Clinical Nutrition 39 (2020) 3512–3519

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Original article

Vitamin A and iron status of children before and after treatment of uncomplicated severe acute malnutrition

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ARTICLE INFO

Article history:
Received 15 January 2020
Accepted 10 March 2020

Keywords:
Vitamin A
Iron
Micronutrient
Severe acute malnutrition
Children
Ready-to-use therapeutic food

SUMMARY

Background & aims: Treatment of children with uncomplicated severe acute malnutrition (SAM) is based on ready-to-use therapeutic foods (RUTF) and aims for quick regain of lost body tissues while providing sufficient micronutrients to restore diminished body stores. Little evidence exists on the success of the treatment to establish normal micronutrient status. We aimed to assess the changes in vitamin A and iron status of children treated for SAM with RUTF, and explore the effect of a reduced RUTF dose.

Methods: We collected blood samples from children 6–59 months old with SAM included in a randomised trial at admission to and discharge from treatment and analysed haemoglobin (Hb) and serum concentrations of retinol binding protein (RBP), ferritin (SF), soluble transferrin receptor (sTfR), C-reactive protein (CRP) and α1-acid glycoprotein (AGP). SF, sTfR and RBP were adjusted for inflammation (CRP and AGP) prior to analysis using internal regression coefficients. Vitamin A deficiency (VAD) was defined as RBP < 0.7 μmol/l, anaemia as Hb < 110 g/l, storage iron deficiency (sID) as SF < 12 μg/l, tissue iron deficiency (tID) as sTfR > 8.3 mg/l and iron deficiency anaemia (IDA) as both anaemia and sID. Linear and logistic mixed models were fitted including research team and study site as random effects and adjusting for sex, age and outcome at admission.

Results: Children included in the study (n = 801) were on average 13 months of age at admission to treatment and the median treatment duration was 56 days [IQR: 35; 91] in both arms. Vitamin A and iron status markers did not differ between trial arms at admission or at discharge. Only Hb was 1.7 g/l lower (95% CI −0.3, 3.7; p = 0.088) in the reduced dose arm compared to the standard dose, at recovery. Mean concentrations of all biomarkers improved from admission to discharge: Hb increased by 12% or 11.6 g/l (95% CI 10.2, 13.0), RBP increased by 13% or 0.12 μmol/l (95% CI 0.09, 0.15), SF increased by 36% or 4.4 μg/l (95% CI 3.1, 5.7) and sTfR decreased by 16% or 1.5 mg/l (95% CI 1.0, 1.9). However, at discharge, micronutrient deficiencies were still common, as 9% had VAD, 55% had anaemia, 35% had sID, 41% had tID and 21% had IDA.

Conclusion: Reduced dose of RUTF did not result in poorer vitamin A and iron status of children. Only haemoglobin seemed slightly lower at recovery among children treated with the reduced dose. While improvement was observed, the vitamin A and iron status remained sub-optimal among children treated successfully for SAM with RUTF. There is a need to reconsider RUTF fortification levels or test other potential strategies in order to fully restore the micronutrient status of children treated for SAM.

1. Introduction

Severe acute malnutrition (SAM), defined as severe wasting, low mid-upper arm circumference (MUAC) and/or oedema, is widespread among children in low-income countries. While incidence

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https://doi.org/10.1016/j.clnu.2020.03.016

Abbreviations

AGP	α1-acid glycoprotein
CMAM	community-based management of acute malnutrition
CRP	C-reactive protein
Hb	haemoglobin
IDA	iron deficiency anaemia
MAM	moderate acute malnutrition
MUAC	mid-upper arm circumference
RBP	retinol binding protein
RUTF	ready-to-use therapeutic food
SAM	severe acute malnutrition
SF	serum ferritin
sID	storage iron deficiency
tID	tissue iron deficiency
sTfR	soluble transferrin receptor
VAD	vitamin A deficiency
WHO	World Health Organisation
WHZ	weight-for-height *z*-score

data are lacking, the number of children with severe wasting alone, at any time, was estimated at 16.6 million children in 2018 [1]. The treatment of SAM without medical complications consists of ready-to-use-therapeutic food (RUTF) and one week of antibiotic treatment [2]. RUTFs are fortified energy-dense pastes designed to fulfil all the nutritional needs of children during recovery from SAM [3]. The aim of the treatment is to enable a rapid regain of lost body tissues while providing sufficient micronutrients to restore diminished body stores. However, little evidence exists on the success of the treatment to restore sufficient micronutrient status by recovery.

Vitamin A deficiency (VAD) affects 190 million children under 5 years of age worldwide and is associated with increased risk of morbidity and mortality, and in the most severe form can lead to blindness [4]. In Brazil 41.2% and in Congo up to 98% of hospitalised malnourished children had VAD [5,6]. RUTFs contain 0.7–1.0 mg of vitamin A per 92 g sachet [3] and each child typically receives 2–3 sachets per day, which translates to 3–8 times the daily recommended intake of 0.4 mg for healthy children in this age group [7]. However, no data exist on the vitamin A status of children with uncomplicated SAM at admission to and discharge from community-based treatment. Ensuring a normal vitamin A status by discharge would seem crucial, considering its role in healthy growth [8–10].

Similarly, anaemia and iron deficiency are common worldwide; WHO estimates that nearly half of all children under 5 years of age are anaemic and/or iron deficient [11]. Iron deficiency in childhood is associated with impaired growth and development [12,13]. Previous studies on children with SAM have reported high prevalence of anaemia at admission: up to 95% in inpatient settings in India [14,15], 80% in Burkina Faso [16], and 49% among uncomplicated cases in Malawi [17]. RUTFs contain 9–11 mg of iron per sachet [3] with 2 sachets equalling the daily recommended intake of 18.6 mg (assuming a low bioavailability) for healthy children [7]. A study in Malawi showed that 25% of children were still anaemic when discharged from SAM treatment [17]. Additionally, they observed that the proportion of children with iron deficiency (defined as soluble transferrin receptor concentrations > 8.3 mg/l when adjusted for inflammation) did not decrease from admission (56%) to discharge (58%). Documenting the response to SAM treatment in different contexts would be important in order to guide possible protocol revisions aimed at improving the iron status of treated children.

The objective of this paper is to assess the change in vitamin A and iron status of children treated for uncomplicated SAM with RUTF, and explore the effect of a reduced RUTF dose on the vitamin A and iron status of recovered children.

2. Methods

2.1. Study area and participants

This study was a cohort study nested in to the MANGO study, a randomised non-inferiority trial testing the efficacy of a reduced RUTF dose compared to standard RUTF dose in the treatment of uncomplicated SAM among children 6–59 months of age [18]. We previously reported a non-inferior weight gain velocity [18] and a similar tissue gain pattern (in press) with a reduced RUTF dose compared to the standard dose.

The study was conducted from October 2016 to December 2018 in the east of Burkina Faso, where the prevalence of wasting was 10% in 2016 [19]. Intestinal parasites are common with 86% of school age children in neighbouring regions infected [20] and up to 16% are estimated to present with haemoglobinopathies [21]. Six-monthly vaccination campaigns with vitamin A supplementation (100 000 IU to 6–11 month olds; 200 000 IU to 12–59 month olds) were organised in the area throughout the study period. Community and health centre level sensitisation on appropriate infant and young child feeding (IYCF) practices including promotion of the use of fortified infant flours were supported by various NGOs working in the area.

As described previously [18], participants were recruited at 10 health centres. Eligibility criteria consisted of having a weight-for-height *z*-score (WHZ) < −3 and/or a MUAC < 115 mm, no oedema and the absence of medical complications as per the national protocol [22]. Children failing the appetite test, having received SAM treatment in the past 6 months, with known peanut or milk allergy, disability affecting food intake or whose caregiver were unable to comply with the weekly visit schedule were excluded.

2.2. Randomisation and study procedures

For a full description of the methodology, please refer to Ref. [18]. In brief, after obtaining caregiver consent, children were randomised individually to receiving either a standard dose of RUTF throughout treatment or a reduced dose of RUTF from the 3rd treatment week onwards (Table 1). The vitamin A and iron content per daily dose and per arm is described in Table 1. The content has been calculated based on RUTF nutrient specifications [3]. Medical treatment was provided for all children per the national protocol [22] including a 7-day course of amoxicillin at admission (50–100 mg/kg/d) and albendazole at the second treatment visit for children ≥ 12 months of age (200 mg to 12–23-month-olds; 400 mg to ≥24-month-olds). Any missed routine vaccinations or 6-monthly vitamin A supplementations were caught-up at admission. Children were treated until reaching anthropometric recovery criteria or until defaulting from treatment, being transferred to inpatient care, diseased or declared non-responding after a maximum of 16 weeks of treatment. Anthropometric recovery was defined as a WHZ ≥ −2 for those admitted with a WHZ < −3 only, MUAC ≥ 125 mm for those admitted with a MUAC < 115 mm only, or WHZ ≥ −2 and MUAC ≥ 125 mm for those admitted with WHZ < −3 and MUAC < 115 mm, on two consecutive visits and absence of any illness.

Venous blood was collected in Vacutainer® (BD, New Jersey, USA) clot activator tubes at admission and at discharge from

Table 1
Vitamin A and iron content per daily RUTF dose with reduced and standard dose.

Weight (kg)	Standard RUTF dose[a]			Reduced RUTF dose[a]		
	RUTF quantity/day (sachets)	Vitamin A/daily RUTF dose (mg)	Iron/daily RUTF dose (mg)	RUTF quantity/day (sachets)	Vitamin A/daily RUTF dose (mg)	Iron/daily RUTF dose (mg)
3.0–3.4	1.1	1.1	11.4	1.0	1.0	10.0
3.5–4.9	1.4	1.4	14.3	1.0	1.0	10.0
5.0–6.9	2.1	2.1	21.4	1.0	1.0	10.0
7.0–9.9	2.9	2.9	28.6	2.0	2.0	20.0
10.0–14.9	4.3	4.3	42.9	2.0	2.0	20.0

RUTF, ready-to-use therapeutic foods.
[a] Reduced dose arm received a standard dose of RUTF for the first 2 weeks and then the reduced dose from 3rd treatment week onwards.

treatment. Two attempts were made for blood collection from a total of 3 possible sites. If infeasible, a finger prick was used for a rapid diagnostic test for malaria (SD Bioline, Abbott, Illinois, USA) and to measure Hb with a HemoCue® 301 device (Hemocue AB, Sweden). The accuracy of the HemoCue® was monitored monthly using commercial controls (Eurotrol, Kentucky, USA) and the values were within certified values. Blood samples were transported in a cold box at 2–8 °C to the field laboratory where samples were stored in a fridge at 2–10 °C for maximum 24 h. Serum was isolated following centrifugation at 3000 rotations per minute for 5 min (EBA 20 S Hettich, Germany) and stored at −20 °C until shipment to VitMin Lab in Willstaedt, Germany for analysis. Serum ferritin (SF), soluble transferrin receptor (sTfR), retinol binding protein (RBP), C-reactive protein (CRP), α1-acid glycoprotein (AGP), were determined using a combined sandwich enzyme linked immunosorbent assay [23].

A thorough medical history and evaluation of morbidities was performed by a study nurse at admission and at each weekly visit. Fever was defined as an arm pit temperature of ≥37.5 °C and led systematically to rapid testing for malaria with a positive result defining malaria. Acute respiratory illness (ARI) was defined as cough reported by caregiver in the past week or diagnosed by study nurse during visit. Diarrhoea included acute, persistent or dysenteric forms and was defined as 3 or more loose stools per day as reported by caregiver in the past week or diagnosed by study nurse. Medical treatment offered included a 3-day course of arthemeter (2 × 20 mg/d) and lumefantrine (2 × 120 mg/d) in case of malaria and a 7-day course of amoxicillin (50–100 mg/kg/d) in case of ARI or diarrhoea.

2.3. Outcomes and adjustment for inflammation

As SF, sTfR and RBP are affected by inflammation they were adjusted prior to analysis using internal regression coefficients as previously described [24–26]. Log-transformation was applied for RBP, SF, sTfR, CRP and AGP due to non-normal distribution. The coefficients were 0.178 for log transformed CRP (logCRP) and 0.167 for log transformed AGP (logAGP) when adjusting log transformed SF, 0.004 for logCRP and 0.228 for logAGP when adjusting log transformed sTfR and −0.071 for logCRP and −0.028 for logAGP when adjusting log transformed RBP. Based upon recommendations from the BRINDA study group [24–26], adjustments were not applied below first deciles of CRP and AGP corresponding to 0.20 mg/l for CRP and 0.43 g/l for AGP in the current data.

Anaemia was defined as Hb < 110 g/L [27], storage iron deficiency (sID) as inflammation adjusted SF (SF_{adj}) < 12 μg/L [28], tissue iron deficiency (tID) as inflammation adjusted sTfR >8.3 mg/l and iron-deficiency anaemia (IDA) as Hb < 110 g/L and SF_{adj} < 12 μg/L. Vitamin A deficiency (VAD) was defined as inflammation adjusted RBP <0.7 μmol/l. For descriptive purposes, inflammation categories were defined as proposed by Thurnham et al. [29].

2.4. Statistical analysis

Data were collected via tablets using Open Data Kit software. All statistical analyses were carried out using Stata 15 (Stata Corp, Texas, USA). Characteristics of the study population were summarized as percentages and means ±SDs or, if not normally distributed as median (IQR). Linear and logistic mixed models were used to assess differences in means and proportions at admission, with study team and health centre included as random effects.

Linear and logistic mixed models were used to evaluate change from admission to discharge in mean biomarker concentrations or proportions of deficiencies, as appropriate. Study team, health centre, and child id were included as random effects. Both unadjusted and adjusted models (including sex and age) were fitted. Similarly, the effect of RUTF dose on mean biomarker concentrations and proportions of deficiencies were analysed with linear and logistic mixed models. Study team and health centre were included in the models as random effects. Unadjusted and adjusted models (including sex, age, and outcome measure at admission) were fitted.

Results were presented as estimated mean differences with 95% CI in means and proportions. Right-skewed outcomes were logarithm-transformed prior to analysis. Subsequently, back transformation was applied to log transformed variables to estimate mean differences in original units [30]. Model checking was based on residual and normal probability plots.

2.5. Ethical considerations

Children not included in the study but diagnosed with SAM were referred to standard care at the health centre. Children who did not recover from SAM within 16 weeks of treatment were subsequently referred to standard care. The study was carried out in accordance with the Declaration of Helsinki. Field registries were kept in a locked facility. The study was approved by the national Ethics Committee of Burkina Faso (deliberation number 2015-12-00) and the national clinical trials board of Burkina Faso (Direction Générale de la Pharmacie, du Médicament et des Laboratoires (DGPML)). The trial was registered in the International Standard Randomized Controlled Trial Number (ISRCTN) registry as ISRCTN50039021.

3. Results

Out of the total 801 children included in the trial, 402 were randomised to the reduced RUTF dose and 399 to the standard dose. Hb was analysed for all admitted children and additional biomarkers including SF, sTfR, RBP, CRP and AGP were analysed for 714 (89%) of admitted children. At discharge, we analysed Hb for 425 (98%) of recovered children and additional biomarkers were analysed for 383 (90%) of recovered children (see Fig. 1).

As previously detailed [18], non-recovered children represented a heterogeneous group of children referred to inpatient care (20%), defaulters (12%), lost to follow-up (0.1%), deaths (0.1%), non-responders (13%) and false discharges (3%). Because of ethical and operational constraints, vitamin A and iron biomarker data were only obtained from 30% of these children, mostly from the non-responders.

Baseline characteristics of children did not differ between the study groups in terms of morbidity, inflammatory markers, and vitamin A and iron status markers (Table 2). For the full cohort, the mean age was 13.4 months at admission and 49% were male. Approximately 78% of children reported or were diagnosed with an illness at admission with 33% presenting with positive malaria rapid test. Most children had elevated serum CRP (42%) or AGP (64%) at admission. The median length of stay in treatment was 56 days [IQR: 35; 91] in both arms.

Mean concentrations of all vitamin A and iron status biomarkers improved from admission to discharge: Hb increased by 12%, RPB by 12% and SF by 36% while sTfR decreased by 16%. These changes resulted in fewer children being under the deficiency cut-offs at discharge (Table 3). Vitamin A deficiency (RBP < 0.7 μmol/l) decreased from 25% at admission to 9% at discharge (Fig. 2). Anaemia (Hb < 110 g/l) decreased from 77% at admission to 55% at discharge. Storage iron deficiency (SF < 12 μg/l) decreased from 50% at admission to 35% at discharge. Tissue iron deficiency (sTfR > 8.3 mg/l) decreased from 55% at admission to 41% at discharge. Iron deficiency anaemia decreased from 42% at admission to 21% at discharge.

At recovery, no differences were found in the mean concentrations of RBP, SF or sTfR or on the percentages of VAD, sID, tID or IDA between the study arms (Table 4). However, the reduced dose was associated with a 1.7 g/l lower haemoglobin concentration and 9% higher anaemia prevalence, although these differences were only marginally significant. Similar results were found when including all discharge categories as opposed to only recovered (S1 Table) or without adjustments for age, sex and outcome measure at admission.

4. Discussion

In this study, a high proportion of children with SAM had sub-optimal vitamin A and iron status at admission and these deficiencies were only partly corrected by discharge with 56% anaemic, 21% IDA and 9% VAD. There was no difference in mean RBP, SF or sTfR at discharge between children who had received the reduced and the standard RUTF dose. However, the mean Hb concentration was slightly lower in children receiving the reduced compared to standard dose, although this difference was only marginally significant.

High rates of anaemia have been reported in previous studies among malnourished children [17,31,32], however few have reported change during treatment. In our study, anaemia decreased significantly from 78% to 56%, similarly to Cichon's observation among children with MAM treated for 12 weeks where anaemia decreased from 70% at admission to 53% at discharge [31].

Iron deficiency explains only half of the anaemia; 40% were IDA while 78% were anaemic at admission, and 21% were IDA and 56% anaemic at discharge. RUTFs contain many nutrients such as vitamins A, C, D, E, B2, B6, B12, folate, copper and zinc, whose deficiency can cause anaemia [33–39]. Thus, treatment with RUTF could correct nutritional anaemia not caused by iron deficiency. Yet, haemoglobinopathies and inflammation may also cause anaemia [36], meaning nutritional supplementation may not fully correct it.

We observed a trend towards 1.7 g/l lower mean Hb and 9-percentage points more anaemia among children who received the reduced compared to standard RUTF dose. A similar trend was

Table 2
Characteristics of children at admission to SAM treatment receiving a reduced or a standard RUTF dose.

Characteristics	n	Reduced RUTF	Standard RUTF	p-value
Age, months	801	13.3 ± 8.6	13.4 ± 8.9	0.79
Male, %	801	49.5 (199)	49.4 (197)	0.97
Morbidity				
Any illness, %	801	79 (316)	78 (311)	0.82
Malaria, %	801	33 (134)	32 (129)	0.75
Acute respiratory illness, %	801	31 (126)	31 (125)	0.94
Diarrhoea, %	801	25 (101)	25 (99)	0.93
Other, %	801	11 (45)	13 (53)	0.34
Fever, %	801	27 (108)	25 (100)	0.57
Inflammation				
C-reactive protein (CRP), mg/l	714	2.5 [0.6–12.6]	3.3 [0.7–13.2]	0.59
>5	714	41 (149)	42 (150)	0.74
α1-acid glycoprotein (AGP), g/l	714	1.3 [0.8–1.9]	1.3 [0.8–2.0]	0.19
>1	714	62 (225)	65 (229)	0.48
Inflammation categories				
CRP ≤ 5 mg/l and AGP ≤ 1 g/l	714	33 (118)	31 (111)	0.72
CRP > 5 mg/l and AGP ≤ 1 g/l	714	5 (18)	4 (13)	0.39
CRP > 5 mg/l and AGP > 1 g/l	714	36 (131)	39 (137)	0.49
CRP ≤ 5 mg/l and AGP > 1 g/l	714	26 (94)	26 (92)	0.99
Vitamin A and iron				
Retinol binding protein, μmol/l	714	0.88 [0.70–1.12]	0.90 [0.71–1.11]	0.94
<0.7 μmol/l, %	714	26 (93)	25 (87)	0.73
Haemoglobin, g/l	801	96.6 ± 16.9	94.7 ± 17.8	0.11
<110 g/l, %	801	78 (314)	79 (314)	0.85
Ferritin, μg/l	714	11.4 [5.1–31.6]	12.9 [5.1–31.2]	0.58
<12 μg/l, %	714	52 (187)	48 (168)	0.26
Soluble transferrin receptor, mg/l	714	9.0 [6.4–13.8]	9.3 [6.5–14.2]	0.50
>8.3 mg/l, %	714	55 (200)	57 (200)	0.76
Iron deficiency anaemia, %	714	43 (156)	40 (141)	0.37

Data are mean ± SD, median [IQR] or proportion (n) with p-value for difference using logistic or linear mixed models with study site and research team as random variables.
RUTF, ready-to-use therapeutic food.

Table 3
Change in vitamin A and iron status biomarkers from admission to discharge from SAM treatment.

Outcome	Admission		Discharge		Change[a]	
	n	values	n	values	mean (95% CI)	p-value
RBP, μmol/l	714	0.89 [0.70–1.11]	473	1.00 [0.83–1.21]	0.12 (0.09; 0.15)	<0.001
Hb, g/l	801	95.7 ± 17.4	537	106.8 ± 13.5	11.6 (10.1; 13.0)	<0.001
SF, μg/l	714	12.1 [5.1–31.6]	474	16.1 [9.6–28.6]	4.4 (3.1; 5.7)	<0.001
sTfR, mg/l	714	9.2 [6.5–14.1]	474	7.8 [6.3–10.7]	−1.5 (−1.9; −1.0)	<0.001

Values are median [IQR] for RBP, SF and sTfR and mean ± SD for Hb.
RBP, retinol binding protein adjusted for inflammation; Hb, haemoglobin; SF, serum ferritin adjusted for inflammation; sTfR, soluble transferrin receptor adjusted for inflammation.
[a] Change in concentrations when adjusting for sex and age and using linear mixed models with id, study site and research team as random effects.

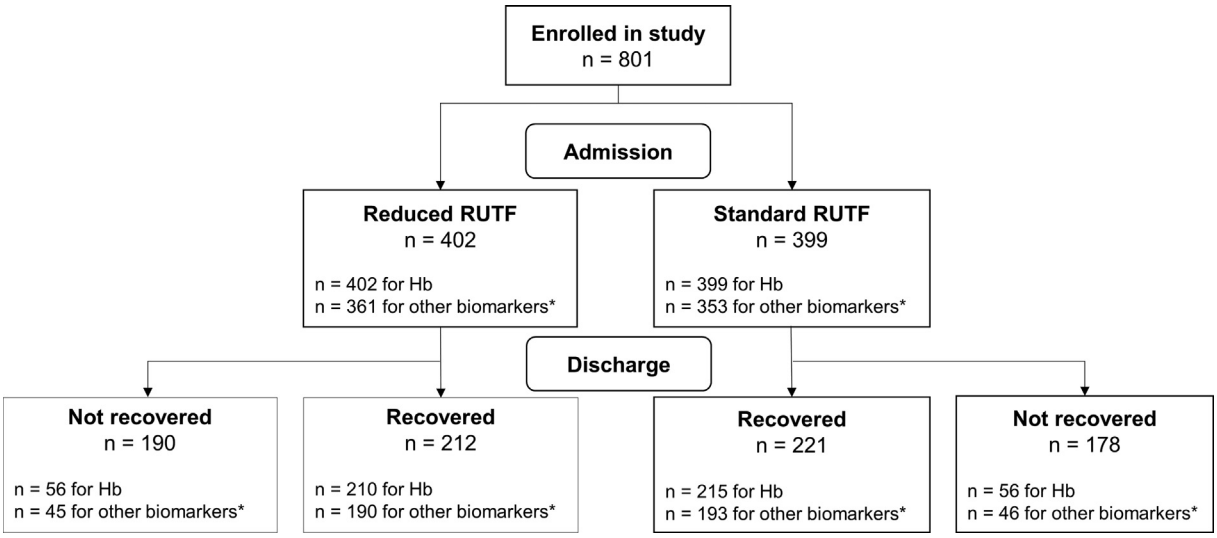

Fig. 1. Patient flow chart. * including serum ferritin (SF), soluble transferrin receptor (sTfR), retinol binding protein (RBP), C-reactive protein (CRP), α1-acid glycoprotein (AGP). Hb, haemoglobin; RUTF, ready-to-use therapeutic food.

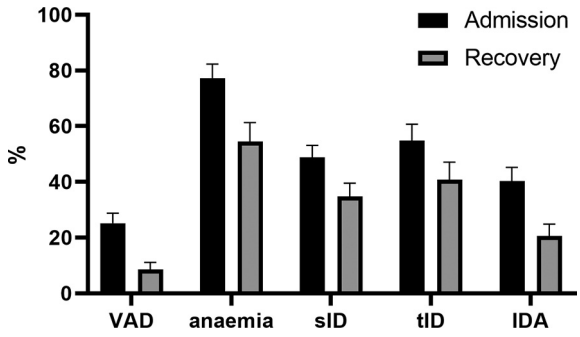

Fig. 2. Deficiencies in vitamin A and iron status biomarkers at admission to and discharge from SAM treatment. Data are means with 95% CI when using logistic mixed models including study team, health centre and id as random factors and adjusting for sex and age. VAD: vitamin A deficiency defined as retinol binding protein adjusted for inflammation < 0.7 μmol/l; sID: storage iron deficiency defined as serum ferritin adjusted for inflammation < 12 μg/l; tID: tissue iron deficiency defined as soluble transferrin receptor adjusted for inflammation > 8.3 mg/l; IDA: iron deficiency anaemia defined as haemoglobin < 110 g/l and serum ferritin adjusted for inflammation < 12 μg/l.

not observed in SF nor sTfR. The observed effect on Hb might be due to other micronutrients in the RUTF that, when given at smaller quantities, become insufficient to correct low Hb.

While we observed a favourable change in all iron status markers, it was insufficient to achieve normal values upon discharge. In Malawi non-standard formulations of RUTF were tested including three times the iron of the standard RUTF [17]. The alternative formulations led to significantly lower rate of anaemia by discharge; 12–18% compared to 25% with standard RUTF [17]. These results combined with our observation of high rates of anaemia and iron deficiency at discharge from standard treatment suggest that children with SAM might benefit from RUTF formulations with higher iron content. Currently, a sachet of RUTF contains 9–11 mg of iron with 2–3 sachets providing a maximum of twice the recommended daily intake (12–19 mg/d) of a healthy 7–59 month old child [7]. Most of the iron requirements for children under 3 years of age are related to growth [7]. Children recovering from malnutrition, with an estimated weight gain velocity 3–5 times higher [40] than normal children [41], would be expected to have proportionally high iron requirements. This said, it is usually accepted that correcting iron deficiency takes 3–6 months [42,43] and therefore post-discharge interventions designed to correct the remaining micronutrient deficiencies should be considered.

However, iron interventions raise several issues. First, potential effects of additional iron on morbidity should be investigated as iron can increase the risk and severity of diarrhoea, fever, vomiting and hospitalisations [44–47]. Second, additional iron might have a negative effect on the microbiota [47–50] that is already altered in malnourished children [51,52]. Third, additional iron might negatively affect growth especially among iron replete children [44,53–55]. Slower weight gain velocity was also observed in the Malawian study among children with SAM receiving RUTF with higher than standard iron content [56]. Fourth, high iron content in

Table 4
Vitamin A and iron status at discharge among children recovered from SAM and treated with a reduced or a standard RUTF dose.

Outcome	n	Reduced RUTF dose	Standard RUTF dose	Difference[a]	
				mean (95% CI)	p-value
RBP, μmol/l	382	0.99 [0.83–1.23]	0.99 [0.83–1.17]	0.02 (−0.03; 0.08)	0.38
<0.7 μmol/l, %	382	10 (18)	9 (17)	0 (−5; 4)	0.87
Hb, g/l	425	107.1 ± 11.8	108.6 ± 11.2	−1.7 (−3.7; 0.3)	0.088
<110 g/l, %	425	59 (124)	51 (110)	9 (−1; 19)	0.074
SF, μg/l	383	16.1 [9.4–27.2]	16.5 [10.0–28.4]	0.8 (−1.8; 3.3)	0.56
<12 μg/l, %	383	36 (69)	31 (60)	2 (−8; 13)	0.65
sTfR, mg/l	383	7.8 [6.4–11.2]	8.1 [6.4–10.8]	−0.1 (−0.8; 0.7)	0.82
>8.3 mg/l, %	383	43 (82)	43 (83)	−3 (−13; 8)	0.63
IDA, %	376	23 (43)	19 (35)	1 (−7; 9)	0.75

Values are median [IQR] for RBP, SF and sTfR, mean ± SD for Hb and proportions (n).
RBP, retinol binding protein adjusted for inflammation; Hb, haemoglobin; SF, serum ferritin adjusted for inflammation; IDA, iron deficiency anaemia (defined as Hb < 110 g/l and SF < 12 μg/l); sTfR, soluble transferrin receptor adjusted for inflammation.
[a] Difference when adjusting for age, sex and outcome measure at admission using linear and logistic mixed models including research team and study site as random factors.

infant formula has been associated with impairment of cognitive development [57,58]. Finally, additional iron could interfere with the absorption of other trace elements such as copper and zinc [59–62]. The choice of the form of iron would also be crucial in minimising the harmful and maximising the positive effects [63].

To our knowledge this is the first study reporting the response of vitamin A status to treatment with RUTF of children with uncomplicated SAM; VAD decreased from 25% at admission to 9% at discharge. Previous studies conducted in inpatient settings have reported high rates of VAD; 41% in Brazil [5] and 81% in Bangladesh [64] had VAD. However, neither study adjusted serum retinol for inflammation and therefore may have overestimated VAD prevalence [65].

That 9% of children remained VAD while discharged as recovered from SAM deserves attention. This was observed despite daily RUTF intake for a mean duration of 2 months and in the context of twice a year high-dose vitamin A supplementation campaigns. The 16-percentage point decrease in VAD during treatment reflects an effective response to RUTF, but the question remains whether a higher vitamin A fortification level of RUTF could eliminate VAD. A sub-optimal vitamin A status is also related to anaemia and iron deficiency and thus eradicating VAD might also help in further decreasing anaemia [36,66].

However, there remains a lot of uncertainty as to the upper safe limit of vitamin A intake: the WHO advises against intakes over 0.9 mg/d [7] among infants but without evidence from studies showing adverse effects, and the IOM gives an upper-limit of 0.6 mg/d for children under 3 years of age [67]. Toxicity has been observed with daily intakes of 0.45 mg/kg for 6 months [68] which would translate to around 4 mg/d for a normal 1 year old. These limits are for healthy children with normal stores but considering that malnourished children seem to start with deficiencies, higher safety cut-offs would probably apply. The vitamin A content of RUTF is about 0.2 mg/100 kcal [3] and following the current dosage recommendations the daily intake would equal 0.4 mg/kg or 3 mg for an average 1-year-old child with SAM. It thus seems unlikely that the current dose of RUTF would result in excessive intakes. Unfortunately serum RBP is not a good marker of excessive vitamin A stores but mainly designed to detect deficiency and thus we could not assess risk of excessive vitamin A among the studied children [65]. Studies aiming at providing evidence on the vitamin A status of children with SAM are advised to use more appropriate methods such as stable isotope techniques to study the full range of vitamin A stores [65]. Looking into actual intake of RUTF and other foods during treatment would also be crucial in determining the potential sources and actual quantity of vitamin A intake during treatment.

The strength of the current study is the high rate of success in obtaining biomarker data. This allows for a confident estimation of vitamin A and iron status at admission to and recovery from treatment. We also had a sufficient sample size to apply adjustment for inflammation using internal regression coefficients recommended by the BRINDA study group [24–26].

Our study also has limitations. First, we only obtained data at discharge from a third of non-recovered children with few data points from referrals or other potentially “worst off” patients. Thus, the discharge data probably overestimates the mean effect among all treated children and underestimates the deficiencies at discharge, representing a “best case” scenario. Second, we did not collect data on the timing of the high-dose vitamin A supplementation campaigns. Therefore, we could not account for the impact of time since high-dose supplementation prior to admission.

In conclusion, reducing the RUTF dose does not seem to have an impact on the vitamin A and iron status of treated children. Only haemoglobin was slightly lower at recovery among children treated with the reduced dose. However, the vitamin A and iron status remained sub-optimal among children treated successfully for SAM with RUTF. Thus, there is a need to carefully reconsider micronutrient fortification levels in RUTF or test other strategies aiming at improving the micronutrient status of children treated for SAM.

Funding sources

This trial was funded by Action Against Hunger France, European Commission's Civil Protection and Humanitarian aid Operations, Children's Investment Fund Foundation, European Commission's Civil Protection and Humanitarian aid Operations Enhanced Response Capacity and Humanitarian Innovation Fund, a programme managed by Enhancing Learning and Research for Humanitarian Assistance. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Data availability

The dataset used and analysed during the current study is available from the Zenodo data repository: https://zenodo.org/record/3582441.

Conflict of interest

HF has received research grants from ARLA Food for Health Centre, Danish Dairy Research Foundation and also has research collaboration with Nutriset. Other authors declare no conflicts of interest.

CRediT authorship contribution statement

Suvi T. Kangas: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization, Project administration, Funding acquisition. **Cécile Salpéteur:** Conceptualization, Writing - review & editing, Project administration, Funding acquisition. **Victor Nikièma:** Data curation, Writing - review & editing, Project administration. **Leisel Talley:** Conceptualization, Writing - review & editing. **André Briend:** Conceptualization, Methodology, Validation, Writing - review & editing, Supervision. **Christian Ritz:** Methodology, Formal analysis, Writing - review & editing. **Henrik Friis:** Conceptualization, Methodology, Validation, Investigation, Writing - review & editing, Supervision. **Pernille Kaestel:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - review & editing, Supervision.

Acknowledgements

We thank the research teams for their everyday efforts in implementing the trial and the study participants and caregivers for participating in the trial.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2020.03.016.

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