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Clinical and Biochemical Responses to Treatment of Uncomplicated Severe Acute Malnutrition: A Multicentric Observational Cohort from the OptiDiag Study

Lishi Deng[1], Alemayehu Argaw[1], Benjamin Guesdon[2], Michael Freemark[3,4], Dominique Roberfroid[5,6], Issa A. Kemokai[7], Md. Rayhan Mostak[8,9], Md. Abdul Alim[10,11], Md. Abdul Hashem Khan[12], Michael Muehlbauer[4], Murad Md. Shamsher Tabris Khan[13], Luke Bawo[14], Nelson K. Dunbar[14], Curtis H. Taylor[15], Helene Fouillet[16], Jean-Francois Huneau[16], Carl Lachat[1], Patrick Kolsteren[1], Trenton Dailey-Chwalibóg*[,1,17]

[1] Department of Food Technology, Safety and Health, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium;
[2] Department of Expertise and Advocacy, Action Contre la Faim, Paris, France;
[3] Division of Pediatric Endocrinology and Diabetes, Duke University Medical Center, Durham, NC 27710, U.S.A;
[4] Duke Molecular Physiology Institute (DMPI), Duke University Medical Center, Durham, NC 27701, U.S.A;
[5] Belgian Health Care Knowledge Centre, Brussels, Belgium;
[6] Medicine Department, Faculty of Medicine, University of Namur, Namur, Belgium;
[7] Action Contre la Faim, Monrovia, Liberia;
[8] Palli Karma-Sahayak Foundation, Dhaka, Bangladesh;
[9] Action Against Hunger, Dhaka, Bangladesh;
[10] Directorate General of Health Services, Bangladesh;
[11] Department of Public Health and Primary Care, Ghent University, Ghent, Belgium;
[12] Community Based Health Care (CBHC), Dhaka, Bangladesh;
[13] National Nutrition Service, Institute of Public Health Nutrition, Dhaka, Bangladesh;
[14] Ministry of Health, Monrovia, Liberia;
[15] Pacific Institute for Research and Evaluation (UL-PIRE), University of Liberia, Monrovia, Liberia;
[16] Université Paris-Saclay, AgroParisTech, INRAE, UMR PNCA, 91120, Palaiseau, France; and
[17] Agence de Formation de Recherche et d'Expertise en Santé pour l'Afrique (AFRICSanté), Bobo-Dioulasso, Burkina Faso

Lishi Deng and Trenton Dailey-Chwalibóg contributed equally to this work
* Correspondence: Trenton Dailey-Chwalibóg, Department of Food Technology, Safety and Health, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, 9000, Ghent, Belgium. [Trenton@Dailey-Chwalibog.com]. +33 6 03 23 36 14

Abbreviations:.

AGP: α-1-acid glycoprotein
BIS: body iron stores
CI: confidence interval
CMAM: community management of acute malnutrition
CRP: C-reactive protein
ELISA: enzyme-linked immunosorbent assays
ES: regression coefficient estimates
HAZ: height-for-age *Z*-score
IMCI: Integrated Management of Childhood Illness
IQR: interquartile range
LMIC: low- and middle- income country

MUAC: mid-upper arm circumference
RBP: retinol-binding protein
RUTF: Ready-to-Use Therapeutic Food
SAM: severe acute malnutrition
SSRZ: sitting/standing ratio *Z*-score
sTfR: soluble transferrin receptor
WHO: World Health Organization
WHZ: weight-for-height *Z*-score

Data Availability*:

Data described in the manuscript, code book, and analytic code will be made available upon request pending. The data will be made available after publication to researchers who provide a methodologically sound proposal for use in achieving the goals of the approved proposal. Proposals should be submitted to Dr. Trenton Dailey-Chwalibóg at Trenton@Dailey-Chwalibog.com.

Abstract

Background: Severe acute malnutrition (SAM) can be diagnosed using weight-for-height *Z*-score (WHZ) and/or mid-upper arm circumference (MUAC). While some favor using MUAC alone, valuing its presumed ability to identify children at greatest need for nutritional care, the functional severity and physiological responses to treatment in children with varying deficits in WHZ and MUAC remain inadequately characterised.

Objective: We aimed to compare clinical and biochemical responses to treatment in children with: (1) both low MUAC and low WHZ; (2) low MUAC only; and (3) low WHZ only.

Methods: A multicentric, observational cohort study was conducted in children aged 6–59 months with nonedematous, uncomplicated SAM in Bangladesh, Burkina Faso, and Liberia. Anthropometric measurements and critical indicators were collected three times during treatment; metrics included clinical status, nutritional status, viability and serum leptin, a biomarker of mortality risk in SAM.

Results: Children with combined MUAC and WHZ deficits had greater increases in leptin levels during treatment than those with low MUAC alone, showing a 34.4% higher rise at the second visit (95% CI: 7.6%, 43.6%; $p = 0.02$) and a 34.3% greater increase at the third visit (95% CI: 13.2%, 50.3%; $p = 0.01$). Similarly, weight gain velocity was higher by 1.56 g/kg/d in the combined deficit group (95% CI: 0.38, 2.75; $p = 0.03$) compared to children with low MUAC only. Children with combined deficits had higher rates of iron deficiency and wasting while those with low WHZ alone and combined deficits had higher rates of tachypnea and pneumonia during treatment.

Conclusions: Given the comparable treatment responses of children with low WHZ alone and those with low MUAC alone, and the greater vulnerability at admission and during treatment in

those with combined deficits, our findings support retaining WHZ as an independent diagnostic and admission criterion of SAM, alongside MUAC.

Clinical Trial Registry number and website where it was obtained. This trial has been registered at www.clinicaltrials.gov/study/NCT03400930 (identifier NCT03400930).

Statement of Significance: Weight gain during treatment was associated with increased leptin and was greatest in children with combined deficits in MUAC and WHZ compared to those with low MUAC alone. Children with low WHZ had higher rates of tachypnea and pneumonia than children with low MUAC alone.

Keywords: severe acute malnutrition; leptin; weight-for-height *Z*-score; mid-upper arm circumference; response to treatment; biomarker.

Introduction

More than 45 million children under age 5 are malnourished (weight-for-height *Z*-score, WHZ <-2 SD) and nearly 13.6 million are severely "wasted" (WHZ <-3 SD); the prevalence is greatest in Africa, Southern Asia, and parts of the Middle East (1,2). Children with severe acute malnutrition (SAM) are predisposed to developmental delays, infectious diseases, and death (2). Medico-nutritional interventions, including use of therapeutic milks and Ready-to-Use Therapeutic Food (RUTF), can support catch-up weight gain, linear growth, and the resolution of edema, but effects on mortality remain unclear (2).

The World Health Organization (WHO) has recommended using WHZ <-3 and mid-upper arm circumference (MUAC) <115 mm as independent anthropometric criteria for admission of children with SAM to therapeutic feeding programs (3–5). WHZ was traditionally used to diagnose children with SAM, but measurements of weight and height of children and assessment of *Z*-scores based on tables or graphs are often challenging in low- and middle-income countries (LMICs), as they require technical and practical experience (6). Therefore, in practice, MUAC is frequently used as a standalone screening and admission criterion (7).

However, adoption of a 115 mm MUAC cut-off as a sole screening tool for SAM, devoid of assessment of WHZ, has important limitations: MUAC varies with age and sex and its use effectively excludes children with low WHZ but MUAC >115 mm from SAM treatment (8–10). Advocates of the use of MUAC as the sole anthropometric criterion for screening and admission contend that children with WHZ <-3 but MUAC >115 mm are at lower risk for morbidity and mortality than children with low MUAC (11). However, a recent investigation by our group (10) showed that malnourished children with low WHZ only at the time of admission to feeding programs have deficits in nutritional status, hydration, and iron balance as or more severe than

57 those in children with low MUAC only, and have lower levels of leptin, a biomarker of mortality
58 risk in SAM (10,12). The objective of this follow-up study was to characterize and compare the
59 changes in weight, MUAC, clinical status, and biochemical markers following medico-
60 nutritional treatment of children with uncomplicated SAM distinguished by three anthropometric
61 phenotypes: (1) both low MUAC and low WHZ (MUAC&WHZ group), (2) low MUAC only
62 (MUAC only group), or (3) low WHZ only (WHZ only group).

63 **Methods**

64 **Study Design and Participants**

65 The OptiDiag study (10) followed a multi-centric design and was conducted in
66 Bangladesh, Burkina Faso, and Liberia. Study clinics were integrated within Community
67 Management of Acute Malnutrition (CMAM) programs at primary health care centers. Children
68 with SAM were identified and referred to outpatient treatment by active and passive screening by
69 community health workers and health staff. Nonedematous children with SAM aged 6-59
70 months without medical complications were recruited into the study at the time of admission to
71 therapeutic feeding programs.

72 Participants were included in the study based on a rhythmic recruitment process in each
73 country: children with SAM were included in consecutive blocks of 12, with 4 patients of each
74 anthropometric phenotype at a time per country. A patient was eligible for recruitment in the
75 ongoing recruitment block only if the quota of 4 patients for this phenotype had not been reached,
76 and each block had to be filled before moving to the next one. In doing this, we ensured an equal
77 representation of anthropometric phenotypes over the one-year implementation period to account
78 for seasonal variability. The first patient was recruited on March 6th, 2017 and the last on June 8th,
79 2018.

Exclusion criteria were: bilateral pitting edema; medical complications requiring inpatient care, including inability to drink or breastfeed, bloody diarrhea, uncontrolled vomiting, convulsions, lethargy, and/or unconsciousness; known peanut and/or milk allergies; congenital malformations affecting food intake; and/or plans to leave the catchment area within the next 6 months. HIV testing was not performed because the prevalence of HIV in the intervention zones was known to be low. Biochemical studies were not performed in the very few recruited children known to be HIV positive.

After enrollment in the therapeutic feeding program, all children with uncomplicated SAM received a standard course of amoxicillin to address minor subclinical infections. Children with malaria were treated following national guidelines (13), typically involving combinations such as artesunate and amodiaquine or artemether and aumefantrine. Participants were asked to return each week until recovery, which was defined as having attained a WHZ $\geq$ -2 for two consecutive visits for children with admission WHZ < -3, and MUAC $\geq$ 125 mm for children with admission MUAC < 115 mm. Patients were expected to meet discharge criteria within 16 weeks of admission. If discharge criteria were not met within 16 weeks, the child was referred for further evaluation and/or inpatient treatment.

The OptiDiag study was registered under clinicaltrials.gov number NCT03400930 and at isrctn.com number ISRCTN50039021 for the cohort in Burkina Faso. Ethical approval was obtained from the ethics committee at the University Hospital of Antwerp and the University of Antwerp; the National Research Ethics Committee at the Bangladesh Medical Research Council; the Institutional Review Board at the University of Liberia; the clinical trials board at the Directorate-General of Pharmacy, Medicines and Laboratories in Burkina Faso; and the Institutional Review Board of the Duke University School of Medicine.

All parents and/or legal guardians of participants were asked to sign an informed consent form and those who were illiterate indicated consent by inked thumbprint in the signature space.

Data collection

Alongside anthropometric measures, blood and urine samples were collected, and clinical examinations were conducted at three visits: admission, around 2 weeks and at a third time point. The third time point differed among countries: in Bangladesh and Liberia the third data collection visit was conducted at 8 weeks; in Burkina Faso, the study was nested into a larger trial – Modelling an Alternative Nutrition Protocol Generalizable for Outpatient (MANGO) – and the third data collection visit occurred on the date of discharge, which ranged from 3 weeks to 16 weeks (median 8 weeks, IQR 6-16 weeks).

Anthropometric measurements (weight, height/length and sitting height) were taken in duplicate and followed standard WHO recommendations for children in this age group (14). WHZ and height-for-age *Z*-scores (HAZ) were calculated using the WHO child growth standards (15).

The primary biochemical parameter of interest was serum leptin, a surrogate measure of white adipose tissue reserves and the most reliable predictive biomarker of mortality in SAM (12). Other biochemical metrics included markers of inflammation [C-reactive protein (CRP) and α-1-acid glycoprotein (AGP)], biomarkers of iron and vitamin A status [serum ferritin, soluble transferrin receptor (sTfR) and body iron stores, retinol-binding protein (RBP)], and urinalyses. Because ferritin and RBP are known to inaccurately reflect nutritional status in the presence of subclinical inflammation, correction factors were calculated to adjust their values (16). The concentrations of AGP, CRP, serum ferritin, sTfR and RBP were measured in duplicate using enzyme-linked immunosorbent assays (ELISA). In instances of significant discrepancies

between duplicate measurements, we conducted a reevaluation, discarding the outlier before calculating the mean values for subsequent analysis.

During clinical examinations, nurses assessed vital signs, including temperature and heart and respiratory rates, and presence or absence of respiratory infection. Dermatoses and brittle or hypopigmented hair are common clinical features of SAM (17). Thus, nurses assessed these signs during clinical examinations. Visible severe wasting, pneumonia, hydration state, and clinical evidence of iron deficiencies were assessed using WHO guidelines (IMCI) and the WHO Training Course on the Management of Severe Malnutrition (18,19). Caretakers were asked to report recent changes in their child's general health.

Study outcomes and covariates

The primary outcome of this study was leptin concentration following treatment for SAM. Secondary outcomes included weight and MUAC gain velocities, the concentrations of biomarkers such as AGP, CRP, sTfR, body iron stores, RBP, and the prevalence of clinical morbidities during treatment. Thresholds for defining abnormal biochemical parameters in blood were: AGP level >1 g/L and CRP level >5 mg/L for inflammation (20). inflammation-adjusted ferritin level <12 µg/L (16,21) and sTfR level >8.3 mg/L (22) for iron deficiency; and adjusted RBP ≤0.7 µmol/L and ≤1.05 µmol/L for vitamin A deficiency and vitamin A insufficiency, respectively (23).

Previous studies showed that age, sex, stunting, and the presence of longer legs, as identified as being in the lower tertile of sitting/standing ratio *Z*-score (SSRZ), may differentially affect the diagnosis of SAM by MUAC and WHZ (24,25). Therefore, age, sex, stunting, and SSRZ were considered as covariates in the analysis. To control for potential confounding caused by multi-centric design, country was added as a covariate in analysis. In addition, biochemical

values upon admission were included in the models as covariates to account for baseline differences.

Statistical Analysis

Our study plan and sample size were designed to compare leptin levels, the primary outcome, among children across the three groups upon admission and throughout the treatment course. We considered an effect size of 0.27 (SD = 4,057 pg/ml) in leptin concentration between groups to be clinically important, given that this difference in leptin levels was accompanied by changes in a broad array of hormones, cytokines, growth factors and metabolites in children with SAM (12). A total sample size of 465 (155 per groups) had a power of 60% to detect the above difference using 95% confidence and with an attrition rate of 20%.

Data handling and analysis were performed using Stata/MP 17.0. Data visualization was conducted in R studio. A two-sided statistical significance was considered at p-value <0.05. Participants' characteristics were summarized as percentages, representing the ratio of participants who tested positive for an indicator to the overall participant count; age was summarized as median and interquartile ranges (IQRs). Our analysis followed a complete cases analysis, including only participants with available information at each clinic visit.

To assess changes in biomarker concentrations across phenotypic groups at each visit, we used mixed-effects linear regression models with the child as a random intercept. These models included fixed effects for phenotypic group, visit, and their interactions, aiming to quantify group differences in biomarker changes over time. We applied log transformation to biomarker concentrations, including leptin, to achieve a normal distribution. Interaction term estimates in the models indicate logarithmic changes in biomarker concentrations from the baseline levels.

For a practical interpretation of these results, we calculated percentage differences in biomarker levels between groups using the formula $(e^{estimate} - 1) \times 100$.

Weight gain velocity (g/kg/d) and MUAC gain velocity (mm/d) were compared among groups using linear regression models. We used linear probability models with robust variance estimation to compare the prevalence of morbidity and abnormal biochemical parameters. This approach enables the estimation of group differences in absolute terms, presented as percentage points (26).

In both unadjusted and adjusted model estimates, country and baseline values of the continuous outcome variable were considered as covariates, whereas adjusted model additionally included age at admission, sex, stunting (HAZ <-2), and SSRZ. In cases where significant overall group differences were found, pairwise comparisons were conducted to identify groups that differed significantly. Pairwise comparisons of phenotypes were adjusted for false discovery rate using the Benjamini-Hochberg method, controlling for multiple comparison.

As a further exploratory analysis, we evaluated the correlation between leptin and anthropometric measurements using Spearman's rank correlation. Additionally, the correlation between changes in biomarkers concentrations and changes in anthropometric measurements were assessed using Pearson product-moment correlation.

Results

Participant Characteristics

From a total of 473 patients recruited into this study, 22 were excluded from analysis because of inclusion error: 4 patients (0.9%) were younger than 6 months at the time of admission, and 18 patients (3.8%) were only moderately malnourished (**Figure 1**). Consequently, 451 patients were analysed at enrollment, comprising 152 (33.7%) children in the MUAC&WHZ

group, 161 (35.7%) children in the MUAC only group, and 138 (30.6%) children in the WHZ only group. Together, 35 patients (7.8%) from all groups were lost to follow-up at the second visit, with 1 death in the MUAC&WHZ group. A total of 123 patients (85.4% of Liberian participants, i.e., 27.3% of total participants) in Liberia lacked follow-up at the third visit owing to unexpected early discharge (i.e., discharge before the designed timepoint, 8 weeks). Otherwise, at the third visit, a total of 46 patients (10.2%) were lost to follow-up, including 1 death in the WHZ only group. Therefore, the analysis at the second visit included 416 patients, while at the third visit, 282 patients were retained. In Burkina Faso, the third visit occurred at a median of 8 weeks (IQR: 6-16 weeks).

Baseline characteristics of study participants are presented in **Table 1**. There were variations in median age, sex, proportion of stunted children and those with longer legs among phenotypic groups. Children in the MUAC only group were younger with higher prevalence of stunting, were more often girls and had a lower proportion of body length in legs compared to children in the WHZ only group. Children in the WHZ only group had higher prevalence of iron deficiency, dehydration, and visible severe wasting than children in the MUAC only group, consistent with our previous findings (10).

Baseline characteristics of participants who attended the third visit (n = 282) and those lost to follow-up (n = 169) are summarized in the **Supplementary Table 1**. While most baseline attributes were similar, notable differences included higher rates of inflammation, cough, sunken eyes, and iron deficiency among children not present for the third visit.

Primary outcome: serum leptin concentration

As illustrated in **Figure 2**, the line plot shows a consistent rise in leptin levels throughout treatment. Mixed-effect models indicated significant differences in both short-term (from

admission to the second visit) and long-term (from admission to the third visit) changes in serum leptin concentration among the three phenotypic groups (p = 0.02, and p = 0.01, respectively). Pairwise comparisons revealed a significantly greater short-term increase in leptin concentration in the MUAC&WHZ group compared with the MUAC only group, with an estimated difference of -0.33 (95% CI: -0.57 to -0.08; p = 0.02). Expressing the adjusted estimate as a percentage difference, the MUAC&WHZ group showed a 34.4% greater increase in leptin levels compared to the MUAC only group at the second visit (95% CI: 7.6% to 43.6%). Similarly, the long-term increase in leptin levels was significantly greater in the MUAC&WHZ group than in the MUAC only group, with an estimated difference of -0.42 (95% CI: -0.70 to -0.14; p = 0.01), translating to a 34.3% higher increase in the MUAC&WHZ group during the third visit (95% CI: 13.2% to 50.3%).

Changes in leptin levels in the WHZ only group fell intermediate between those of the other two groups, but differences did not reach statistical significance. The median (IQR) leptin concentrations are summarized in **Supplementary Table 2**.

Due to missing data from Liberia during the third visit, we performed an analysis with mixed-effect models on the primary outcome (serum leptin) using the complete data from Bangladesh and Liberia (**Supplementary Figure 1**) and conducted a separate analysis on the primary outcome using data from the second visit, excluding missing entries at the third visit (**Supplementary Figure 2**). Similar results were obtained when data from Liberia were excluded from the analysis; likewise, results were similar when the analysis included only subjects with data at all three visits.

Secondary outcomes

At the second visit, the MUAC only group exhibited a lower average weight gain compared to the MUAC&WHZ group [ES (95% CI): -1.56 (-2.75 to -0.38) g/kg/d, $p = 0.03$]; no significant difference was observed at the third visit. MUAC gain velocities were similar across groups at both visits (**Figure 3**). Mean ± SD for weight and MUAC gain velocities are provided in **Supplementary Table 3**.

Overall, no significant differences in biomarkers other than leptin were observed among groups (**Figure 4**). Line plots displayed a progressive decrease in the inflammatory markers AGP and CRP in all groups. There were insignificant trends in biomarkers related to iron status: body iron stores and sTfR trended downward while serum ferritin generally trended higher during the initial treatment. Likewise, RBP, an indicator of vitamin A status, trended higher during the initial treatment phase in all groups. The median (IQR) of biomarker concentrations are summarised in Supplementary Table 2.

The prevalence of inflammation, iron deficiency, vitamin A insufficiency and visible severe wasting remained high among all groups during treatment (**Tables 2**). Most of the morbidity outcomes were comparable among three groups. The major differences at the second visit included higher rates of iron deficiency and severe/extreme wasting in the MUAC&WHZ group compared to the other groups, and a higher rate of leukocyturia in the MUAC&WHZ group compared to the WHZ only group. At the third visit, rates of tachypnea and pneumonia were higher in the MUAC&WHZ and the WHZ only groups than in the MUAC only group. Moreover, the rate of dehydration was higher in the MUAC&WHZ group than in the MUAC only group. Pairwise comparisons confirmed these findings (**Supplementary Table 4 and Supplementary Table 5**).

Correlation of Biomarkers and Anthropometry

Leptin levels among all patients correlated more strongly with WHZ than with MUAC at admission (WHZ $R = 0.32$, $p < 0.001$; MUAC $R = 0.09$, $p = 0.06$), at the second visit (WHZ $R = 0.35$, $p < 0.001$; MUAC $R = 0.21$, $p < 0.001$), and at the third visit (WHZ $R = 0.40$, $p < 0.001$; MUAC $R = 0.23$, $p = 0.0015$; **Figure 5**). The stronger association of leptin with WHZ was confirmed by multivariate regression analyses in which WHZ and MUAC were employed as independent variables and covariates included sex and age at admission.

During the early stages of treatment, gains in weight and MUAC demonstrated similar correlations with changes in serum leptin (weight gain $R = 0.23$; MUAC gain $R = 0.22$). However, during long-term treatment, changes in leptin concentration correlated slightly more strongly with MUAC gain ($R = 0.4$) than with weight gain ($R = 0.32$; **Figure 6**).

Discussion

Advocates for the use of MUAC as the sole anthropometric criterion (without consideration of WHZ) for SAM screening and admission to therapeutic treatment value its simplicity and maintain that it identifies children most vulnerable and in greatest need of nutritional rehabilitation (11). Previous studies and our recent investigations, however, note the failure of MUAC-only screening to identify a large number of malnourished children with low WHZ, whose risks of morbidity and mortality equal or exceed those with low MUAC (10,27–29). Controversy regarding screening is fueled in part by the lack of data characterizing the pathophysiology and functional severity associated with different anthropometric phenotypes of SAM (30). By identifying differences in baseline status and response to treatment among children with low WHZ alone, low MUAC alone, and both low MUAC and low WHZ, we provide novel insights integral to screening deliberations and therapeutic decision making.

Our previous analysis (10) revealed that all children with SAM, regardless of phenotype, presented with clinical evidence of nutritional deprivation and micronutrient deficiencies; children with low WHZ alone displayed deficits in nutritional status, hydration, and iron balance at baseline equal to or more severe than those with low MUAC alone, and had lower levels of leptin, a marker of mortality risk in SAM. Here, we examined longitudinal changes in leptin, weight, MUAC and various other biochemical and clinical parameters in response to treatment.

We present three novel findings. First, leptin levels increased more dramatically during treatment in children with combined deficits in MUAC and WHZ than in children with low MUAC only. Second, short-term weight gain during treatment was greatest in children with combined deficits in MUAC and WHZ than in children with low MUAC alone. Finally, the rates of iron deficiency and wasting were higher in children with combined deficits in MUAC and WHZ at the second visit, while rates of tachypnea and pneumonia were higher in children with low WHZ alone and those with combined deficits in MUAC and WHZ at the third visit.

Levels of the adipocyte hormone leptin increased markedly during nutritional treatment. A previous investigation reported that low levels of leptin associate with, and may predict, mortality in infants and toddlers with SAM prior to and during treatment (12). Studies in children with protein-energy malnutrition have also reported rapid increases in leptin levels during recovery (12,31–33). In the current investigation, a more striking rise in leptin was observed in children with combined deficits in MUAC and WHZ than those with low MUAC alone; the changes in leptin levels in the WHZ only group fell intermediate between those of the other two groups, although the differences among groups failed to reach statistical significance. This finding suggests preferential deposition of fat during nutritional recovery, perhaps as an adaptation to increase potential for survival under conditions of severe stress (12). Indeed,

children with combined deficits in WHZ and MUAC and those with low WHZ alone had higher rates of pneumonia than children with low MUAC alone.

Change in serum levels of leptin during treatment correlated strongly with weight and MUAC gain velocities, the two indicators commonly used to track treatment progress of SAM (34). Weight and MUAC increased in all children during treatment. However, children with combined deficits in MUAC and WHZ exhibited higher rates of weight gain than children with low MUAC alone during the early treatment. This observation is consistent with the results from a study in rural Niger (27). Increases in weight and MUAC during treatment, in parallel with increases in leptin, likely reflect increases in lean body mass as well as fat mass; the rate of recovery appears most striking in those with greatest nutritional deficits at baseline.

We found that the nutritional deprivation and micronutrient deficiencies are only partially corrected by therapeutic refeeding, with persistent elevation of inflammatory markers in 32% of children, iron deficiency (defined as sTfR >8.3 mg/l) in 45%, and vitamin A insufficiency in 58% (with 11% classified as deficient). These deficits did not differ significantly among the three anthropometric phenotypes. In children from LMICs, multiple-micronutrient insufficiencies often coexist with acute and chronic inflammation (20). Previous studies have reported high rates of iron and vitamin A deficiencies and the challenges associated with optimizing their levels among malnourished children (35,36).

Our study has a number of strengths, including comprehensive examinations of both biochemical and clinical profiles, and a multicentric design covering representative countries in West Africa and South Asia. Limitations include the lack of data from Liberia at the third visit, the variations in time of analysis in Burkina Faso, and observed higher rates of morbidities at baseline in those lost to follow up at the third visit. The conclusions drawn from the results

observed at the third visit should be interpreted with caution. Nevertheless, similar results were obtained when data from Liberia were excluded from the analysis; likewise, results were similar when the analysis included only subjects with data at all three visits.

Use of MUAC has important advantages in settings where resources and trained health professionals are scarce (7). Thus, there has been gradual acceptance and promotion of using MUAC alone as a sole anthropometric tool for identifying children requiring therapeutic feeding (7,11,37,38). However, using MUAC as the sole anthropometric criterion for screening and admission has limitations, as it may fail to identify malnourished children with low WHZ and normal (>115 mm) MUAC (10), and its interpretation is complicated by variations in age, sex and stunting (25). Moreover, the notion that MUAC alone identifies children at highest risk has been challenged by previous and recent investigations from our group (10) and others. In a study of severely malnourished children in 18 African countries, children with low WHZ had higher mortality rates than those with low MUAC (8,28,39). Another study showed no differences in clinical and laboratory characteristics or discharge outcomes between children with low MUAC and those with low WHZ only (27). Thus, malnourished children with low WHZ require intensive nutritional assessment and intervention, even if MUAC exceeds 115 mm. Furthermore, results from the present study suggest that highest priority should be assigned to children with combined deficits in MUAC and WHZ, who appear to be at highest risk and in urgent need of intervention.

Given the associations between low WHZ, hypoleptinemia, and co-morbidities including pneumonia and iron deficiency, the comparable responses to treatment of children with low WHZ only and those with low MUAC only, and the higher vulnerability at admission and during treatment in children with combined low MUAC and low WHZ, our previous study (10) and

current findings provide support for retaining WHZ as an independent diagnostic and admission criterion of SAM, alongside MUAC.

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Table 1 Baseline characteristics of study participants.

Characteristics	MUAC&WHZ (*n* = 152)	MUAC only (*n* = 161)	WHZ only (*n* = 138)
Study sites [1]			
Bangladesh (*n* = 142)	32 (45/142)	34 (48/142)	35 (49/142)
Burkina Faso (*n* = 165)	32 (53/165)	39 (64/165)	29 (48/165)
Liberia (*n* = 144)	38 (54/144)	34 (49/144)	28 (41/144)
Age, mo	10.21 (7.36, 16.47)	7.99 (6.71, 11.80)	15.48 (10.55, 25.61)
Male sex	45 (68/152)	33(53/161)	68 (94/138)
HAZ < -2	59 (89/152)	71 (113/161)	43 (59/138)
With longer legs [2]	31 (45/147)	20 (32/159)	51 (70/138)
Biochemical features			
Serum leptin <35 pg/ml	10 (14/142)	3 (5/156)	5 (7/129)
Inflammation			
AGP >1 mg/l	66 (94/142)	56 (87/154)	66 (85/128)
CRP >5 mg/l	32 (45/142)	38 (59/154)	34 (44/128)
AGP >1 mg/l and/or CRP >5 mg/l	69 (98/142)	62 (96/154)	67 (86/128)
Iron deficiency			
Body iron stores <0 mg/kg bw	17 (24/142)	14 (21/154)	25 (32/128)
Adjusted serum ferritin level <12 µg/l	30 (42/142)	32 (49/154)	45 (57/128)
sTfR level >8.3 mg/l	54 (76/142)	57 (88/154)	52 (67/128)
Vitamin A insufficiency or deficiency			
Adjusted RBP level <1.05 µmol/l	58 (83/142)	56 (87/154)	55 (71/128)
Adjusted RBP level <0.7 µmol/l	15 (22/142)	19 (29/154)	20 (25/128)
Urinalysis			
Ascorbic acid excretion, ≥0.2 g/l (20 mg/dl)	53 (49/92)	48 (41/86)	44 (39/88)
Bilirubinuria, ≥15 µmol/l (1mg/dl)	18 (17/92)	6 (5/86)	19 (17/88)
Hematuria, ≥5-10 erythrocytes per µl	17 (15/90)	19 (16/86)	9 (8/88)
Glycosuria, ≥2.8 mol/l (50 mg/dl)	3 (3/91)	1(1/85)	1 (1/88)
Ketonuria, ≥1 mmol/l (10mg/dl)	2 (2/92)	2 (2/84)	7 (6/88)
Leukocyturia, ≥25 leukocytes per µl	30 (28/92)	22 (19/85)	14 (12/88)
Nitrituria	31 (28/91)	40 (34/86)	25 (22/88)
Proteinuria	10 (9/92)	8 (7/85)	8 (7/88)
Urobilinogenuria, ≥35 µmol/l (2 mg/dl)	1 (1/92)	1 (1/86)	3 (3/88)
Clinical features			
Cough or difficult breathing			
Cough/difficult breathing	57 (86/152)	45 (73/161)	48 (66/138)
Nasal discharge	37 (56/152)	28 (45/161)	31 (43/137)
Tachypnea	25 (38/152)	21 (34/161)	17 (24/138)
Subcostal indrawing	2 (3/152)	1 (2/161)	2 (3/138)
Stridor	3 (4/152)	4 (7/161)	1 (2/138)
WHO IMCI: pneumonia or severe pneumonia	28 (43/152)	25 (40/161)	20 (28/138)
Dehydration			
Slow or very slow skin pinch	13 (19/150)	2 (4/161)	11 (15/138)
Sunken eyes	20 (30/152)	9 (15/161)	22 (30/138)
Restlessness and/or irritability	11 (16/152)	2 (3/161)	6 (8/138)
WHO IMCI: some or severe dehydration	13 (20/150)	4 (6/160)	12 (17/138)
Dermatosis	18 (27/152)	7 (12/161)	9 (12/138)
Hair change	4 (6/150)	7 (11/157)	9 (13/138)
Iron deficiency			
Conjunctival and/or palmar pallor	41 (62/151)	27 (43/161)	34 (47/138)
Fever	18 (28/152)	18 (29/161)	15 (21/138)
Malaria	14 (20/148)	21 (33/157)	8 (11/136)
Visible severe wasting			
Visible front ribs	49 (75/152)	32 (52/161)	43 (60/138)
Loose skin on arms or thighs	22 (33/152)	8 (13/161)	14 (19/138)
Visible back ribs or shoulders	45 (68/152)	28 (45/161)	38 (51/138)
Flesh missing or folds of skin on buttocks	11 (17/152)	2 (3/161)	4 (5/138)
WHO IMCI: severe or extreme wasting	50 (76/152)	35 (56/161)	43 (60/138)
Recent health histories, caretaker reported			
Diarrhea	31 (40/130)	25(33/134)	26 (31/117)
Vomiting	26 (34/129)	19 (25/133)	16 (18/114)
Fever	70 (89/128)	59 (78/132)	64 (74/115)
Cough and/or difficult breathing	55 (71/130)	52 (70/134)	53 (62/117)

Values are percentage (%) (n/N, n is the number of patients positive for the indicator; N is the total number of patients received test.) for binary variables and median (P25, P75) for age.

[1] Percentage in anthropometric phenotypes reflect proportion of each phenotype per country. [2] Lowest tertial of a low sitting/standing ratio *Z*-score (SSRZ). HAZ: height-for-age *Z*-score; AGP: α-1-acid glycoprotein; CRP: C-reactive protein; sTfR: soluble transferrin receptor; RBP: retinol-binding protein; IMCI: integrated management of childhood illness; MUAC, mid-upper arm circumference; WHZ, weight-for-height *Z*-score.

Table 2 Clinical and biochemical profiles of participants by anthropometric phenotypes

	At the second visit				At the third visit			
	MUAC&WHZ (*n* = 137)	MUAC only (*n* = 151)	WHZ only (*n* = 128)	*p*	MUAC&WHZ (*n* = 90)	MUAC only (*n* = 103)	WHZ only (*n* = 89)	*p*
SAM, %	27 (37/137)	30 (46/151)	27 (35/128)	0.67	11 (10/90)	22 (23/103)	22 (20/89)	0.047
Biochemical features								
Serum leptin <35 pg/ml	2 (2/125)	0 (0/135)	0 (0/109)	0.36	0 (0/76)	0 (0/96)	0 (0/80)	-
Inflammation								
AGP >1 mg/l	39 (46/119)	33 (44/134)	34 (37/110)	0.61	24 (19/78)	31 (30/98)	24 (20/83)	0.53
CRP >5 mg/l	18 (22/119)	21 (28/134)	15 (16/110)	0.48	17 (13/78)	24 (24/98)	17 (14/83)	0.37
AGP >1 mg/l and/or CRP >5 mg/l	39 (47/119)	39 (52/134)	36 (40/110)	0.99	27 (21/78)	39 (38/98)	29 (24/83)	0.22
Iron deficiency								
Body iron stores <0 mg/kg bw	20 (24/119)	19 (25/134)	25 (28/110)	0.24	15 (12/78)	15 (15/98)	16 (13/83)	0.97
Adjusted serum ferritin level <12 µg/l	27 (32/119)	24 (32/134)	31 (34/110)	0.41	27 (21/78)	31 (30/98)	29 (24/83)	0.87
sTfR level >8.3 mg/l	60 (71/119)	61 (82/134)	46 (51/110)	0.12	41 (32/78)	53 (52/98)	39 (32/83)	0.22
Vitamin A insufficiency or deficiency								
Adjusted RBP level <1.05 µmol/l	45 (54/119)	51 (69/134)	37 (41/110)	0.13	59 (46/78)	61 (60/98)	54 (45/83)	0.80
Adjusted RBP level <0.7 µmol/l	4 (5/119)	9 (12/134)	5 (6/110)	0.32	15 (12/78)	9 (9/98)	10 (8/83)	0.32
Urinalysis								
Ascorbic acid excretion, ≥0.2 g/l (20 mg/dl)	63 (54/86)	63 (55/88)	56 (48/96)	0.60	56 (27/48)	51 (26/51)	61 (35/57)	0.55
Bilirubinuria, ≥15 µmol/l (1mg/dl)	8 (7/86)	7 (6/88)	6 (5/85)	0.84	8 (4/48)	4 (2/51)	11 (6/57)	0.36
Hematuria, ≥5-10 erythrocytes per µl	14 (12/85)	18 (16/88)	9 (8/86)	0.54	10 (5/48)	12 (6/50)	11 (6/56)	0.98
Glycosuria, ≥2.8 mol/l (50 mg/dl)	2 (2/86)	2 (2/88)	6 (5/85)	0.54	2 (1/48)	4 (2/50)	2 (1/56)	0.82
Ketonuria, ≥1 mmol/l (10mg/dl)	2 (2/85)	3 (3/88)	6 (5/85)	0.53	0 (0/48)	2 (1/51)	2 (1/57)	0.38
Leukocyturia, ≥25 leukocytes per µl	22 (19/86)	17 (15/88)	8 (7/86)	0.02	13 (6/48)	20 (10/51)	14 (8/57)	0.65
Nitrituria, nitrites present in urine	31 (27/86)	34 (30/88)	21 (18/86)	0.24	29 (14/48)	31 (16/51)	28 (16/57)	0.98
Proteinuria, protein present in urine	5 (4/86)	1 (1/88)	8 (7/86)	0.07	4 (2/48)	2 (1/51)	5 (3/57)	0.60
Urobilinogenuria, ≥35 µmol/l (2 mg/dl)	1 (1/86)	1 (1/88)	0 (0/86)	0.37	2 (1/47)	4 (2/51)	0 (0/57)	0.22
Clinical features								
Cough or difficult breathing								
Cough/difficult breathing	47 (47/101)	41 (43/106)	40 (35/88)	0.69	37 (17/46)	44 (21/48)	41 (16/39)	0.99
Nasal discharge	23 (29/127)	15 (21/137)	21 (25/121)	0.31	15 (11/73)	24 (18/75)	18 (13/71)	0.49
Tachypnea	25 (32/129)	17 (23/136)	18 (22/122)	0.23	20 (15/74)	5 (4/75)	19 (14/73)	0.003
Subcostal indrawing	0 (0/83)	1 (1/91)	0 (0/73)	0.61	3 (1/35)	0 (0/33)	0 (0/28)	0.53
WHO IMCI: pneumonia or severe pneumonia	27 (35/129)	19 (26/137)	20 (24/122)	0.19	24 (18/74)	7 (5/75)	19 (14/72)	0.002
Dehydration								
Slow or very slow skin pinch	2 (2/127)	2 (3/136)	2 (3/122)	0.94	4 (3/74)	3 (2/74)	0 (0/71)	0.08
Sunken eyes	8 (10/128)	2 (3/135)	8 (10/122)	0.03	0 (0/28)	0 (0/24)	0 (0/20)	-
Restlessness and/or irritability	4 (5/127)	1 (2/135)	4 (5/122)	0.36	8 (6/74)	0 (0/74)	1 (1/72)	0.03
WHO IMCI: some or severe dehydration	1 (1/126)	1 (1/137)	4 (5/122)	0.09	0 (0/74)	1 (1/75)	0 (0/72)	0.61
Dermatosis	6 (8/128)	6 (8/137)	3 (4/122)	0.56	4 (3/74)	3 (2/75)	6 (4/72)	0.63
Hair change	4 (5/127)	2 (3/135)	2 (2/120)	0.59	1 (1/71)	1(1/74)	3 (2/70)	0.78
Iron deficiency								
Conjunctival and/or palmar pallor	32 (41/127)	16 (22/135)	20 (24/120)	0.005	16 (12/73)	14(10/74)	15 (11/71)	0.81
Fever	10 (13/129)	10 (14/137)	7 (9/122)	0.59	15 (11/74)	9 (7/75)	12 (9/73)	0.65
Malaria	20 (17/86)	9 (7/82)	9 (7/74)	0.05	20 (11/56)	10 (5/49)	11 (6/53)	0.60
Visible severe wasting								
Visible front ribs	41 (45/109)	25 (28/110)	37 (39/105)	0.11	47 (28/60)	40 (21/53)	45 (25/55)	0.40
Loose skin on arms or thighs	10 (10/103)	3 (3/107)	7 (7/104)	0.29	5 (3/59)	4 (2/53)	7 (4/55)	0.83
Visible back ribs or shoulders	38 (41/109)	19 (21/108)	31 (32/102)	0.06	33 (19/58)	25 (13/52)	34 (18/53)	0.69
Flesh missing or folds of skin on buttocks	4 (4/103)	0 (0/107)	0 (0/100)	0.12	2 (1/57)	0 (0/52)	0 (0/53)	0.58
WHO IMCI: severe or extreme wasting	45 (49/110)	29 (32/112)	38 (40/106)	0.02	49 (29/59)	40 (21/53)	49 (27/55)	0.13
Recent health histories, caretaker reported								
Diarrhea	19 (18/97)	21 (20/96)	12 (10/82)	0.47	23 (13/56)	24 (14/59)	14 (9/63)	0.22
Vomiting	13 (13/97)	12 (12/97)	10 (8/82)	0.64	11 (6/55)	17 (10/59)	13 (8/63)	0.64
Fever	49 (48/97)	37 (36/97)	33 (27/82)	0.07	54 (30/56)	46 (27/59)	38 (24/63)	0.26
Cough and/or difficult breathing	51 (49/97)	40 (39/97)	38 (31/81)	0.25	30 (17/56)	42 (25/59)	35 (22/63)	0.44

Values are percentage (%) (*n*/*N*, *n* is the number of patients positive for the indicator; *N* is the total number of patients received test.) -, not applicable
Group comparison by linear probability model with robust variance estimates and with age, sex, stunting, SSRZ and country as covariates.
SAM: severe acute malnutrition; AGP: α-1-acid glycoprotein; CRP: C-reactive protein; sTfR: soluble transferrin receptor; RBP: retinol-binding protein; IMCI: integrated management of childhood illness; MUAC, mid-upper arm circumference; WHZ, weight-for-height *Z*-score; SSRZ, sitting/standing ratio *Z*-score.

Figure Legend

Figure 1: Flow diagram of recruitment, enrollment, and follow-up of children who participated in OptiDiag study. MUAC, mid-upper arm circumference; WHZ, weight-for-height *Z*-score.

Figure 2: **Trend of change in log-transformed leptin levels (pg/ml). A.** The *p*-values report the level of significant difference for overall comparison of short-term (i.e., from admission to the second visit, $n = 416$) followed by long-term (i.e., from admission to the third visit, $n = 282$) changes. **B**. Pairwise comparison with adjusted estimates and *p*-values report the difference between the MUAC group and the MUAC&WHZ group estimated using mixed-effects linear regression model with random intercept child. The adjusted estimate was controlled for the covariates age, sex, stunting, SSRZ, country and the baseline value of leptin. Correction for false discovery rate were applied using the Benjamini-Hochberg method. ES: regression coefficient estimates; CI: confidence interval; MUAC, mid-upper arm circumference; WHZ, weight-for-height *Z*-score; SSRZ, sitting/standing ratio *Z*-score.

Figure 3: Weight gain velocity (g/kg/d) and MUAC gain velocity (mm/d) over the second ($n = 416$) and third visit ($n = 282$). The *p*-values at left top report the level of significant difference for overall comparison. The pairwise comparison with adjusted estimates and *p*-value report the difference between the MUAC group and the MUAC&WHZ group estimated using linear regression model controlled for covariates age, sex, stunting, SSRZ, country and the baseline values of weight or MUAC. Correction for false discovery rate were applied using the Benjamini-Hochberg method. ES: regression coefficient estimates; CI: confidence interval; MUAC, mid-upper arm circumference; WHZ, weight-for-height *Z*-score; SSRZ, sitting/standing ratio *Z*-score.

Figure 4: Trend of change in log-transformed biomarkers over the second (*n* = 416) and third visit (*n* = 282). The *p*-values report the level of significant difference for overall comparison using mixed effect models controlled for covariates age, sex, stunting, SSRZ, country and baseline biomarker values. Ferritin and RBP levels were corrected for inflammation. AGP: α-1-acid glycoprotein; CRP: C-reactive protein; BIS: body iron stores; sTfR: soluble transferrin receptor; RBP: retinol-binding protein; MUAC, mid-upper arm circumference; WHZ, weight-for-height *Z*-score; SSRZ, sitting/standing ratio *Z*-score.

Figure 5: Serum leptin (pg/ml) association with WHZ and MUAC (mm) at various visits. Solid lines depict linear regression with standard errors. *R* values represent Spearman's rank correlation. ES (95%CI) and *p*-values reflect multivariate regression with covariates sex and age. *R*: correlation coefficient; ES: regression coefficient estimates; CI: confidence interval; MUAC: mid-upper arm circumference; WHZ: weight-for-height *Z*-score.

Figure 6: Correlation of changes in biomarker concentrations and gains in weight and MUAC. Short-term changes indicate the changes from admission to the second visit. Long-term changes indicate the changes from admission to the third visit. The values report the correlation coefficients by Pearson. The red colors indicate negative correlation and the blue indicate positive correlation. The symbols × indicate insignificant correlations. Ferritin and RBP levels were corrected for inflammation. AGP: α-1-acid glycoprotein; CRP: C-reactive protein; BIS: body iron stores; sTfR: soluble transferrin receptor; RBP: retinol-binding protein; MUAC, mid-upper arm circumference.