Severe Acute Malnutrition in Childhood: Hormonal and Metabolic Status at Presentation, Response to Treatment, and Predictors of Mortality

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Objective: Malnutrition is a major cause of childhood morbidity and mortality. To identify and target those at highest risk, there is a critical need to characterize biomarkers that predict complications prior to and during treatment.

Methods: We used targeted and nontargeted metabolomic analysis to characterize changes in a broad array of hormones, cytokines, growth factors, and metabolites during treatment of severe childhood malnutrition. Children aged 6 months to 5 years were studied at presentation to Mulago Hospital and during inpatient therapy with milk-based formulas and outpatient supplementation with ready-to-use food. We assessed the relationship between baseline hormone and metabolite levels and subsequent mortality.

Results: Seventy-seven patients were enrolled in the study; a subset was followed up from inpatient treatment to the outpatient clinic. Inpatient and outpatient therapies increased weight/height z scores and induced striking changes in the levels of fatty acids, amino acids, acylcarnitines, inflammatory cytokines, and various hormones including leptin, insulin, GH, ghrelin, cortisol, IGF-I, glucagon-like peptide-1, and peptide YY. A total of 12.2% of the patients died during hospitalization; the major biochemical factor predicting mortality was a low level of leptin ($P = .0002$), a marker of adipose tissue reserve and a critical modulator of immune function.

Conclusions: We have used metabolomic analysis to provide a comprehensive hormonal and metabolic profile of severely malnourished children at presentation and during nutritional rehabilitation. Our findings suggest that fatty acid metabolism plays a central role in the adaptation to acute malnutrition and that low levels of the adipose tissue hormone leptin associate with, and may predict, mortality prior to and during treatment. **(*J Clin Endocrinol Metab* 99: 2128–2137, 2014)**

Nearly 8 million of the world's children under the age of 5 years die every year; undernutrition is the underlying factor in 35% of these cases (1–3). Rates of death from diarrhea, pneumonia, measles, and malaria are increased greatly in malnourished children (4); infants and toddlers remain at high risk despite recent advances including micronutrient supplementation and the use of ready-to-use therapeutic food (RUTF) (5–12). Malnutrition in childhood is associated with a decreased adult height and chronic disease that may limit weight gain dur-

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Abbreviations: ALT, alanine aminotransferase; CRP, C-reactive protein; GI, gastrointestinal; GLP-1, glucagon-like peptide-1; GM-CSF, granulocyte macrophage colony-stimulating factor; HMW, high molecular weight; MUAC, mid-upper arm circumference; NEFA, nonesterified fatty acid; PYY, peptide YY; RUTF, ready-to-use therapeutic food; WHO, World Health Organization; W/H z, weight for height z score.

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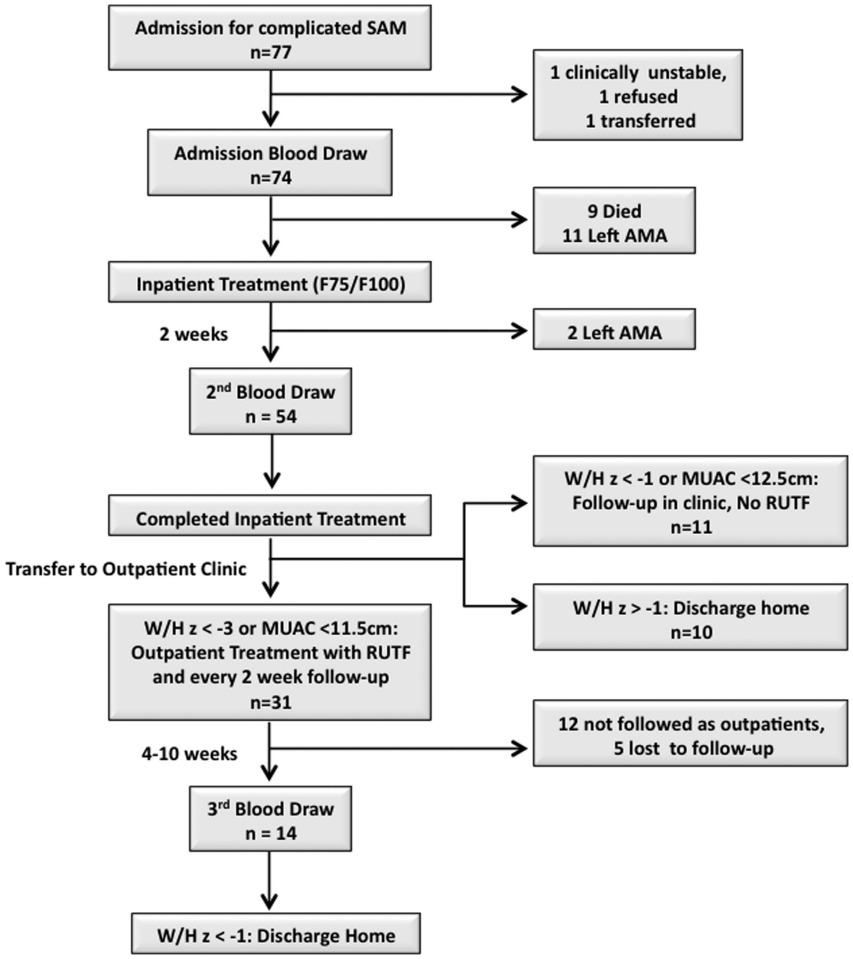

Figure 1. Diagnosis and treatment of severe acute malnutrition in Mwanamugimu Nutrition Unit. AMA, against medical advice.

ing pregnancy and impair the growth of the developing fetus; in females, this may create a vicious intergenerational cycle (13).

Classic studies by Taylor and Keys (14), Cahill (15), Waterlow and Alleyne (16, 17), Whitehead and colleagues (18, 19), Badaloo and colleagues (20, 21), and Manary et al (22, 23) analyzed the levels of selected hormones and metabolites in malnourished children and healthy adults subjected to prolonged fasting. Yet the pathogenesis of acute malnutrition remains poorly understood. In this study we used targeted and nontargeted metabolomic analysis to characterize changes in a broad array of hormones, growth factors, cytokines, and metabolites during nutritional therapy of malnourished infants and children. We hypothesized that hormonal and metabolic factors measured at baseline would associate with, or predict, subsequent mortality during treatment.

Materials and Methods

Study cohort

The study was conducted at Mwanamugimu Nutrition Unit at Mulago Hospital (Kampala, Uganda). All children aged 6 months to 5 years referred to the unit for severe acute malnutrition were eligible for enrollment. Referrals came from the Mulago pediatric acute ward and community clinics. Severe acute malnutrition was defined by one of three criteria: 1) weight-for-height z-score (W/H z) less than −3 according to World Health Organization (WHO) growth standards; 2) mid-upper arm circumference (MUAC) less than 110 mm; and/or 3) the concurrence of edema and malnutrition. We excluded patients who had previously received blood transfusions or who were deemed too unstable for additional blood draws. Written consent to participate in the study was obtained from all guardians. Each patient received an insecticide-treated bed net at enrollment. As compensation for participation, the caregivers received transportation money at the time of patient discharge or death.

At presentation, children were screened for medical complications and triaged to either the inpatient facility or the outpatient clinic (Figure 1). Admission triage was performed according to the Mwanamugimu Nutrition Unit's protocols, which follow Uganda Ministry of Health's guidelines (24). A medical and limited diet history, sociodemographic profile, and physical examination were completed at enrollment. Diet histories focused on current or previous breast-feeding and intake of fruits, vegetables, and protein. Malaria smears were performed on all study subjects; patients with positive smears were treated. HIV status was assessed with a HIV rapid antibody test (Determine; Abbott) in patients older than 18 months and by HIV DNA PCR (AMPLICOR HIV-1 Monitor Test 1.5; Roche) in patients younger than 18 months.

Study interventions

Inpatient nutritional rehabilitation was carried out by medical house officers according to Mwanamugimu Nutrition Unit protocols, which follow WHO guidelines for the inpatient treatment of severely malnourished children [Supplemental Appendix (25)]. All children received equivalent care, regardless of participation in the study. Inpatient therapy was administered in two phases: an initial stabilization phase during which acute medical conditions are managed and a longer rehabilitation phase. The first phase included bolus-feed therapy with milk-based liquid formula (F75) containing 75 kcal/100 mL and 0.9 g protein per 100 mL (total daily caloric intake 97 kcal/kg · d). A standardized packet of micronutrients was added to feeds to correct deficiencies of vitamin A, folic acid, zinc, and iron. All patients received treatment for parasitic infection and antibiotics according to WHO guidelines [Supplemental Appendix (25)]. When the clinical condition and appetite improved, children were advanced to the second phase, which consisted of therapy with milk-based liquid (F100) containing 100 kcal/100 mL and 2.9 g protein per 100 mL (total daily caloric intake 130 kcal/kg · d).

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Weights, feeding regimen, and pertinent events were recorded daily. Patients were followed up from the time of enrollment until death or discharge from the inpatient unit. Discharged patients were transferred to the outpatient RUTF clinic if they were free of edema and medically stable but still had W/H z less than −3 or MUAC less than 110 mm. If the W/H z was greater than −3 but still less than −1, they were transferred to the outpatient Moderate Acute Malnutrition Clinic. If the W/H z was greater than −1, patients were discharged home.

A subset of patients was followed up from inpatient hospitalization through treatment in the outpatient RUTF clinic. Outpatient nutritional rehabilitation using RUTF was administered according to Mwanamugimu Nutrition Unit protocols (24). Children received 2-week rations of RUTF in quantities that provided 175 kcal/kg · d. Caretakers and children then returned to the clinic for reassessment every 2 weeks. Weight and length were measured at each visit, and the caretaker was asked about intercurrent illnesses. If children still met criteria for treatment, they received additional 2-week rations of RUTF. Patients were discharged as cured if the child had spent a minimum of 4 weeks in the program, had W/H z greater than −1 for two consecutive visits, and appeared clinically well.

Blood sampling and analysis

Blood samples (maximum 5 mL) were collected at time of enrollment (within 24 h of admission). A second blood sample was collected after 2 weeks of inpatient treatment or at the time of discharge from the inpatient unit, whichever occurred first. In children followed up at the outpatient clinic, a third blood sample was collected after 4–10 weeks of outpatient treatment. Aprotinin (500 KIU/mL of blood; Sigma-Aldrich) was added to prevent enzymatic protein degradation. Blood samples were collected on ice and processed promptly; EDTA plasma was stored at −70°C. All samples were then shipped in bulk to Duke University's Stedman Nutrition Center for analysis. Detailed methods describing the metabolic and hormonal analyses are presented in the Supplemental Appendix.

Statistical analysis

Sample size was based on commonly reported concentrations and variability of classical hormones (insulin, GH, cortisol) in infants and children. The unit of observation was the child. We used standard summary statistics (mean ± SD and counts) to describe the study variables. We evaluated pretreatment anthropometric variables and biomarkers as well as their absolute change during the study treatment. We compared distributions of anthropometric variables and biomarkers in patients with and without the outcomes of interest using the nonparametric Wilcoxon rank-sum test and performed univariable logistic and linear regression to evaluate the association between anthropometric variables and biomarker levels and the outcomes of interest. We performed a multivariable logistic regression controlling for HIV status and admission W/H z to evaluate the association between biomarker levels and mortality. Changes in anthropometric variables and biomarker levels before and after inpatient treatment and before and after outpatient treatment were compared separately using the nonparametric Wilcoxon signed-rank test. All analyses were conducted using Stata 12 (Stata Corp); a two-sided value of $P < .05$ was considered statistically significant for all tests.

Table 1. Patient Characteristics at Presentation

Variable	Mean ± SD
Age, mo	16.3 ± 8.9
W/H z (nonedematous patients)	−4.2 ± 1.4
Weight z-score (nonedematous patients)	−4.8 ± 1.5
Length z-score (all patients)	−3.2 ± 1.5
Days of treatment	25 ± 10
	Number, %
Male sex	43/75 (57.3)
HIV positive	18/75 (24.0)
Edema present	42/74 (56.8)
Malaria positive	7/74 (9.5)
Mortality	9/74 (12.2)

Informed consent

The study was approved by the Duke University Institutional Review Board, Makerere University School of Public Health Institutional Review Board, and the Uganda National Council for Science and Technology. All guardians signed an informed consent in English or Luganda.

Role of the funding agency

Sponsors of the study assisted with data interpretation but had no role in study design, data collection, or data analysis.

Results

Study population

Seventy-seven patients were referred to the Mwanamugimu Inpatient Nutrition Unit and screened for enrollment into the study between December 2010 and March 2011. Of the 77 inpatients, one patient was deemed clinically unstable by the medical house officer for extra blood draws and one patient refused to participate. Another inpatient was transferred from the ward soon after age and sex were recorded and blood drawn. Therefore, 75 patients had known HIV status, 74 had complete admission anthropometry, and 74 completed malaria screening.

The patient population is described in Table 1. Edema-free inpatients had a mean initial weight 5.6 ± 1.5 kg (mean ± SD), W/H z −4.2 ± 1.4, weight z −4.8 ± 1.5, and MUAC 99 ± 12 mm. Average length z for all patients was −3.2 ± 1.5. Of those who successfully completed inpatient treatment (53 of 74), average length of stay on the ward was 24 ± 10 days and discharge W/H z was −2.1 ± 1.8. Overall mortality was 12.2%. Of the surviving patients, 81.5% were followed until discharge from the ward, and 18.5% left the ward against medical advice before achieving nutritional stability.

Metabolic status at time of enrollment

Metabolomic profiles at baseline (Table 2 and Supplemental Tables 1 and 2) showed free fatty acidemia,

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Table 2. Laboratory Measurements and Changes Over the Course of Treatment

Measurement	Inpatient Baseline (a) (n = 62)	Inpatient (After 2 wk of F75/F100) (b) (n = 54)	Wilcoxon *P* Value (a vs b)	Subsequent Outpatient (After 4–10 wk of RUTF) (c) (n = 14)	Wilcoxon *P* Value (b vs c)
Anthropometric data (n = 75)					
W/H z (nonedematous)	−4.2 ± 1.4	−2.6 ± 1.9	**.012**	−1.6 ± 1.9	**.002**
Weight z-score (nonedematous)	−4.8 ± 1.5	−3.6 ± 1.8	**.040**	−2.5 ± 1.5	**.001**
Length z-score	−3.2 ± 1.5	n/a	n/a	n/a	n/a
Fatty acid metabolites					
NEFAs, mmol/L	0.56 ± 0.41	0.34 ± 0.24	**.0037**	0.26 ± 0.21	.778
Total ketones, μmol/L	528 ± 774	153 ± 341	**.0016**	65 ± 93	.975
Hormones					
Insulin, μIU/mL	2.28 ± 2.78	3.59 ± 3.70	**.031**	5.44 ± 5.51	.683
IGF-1, ng/mL	10.39 ± 13.51	27.57 ± 23.73	**<.0001**	61.00 ± 49.96	**.019**
Leptin, pg/mL	248.87 ± 321.48	744.80 ± 1188.10	**.0001**	1860.31 ± 4057.11	.221
GH, ng/mL	11.35 ± 9.41	9.02 ± 9.86	**.025**	3.74 ± 4.44	.300
Cortisol, μg/dL	48.03 ± 17.49	38.94 ± 20.31	**.002**	22.00 ± 20.02	.221
Ghrelin, pg/mL	3920 ± 2183	2608 ± 1987	**<.0001**	1499 ± 1000	**.036**
GLP-1, pg/mL	104.47 ± 86.82	97.20 ± 103.43	.554	26.31 ± 16.27	**.011**
PYY, pg/mL	1200.0 ± 686.8	978.9 ± 505.2	.126	376.9 ± 175.1	**.001**
Adipocytokines					
Total adiponectin, ng/mL	14 002 ± 7596	19 259 ± 8953	**<.0001**	14 664 ± 3860	.397
HMW adiponectin, ng/mL	8479 ± 5141	13 679 ± 8205	**<.0001**	7385 ± 2928	**.013**
MCP-1, pg/mL	917 ± 1209	1113 ± 938	**.002**	654 ± 267	.198
PAI-1, pg/mL	208 816 ± 192325	159 168 ± 117446	.181	207 601 ± 161433	.683
Inflammatory cytokines					
GM-CSF, pg/mL	11.2 ± 39.7	4.3 ± 12.3	**.011**	11.8 ± 33.3	.660
IFN-γ pg/mL	12.7 ± 30.6	14.1 ± 17.3	.213	5.9 ± 4.3	**.048**
IL-10, pg/mL	33.1 ± 38.8	67.9 ± 180.1	.902	11.5 ± 9.3	**.048**
IL-12p70, pg/mL	10.5 ± 35.3	41.7 ± 203.4	.442	3.1 ± 2.6	.221
IL-1β, pg/mL	1.4 ± 5.0	0.9 ± 1.6	.125	1.6 ± 4.3	.594
IL-2, pg/mL	4.7 ± 9.0	1.9 ± 1.6	.148	3.1 ± 3.7	.084
IL-6, pg/mL	43.9 ± 151.5	7.0 ± 16.7	**.003**	4.9 ± 6.8	.331
IL-8, pg/mL	133.1 ± 419.6	39.5 ± 27.0	.208	45.6 ± 72.9	.272
TNF-α, pg/mL	38.8 ± 56.7	32.9 ± 27.9	.985	46.7 ± 66.8	.683
Amino acids					
Molar sum, μmol/L	1200 ± 323	1927 ± 518	**<.0001**	1820 ± 345	.433
Glycine, μmol/L	237 ± 85	306 ± 88	**<.0001**	288 ± 44	.925
Alanine, μmol/L	200 ± 112	416 ± 181	**<.0001**	372 ± 112	.140
Serine, μmol/L	109 ± 38	151 ± 47	**<.0001**	123 ± 22	.140
Proline, μmol/L	153 ± 57	284 ± 134	**<.0001**	180 ± 62	**.026**
Valine, μmol/L	82 ± 44	148 ± 67	**<.0001**	151 ± 47	.875
Leucine/isoleucine, μmol/L	73 ± 36	128 ± 48	**<.0001**	140 ± 46	.975
Methionine, μmol/L	16 ± 7	24 ± 11	**<.0001**	22 ± 8	.470
Histidine, μmol/L	27 ± 29	51 ± 18	.795	53 ± 12	.198
Phenylalanine, μmol/L	52 ± 34	54 ± 17	.820	59 ± 20	.975
Tyrosine, μmol/L	25 ± 18	51 ± 33	**<.0001**	62 ± 30	.177
Aspartic acid, μmol/L	37 ± 19	51 ± 33	**<.0001**	91 ± 25	**.002**
Glutamic acid, μmol/L	96 ± 42	149 ± 55	**<.0001**	141 ± 30	.300
Ornithine, μmol/L	27 ± 14	46 ± 22	**<.0001**	59 ± 22	.140
Citrulline, μmol/L	8 ± 5	19 ± 11	**<.0001**	25 ± 8	.124
Arginine, μmol/L	28 ± 13	49 ± 22	**<.0001**	48 ± 17	.638

(*Continued*)

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Table 2. Continued

Measurement	Inpatient Baseline (a) (n = 62)	Inpatient (After 2 wk of F75/F100) (b) (n = 54)	Wilcoxon *P* Value (a vs b)	Subsequent Outpatient (After 4–10 wk of RUTF) (c) (n = 14)	Wilcoxon *P* Value (b vs c)
Other					
Glucose, mg/dL	83 ± 28	77 ± 14	.069	78 ± 18	.615
CRP, mg/L	36.3 ± 46.4	10.1 ± 24.0	**<.0001**	5.5 ± 7.0	.783
Albumin, g/dL	2.0 ± 0.8	2.5 ± 0.8	**<.0001**	3.2 ± 0.5	**.004**
Triglycerides, mg/dL	137 ± 77	127 ± 66	.844	99 ± 56	**.01**
BUN, mg/dL	7.7 ± 6.7	9.3 ± 6.7	.821	8.2 ± 3.8	.306
Creatinine, mg/dL	0.28 ± 0.18	0.34 ± 0.39	.066	0.25 ± 0.07	.209
Phosphorus	3.23 ± 1.11	4.47 ± 0.86	**<.001**	5.52 ± 1.56	**.003**
ALT, IU/L	49.5 ± 82.9	33.4 ± 23.6	.367	14.0 ± 5.4	**.002**
Lactate, mmol/L	2.6 ± 1.2	2.7 ± 1.1	.972	3.1 ± 1.2	.638

Abbreviations: (a), inpatient baseline; (b), inpatient (after 2 wk of F75/F100); BUN, blood urea nitrogen; (c), subsequent outpatient (after 4–10 wk of RUTF); IFN, interferon; MCP-1, macrophage chemoattractant protein-1; n/a, not available; PAI-1, plasminogen activator inhibitor-1. Values represent means ± SD. Bold print means significantly different than preceding value.

ketonemia, high levels of even-chain acylcarnitines, hypoalbuminemia (2.0 ± 0.8 g/dL), and hypoaminoacidemia. Blood glucose (83 ± 28 mg/dL, mean ± SD), lactate (2.6 ± 1.2 mmol/L), and creatinine (0.28 ± 0.18 mg/dL) were normal, but phosphorus was low (3.23 ± 1.11 mg/dL); triglycerides (137 ± 77), alanine aminotransferase (ALT; 49.5 ± 82.9 IU/L), and several inflammatory markers including C-reactive protein (CRP), IL-6, and granulocyte macrophage colony-stimulating factor (GM-CSF) were elevated. Although variable at baseline, the mean levels of insulin (2.28 ± 2.78 μIU/mL), IGF-I (10.39 ± 13.51 ng/mL), and leptin (248.87 ± 321.48 pg/mL) were low, whereas GH (11.35 ± 9.41 ng/mL), cortisol (48.03 ± 17.49 μg/dL), ghrelin (3920 ± 2183 pg/mL), glucagon-like peptide-1 (GLP-1; 104.47 ± 86.82 pg/mL), and peptide YY (PYY; 1200.0 ± 686.8 pg/mL) were high (compare levels in references 26–29).

Response to inpatient formula feeding

After 2 weeks of inpatient treatment with F75 and/or F100, the average W/H z in nonedematous patients increased from −4.2 to −2.6 ($P = .012$) (Supplemental Figure 1). There were striking changes in a number of metabolites assayed by conventional clinical chemistries and by targeted metabolomics (Table 2, Supplemental Table 1, and Figure 2). Phosphorus and albumin levels rose and fatty acid metabolites including nonesterified fatty acids (NEFAs) and total ketones declined; most serum amino acids increased dramatically. Most even-numbered acylcarnitines, which are products of fatty acid oxidation, de-

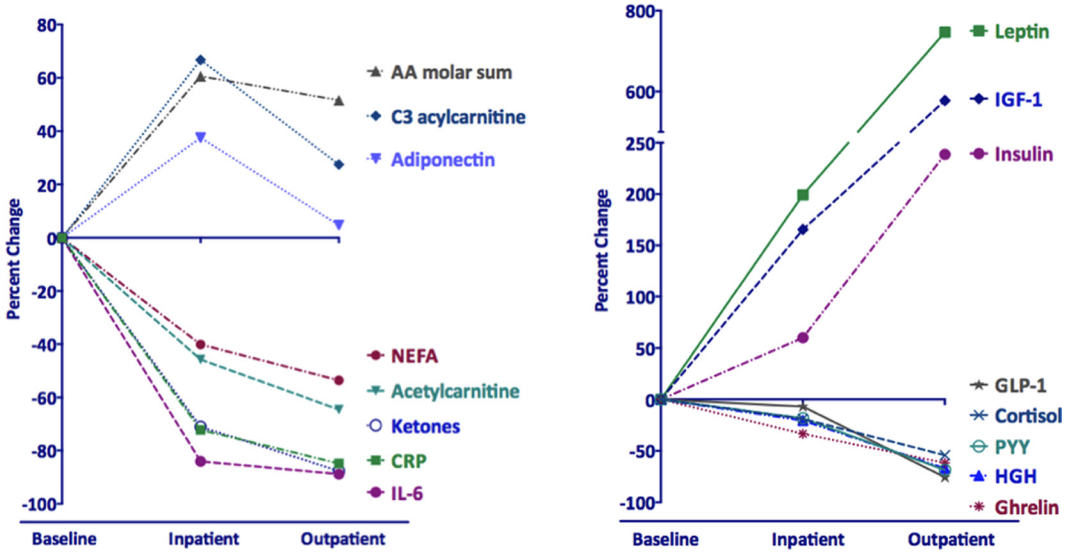

Figure 2. Percentage change in metabolite levels from baseline. Values represent the means of 62 inpatients at baseline, 54 inpatients after 2 weeks of formula feeding, and 14 outpatients after 4–10 weeks of RUTF. Adiponectin refers to total adiponectin; similar trends were noted in HMW adiponectin. AA, amino acids; HGH, human GH.

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creased during inpatient treatment, whereas C3 acyl (propionyl) carnitine, a product of branched chain amino acid oxidation, increased.

Nontargeted metabolomics (Supplemental Table 2) confirmed increases during inpatient treatment of several amino acids (alanine, valine, proline, leucine, isoleucine, and ornithine) as well as threonine, cysteine, tryptophan, an isomeric group comprising 2-aminobutanoic acid and N-methylalanine, and the polyamine, putrescine. Levels of α- and β-tocopherol also rose. Consistent with targeted data on total ketones and NEFAs, inpatient treatment reduced β-hydroxybutyric acid and three individual NEFA species (myristate, palmitoleate, and oleate). Decreased lipolysis was also suggested by a drop in ethanolamine. Several minor sugars and sugar alcohols fell with feeding, including aldopentoses and two cyclic polyols, 1,5-anhydroglucitol and myoinositol.

Insulin levels rose 50% during formula feeding, whereas leptin and IGF-I levels increased nearly 3-fold. GH fell by 21% ($P = .025$), whereas ghrelin decreased 35% ($P < .001$). Cortisol levels also declined (−20%, $P = .002$), but there were no significant changes in glucose, creatinine, GLP-1, or PYY. Total and high-molecular-weight (HMW) adiponectin increased by 37% and 61%, respectively. There were downward trends in all inflammatory cytokines except interferon-γ, IL-10, and IL-12p70; however, significant reductions were noted only for GM-CSF and IL-6. The levels of inflammatory cytokines and CRP did not correlate with HIV or malaria positivity and declined during treatment.

Response to outpatient feeding with RUTF

The 14 patients followed from inpatient treatment through outpatient clinic treatment (Table 2, Supplemental Tables 1 and 2, and Figure 2), showed progressive increases in W/H z, albumin, and phosphorus. In contrast, ALT decreased by 57%. There were no further changes in markers of fatty acid metabolism such as NEFAs, total ketones, and most acylcarnitine levels. Most serum amino acids also stabilized.

Outpatient weight gain was accompanied by a further doubling of leptin and IGF-I levels. This was associated with a nonsignificant 50% decrease in GH but a larger and statistically significant decline in the levels of ghrelin, PYY, and GLP-1. Insulin levels rose by an additional 50%, whereas adiponectin (total and HMW) levels declined slightly. Inflammatory markers such as CRP and cortisol showed downward, but statistically insignificant, trends.

Baseline anthropometric values for the 14 children followed from admission through the completion of outpatient therapy were comparable with the remainder of the initial group except for a lower prevalence of edema (2 of 14 vs 39 of 61). There were no significant differences in baseline or follow-up biomarker levels between the 14 followed up through the final stage of the study and the other 40 patients studied only during the inpatient phase.

Predictors of mortality during inpatient treatment

Mortality rates were higher for HIV seropositive (33.3%) than for seronegative (5.4%) children (odds ratio 8.82). Age, malaria status, and edema were not associated with increased mortality risk. In nonedematous inpatients, anthropometric risk factors for mortality on admission were low W/H z ($P = .0221$), low weight z-score ($P = .0141$), and low MUAC ($P = .0016$).

In the cohort as a whole (edematous and nonedematous patients), the biochemical factors at baseline associated with subsequent mortality were hypoleptinemia ($P = .0002$) (Figure 3), low levels of HMW adiponectin ($P = .0149$), and high levels of PYY ($P = .0087$), IL-2 ($P = .0004$), IL-6 ($P = .004$), and TNF-α ($P = .0198$). Mortality did not vary with other baseline measures including total ketones, NEFAs, phosphorus, albumin, creatinine, cortisol, CRP, or IGF-I. In further analysis of only edematous patients, hypoleptinemia remained a significant predictor of mortality ($P = .0169$). The highest leptin value of those who died was 35 pg/mL. Of those who survived, only 9 of 54 (16.6%) had leptin levels of 35 pg/mL or less. There were no significant differences in age, sex, W/H z, edema status, HIV status, or biochemical status between the nine patients who survived with leptin levels of 35 pg/mL or less and the nine who died.

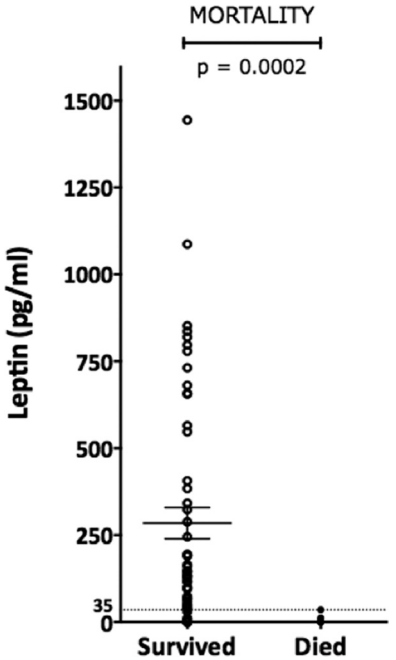

Figure 3. Baseline (pretreatment) leptin levels in children who survived or died during inpatient hospitalization. Horizontal bars represent mean ± SEM.

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Table 3. Multivariate Logistic Regression Analysis

Measurement	OR	*P* Value	95% CI
Weight/height z-score	0.546	.214	0.211–1.417
HIV status	116.845	**.022**	2.005–6809.128
Leptin	0.906	**.035**	0.827–0.993
HMW adiponectin	1.000	.184	0.999–1.001

Abbreviations: CI, confidence interval; OR, odds ratio for mortality.
Bold print indicates statistically significant.

Multivariate logistic regression analysis (Table 3) controlling for HIV status and admission W/H z showed that baseline hypoleptinemia remained a significant predictor of mortality ($P = .035$). A model combining admission W/H z, HIV, and baseline leptin appeared to explain 67% of the variation in mortality (adjusted $R^2 = 0.67$).

Discussion

Despite improvements in therapy, there remain fundamental questions regarding the pathogenesis of severe acute childhood malnutrition. In this study we used targeted and nontargeted metabolomic analysis to provide a comprehensive hormonal and metabolic profile of severely malnourished infants and children prior to and during nutritional rehabilitation. We then assessed the relationship between baseline hormonal and metabolic factors and subsequent mortality. Our analysis suggests that fatty acid metabolism plays a central role in the adaptation to acute childhood malnutrition and that low levels of the adipose tissue hormone leptin associate with, and may predict, mortality during treatment.

Regardless of HIV or edema status, malnourished children in this study presented with fat and protein depletion associated with hypoalbuminemia and hypoaminoacidemia. Blood glucose and lactate levels were normal, but free fatty acids, ketones, and even-numbered acylcarnitines were markedly elevated. These findings, consistent with more limited studies in marasmus and prolonged fasting (14, 15, 21), suggest that hydrolysis of lipid stores and oxidation of fatty acids are critical components of the metabolic response to severe acute childhood malnutrition.

The etiology of hypoalbuminemia and hypoaminoacidemia in severe malnutrition is complex; previous investigations (16–18, 20, 22, 23) suggested that hypoalbuminemia results from inadequate protein intake, gastrointestinal (GI) malabsorption, albumin catabolism, and, in edematous patients, diminished albumin synthesis. Interestingly, turnover of muscle protein and oxidation of leucine are suppressed in malnourished children, particularly in those with edema (17, 20, 22, 23). This may explain the hypoaminoacidemia and low levels of C3 acylcarnitine in our study cohort at presentation. Rates of proteolysis are higher in nonedematous malnourished and infected patients (17, 20, 22, 23). Amino acids released from muscle and glycerol from fat serve as substrates for hepatorenal glucose production, whereas energy provided by fatty acid oxidation sustains gluconeogenesis and cardiopulmonary function. The lack of a rise in serum lactate in our study participants is consistent with preferential use of fatty acids as an energy source in the acutely malnourished state.

In malnourished patients, fatty acids are derived from lipolytic breakdown of adipose triglyceride. Levels of leptin, which correlate with white adipose mass, were low in our cohort at baseline but varied among patients; this reflects variability in preexisting white adipose reserves. Baseline insulin and IGF-I were also low, whereas GH, cortisol, and ghrelin were high. The high levels of GH, resulting from hyperghrelinemia and lack of IGF-I feedback (30, 31), promote lipolysis and maintain blood glucose through hepatic gluconeogenesis (32); these actions are facilitated by hypoinsulinemia and hypercortisolemia. The lipolytic and gluconeogenic effects of GH are independent of IGF-I (33); thus, malnutrition directs GH action toward fat catabolism and glucose production and away from energy- and IGF-I-dependent linear growth.

The catabolic state at presentation was reversed after 2 weeks of formula feeding, which increased the levels of leptin, insulin, and IGF-I and reduced levels of GH and cortisol. These hormonal changes were associated with sharp reductions in free fatty acids, ketones, and even-numbered acylcarnitines, which are derived from fatty acid oxidation. Decreases in myoinositol and ethanolamine also suggested lower turnover of complex lipids. The levels of albumin and most amino acids rose sharply, and there was a rise in levels of C3 acylcarnitine. There were also increases in carnitine esters of C18:2 and C20:4, which are derivatives of the essential linoleic and arachidonic fatty acids, and a few medium-chain acylcarnitine species, such as C5:1 and C8:1, which may reflect the high concentrations of their precursor fatty acids in milk-based formulas.

The inverse relationship between even-numbered acylcarnitines, products of fatty acid oxidation, and propionylcarnitine (C3), derived from oxidation of branched chain amino acids and methionine, suggests striking changes in substrate use in response to therapy. Prior to treatment, energy is provided primarily by oxidation of fatty acids. During nutritional recovery, the increased availability of dietary protein allows for the oxidation of amino acids; the oxidation of fatty acids is thereby cur-

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tailed, promoting fat deposition and weight gain. Continuing increases in nutrient availability during outpatient treatment with RUTF, progressive increases in insulin and IGF-I, and decreases in cortisol limit protein catabolism and permit accretion of muscle protein.

The roles of GI hormones in the pathogenesis of malnutrition are unclear. GLP-1 is released from the small intestine in response to nutrients; it stimulates glucose-dependent insulin secretion, slows gastric emptying, and inhibits food intake. PYY is secreted by enteroendocrine cells in response to intraluminal nutrients, particularly fats. Its functions include inhibition of gastric emptying and induction of satiety (34). Given their anorexigenic effects, we hypothesized that the levels of GLP-1 and PYY would be low in malnourished infants at baseline and would rise with treatment. Unexpectedly, levels of GLP-1 and PYY at baseline were considerably higher than those in normal infants and children (26–29) and declined sharply during outpatient RUTF treatment. It is conceivable that the rise in GI hormone levels at presentation serves to reduce gastric emptying and thereby enhance nutrient absorption. This hypothesis concords with findings in adolescents with anorexia nervosa, who have high PYY levels (35), and obese teens and adults, who have low levels of PYY (36). GLP-1 and PYY are also elevated in critically ill patients (37, 38) for reasons unknown. Whether this explains the high levels of GLP-1 and PYY in our study participants at baseline is unclear, but levels of the hormones failed to decline significantly during inpatient hospitalization despite partial clinical recovery.

Mortality rates remain quite high among infants with severe acute malnutrition. Factors that increase the risk of death include HIV, coexisting infection, and the severity of caloric deprivation (1–4). In our study, age, malaria, and edema were not associated with increased mortality. However, the levels of certain hormones and cytokines at presentation predicted mortality during inpatient hospitalization; these included leptin, HMW adiponectin, PYY, IL-2, IL-6, and TNF∝.

Among these, leptin is particularly interesting because it reflects the adequacy of white adipose tissue (particularly sc) reserve. Those with the lowest leptin were at highest risk of death: baseline leptin predicted mortality in edematous and nonedematous patients in a multivariate analysis controlling for HIV status and admission W/H z. In contrast to leptin, neither baseline NEFAs nor ketones predicted mortality. We speculate that hypoleptinemic children, who have limited reserves of white adipose tissue, can generate and oxidize free fatty acids acutely but deplete their adipose reserves under continuing stress. Depletion of white adipose stores is postulated to limit the ability of a child to sustain energy production during the course of the illness; this would increase the child's risk of death. Alternatively, hypoleptinemia may reduce viability through effects on glucose and energy homeostasis (39–41) or immune competence (42, 43). In either case, leptin levels may prove clinically useful because other markers of mortality risk including W/H z are difficult to interpret in the presence of edema.

There are several limitations to our study. Blood samples were not obtained under fasted conditions or at precisely timed intervals; we did not think that fasting could be justified in critically ill patients at risk for hypoglycemia and metabolic decompensation. The cohort size was relatively small. The loss to follow-up in the inpatient portion of the study was only 16% of the original 75 recruited and unlikely to have biased our results. However, 26% of the 19 patients managed in the outpatient clinic were lost to follow-up. Comparable dropout rates are found in many malnutrition clinics in the developing world and present major challenges to high-risk children and their health care providers.

Finally, given the available clinical resources and the lack of autopsies, we were unable to determine the causes of death in our cohort. In addition to energy depletion, the possibilities include sepsis and other infectious complications and the refeeding syndrome. Refeeding syndrome (44) is classically associated with a precipitous decline in serum phosphorus (and in some cases potassium and magnesium) soon after beginning nutrient supplements; the WHO formulas and feeding protocols are designed to minimize the risks of hypophosphatemia and refeeding syndrome. In our study, serum phosphorus was relatively low at baseline but rose significantly in the surviving patients during treatment. Moreover, baseline phosphorus levels did not correlate with mortality. Nevertheless, we did not measure serum phosphorus (or any other hormones or metabolites) at the time of death; it is possible that a fall in phosphorus, potassium, or magnesium levels might have contributed to death in a subset of our patients.

The results of our studies provide new insight into the pathogenesis of childhood malnutrition. The identification of leptin as a biomarker that predicts mortality in both edematous and nonedematous patients may have therapeutic implications and may permit the targeting of particularly high-risk patients for more aggressive nutritional management and support.

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The author contributions included the following: S.B. conceptualized and designed the study, coordinated the data collection, assisted in the data analysis, drafted the initial manuscript, and approved the final manuscript as submitted; M.F. conceptualized and designed the study, assisted in the data analysis, reviewed and revised the manuscript, and approved the final manuscript as submitted; A.M. supervised the data collection, assisted in the data analysis, and approved the final manuscript as submitted; C.H. performed the data analysis and approved the final manuscript as submitted; R.S. and J.B. performed the acylcarnitine and nontargeted metabolic assays, respectively and approved the final manuscript as submitted; M.M. performed the hormonal and cytokine assays and approved the final manuscript as submitted; T.K. assisted in the data collection and approved the final manuscript as submitted; E.K. assisted in the data collection and approved the final manuscript as submitted; R.S. performed the amino acid assay and approved the final manuscript as submitted; J.B. assisted with the site coordination and approved the final manuscript as submitted; J.V.S.P. assisted with the data analysis and approved the final manuscript as submitted; and C.N. assisted with the metabolic assays and the data analysis and approved the final manuscript as submitted.

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References

1. **Black RE, Cousens S, Johnson HL, et al.** Global, regional, and national causes of child mortality in 2008: a systematic analysis. *Lancet*. 2010;375(9730):1969–1987.
2. **Black RE, Allen LH, Bhutta ZA, et al.** Maternal and child undernutrition: global and regional exposures and health consequences. *Lancet*. 2008;371(9608):243–260.
3. **Lozano R, Mohsen N, Foreman K, et al.** Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*. 2012;380(9859):2095–2128.
4. **Caulfield LE, de Onis M, Blossner M, Black RE.** Undernutrition as an underlying cause of child deaths associated with diarrhea, pneumonia, malaria, and measles. *Am J Clin Nutr*. 2004;80(1):193–198.
5. **Jones G, Steketee RW, Black RE, Bhutta ZA, Morris SS, Bellagio Child Survival Study Group.** How many child deaths can we prevent this year? *Lancet*. 2003;362(9377):65–71.
6. **World Health Organization.** Effect of breastfeeding on infant and child mortality due to infectious diseases in less developed countries: a pooled analysis. WHO Collaborative Study Team on the Role of Breastfeeding on the Prevention of Infant Mortality. *Lancet*. 2000; 355(9202):451–455.
7. **Brown KH, Peerson JM, Rivera J, Allen LH.** Effect of supplemental zinc on the growth and serum zinc concentrations of prepubertal children: a meta-analysis of randomized controlled trials. *Am J Clin Nutr*. 2002;75(6):1062–1071.
8. **Bhutta ZA, Ahmed T, Black RE, et al.** What works? Interventions for maternal and child undernutrition and survival. *Lancet*. 2008; 371(9610):417–440.
9. **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.
10. **Lagrone L, Cole S, Schondelmeyer A, Maleta K, Manary MJ.** Locally produced ready-to-use supplementary food is an effective treatment of moderate acute malnutrition in an operational setting. *Ann Trop Paediatr*. 2010;30(2):103–108.
11. **Linneman Z, Matilsky D, Ndekha M, Maleta K, Manary MJ.** 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.
12. **Patel MP, Sandige HL, Ndekha MJ, Briend A, Ashorn P, Manary MJ.** Supplemental feeding with ready-to-use therapeutic food in Malawian children at risk of malnutrition. *J Health Popul Nutr*. 2005;23(4):351–357.
13. **Victora CG, Adair L, Fall C, et al.** Maternal and child undernutrition: consequences for adult health and human capital. *Lancet*. 2008;371(9609):340–357.
14. **Taylor HL, Keys A.** Adaptation to caloric restriction. *Science*. 1950; 112(2904):215–218.
15. **Cahill GF Jr.** Starvation in man. *N Engl J Med*. 1970;282(12):668–675.
16. **Waterlow JC, Alleyne GA.** Protein malnutrition in children: advances in knowledge in the last ten years. *Adv Protein Chem*. 1971; 25:117–241.
17. **Tomkins AM, Garlick PJ, Schofield WN, Waterlow JC.** The combined effects of infection and malnutrition on protein metabolism in children. *Clin Sci (Lond)*. 1983;65(3):313–324.
18. **Lunn PG, Whitehead RG, Coward WA.** Two pathways to kwashiorkor? *Trans R Soc Trop Med Hyg*. 1979;73(4):438–444.
19. **Whitehead RG, Lunn PG.** Endocrines in protein-energy malnutrition. *Proc Nutr Soc*. 1979;38(1):69–76.
20. **Jahoor F, Badaloo A, Reid M, Forrester T.** Protein metabolism in severe childhood malnutrition. *Ann Trop Paediatr*. 2008;28(2):87–101.
21. **Badaloo AV, Forrester T, Reid M, Jahoor F.** Lipid kinetic differences between children with kwashiorkor and those with marasmus. *Am J Clin Nutr*. 2006;83(6):1283–1288.
22. **Manary MJ, Yarasheski KE, Berger R, Abrams ET, Hart CA, Broadhead RL.** Whole-body leucine kinetics and the acute phase response during acute infection in marasmic Malawian children. *Pediatr Res*. 2004;55(6):940–946.
23. **Manary MJ, Brewster DR, Broadhead RL, Crowley JR, Fjeld CR, Yarasheski KE.** Protein metabolism in children with edematous malnutrition and acute lower respiratory infection. *Am J Clin Nutr*. 1997;65(4):1005–1010.
24. **Bachou H.** *Outpatient care of children with severe acute malnutri-*

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tion. Republic of Uganda Ministry of Health; Kampala, Uganda, 2007.
25. **Ashworth A, Khanum S, Jackson A, Schofield C.** Guidelines for the inpatient treatment of severely malnourished children. Geneva: World Health Organization; 2003:1–48.
26. **Feigerlová E, Diene G, Conte-Auriol F, et al.** Hyperghrelinemia precedes obesity in Prader-Willi syndrome. *J Clin Endocrinol Metab*. 2008;93(7):2800–2805.
27. **Higgins PB, Férnández JR, Garvey WT, Granger WM, Gower BA.** Entero-insular axis and postprandial insulin differences in African American and European American children. *Am J Clin Nutr*. 2008; 88(5):1277–1283.
28. **Lomenick JP, Melguizo MS, Mitchell SL, Summar ML, Anderson JW.** Effects of meals high in carbohydrate, protein, and fat on ghrelin and peptide YY secretion in prepubertal children. *J Clin Endocrinol Metab*. 2009;94(11):4463–4471.
29. **Lomenick JP, Clasey JL, Anderson JW.** Meal-related changes in ghrelin, peptide YY, and appetite in normal weight and overweight children. *Obesity (Silver Spring)*. 2008;16(3):547–552.
30. **Veldhuis JD, Bowers CY.** Sex-steroid modulation of growth hormone (GH) secretory control: three-peptide ensemble regulation under dual feedback restraint by GH and IGF-I. *Endocrine*. 2003; 22(1):25–40.
31. **Scacchi M, Pincelli AI, Cavagnini F.** Nutritional status in the neuroendocrine control of growth hormone secretion: the model of anorexia nervosa. *Front Neuroendocrinol*. 2003;24(3):200–224.
32. **Li RL, Sherbet DP, Elsbernd BL, Goldstein JL, Brown MS, Zhao TJ.** Profound hypoglycemia in starved, ghrelin-deficient mice is caused by decreased gluconeogenesis and reversed by lactate or fatty acids. *J Biol Chem*. 2012;287(22):17942–17950.
33. **Vijayakumar A, Novosyadlyy R, Wu Y, Yakar S, LeRoith D.** Biological effects of growth hormone on carbohydrate and lipid metabolism. *Growth Horm IGF Res*. 2010;20(1):1–7.
34. **De Silva A, Bloom SR.** Gut hormones and appetite control: a focus on PYY and GLP-1 as therapeutic targets in obesity. *Gut Liver*. 2012;6(1):10–20.
35. **Misra M, Miller KK, Tsai P, et al.** Elevated peptide YY levels in adolescent girls with anorexia nervosa. *J Clin Endocrinol Metab*. 2006;91(3):1027–1033.
36. **Batterham RL, Cohen MA, Ellis SM, et al.** Inhibition of food intake in obese subjects by peptide YY3–36. *N Engl J Med*. 2003;349(10): 941–948.
37. **Nguyen NQ, Fraser RJ, Chapman M, et al.** Fasting and nutrient-stimulated plasma peptide-YY levels are elevated in critical illness and associated with feed intolerance: an observational, controlled study. *Crit Care*. 2006;10(6):R175.
38. **Deane A, Chapman MJ, Fraser RJ, Horowitz M.** Bench-to-bedside review: the gut as an endocrine organ in the critically ill. *Crit Care*. 2010;14(5):228.
39. **Baskin, DG, Blevins JE, Schwartz MW.** How the brain regulates food intake and body weight: the role of leptin. *J Pediatr Endocrinol Metab*. 2001;14(suppl 6):1417–1429.
40. **Mizuno TM, Kleopoulos SP, Bergen HT, Roberts JL, Priest CA, Mobbs CV.** Hypothalamic pro-opiomelanocortin mRNA is reduced by fasting and [corrected] in ob/ob and db/db mice, but is stimulated by leptin. *Diabetes*. 1998;47(2):294–297.
41. **Ahima RS, Prabakaran D, Mantzoros C, et al.** Role of leptin in the neuroendocrine response to fasting. *Nature*. 1996;382(6588):250–252.
42. **Procaccini C, Jirill, E, Matarese G.** Leptin as an immunomodulator. *Mol Aspects Med*. 2012;33(1):35–45.
43. **Saucillo DC, Gerriets VA, Sheng J, Rathmell JC, Maciver NJ.** Leptin metabolically licenses T cells for activation to link nutrition and immunity. *J Immunol*. 2014;192(1):136–144.
44. **Fuentebella J, Kerner JA.** Refeeding syndrome. *Pediatr Clin North Am*. 2009;56(5):1201–1210.

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