role of complement system in adipose tissue-related inflammation.** *Immunol Res.* 2016; **64**(3): 653–664.
PubMed Abstract | Publisher Full Text

94. Choy LN, Spiegelman BM: **Regulation of alternative pathway activation and C3a production by adipose cells.** *Obes Res.* 1996; **4**(6): 521–532.
PubMed Abstract | Publisher Full Text

95. Choy LN, Rosen BS, Spiegelman BM: **Adipsin and an endogenous pathway of complement from adipose cells.** *J Biol Chem.* 1992; **267**(18): 12736–12741.
PubMed Abstract | Publisher Full Text

96. Peake PW, O'Grady S, Pussell BA, *et al.*: **Detection and quantification of the control proteins of the alternative pathway of complement in 3T3-L1 adipocytes.** *Eur J Clin Invest.* 1997; **27**(11): 922–927.
PubMed Abstract | Publisher Full Text

97. Pomeroy C, Mitchell J, Eckert E, *et al.*: **Effect of body weight and caloric restriction on serum complement proteins, including Factor D/adipsin: studies in anorexia nervosa and obesity.** *Clin Exp Immunol.* 1997; **108**(3): 507–515.
PubMed Abstract | Publisher Full Text | Free Full Text

98. Hernández-Mijares A, Bañuls C, Bellod L, *et al.*: **Effect of weight loss on C3 and C4 components of complement in obese patients.** *Eur J Clin Invest.* 2012; **42**(5): 503–509.
PubMed Abstract | Publisher Full Text

99. Rytter MJ, Kolte L, Briend A, *et al.*: **The immune system in children with malnutrition--a systematic review.** *PLoS One.* 2014; **9**(8): e105017.
PubMed Abstract | Publisher Full Text | Free Full Text

100. Russell ST, Zimmerman TP, Domin BA, *et al.*: **Induction of lipolysis *in vitro* and loss of body fat *in vivo* by zinc-alpha$_2$-glycoprotein.** *Biochim Biophys Acta.* 2004; **1636**(1): 59–68.
PubMed Abstract | Publisher Full Text

101. Bao Y, Bing C, Hunter L, *et al.*: **Zinc-alpha$_2$-glycoprotein, a lipid mobilizing factor, is expressed and secreted by human (SGBS) adipocytes.** *FEBS Lett.* 2005; **579**(1): 41–47.
PubMed Abstract | Publisher Full Text

102. Hirai K, Hussey HJ, Barber MD, *et al.*: **Biological Evaluation of a Lipid-mobilizing Factor Isolated from the Urine of Cancer Patients.** *Cancer Res.* 1998; **58**(11): 2359–2365.
PubMed Abstract

103. Maffei M, Halaas J, Ravussin E, *et al.*: **Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects.** *Nat Med.* 1995; **1**(11): 1155–1161.
PubMed Abstract | Publisher Full Text

104. Friedman JM, Halaas JL: **Leptin and the regulation of body weight in mammals.** *Nature.* 1998; **395**(6704): 763–770.
PubMed Abstract | Publisher Full Text

105. Halaas JL, Boozer C, Blair-West J, *et al.*: **Physiological response to long-term peripheral and central leptin infusion in lean and obese mice.** *Proc Natl Acad Sci U S A.* 1997; **94**(16): 8878–8883.
PubMed Abstract | Publisher Full Text | Free Full Text

106. Halaas JL, Gajiwala KS, Maffei M, *et al.*: **Weight-reducing effects of the plasma protein encoded by the obese gene.** *Science.* 1995; **269**(5223): 543–546.
PubMed Abstract | Publisher Full Text

107. Lord GM, Matarese G, Howard GK, *et al.*: **Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression.** *Nature.* 1998; **394**(6696): 897–901.
PubMed Abstract | Publisher Full Text

108. Sánchez-Margalet V, Martín-Romero C, Santos-Alvarez J, *et al.*: **Role of leptin as an immunomodulator of blood mononuclear cells: mechanisms of action.** *Clin Exp Immunol.* 2003; **133**(1): 11–19.
PubMed Abstract | Publisher Full Text | Free Full Text

109. Santos-Alvarez J, Goberna R, Sánchez-Margalet V: **Human leptin stimulates proliferation and activation of human circulating monocytes.** *Cell Immunol.* 1999; **194**(1): 6–11.
PubMed Abstract | Publisher Full Text

110. Kryukov GV, Castellano S, Novoselov SV, *et al.*: **Characterization of Mammalian Selenoproteomes.** *Science.* 2003; **300**(5624): 1439–1443.
PubMed Abstract | Publisher Full Text

111. Yamamoto Y, Takahashi K: **Glutathione peroxidase isolated from plasma reduces phospholipid hydroperoxides.** *Arch Biochem Biophys.* 1993; **305**(2): 541–545.
PubMed Abstract | Publisher Full Text

112. Maddipati KR, Marnett LJ: **Characterization of the major hydroperoxide-reducing activity of human plasma. Purification and properties of a selenium-dependent glutathione peroxidase.** *J Biol Chem.* 1987; **262**(36): 17398–17403.
PubMed Abstract | Publisher Full Text

113. Steinbrenner H, Micoogullari M, Anh Hoang N, *et al.*: **Selenium-binding protein 1 (SELENBP1) is a marker of mature adipocytes.** *Redox Biol.* 2019; **20**: 489–495.
PubMed Abstract | Publisher Full Text | Free Full Text

114. Lee YS, Kim AY, Choi JW, *et al.*: **Dysregulation of adipose glutathione peroxidase 3 in obesity contributes to local and systemic oxidative stress.** *Mol Endocrinol.* 2009; **22**(9): 2176–2189.
PubMed Abstract | Publisher Full Text | Free Full Text

115. Hauffe R, Stein V, Chudoba C, *et al.*: **GPx3 dysregulation impacts adipose tissue insulin receptor expression and sensitivity.** *JCI Insight.* 2020; **5**(11): e136283.
PubMed Abstract | Publisher Full Text | Free Full Text

116. Park PJ,Kong SW, Tebaldi T, *et al.*: **Integration of heterogeneous expression data sets extends the role of the retinol pathway in diabetes and insulin resistance.** *Bioinformatics.* 2009; **25**(23): 3121–3127.
PubMed Abstract | Publisher Full Text | Free Full Text

117. Flehmig G, Scholz M, Klöting N, *et al.*: **Identification of Adipokine Clusters Related to Parameters of Fat Mass, Insulin Sensitivity and Inflammation.** *PLoS One.* 2014; **9**(6): e99785.
PubMed Abstract | Publisher Full Text | Free Full Text

118. Wernstedt Asterholm I, Tao C, Morley TS, *et al.*: **Adipocyte inflammation is essential for healthy adipose tissue expansion and remodeling.** *Cell Metab.* 2014; **20**(1): 103–118.
PubMed Abstract | Publisher Full Text | Free Full Text

119. Lourenço BH, Cardoso MA; ACTION Study Team: **C-reactive protein concentration predicts change in body mass index during childhood.** *PLoS One.* 2014; **9**(3): e90357.
PubMed Abstract | Publisher Full Text | Free Full Text

120. Holz T, Thorand B, Döring A, *et al.*: **Markers of inflammation and weight change in middle-aged adults: results from the prospective MONICA/KORA S3/F3 study.** *Obesity (Silver Spring).* 2010; **18**(12): 2347–2353.
PubMed Abstract | Publisher Full Text

121. Fabiansen C, Phelan KPQ, Cichon B, *et al.*: **Short Malnourished Children and Fat Accumulation With Food Supplementation.** *Pediatrics.* 2018; **142**(3): e20180679.
PubMed Abstract | Publisher Full Text

122. Koch RM, Swiger LA, Chambers D, *et al.*: **Efficiency of Feed Use in Beef Cattle.** *J Anim Sci.* 1963; **22**(2): 486–494.
Publisher Full Text

123. Fonseca LD, Eler JP, Pereira MA, *et al.*: **Liver proteomics unravel the metabolic pathways related to Feed Efficiency in beef cattle.** *Sci Rep.* 2019; **9**: 5364–5364.
PubMed Abstract | Publisher Full Text | Free Full Text

124. Alexandre PA, Kogelman LJA, Santana MHA, *et al.*: **Liver transcriptomic networks reveal main biological processes associated with feed efficiency in beef cattle.** *BMC Genomics.* 2015; **16**: 1073.
PubMed Abstract | Publisher Full Text | Free Full Text

125. Jégou M, Gondret F, Vincent A, *et al.*: **Whole Blood Transcriptomics Is Relevant to Identify Molecular Changes in Response to Genetic Selection for Feed Efficiency and Nutritional Status in the Pig.** *PLoS One.* 2016; **11**(1): e0146550.
PubMed Abstract | Publisher Full Text | Free Full Text

Open Peer Review

Current Peer Review Status: ✔ ✔

Version 2

Reviewer Report 23 April 2021

https://doi.org/10.21956/wellcomeopenres.18425.r43091

✔ **Lubaina Ehsan**
University of Virginia, Charlottesville, VA, USA
Sana Syed
Division of Pediatric Gastroenterology, Hepatology and Nutrition, Department of Pediatrics, School of Medicine, University of Virginia, Charlottesville, VA, USA

The authors have responded to all my concerns. I have no further comment and approve this manuscript.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Gastroenterology, Nutrition, Data Science

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 22 March 2021

https://doi.org/10.21956/wellcomeopenres.18425.r43090

✔ **Keith P. West**
Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD, USA

The paper reads well and the authors have responded fully to my concerns. I have no further comments and approve the manuscript. A minor query that does not affect my approval is that the 1st “D” in the acronyms DWAD, DMAD and DHAD presumably refers to “delta”, but this is not

explicit. As each is defined as a "change" in weight, MUAC or height, absolute difference, might these be respecified to CWAD, CMAD and CHAD? Alternatively, I may have also missed something and nothing needs to be altered.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Nuritional epidemiology, applied proteomics, nutrition interventions, child growth, maternal nutrition.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 09 December 2020

https://doi.org/10.21956/wellcomeopenres.17953.r41116

?

Keith P. West
Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD, USA

This study examines the potential attenuating influence of inflammation at time of discharge on subsequent linear and ponderal growth of young children initially hospitalized for severe complicated, non-HIV-related malnutrition. Inflammation at discharge was assessed by a combined assessment of cytokines (IL-2, IL17a and SAP) and relative abundance of plasma protein biomarkers, including those whose expression covaries with phases of the inflammatory response. The study is an important follow-up from a previous case-control study (Njunge et al Sci Reports 2019[1]) but also because it adds new insights amidst a sparsity of studies harnessing the potential of plasma proteomics to reveal metabolic alterations and pathways that may be affecting recovery-related (catch-up) child growth following severe malnutrition.

Comments:

The methods would benefit from slightly more essential study details that can render this paper independent of a previous paper (Njunge et al., Sci Reports 2019[1]) as I found myself needing to return to that publication to better understand aspects of study design: please clarify when children were phlebotomized (presumably just prior to discharge that constitute enrolment samples), which is also equivalent to baseline.

Notwithstanding important details provided about the analytical methods, thresholds at which associations between DMAD and DWAD and protein relative abundance/cytokine concentrations

are considered chance-adjusted, statistically significant are not specified. Is there a p- or q-value considered to be SS, and how were family-wise error probabilities from multiple comparisons managed? P-values for EN models for DWAD and DMAD are given in Table 3 but its not clear what these are testing or how derived.

Children were followed monthly through 6 mo and bi-monthly thereafter to 12 months. Given the measurements are available through 1-year post discharge, and partly reported as 60-day increments to 6 months of age in Table 2, the rationale for restricting evaluation of biomarker-growth deficit recovery to the 1st 60 days of follow-up, as opposed to longer, would benefit from clearer argument. It would be easier to follow if all data were restricted to a 60-day period for this paper, which aligns with the survival findings reported in Njunge et al 2019[1]. That said, reporting associations between discharge inflammation status and growth over longer intervals would be an important contribution, as little is known about extended influences of inflammation and longer term growth in undernourished populations. One population cohort-based plasma proteomics study in Nepal has shown head size at birth (Lee SE et al Sci Reports 2018[2]) and attained HAZ and WAZ at 6-8 years of age (Lee SE et al J Nutr 2017[3]), to be associated with the relative abundance of a wide array of plasma proteins, including S100 calprotectins (A9 and A12 isomers, respectively), assessed at 6-8 years. The few such studies that exist suggest persistent (long term), bidirectional associations between child growth and metabolic pathways, as expressed through the plasma proteome, observations that pique interest to explore protein biomarker associations with extended growth endpoints, in this paper or a subsequent treatise.

To the extent time points are maintained, can the authors clarify if children were measured at exactly 60, 120 and 180 days post-enrollment? If not, how were growth intervals standardized to 60-day periods?

DHAD data are summarized in Table 2. Why was it not also a primary outcome?

Table 1: In the 1st row, it would be informative to have a summary [median (IQR)] of ages of children at 60 days (removing “at enrolment” in the row label).

Table 3: The title is long and difficult to understand. Mention is made of correlation, but R^2 is reported. Please shorten and articulate specifics in the footnotes, including the exposure protein variables in each model (assuming these are the proteins reported in Figure 1 and b?).

Figure 1: Please clarify the x-axis label in a and b as regression coefficient and in the legend, specify measurement unit for each. Findings from Figures 1e and 1f, presenting Inclusion Rates associated with DWAD and DMAD, are presently not mentioned in the Results.

Discussion

Three-quarters down the 1st paragraph, noting the height deficit did not show evidence of recovery, could this be placing too high an expectation on long bone growth recovery commensurate with regaining soft tissue accretion within only 60 days?

Few typos:
Figure 1: Within the parentheses (d and f) should be (e and f);
In discussion – LBP is a plasma protein that binds…

References
1. Njunge J, Gwela A, Kibinge N, Ngari M, et al.: Biomarkers of post-discharge mortality among children with complicated severe acute malnutrition. *Scientific Reports*. 2019; **9** (1). Publisher Full Text
2. Lee S, West K, Cole R, Schulze K, et al.: Novel Plasma Proteins in Nepalese School-aged Children are Associated with a Small Head Size at Birth. *Scientific Reports*. 2018; **8** (1). Publisher Full Text
3. Lee SE, Stewart CP, Schulze KJ, Cole RN, et al.: The Plasma Proteome Is Associated with Anthropometric Status of Undernourished Nepalese School-Aged Children.*J Nutr*. **147** (3): 304-313 PubMed Abstract | Publisher Full Text

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Nuritional epidemiology, applied proteomics, nutrition interventions, child growth, maternal nutrition.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 05 Mar 2021

James Njunge, The Childhood Acute Illness & Nutrition (CHAIN) Network, Nairobi, Kenya

Reviewer 1

Sana Syed, Division of Pediatric Gastroenterology, Hepatology and Nutrition, Department of Pediatrics, School of Medicine, University of Virginia, Charlottesville, VA, USA.
Lubaina Ehsan, University of Virginia, Charlottesville, VA, USA

Approved With Reservations

The manuscript submitted by Dr. Njunge and colleagues aims to investigate the association of systemic inflammation among early post-discharge children who are severely malnourished and have had an acute illness. Overall, this manuscript presents results that would be of interest to the community of scientists and clinicians concerned with this problem, major strengths of their work are:

- **Well-written and coherent manuscript which clearly outlines the work that was done.**
- **Strong statistical analysis of not only the weight/MUAC gain but also the absolute deficits in these measurements.**
- **A discussion that outlines each significant result clearly with extensive literature being referenced.**
- **The assessment of potential markers for growth being associated with inflammation which can pave the way for revised standards of anthropometric measurement among children within the same cohort.**

However, there are a few issues in this manuscript that prevent us from recommending that this manuscript be indexed in its current state:

Overall

There is a mismatch between the introduction and the discussion. While the introduction mentions RUTF and GH/IGF1 axis, we also suggest adding the background behind the assessment of the relationship of plasma proteomic and cytokine profiles with growth. The discussion then excellently discussed the individual results for cytokines and other markers although, the background for these measurements being part of the introduction will make the foundation of why this work was done even more strong.

- Studies detailing the relationship between plasma proteomics and cytokine profiles on catch-up growth after an acute illness are very limited among undernourished children. We have added additional citations on linear growth. "*In Nepal, birth size was inversely associated with low-grade, chronic inflammation during pregnancy as indicated by serum AGP*[1,2]*. Further, head size at birth among Nepalese*[3] *and attained z scores for height (HAZ) and weight-for-age (WAZ) at 6-8 years of age*[4] *were associated with a wide array of plasma proteins, including S100 calprotectin subunits, assessed at 6-8 years. In community settings, SI is elevated in 17-34% children in LMIC*[5] *(C-reactive protein >5mg/L or α1-antichymotrypsin >0.6g/L) and is associated with reduced linear growth*[6-10]*."* However the literature is quite limited[11].
- **The proteomics and cytokines measurements in plasma were measured using liquid chromatography tandem mass spectrometry and targeted cytokines, chemokines, leptin and soluble CD14 by Luminex and ELISA; however, cytokines are known to be variable and fluctuate in different physiological locations and environments, which is a limitation of the study. We would suggest the authors describe how they overcame this limitation.**

  We thank the reviewers for pointing out this limitation which we highlight in our study. We have added a statement in the main text about other unmeasured factors causing fluctuations in cytokine levels *"we were unable to control for other factors that*

may influence cytokine levels". Further, we lacked serial measurements of inflammatory markers. However, it has been shown that "*in high income settings, survivors of sepsis have elevated markers of SI for up to 1 year and retain an increased mortality risk*[12-16]*. After sepsis, systemic levels of C-reactive protein and soluble programmed death ligand 1 (a marker of immunosuppression) are elevated for up to 1 year in patients while IL6 and IL10 persists for several months in human and experimental models*[12-14]*."* These data implicate SI in both short and longer-term growth outcomes post-discharge. We also agree with Reviewer 2 who points out that "reporting associations between discharge inflammation status and growth over longer intervals would be an important contribution, as little is known about extended influences of inflammation and longer-term growth in undernourished populations".

Methods

- **It is mentioned that the data was from a nested case control study within a clinical trial (NCT00934492) that tested the efficacy of daily co-trimoxazole prophylaxis. However, there was no comment found on the potential effect of co-trimoxazole prophylaxis as a confounder on the results for systematic inflammation. We suggest that a short explanation is added, whether in the main manuscript or supplemental material.**

  This was conducted and is indicated under the bioinformatics and statistics section of the manuscript. We adjusted for randomisation in the regression models to account for the potential effect of co-trimoxazole prophylaxis.

- **The authors have used a 60-day cut-off post-discharge – was there a particular reason for selecting this and not any earlier/later time-point and any literature supporting assessment of growth at 60 days post-discharge?**

  We selected the 60-day post-discharge period because it's when catch up in weight deficit was at its greatest and allows for short term convalescence. We also wished to retain the time-period at 60 days post-discharge as was the original design of the case-control mortality analysis[17]. This is also the period most likely to be related to biological factors measured at discharge unlike the later time-points. Following the reviewers' suggestions on the lack of data regarding extended influences of inflammation and longer-term growth in undernourished populations, we have conducted further analysis with growth deficits (weight, MUAC, and height) at 1 year and presented these results in the main text. We believe that this analysis on longer-term impact on growth is a valuable contribution to the field where the data is quite limited. Future analysis utilising other cohorts including the Childhood Acute Illness and Nutrition (CHAIN) Network Cohort in Kenya, Uganda, Malawi, Burkina Faso, Bangladesh and Pakistan that is examining mortality, readmission and growth among acutely ill children below 2 years[18] will focus on longer time periods post-discharge. This was a pilot study and there is no literature to the best of our knowledge that guides assessment of growth at 60 days post-discharge. However, most community-based feeding studies have assessed growth between 1 and 6 months[19-26].

- **We suggested clarifying how 'absolute deficit' was calculated.** Absolute deficit was defined as the median value for age according to WHO growth charts minus the child's measured value. We have added text to indicate that *"It was calculated as the difference between the measured weight, height, or MUAC and the median age- and sex-specific value obtained from the WHO 2006 growth standards[27-29]".*

Results

- **Was there a difference noted for patients that were born prematurely or underweight since the eventual weight gain may have been due to regression to the mean? Any analysis that was done after excluding these patients?** Regression to the mean was addressed by including enrolment anthropometric values in the regression models. Except for one child, all children born premature were also underweight. The model outcomes did not change when we included the underweight variable in the model.

Discussion

- **A few comments are mentioned under the 'overall' heading.**
  Any future directions for the current work?
- Yes. "*Future work should investigate effects over a longer time period post-discharge and compare with community participants/children without acute illness to establish community norms.*" We have added this to the main text.

- **We also briefly suggest adding strengths of the study, although not necessary.** Strengths included robust and standardised data collection within a clinical trial, follow-up for one year and very low loss to follow-up. This has been added on the main text.

Reviewer 2.
Keith P. West, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD, USA

Approved With Reservations
This study examines the potential attenuating influence of inflammation at time of discharge on subsequent linear and ponderal growth of young children initially hospitalized for severe complicated, non-HIV-related malnutrition. Inflammation at discharge was assessed by a combined assessment of cytokines (IL-2, IL17a and SAP) and relative abundance of plasma protein biomarkers, including those whose expression covaries with phases of the inflammatory response. The study is an important follow-up from a previous case-control study (Njunge et al Sci Reports 2019[1]) but also because it adds new insights amidst a sparsity of studies harnessing the potential of plasma proteomics to reveal metabolic alterations and pathways that may be affecting recovery-related (catch-up) child growth following severe malnutrition.

Comments:

The methods would benefit from slightly more essential study details that can render this paper independent of a previous paper (Njunge et al., Sci Reports 2019[1]) as I found myself needing to return to that publication to better understand aspects of study

design: please clarify when children were phlebotomized (presumably just prior to discharge that constitute enrolment samples), which is also equivalent to baseline.
We have adjusted the text to indicate that *"Samples were collected from study participants prior to initiation of the investigational product: co-trimoxazole or placebo at discharge and constitute enrolment samples".*

Notwithstanding important details provided about the analytical methods, thresholds at which associations between DMAD and DWAD and protein relative abundance/cytokine concentrations are considered chance-adjusted, statistically significant are not specified. Is there a p- or q-value considered to be SS, and how were family-wise error probabilities from multiple comparisons managed? P-values for EN models for DWAD and DMAD are given in Table 3 but its not clear what these are testing or how derived.
We did not infer associations between DMAD or DWAD with individual proteins. Instead, we directly performed a penalised multivariate analysis to model the association between all proteins and growth outcomes. Hence, only 2 multivariate models were made, resulting to the p-values reported in Table 3. Adjustment for multiple comparison is not typically used for these types of models and the elastic net method utilised aggressively penalised redundant and non-predictive protein features. The p-values for the EN models in Table 3 are the association between the actual DWAD/DMAD to the predicted DWAD/DMAD of the model. Therefore, the r value therefore represents the variance explained by the model, in which case our models captured 51% and 57% of the variance for DWAD and DMAD at month two, respectively.

Children were followed monthly through 6 mo and bi-monthly thereafter to 12 months. Given the measurements are available through 1-year post discharge, and partly reported as 60-day increments to 6 months of age in Table 2, the rationale for restricting evaluation of biomarker-growth deficit recovery to the 1st 60 days of follow-up, as opposed to longer, would benefit from clearer argument. It would be easier to follow if all data were restricted to a 60-day period for this paper, which aligns with the survival findings reported in Njunge et al 2019[1]. That said, reporting associations between discharge inflammation status and growth over longer intervals would be an important contribution, as little is known about extended influences of inflammation and longer term growth in undernourished populations. One population cohort-based plasma proteomics study in Nepal has shown head size at birth (Lee SE et al Sci Reports 2018[2]) and attained HAZ and WAZ at 6-8 years of age (Lee SE et al J Nutr 2017[3]), to be associated with the relative abundance of a wide array of plasma proteins, including S100 calprotectins (A9 and A12 isomers, respectively), assessed at 6-8 years. The few such studies that exist suggest persistent (long term), bidirectional associations between child growth and metabolic pathways, as expressed through the plasma proteome, observations that pique interest to explore protein biomarker associations with extended growth endpoints, in this paper or a subsequent treatise.
We thank both reviewers for questioning the rationale for restricting evaluation of biomarker-growth deficit recovery to the 1st 60 days of follow-up, as opposed to longer. We have addressed this under reviewer 1 comment 2 in the methods section. We agree that reporting associations between discharge inflammation status and growth over longer intervals would be an important contribution, as little is known about extended influences

of inflammation and longer-term growth in undernourished populations. We have therefore conducted further analysis with changes in growth deficits (weight, MUAC, and height) during one-year from enrolment, presented the results in the main text and discussed the findings. An interesting and strong association between Glutathione peroxidase 3 and one-year weight and MUAC deficit changes emerged. To extend this work, our next step is a planned detailed longitudinal analysis of the relationship between systemic inflammation and growth outcomes post-discharge among acutely ill children right across the nutritional spectrum using data and samples from a large recently completed cohort study in Africa and Asia[18].

To the extent time points are maintained, can the authors clarify if children were measured at exactly 60, 120 and 180 days post-enrollment? If not, how were growth intervals standardized to 60-day periods?
The mean number of days from enrolment to month two was 61.7 days (SD; 1.98), median [IQR] was 61 [61-63] days. The mean (SD) and median [IQR] number of days between months 2 – 4 and months 4 – 6 were: 61.5 (3.40); 61 [60-63] and 61.5 (6.41); 61 [59-63] respectively. Calculations on growth velocity were based on the difference between the measured values at enrolment and month two divided by the difference in the actual number of days between enrolment and the month two visit and subsequent visits.

DHAD data are summarized in Table 2. Why was it not also a primary outcome?
Various studies including ours[30] have shown that children initially gain weight before height therefore we did not expect major differences in height gain in the first 60 days. When wasting and stunting co-exist in the same child, it has been observed that, whereas rapid catch-up in tissue growth commences as soon as treatment is initiated, the peak velocity for catch-up in linear growth occurs 1 to 3 months later[31]. This delay coincides with the time taken by children to attain a weight that is appropriate for their length[31]. The early post discharge period (within 2 months) is normally accompanied by high gains in weight compared to later time points. We also selected weight gain as primary analysis since the original trial design used severe wasting (mid-upper-arm circumference (MUAC) <11.5cm if aged ≥6 months and <11.0cm if aged 2–5 months or had oedematous malnutrition) as an enrolment criteria and not severe stunting[32]. We have however included changes in height in the new one-year analysis.

Table 1: In the 1st row, it would be informative to have a summary [median (IQR)] of ages of children at 60 days (removing "at enrolment" in the row label). This has been edited and the median [IQR] of ages of children at 60 days included.

Table 3: The title is long and difficult to understand. Mention is made of correlation, but R^2 is reported. Please shorten and articulate specifics in the footnotes, including the exposure protein variables in each model (assuming these are the proteins reported in Figure 1 and b?).
The title has been changed to read *"Elastic Net regression model optimal alpha parameters and performance of proteins associated with change in growth deficits within 60 days"*. We have also changed R^2 to r. We have added a footnote that reads "*Optimal alpha parameter and correlation coefficients for the EN model enumerating the correlation between DWAD and DMAD at two months and exposure protein variables (untargeted plasma proteome, and targeted*

cytokine/chemokines, leptin, sCD14, and CRP) extracted by the multivariate regularized models."

Figure 1: Please clarify the x-axis label in a and b as regression coefficient and in the legend, specify measurement unit for each. Findings from Figures 1e and 1f, presenting Inclusion Rates associated with DWAD and DMAD, are presently not mentioned in the Results.
The x-axis label in Figure 1a and 1b now reads regression coefficient. Legend has been adjusted to read "Untargeted liquid chromatography tandem mass spectrometry plasma proteins, and targeted cytokines/chemokines, Leptin, sCD14, and CRP associated with DWAD (***a***) and DMAD (***b***) in multivariate elastic net (EN) regularized linear regression models at two months. Log normalised protein values were used in the analysis and regression models were adjusted for age, randomisation arm, sex, respective enrolment growth deficits, and site."

The findings from Figure 1e and 1f are discussed under the subheading "***Bootstrap analysis***. *After 1000 bootstrap iterations, only IL17a was identified in >60% of the DWAD model repetitions (Figure 1e) indicating that this was the most robust feature associated with weight gain. Using similar iterations during bootstrap validation for the DMAD model, no features were extracted at the 60% threshold and the most frequently selected features were IL17a (55%), B2M (55%), AGT (49%), SAP (48%), and sCD14 (48%) as shown (Figure 1f)*."

Discussion
Three-quarters down the 1st paragraph, noting the height deficit did not show evidence of recovery, could this be placing too high an expectation on long bone growth recovery commensurate with regaining soft tissue accretion within only 60 days?
Our group[30] and others have observed that during rehabilitation of severely malnourished children who were both wasted and stunted, weight gain is rapid and appetite is high until when such a child attain a weight appropriate for their length height/length gain[31].

Few typos:
Figure 1: Within the parentheses (d and f) should be (e and f);
This has now been corrected as suggested.

In discussion – LBP is a plasma protein that binds…
This has now been corrected as suggested.

References
1. Njunge J, Gwela A, Kibinge N, Ngari M, et al.: Biomarkers of post-discharge mortality among children with complicated severe acute malnutrition. *Scientific Reports*. 2019; **9** (1). Publisher Full Text
2. Lee S, West K, Cole R, Schulze K, et al.: Novel Plasma Proteins in Nepalese School-aged Children are Associated with a Small Head Size at Birth. *Scientific Reports*. 2018; **8** (1). Publisher Full Text
3. Lee SE, Stewart CP, Schulze KJ, Cole RN, et al.: The Plasma Proteome Is Associated with Anthropometric Status of Undernourished Nepalese School-Aged Children.*J Nutr*. **147** (3): 304-313 PubMed Abstract | Publisher Full Text

1 Sauder, M. W. *et al.* Inflammation throughout pregnancy and fetal growth restriction in rural Nepal. *Epidemiology and Infection* **147**, e258, doi:10.1017/S0950268819001493 (2019).
2 Hindle, L. J. *et al.* Effect of multiple micronutrient supplementation during pregnancy on inflammatory markers in Nepalese women. *The American journal of clinical nutrition* **84**, 1086-1092, doi:10.1093/ajcn/84.5.1086 (2006).
3 Lee, S. E. *et al.* Novel Plasma Proteins in Nepalese School-aged Children are Associated with a Small Head Size at Birth. *Scientific reports* **8**, 6390, doi:10.1038/s41598-018-24640-4 (2018).
4 Lee, S. E. *et al.* The Plasma Proteome Is Associated with Anthropometric Status of Undernourished Nepalese School-Aged Children. *The Journal of nutrition* **147**, 304-313, doi:10.3945/jn.116.243014 (2017).
5 Muriuki, J. M. *et al.* Estimating the burden of iron deficiency among African children. *BMC Medicine* **18**, 31, doi:10.1186/s12916-020-1502-7 (2020).
6 Rogawski, E. T. *et al.* Use of quantitative molecular diagnostic methods to investigate the effect of enteropathogen infections on linear growth in children in low-resource settings: longitudinal analysis of results from the MAL-ED cohort study. *The Lancet. Global health* **6**, e1319-e1328, doi:10.1016/s2214-109x(18)30351-6 (2018).
7 Prendergast, A. J. *et al.* Stunting Is Characterized by Chronic Inflammation in Zimbabwean Infants. *PLOS ONE* **9**, e86928, doi:10.1371/journal.pone.0086928 (2014).
8 Campbell, D. I., Elia, M. & Lunn, P. G. Growth faltering in rural Gambian infants is associated with impaired small intestinal barrier function, leading to endotoxemia and systemic inflammation. *The Journal of nutrition* **133**, 1332-1338, doi:10.1093/jn/133.5.1332 (2003).
9 Urlacher, S. S. *et al.* Tradeoffs between immune function and childhood growth among Amazonian forager-horticulturalists. *Proceedings of the National Academy of Sciences* **115**, E3914-E3921, doi:10.1073/pnas.1717522115 (2018).
10 Jones, K. D. J. *et al.* Mesalazine in the initial management of severely acutely malnourished children with environmental enteric dysfunction: a pilot randomized controlled trial. *BMC Medicine* **12**, 133, doi:10.1186/s12916-014-0133-2 (2014).
11 Lee, S. E., Schulze, K. & West, K. P. Rainer Gross Award Lecture 2018: The Childhood Plasma Proteome: Discovering its Applications in Public Health Nutrition. *Food and nutrition bulletin* **40**, 144-150, doi:10.1177/0379572119847851 (2019).
12 Yende, S. *et al.* Inflammatory markers at hospital discharge predict subsequent mortality after pneumonia and sepsis. *American journal of respiratory and critical care medicine* **177**, 1242-1247, doi:10.1164/rccm.200712-1777OC (2008).
13 Kaynar, A. M. *et al.* Effects of intra-abdominal sepsis on atherosclerosis in mice. *Crit Care* **18**, 469, doi:10.1186/s13054-014-0469-1 (2014).
14 Yende, S. *et al.* Long-term Host Immune Response Trajectories Among Hospitalized Patients With Sepsis. *JAMA Network Open* **2**, e198686-e198686, doi:10.1001/jamanetworkopen.2019.8686 (2019).
15 Rahmel, T. *et al.* Long-term mortality and outcome in hospital survivors of septic shock, sepsis, and severe infections: The importance of aftercare. *PLOS ONE* **15**, e0228952, doi:10.1371/journal.pone.0228952 (2020).

16 Póvoa, P. *et al.* C-reactive protein and albumin kinetics after antibiotic therapy in community-acquired bloodstream infection. *International Journal of Infectious Diseases* **95**, 50-58, doi:https://doi.org/10.1016/j.ijid.2020.03.063 (2020).
17 Njunge, J. M. *et al.* Biomarkers of post-discharge mortality among children with complicated severe acute malnutrition. *Scientific reports* **9**, 5981, doi:10.1038/s41598-019-42436-y (2019).
18 Childhood Acute Illness and Nutrition (CHAIN) Network: a protocol for a multi-site prospective cohort study to identify modifiable risk factors for mortality among acutely ill children in Africa and Asia. *BMJ open* **9**, e028454, doi:10.1136/bmjopen-2018-028454 (2019).
19 Sandige, H., Ndekha, M. J., Briend, A., Ashorn, P. & Manary, M. J. Home-Based Treatment of Malnourished Malawian Children with Locally Produced or Imported Ready-to-Use Food. *Journal of Pediatric Gastroenterology and Nutrition* **39**, 141-146 (2004).
20 Yebyo, H. G., Kendall, C., Nigusse, D. & Lemma, W. Outpatient therapeutic feeding program outcomes and determinants in treatment of severe acute malnutrition in tigray, northern ethiopia: a retrospective cohort study. *PLoS One* **8**, e65840, doi:10.1371/journal.pone.0065840 (2013).
21 Kangas, S. T. *et al.* Impact of reduced dose of ready-to-use therapeutic foods in children with uncomplicated severe acute malnutrition: A randomised non-inferiority trial in Burkina Faso. *PLoS medicine* **16**, e1002887, doi:10.1371/journal.pmed.1002887 (2019).
22 Linneman, Z. *et al.* A large-scale operational study of home-based therapy with ready-to-use therapeutic food in childhood malnutrition in Malawi. *Maternal & Child Nutrition* **3**, 206-215, doi:10.1111/j.1740-8709.2007.00095.x (2007).
23 Aguayo, V. M. *et al.* Community management of acute malnutrition (CMAM) programme in Pakistan effectively treats children with uncomplicated severe wasting. *Matern Child Nutr* **14 Suppl 4**, e12623, doi:10.1111/mcn.12623 (2018).
24 James, P. T. *et al.* Low-dose RUTF protocol and improved service delivery lead to good programme outcomes in the treatment of uncomplicated SAM: a programme report from Myanmar. *Matern Child Nutr* **11**, 859-869, doi:10.1111/mcn.12192 (2015).
25 Burza, S. *et al.* Community-based management of severe acute malnutrition in India: new evidence from Bihar. *Am J Clin Nutr* **101**, 847-859, doi:10.3945/ajcn.114.093294 (2015).
26 Das, J. K., Salam, R. A., Saeed, M., Kazmi, F. A. & Bhutta, Z. A. Effectiveness of Interventions for Managing Acute Malnutrition in Children under Five Years of Age in Low-Income and Middle-Income Countries: A Systematic Review and Meta-Analysis. *Nutrients* **12**, 116 (2020).
27 Leroy, J. L., Ruel, M., Habicht, J.-P. & Frongillo, E. A. Linear Growth Deficit Continues to Accumulate beyond the First 1000 Days in Low- and Middle-Income Countries: Global Evidence from 51 National Surveys. *The Journal of Nutrition* **144**, 1460-1466, doi:10.3945/jn.114.191981 (2014).
28 Leroy, J. L., Ruel, M., Habicht, J.-P. & Frongillo, E. A. Using height-for-age differences (HAD) instead of height-for-age z-scores (HAZ) for the meaningful measurement of population-level catch-up in linear growth in children less than 5 years of age. *BMC Pediatr* **15**, 145-145, doi:10.1186/s12887-015-0458-9 (2015).
29 Group, W. M. G. R. S. WHO Child Growth Standards based on length/height, weight and age. *Acta Paediatrica (Oslo, Norway: 1992). Supplement* **450**, 76-85 (2006).
30 Ngari, M. M. *et al.* Linear growth following complicated severe malnutrition: 1-year follow-up cohort of Kenyan children. *Archives of Disease in Childhood* **104**, 229-235,

doi:10.1136/archdischild-2018-315641 (2019).
31 Ashworth, A. & Millward, D. Catch-up growth in children. *Nutrition reviews (USA)* (1986).
32 Berkley, J. A. *et al.* Daily co-trimoxazole prophylaxis to prevent mortality in children with complicated severe acute malnutrition: a multicentre, double-blind, randomised placebo-controlled trial. *Lancet Glob Health* **4**, e464-473, doi:10.1016/S2214-109X(16)30096-1 (2016).

Competing Interests: None

Reviewer Report 04 December 2020

https://doi.org/10.21956/wellcomeopenres.17953.r41367

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Sana Syed
Division of Pediatric Gastroenterology, Hepatology and Nutrition, Department of Pediatrics, School of Medicine, University of Virginia, Charlottesville, VA, USA
Lubaina Ehsan
University of Virginia, Charlottesville, VA, USA

The manuscript submitted by Dr. Njunge and colleagues aims to investigate the association of systemic inflammation among early post-discharge children who are severely malnourished and have had an acute illness. Overall, this manuscript presents results that would be of interest to the community of scientists and clinicians concerned with this problem, major strengths of their work are:

- Well-written and coherent manuscript which clearly outlines the work that was done.
- Strong statistical analysis of not only the weight/MUAC gain but also the absolute deficits in these measurements.
- A discussion that outlines each significant result clearly with extensive literature being referenced.
- The assessment of potential markers for growth being associated with inflammation which can pave the way for revised standards of anthropometric measurement among children within the same cohort.

However, there are a few issues in this manuscript that prevent us from recommending that this manuscript be indexed in its current state:

Overall

- There is a mismatch between the introduction and the discussion. While the introduction mentions RUTF and GH/IGF1 axis, we also suggest adding the background behind the assessment of the relationship of plasma proteomic and cytokine profiles with growth. The discussion then excellently discussed the individual results for cytokines and other markers

although, the background for these measurements being part of the introduction will make the foundation of why this work was done even more strong.

- The proteomics and cytokines measurements in plasma were measured using liquid chromatography tandem mass spectrometry and targeted cytokines, chemokines, leptin and soluble CD14 by Luminex and ELISA; however, cytokines are known to be variable and fluctuate in different physiological locations and environments, which is a limitation of the study. We would suggest the authors describe how they overcame this limitation.

Methods

- It is mentioned that the data was from a nested case control study within a clinical trial (NCT00934492) that tested the efficacy of daily co-trimoxazole prophylaxis. However, there was no comment found on the potential effect of co-trimoxazole prophylaxis as a confounder on the results for systematic inflammation. We suggest that a short explanation is added, whether in the main manuscript or supplemental material.

- The authors have used a 60 day cut-off post-discharge – was there a particular reason for selecting this and not any earlier/later time-point and any literature supporting assessment of growth at 60 days post-discharge?

- We suggested clarifying how 'absolute deficit' was calculated.

Results

- Was there a difference noted for patients that were born prematurely or underweight since the eventual weight gain may have been due to regression to the mean? Any analysis that was done after excluding these patients?

Discussion

- A few comments are mentioned under the 'overall' heading.
- Any future directions for the current work?
- We also briefly suggest adding strengths of the study, although not necessary.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Gastroenterology, Nutrition, Data Science

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.

Author Response 05 Mar 2021

James Njunge, The Childhood Acute Illness & Nutrition (CHAIN) Network, Nairobi, Kenya

Reviewer 1
Sana Syed, Division of Pediatric Gastroenterology, Hepatology and Nutrition, Department of Pediatrics, School of Medicine, University of Virginia, Charlottesville, VA, USA.
Lubaina Ehsan, University of Virginia, Charlottesville, VA, USA

Approved With Reservations
The manuscript submitted by Dr. Njunge and colleagues aims to investigate the association of systemic inflammation among early post-discharge children who are severely malnourished and have had an acute illness. Overall, this manuscript presents results that would be of interest to the community of scientists and clinicians concerned with this problem, major strengths of their work are:

- **Well-written and coherent manuscript which clearly outlines the work that was done.**
- **Strong statistical analysis of not only the weight/MUAC gain but also the absolute deficits in these measurements.**
- **A discussion that outlines each significant result clearly with extensive literature being referenced.**
- **The assessment of potential markers for growth being associated with inflammation which can pave the way for revised standards of anthropometric measurement among children within the same cohort.**

However, there are a few issues in this manuscript that prevent us from recommending that this manuscript be indexed in its current state:

Overall
There is a mismatch between the introduction and the discussion. While the introduction mentions RUTF and GH/IGF1 axis, we also suggest adding the background behind the assessment of the relationship of plasma proteomic and cytokine profiles with growth. The discussion then excellently discussed the individual results for cytokines and other markers although, the background for these measurements being part of the introduction will make the foundation of why this work was done even more strong.

- Studies detailing the relationship between plasma proteomics and cytokine profiles on catch-up growth after an acute illness are very limited among undernourished children. We have added additional citations on linear growth. "*In Nepal, birth size was inversely associated with low-grade, chronic inflammation during pregnancy as indicated*

by serum AGP[1,2]. Further, head size at birth among Nepalese[3] and attained z scores for height (HAZ) and weight-for-age (WAZ) at 6-8 years of age[4] were associated with a wide array of plasma proteins, including S100 calprotectin subunits, assessed at 6-8 years. In community settings, SI is elevated in 17-34% children in LMIC[5] (C-reactive protein >5mg/L or α1-antichymotrypsin >0.6g/L) and is associated with reduced linear growth[6-10]." However the literature is quite limited[11].

- **The proteomics and cytokines measurements in plasma were measured using liquid chromatography tandem mass spectrometry and targeted cytokines, chemokines, leptin and soluble CD14 by Luminex and ELISA; however, cytokines are known to be variable and fluctuate in different physiological locations and environments, which is a limitation of the study. We would suggest the authors describe how they overcame this limitation.**

  We thank the reviewers for pointing out this limitation which we highlight in our study. We have added a statement in the main text about other unmeasured factors causing fluctuations in cytokine levels *"we were unable to control for other factors that may influence cytokine levels".* Further, we lacked serial measurements of inflammatory markers. However, it has been shown that "*in high income settings, survivors of sepsis have elevated markers of SI for up to 1 year and retain an increased mortality risk[12-16]. After sepsis, systemic levels of C-reactive protein and soluble programmed death ligand 1 (a marker of immunosuppression) are elevated for up to 1 year in patients while IL6 and IL10 persists for several months in human and experimental models[12-14]."* These data implicate SI in both short and longer-term growth outcomes post-discharge. We also agree with Reviewer 2 who points out that "reporting associations between discharge inflammation status and growth over longer intervals would be an important contribution, as little is known about extended influences of inflammation and longer-term growth in undernourished populations".

Methods

- **It is mentioned that the data was from a nested case control study within a clinical trial (NCT00934492) that tested the efficacy of daily co-trimoxazole prophylaxis. However, there was no comment found on the potential effect of co-trimoxazole prophylaxis as a confounder on the results for systematic inflammation. We suggest that a short explanation is added, whether in the main manuscript or supplemental material.**

  This was conducted and is indicated under the bioinformatics and statistics section of the manuscript. We adjusted for randomisation in the regression models to account for the potential effect of co-trimoxazole prophylaxis.

- **The authors have used a 60-day cut-off post-discharge – was there a particular reason for selecting this and not any earlier/later time-point and any literature supporting assessment of growth at 60 days post-discharge?**

  We selected the 60-day post-discharge period because it's when catch up in weight deficit was at its greatest and allows for short term convalescence. We also wished to retain the time-period at 60 days post-discharge as was the original design of the

case-control mortality analysis[17]. This is also the period most likely to be related to biological factors measured at discharge unlike the later time-points. Following the reviewers' suggestions on the lack of data regarding extended influences of inflammation and longer-term growth in undernourished populations, we have conducted further analysis with growth deficits (weight, MUAC, and height) at 1 year and presented these results in the main text. We believe that this analysis on longer-term impact on growth is a valuable contribution to the field where the data is quite limited. Future analysis utilising other cohorts including the Childhood Acute Illness and Nutrition (CHAIN) Network Cohort in Kenya, Uganda, Malawi, Burkina Faso, Bangladesh and Pakistan that is examining mortality, readmission and growth among acutely ill children below 2 years[18] will focus on longer time periods post-discharge. This was a pilot study and there is no literature to the best of our knowledge that guides assessment of growth at 60 days post-discharge. However, most community-based feeding studies have assessed growth between 1 and 6 months[19-26].

- **We suggested clarifying how 'absolute deficit' was calculated.** Absolute deficit was defined as the median value for age according to WHO growth charts minus the child's measured value. We have added text to indicate that *"It was calculated as the difference between the measured weight, height, or MUAC and the median age- and sex-specific value obtained from the WHO 2006 growth standards[27-29]".*

Results

- **Was there a difference noted for patients that were born prematurely or underweight since the eventual weight gain may have been due to regression to the mean? Any analysis that was done after excluding these patients?** Regression to the mean was addressed by including enrolment anthropometric values in the regression models. Except for one child, all children born premature were also underweight. The model outcomes did not change when we included the underweight variable in the model.

Discussion

- **A few comments are mentioned under the 'overall' heading.**
  Any future directions for the current work?
- Yes. "*Future work should investigate effects over a longer time period post-discharge and compare with community participants/children without acute illness to establish community norms.*" We have added this to the main text.

- **We also briefly suggest adding strengths of the study, although not necessary.** Strengths included robust and standardised data collection within a clinical trial, follow-up for one year and very low loss to follow-up. This has been added on the main text.

Reviewer 2.
Keith P. West, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD, USA

Approved With Reservations
This study examines the potential attenuating influence of inflammation at time of

discharge on subsequent linear and ponderal growth of young children initially hospitalized for severe complicated, non-HIV-related malnutrition. Inflammation at discharge was assessed by a combined assessment of cytokines (IL-2, IL17a and SAP) and relative abundance of plasma protein biomarkers, including those whose expression covaries with phases of the inflammatory response. The study is an important follow-up from a previous case-control study (Njunge et al Sci Reports 2019[1]) but also because it adds new insights amidst a sparsity of studies harnessing the potential of plasma proteomics to reveal metabolic alterations and pathways that may be affecting recovery-related (catch-up) child growth following severe malnutrition.

Comments:

The methods would benefit from slightly more essential study details that can render this paper independent of a previous paper (Njunge et al., Sci Reports 2019[1]) as I found myself needing to return to that publication to better understand aspects of study design: please clarify when children were phlebotomized (presumably just prior to discharge that constitute enrolment samples), which is also equivalent to baseline.
We have adjusted the text to indicate that *"Samples were collected from study participants prior to initiation of the investigational product: co-trimoxazole or placebo at discharge and constitute enrolment samples".*

Notwithstanding important details provided about the analytical methods, thresholds at which associations between DMAD and DWAD and protein relative abundance/cytokine concentrations are considered chance-adjusted, statistically significant are not specified. Is there a p- or q-value considered to be SS, and how were family-wise error probabilities from multiple comparisons managed? P-values for EN models for DWAD and DMAD are given in Table 3 but its not clear what these are testing or how derived.
We did not infer associations between DMAD or DWAD with individual proteins. Instead, we directly performed a penalised multivariate analysis to model the association between all proteins and growth outcomes. Hence, only 2 multivariate models were made, resulting to the p-values reported in Table 3. Adjustment for multiple comparison is not typically used for these types of models and the elastic net method utilised aggressively penalised redundant and non-predictive protein features. The p-values for the EN models in Table 3 are the association between the actual DWAD/DMAD to the predicted DWAD/DMAD of the model. Therefore, the r value therefore represents the variance explained by the model, in which case our models captured 51% and 57% of the variance for DWAD and DMAD at month two, respectively.

Children were followed monthly through 6 mo and bi-monthly thereafter to 12 months. Given the measurements are available through 1-year post discharge, and partly reported as 60-day increments to 6 months of age in Table 2, the rationale for restricting evaluation of biomarker-growth deficit recovery to the 1st 60 days of follow-up, as opposed to longer, would benefit from clearer argument. It would be easier to follow if all data were restricted to a 60-day period for this paper, which aligns with the survival findings reported in Njunge et al 2019[1]. That said, reporting

associations between discharge inflammation status and growth over longer intervals would be an important contribution, as little is known about extended influences of inflammation and longer term growth in undernourished populations. One population cohort-based plasma proteomics study in Nepal has shown head size at birth (Lee SE et al Sci Reports 2018[2]) and attained HAZ and WAZ at 6-8 years of age (Lee SE et al J Nutr 2017[3]), to be associated with the relative abundance of a wide array of plasma proteins, including S100 calprotectins (A9 and A12 isomers, respectively), assessed at 6-8 years. The few such studies that exist suggest persistent (long term), bidirectional associations between child growth and metabolic pathways, as expressed through the plasma proteome, observations that pique interest to explore protein biomarker associations with extended growth endpoints, in this paper or a subsequent treatise.
We thank both reviewers for questioning the rationale for restricting evaluation of biomarker-growth deficit recovery to the 1st 60 days of follow-up, as opposed to longer. We have addressed this under reviewer 1 comment 2 in the methods section. We agree that reporting associations between discharge inflammation status and growth over longer intervals would be an important contribution, as little is known about extended influences of inflammation and longer-term growth in undernourished populations. We have therefore conducted further analysis with changes in growth deficits (weight, MUAC, and height) during one-year from enrolment, presented the results in the main text and discussed the findings. An interesting and strong association between Glutathione peroxidase 3 and one-year weight and MUAC deficit changes emerged. To extend this work, our next step is a planned detailed longitudinal analysis of the relationship between systemic inflammation and growth outcomes post-discharge among acutely ill children right across the nutritional spectrum using data and samples from a large recently completed cohort study in Africa and Asia[18].

To the extent time points are maintained, can the authors clarify if children were measured at exactly 60, 120 and 180 days post-enrollment? If not, how were growth intervals standardized to 60-day periods?
The mean number of days from enrolment to month two was 61.7 days (SD; 1.98), median [IQR] was 61 [61-63] days. The mean (SD) and median [IQR] number of days between months 2 – 4 and months 4 – 6 were: 61.5 (3.40); 61 [60-63] and 61.5 (6.41); 61 [59-63] respectively. Calculations on growth velocity were based on the difference between the measured values at enrolment and month two divided by the difference in the actual number of days between enrolment and the month two visit and subsequent visits.

DHAD data are summarized in Table 2. Why was it not also a primary outcome?
Various studies including ours[30] have shown that children initially gain weight before height therefore we did not expect major differences in height gain in the first 60 days. When wasting and stunting co-exist in the same child, it has been observed that, whereas rapid catch-up in tissue growth commences as soon as treatment is initiated, the peak velocity for catch-up in linear growth occurs 1 to 3 months later[31]. This delay coincides with the time taken by children to attain a weight that is appropriate for their length[31]. The early post discharge period (within 2 months) is normally accompanied by high gains in weight compared to later time points. We also selected weight gain as primary analysis since the original trial design used severe wasting (mid-upper-arm circumference (MUAC) <11.5cm if aged ≥6 months and <11.0cm if aged 2–5 months or had oedematous malnutrition) as an

enrolment criteria and not severe stunting[32]. We have however included changes in height in the new one-year analysis.

Table 1: In the 1st row, it would be informative to have a summary [median (IQR)] of ages of children at 60 days (removing "at enrolment" in the row label). This has been edited and the median [IQR] of ages of children at 60 days included.

Table 3: The title is long and difficult to understand. Mention is made of correlation, but R^2 is reported. Please shorten and articulate specifics in the footnotes, including the exposure protein variables in each model (assuming these are the proteins reported in Figure 1 and b?).
The title has been changed to read *"Elastic Net regression model optimal alpha parameters and performance of proteins associated with change in growth deficits within 60 days"*. We have also changed R^2 to r. We have added a footnote that reads "*Optimal alpha parameter and correlation coefficients for the EN model enumerating the correlation between DWAD and DMAD at two months and exposure protein variables (untargeted plasma proteome, and targeted cytokine/chemokines, leptin, sCD14, and CRP) extracted by the multivariate regularized models*."

Figure 1: Please clarify the x-axis label in a and b as regression coefficient and in the legend, specify measurement unit for each. Findings from Figures 1e and 1f, presenting Inclusion Rates associated with DWAD and DMAD, are presently not mentioned in the Results.
The x-axis label in Figure 1a and 1b now reads regression coefficient. Legend has been adjusted to read "Untargeted liquid chromatography tandem mass spectrometry plasma proteins, and targeted cytokines/chemokines, Leptin, sCD14, and CRP associated with DWAD (***a***) and DMAD (***b***) in multivariate elastic net (EN) regularized linear regression models at two months. Log normalised protein values were used in the analysis and regression models were adjusted for age, randomisation arm, sex, respective enrolment growth deficits, and site."

The findings from Figure 1e and 1f are discussed under the subheading "***Bootstrap analysis***. *After 1000 bootstrap iterations, only IL17a was identified in >60% of the DWAD model repetitions (Figure 1e) indicating that this was the most robust feature associated with weight gain. Using similar iterations during bootstrap validation for the DMAD model, no features were extracted at the 60% threshold and the most frequently selected features were IL17a (55%), B2M (55%), AGT (49%), SAP (48%), and sCD14 (48%) as shown (Figure 1f)*."

Discussion
Three-quarters down the 1st paragraph, noting the height deficit did not show evidence of recovery, could this be placing too high an expectation on long bone growth recovery commensurate with regaining soft tissue accretion within only 60 days?
Our group[30] and others have observed that during rehabilitation of severely malnourished children who were both wasted and stunted, weight gain is rapid and appetite is high until when such a child attain a weight appropriate for their length height/length gain[31].

Few typos:

Figure 1: Within the parentheses (d and f) should be (e and f);
This has now been corrected as suggested.

In discussion – LBP is a plasma protein that binds...
This has now been corrected as suggested.

References

1. Njunge J, Gwela A, Kibinge N, Ngari M, et al.: Biomarkers of post-discharge mortality among children with complicated severe acute malnutrition. *Scientific Reports*. 2019; **9** (1). Publisher Full Text
2. Lee S, West K, Cole R, Schulze K, et al.: Novel Plasma Proteins in Nepalese School-aged Children are Associated with a Small Head Size at Birth. *Scientific Reports*. 2018; **8** (1). Publisher Full Text
3. Lee SE, Stewart CP, Schulze KJ, Cole RN, et al.: The Plasma Proteome Is Associated with Anthropometric Status of Undernourished Nepalese School-Aged Children.*J Nutr*. **147** (3): 304-313 PubMed Abstract | Publisher Full Text

1 Sauder, M. W. *et al.* Inflammation throughout pregnancy and fetal growth restriction in rural Nepal. *Epidemiology and Infection* **147**, e258, doi:10.1017/S0950268819001493 (2019).
2 Hindle, L. J. *et al.* Effect of multiple micronutrient supplementation during pregnancy on inflammatory markers in Nepalese women. *The American journal of clinical nutrition* **84**, 1086-1092, doi:10.1093/ajcn/84.5.1086 (2006).
3 Lee, S. E. *et al.* Novel Plasma Proteins in Nepalese School-aged Children are Associated with a Small Head Size at Birth. *Scientific reports* **8**, 6390, doi:10.1038/s41598-018-24640-4 (2018).
4 Lee, S. E. *et al.* The Plasma Proteome Is Associated with Anthropometric Status of Undernourished Nepalese School-Aged Children. *The Journal of nutrition* **147**, 304-313, doi:10.3945/jn.116.243014 (2017).
5 Muriuki, J. M. *et al.* Estimating the burden of iron deficiency among African children. *BMC Medicine* **18**, 31, doi:10.1186/s12916-020-1502-7 (2020).
6 Rogawski, E. T. *et al.* Use of quantitative molecular diagnostic methods to investigate the effect of enteropathogen infections on linear growth in children in low-resource settings: longitudinal analysis of results from the MAL-ED cohort study. *The Lancet. Global health* **6**, e1319-e1328, doi:10.1016/s2214-109x(18)30351-6 (2018).
7 Prendergast, A. J. *et al.* Stunting Is Characterized by Chronic Inflammation in Zimbabwean Infants. *PLOS ONE* **9**, e86928, doi:10.1371/journal.pone.0086928 (2014).
8 Campbell, D. I., Elia, M. & Lunn, P. G. Growth faltering in rural Gambian infants is associated with impaired small intestinal barrier function, leading to endotoxemia and systemic inflammation. *The Journal of nutrition* **133**, 1332-1338, doi:10.1093/jn/133.5.1332 (2003).
9 Urlacher, S. S. *et al.* Tradeoffs between immune function and childhood growth among Amazonian forager-horticulturalists. *Proceedings of the National Academy of Sciences* **115**, E3914-E3921, doi:10.1073/pnas.1717522115 (2018).
10 Jones, K. D. J. *et al.* Mesalazine in the initial management of severely acutely

malnourished children with environmental enteric dysfunction: a pilot randomized controlled trial. *BMC Medicine* **12**, 133, doi:10.1186/s12916-014-0133-2 (2014).
11 Lee, S. E., Schulze, K. & West, K. P. Rainer Gross Award Lecture 2018: The Childhood Plasma Proteome: Discovering its Applications in Public Health Nutrition. *Food and nutrition bulletin* **40**, 144-150, doi:10.1177/0379572119847851 (2019).
12 Yende, S. *et al.* Inflammatory markers at hospital discharge predict subsequent mortality after pneumonia and sepsis. *American journal of respiratory and critical care medicine* **177**, 1242-1247, doi:10.1164/rccm.200712-1777OC (2008).
13 Kaynar, A. M. *et al.* Effects of intra-abdominal sepsis on atherosclerosis in mice. *Crit Care* **18**, 469, doi:10.1186/s13054-014-0469-1 (2014).
14 Yende, S. *et al.* Long-term Host Immune Response Trajectories Among Hospitalized Patients With Sepsis. *JAMA Network Open* **2**, e198686-e198686, doi:10.1001/jamanetworkopen.2019.8686 (2019).
15 Rahmel, T. *et al.* Long-term mortality and outcome in hospital survivors of septic shock, sepsis, and severe infections: The importance of aftercare. *PLOS ONE* **15**, e0228952, doi:10.1371/journal.pone.0228952 (2020).
16 Póvoa, P. *et al.* C-reactive protein and albumin kinetics after antibiotic therapy in community-acquired bloodstream infection. *International Journal of Infectious Diseases* **95**, 50-58, doi:https://doi.org/10.1016/j.ijid.2020.03.063 (2020).
17 Njunge, J. M. *et al.* Biomarkers of post-discharge mortality among children with complicated severe acute malnutrition. *Scientific reports* **9**, 5981, doi:10.1038/s41598-019-42436-y (2019).
18 Childhood Acute Illness and Nutrition (CHAIN) Network: a protocol for a multi-site prospective cohort study to identify modifiable risk factors for mortality among acutely ill children in Africa and Asia. *BMJ open* **9**, e028454, doi:10.1136/bmjopen-2018-028454 (2019).
19 Sandige, H., Ndekha, M. J., Briend, A., Ashorn, P. & Manary, M. J. Home-Based Treatment of Malnourished Malawian Children with Locally Produced or Imported Ready-to-Use Food. *Journal of Pediatric Gastroenterology and Nutrition* **39**, 141-146 (2004).
20 Yebyo, H. G., Kendall, C., Nigusse, D. & Lemma, W. Outpatient therapeutic feeding program outcomes and determinants in treatment of severe acute malnutrition in tigray, northern ethiopia: a retrospective cohort study. *PLoS One* **8**, e65840, doi:10.1371/journal.pone.0065840 (2013).
21 Kangas, S. T. *et al.* Impact of reduced dose of ready-to-use therapeutic foods in children with uncomplicated severe acute malnutrition: A randomised non-inferiority trial in Burkina Faso. *PLoS medicine* **16**, e1002887, doi:10.1371/journal.pmed.1002887 (2019).
22 Linneman, Z. *et al.* A large-scale operational study of home-based therapy with ready-to-use therapeutic food in childhood malnutrition in Malawi. *Maternal & Child Nutrition* **3**, 206-215, doi:10.1111/j.1740-8709.2007.00095.x (2007).
23 Aguayo, V. M. *et al.* Community management of acute malnutrition (CMAM) programme in Pakistan effectively treats children with uncomplicated severe wasting. *Matern Child Nutr* **14 Suppl 4**, e12623, doi:10.1111/mcn.12623 (2018).
24 James, P. T. *et al.* Low-dose RUTF protocol and improved service delivery lead to good programme outcomes in the treatment of uncomplicated SAM: a programme report from Myanmar. *Matern Child Nutr* **11**, 859-869, doi:10.1111/mcn.12192 (2015).
25 Burza, S. *et al.* Community-based management of severe acute malnutrition in India: new evidence from Bihar. *Am J Clin Nutr* **101**, 847-859, doi:10.3945/ajcn.114.093294 (2015).

26 Das, J. K., Salam, R. A., Saeed, M., Kazmi, F. A. & Bhutta, Z. A. Effectiveness of Interventions for Managing Acute Malnutrition in Children under Five Years of Age in Low-Income and Middle-Income Countries: A Systematic Review and Meta-Analysis. *Nutrients* **12**, 116 (2020).
27 Leroy, J. L., Ruel, M., Habicht, J.-P. & Frongillo, E. A. Linear Growth Deficit Continues to Accumulate beyond the First 1000 Days in Low- and Middle-Income Countries: Global Evidence from 51 National Surveys. *The Journal of Nutrition* **144**, 1460-1466, doi:10.3945/jn.114.191981 (2014).
28 Leroy, J. L., Ruel, M., Habicht, J.-P. & Frongillo, E. A. Using height-for-age differences (HAD) instead of height-for-age z-scores (HAZ) for the meaningful measurement of population-level catch-up in linear growth in children less than 5 years of age. *BMC Pediatr* **15**, 145-145, doi:10.1186/s12887-015-0458-9 (2015).
29 Group, W. M. G. R. S. WHO Child Growth Standards based on length/height, weight and age. *Acta Paediatrica (Oslo, Norway: 1992). Supplement* **450**, 76-85 (2006).
30 Ngari, M. M. *et al.* Linear growth following complicated severe malnutrition: 1-year follow-up cohort of Kenyan children. *Archives of Disease in Childhood* **104**, 229-235, doi:10.1136/archdischild-2018-315641 (2019).
31 Ashworth, A. & Millward, D. Catch-up growth in children. *Nutrition reviews (USA)* (1986).
32 Berkley, J. A. *et al.* Daily co-trimoxazole prophylaxis to prevent mortality in children with complicated severe acute malnutrition: a multicentre, double-blind, randomised placebo-controlled trial. *Lancet Glob Health* **4**, e464-473, doi:10.1016/S2214-109X(16)30096-1 (2016).

Competing Interests: None