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Cichon, B; Fabiansen, C; Iuel-Brockdorf, AS; Yaméogo, CW; Ritz, C; Christensen, VB; Filteau, S; Briend, A; Michaelsen, KF; Friis, H; (2018) Impact of food supplements on hemoglobin, iron status, and inflammation in children with moderate acute malnutrition: a 2 x 2 x 3 factorial randomized trial in Burkina Faso. *The American journal of clinical nutrition*, 107 (2). pp. 278-286. ISSN 0002-9165
DOI: <https://doi.org/10.1093/ajcn/nqx050>

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Impact of food supplements on hemoglobin, iron status and inflammation in children with moderate acute malnutrition: a 2x2x3 factorial randomized trial in Burkina Faso

Bernardette Cichon, Christian Fabiansen, Ann-Sophie Iuel-Brockdorf, Charles W Yaméogo, Christian Ritz, Vibeke B Christensen, Suzanne Filteau, André Briend, Kim F Michaelsen, Henrik Friis.

Department of Nutrition, Exercise and Sports, University of Copenhagen, Rolighedsvej 30, DK-1958 Frederiksberg C, Denmark (B Cichon PhD, C Fabiansen MD, PhD, C W Yaméogo MSc, A Iuel-Brockdorf PhD, Prof A Briend MD, PhD, C Ritz PhD, Prof K F Michaelsen DMSc, Prof H Friis PhD).

Médecins Sans Frontières – Denmark, Dronningensgade 68, 3. 1420 København K, Denmark (B Cichon PhD, C Fabiansen PhD, A Iuel-Brockdorf PhD, V B Christensen DMSc).

Département Biomédical et Santé Publique, Institut de Recherche en Sciences de la Santé, 03 BP 7047 Ouagadougou 03, Burkina Faso (C W Yaméogo MSc).

Center for Child Health Research, University of Tampere School of Medicine and Tampere University Hospital, Lääkärintäti 1, 33014 University of Tampere, Finland (Prof A Briend MD, PhD).

London School of Hygiene and Tropical Medicine, Faculty of Epidemiology and Population Health; Keppel Street, London, WC1E 7HT (Prof S Filteau PhD).

Department of Paediatrics, Rigshospitalet, Blegdamsvej 9, 2100, Copenhagen, Denmark (V B Christensen DMSc)

PubMed indexing: Cichon, Fabiansen, Iuel-Brockdorf, Yaméogo, Ritz, Christensen, Filteau, Briend, Michaelsen, Friis.

Address for correspondence:

Bernardette Cichon

Department of Nutrition, Exercise and Sports

University of Copenhagen,

Rolighedsvej 30,

1958 Frederiksberg C, Denmark

Email: Bernardette.Cichon@gmail.com

Tel: 0044 7966644069

Sources of support: The study was funded by Danish International Development Assistance (09-097LIFE) (KFM); Médecins Sans Frontières (Denmark, Norway); Arvid Nilsson's Foundation; The World Food Program; the Alliance for International Medical Action; and the European Union's humanitarian aid funds, in partnership with Action Contre la Faim. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

This document covers humanitarian aid activities implemented with the financial assistance of the European Union. The views expressed herein should not be taken, in any way, to reflect the official opinion of the European Union, and the European Commission is not responsible for any use that can be made of the information it contains.

Short running head: Impact of supplementary foods on hemoglobin, iron status and inflammation.

List of abbreviations: corn-soy blend (CSB); dehulled soy (DS); **dry-skimmed milk (DSM)**; hemoglobin (Hb); iron-deficiency anemia (IDA); intention-to-treat (ITT); per protocol (PP); lipid-based nutrient supplements (LNS); moderate acute malnutrition (MAM); mid-upper-arm circumference (MUAC); ready-to-use therapeutic foods (RUTF); serum α_1 -acid glycoprotein (AGP); serum c-reactive protein (CRP); serum ferritin (SF); serum ferritin adjusted for inflammation (SFAI); serum soluble transferrin receptor (sTfR); severe acute malnutrition (SAM); soy isolate (SI); weight-for-height z-score (WHZ).

Trial registration: The trial is registered at www.controlled-trials.com (ISRCTN42569496).

Abstract

1 **Background:** Children with moderate acute malnutrition (MAM) are treated with lipid-based
2 nutrient supplements (LNS) or corn-soy-blends (CSB) but little is known about their impact
3 on hemoglobin (Hb), iron status and inflammation.

4 **Objective:** The objective was to investigate the impact of supplementary foods for treatment
5 of MAM on Hb, iron status, inflammation and malaria.

6 **Design:** A randomized 2x2x3 factorial trial was conducted in Burkina Faso. Children aged 6-
7 23 months with MAM received 500 kcal/day as LNS or CSB, containing either dehulled soy
8 (DS) or soy isolate (SI) and different quantities of dry skimmed milk (0, 20 or 50% of total
9 protein) for 12 weeks. The trial was double-blind with regard to quality of soy and quantity of
10 milk, but not matrix (CSB vs LNS). Hb, serum ferritin (SF), serum soluble transferrin
11 receptor (sTfR), serum C-reactive protein (CRP), serum α_1 -acid glycoprotein (AGP) and
12 malaria antigens were measured at inclusion and after supplementation (ISRCTN42569496).

13 **Results:** Between September 2013 and August 2014, 1609 children were enrolled. Among
14 these, 61 (3.8%) were lost to follow-up. During the 12-week supplementation period,
15 prevalence of anemia, low SF adjusted for inflammation (SFAI), elevated sTfR and iron
16 deficiency anemia decreased by 16.9, 8.7, 12.6 and 10.5 percentage points. Children who
17 received LNS compared to CSB had higher Hb (2 g/L, 95% CI: 1, 4), SFAI (4.2 μ g/L, 95%
18 CI: 2.9, 5.5), and CRP (0.8 mg/L, 95% CI: 0.4, 1.2) and lower sTfR (-0.9 mg/L, 95% CI: -
19 1.3, -0.6) after the intervention. Replacing dehulled soy with soy isolate or increasing milk
20 content, did not affect Hb, SFAI, sTfR or CRP.

21 **Conclusion:** Supplementation with LNS compared to CSB led to better Hb and iron status,
22 but overall prevalence of anemia remained high. The higher concentrations of acute phase
23 proteins in children who received LNS requires further investigation.

24

- 25 **Key words:** Acute phase proteins, Africa, anemia, corn-soy blends, young children, iron
- 26 status, inflammation, lipid-based nutrient supplements, malaria, moderate acute malnutrition.

27 **Introduction**

28

29 Moderate acute malnutrition (MAM) is defined by a weight-for-height z-score (WHZ) <-2

30 and \geq -3 (moderate wasting) and/or a mid-upper arm circumference <125 mm and \geq 115mm

31 (1). While the number of children with MAM based on the above definition is unknown, it

32 has been estimated that 33 million children suffer from moderate wasting alone (2). **MAM**

33 **occurs in both non-emergency and emergency settings. In non-emergency settings it may be**

34 **possible to improve nutritional status through nutrition counselling and optimizing intake of**

35 **family foods.** In emergency settings however, where energy and nutrient needs cannot be met

36 using local foods, MAM is treated with supplementary foods either in the form of fortified

37 blended foods, such as corn-soy blends, or lipid-based nutrient supplements (LNS) (3). To

38 date there are still questions regarding the effectiveness of MAM programs in emergencies

39 (3). In 2012, WHO published a proposed nutrient composition for supplementary foods for

40 children with MAM but called for more research (4).

41

42 Studies investigating different food supplements for MAM treatment have mainly assessed

43 anthropometric outcomes (5–11). However, anthropometric deficits are likely to be

44 accompanied by micronutrient deficiencies and the return to anthropometric measurements in

45 the normal range does not necessarily mean that children are well-nourished in terms of

46 micronutrient status. Anemia affects an estimated 71% of under 5 year old children in west

47 and central Africa (12). Anemia leads to shortness of breath, fatigue and has been associated

48 with poor cognitive development, impaired work capacity and increased susceptibility to

49 infections (13). Two of its predominant causes, namely iron deficiency and infection,

50 especially malaria, are common in children with MAM in Burkina Faso (14,15).

51

52 We have previously described the impact of food supplements either in the form of LNS or
53 corn-soy blends (CSB), with either soy isolate (SI) or dehulled soy (DS) and **with** different
54 quantities of **dry skimmed** milk (**DSM**) on anthropometric outcomes and accretion of fat-free
55 tissue in children with MAM (16). The objective of this paper was to investigate the impact
56 of these supplements on hemoglobin, iron status, inflammation and malaria.

57

58 **Subjects and methods**

59 *Study area and participants*

60 This study was part of the Treatfood trial, a randomized trial with a 2x2x3 factorial design,
61 investigating the effectiveness of food supplements for the treatment of MAM. **As previously**
62 **described (16)**, research sites were constructed at **5** governmental health centers
63 (Gomponsom, Latoden, Bagaré, Bokin and Samba) in the Province du Passoré, Northern
64 Region, Burkina Faso and staffed by the Alliance for International Medical Action. The
65 catchment area covered 143 villages with a total population of ~258,000.

66

67 Screening for participants took place in villages either by community health workers using
68 mid-upper arm circumference (MUAC) tapes or by designated screening teams using both
69 MUAC and WHZ. Children could also present at the site based on the caregiver's initiative or
70 be referred from a health center. A final assessment for eligibility was carried out by study
71 staff at the sites. Children with MAM were enrolled in the trial if they were aged 6-23
72 months, resident in the catchment area and their parents/caregivers had given informed
73 consent for the children to participate. Children who were enrolled in another nutritional
74 program, had been treated for severe acute malnutrition (SAM) or been hospitalized in the
75 last two months, had an illness requiring hospitalization, a hemoglobin <50 g/L, a suspected

76 allergy to milk, peanuts, CSB or LNS, or a severe disability were not eligible. Recruitment
77 took place from September 2013 until August 2014.

78

79 *Randomization and supplementary foods*

80 Stratified, block randomization was used to allocate participants to one out of 12
81 supplements, where stratification was done by site and block sizes were either 12 or 24.
82 Blocked randomization was used to ensure that children were allocated evenly to the trial
83 arms and different block sizes were used to make the allocation process less predictable.
84 Random sequences were created by a person otherwise not involved in the trial using
85 www.randomization.com.

86

87 Supplements were either a LNS or a CSB (referred to as the matrix) with either DS or SI and
88 either 0%, 20% or 50% of protein from **DSM** (M0, M20 or M50) (**Table 1**). The trial was
89 double-blind with respect to soy quality and milk content, but not matrix. Supplements were
90 designated by a 1-letter code by the manufacturer, and a code-key was kept in a sealed
91 envelope in a safe. The supplements were packed in individual boxes containing a full 12-
92 week treatment for 1 participant (either 6 bags of CSB or 84 sachets of LNS). During
93 production, each box, bag and sachet was labelled with a 12-letter sequence containing the
94 relevant 1-letter code in a fixed position and the 11 remaining letters in random order. Only
95 one individual in Burkina Faso, otherwise not involved in recruitment and data collection was
96 aware of the position of the 1-letter code. This individual relabelled boxes and supplements
97 with individual study **identification numbers (IDs)**. At enrolment, children were given a study
98 ID by staff without access to the random sequences or supplements.

99

100 Each participant received the allocated supplement for a 12-week period, **even if they**
101 **recovered from MAM before**. LNS products were provided in individual sachets of 92 g per
102 daily ration and CSB products were provided in 1.7 kg bags per 14-day ration. All
103 supplements consisted of 500 kcal per daily serving (120 g of CSB or 92 g of LNS). LNS
104 products were ready-to-use and CSB products **needed** to be cooked using water and
105 consumed as a porridge. Supplements were manufactured by GC Rieber Compact A/S
106 (Bergen, Norway), who were otherwise not involved in the trial design or interpretation of
107 data. Nutrient composition of products complied with WHO's technical note for the
108 management of MAM (4). The recipes (16) and micronutrient composition (17) of these
109 products have previously been published. Briefly, the supplements contained approximately
110 12 mg of elemental iron added in the form of ferrous gluconate, 14 mg of zinc (**as zinc**
111 **gluconate**) and 1.15 mg of copper **in the form of copper gluconate**. Content of water soluble
112 vitamins was higher in CSBs to account for degradation during cooking. Vitamin C content
113 for example was doubled in CSB compared to LNS (188 mg vs 94 mg) and vitamin B₁₂ was
114 4.1 µg in CSB products and 3.2 µg in LNS.

115

116 **Data collection**

117 During the intervention period children visited the health center every 2 weeks. Children
118 who missed scheduled visits were visited by community health workers and encouraged to
119 return for follow-up. At baseline, study nurses collected information about sociodemographic
120 characteristics, 2-week retrospective morbidity as well as vaccination status and carried out a
121 clinical examination. Children who were not up-to-date with vaccinations were referred to a
122 health center. Children received albendazole (200 mg if < 8 kg; 400 mg > 8 kg) and vitamin
123 A (100,000 IU if 4-8 kg; 200,000 IU if >8 kg) if they had not received a supplement in the
124 previous 6 months. Weight was measured to the nearest 100 g using an electronic scale with

125 double weighing function (Seca model 881 1021659). Length was measured to the nearest 1
126 mm with a wooden height board once a month. WHZ was determined at sites using WHO
127 field tables and later recalculated using the package “zscore06” in STATA 12 (College
128 Station, Texas, USA). MUAC was measured on the left arm to the nearest 1 mm using a
129 standard measuring tape. Anthropometric measurements were taken in duplicate by trained
130 staff. A qualitative 24-hour recall was used to collect dietary data. Venous blood was
131 collected from the arm at baseline and after the supplementation period. One drop was used
132 for diagnosis of malaria (*Plasmodium falciparum*) using a rapid diagnostic test (SD Bioline
133 Malaria Ag Pf) and one drop was used to measure hemoglobin (Hb) on site using a HemoCue
134 device (Hb 301, Ängelholm, Sweden). The HemoCue was calibrated at the end of every
135 month with a control solution. The remaining blood was put into a sample tube with clot
136 activator (BD reference #368492) and transported to the trial laboratory in a cold box at 2-
137 8°C. Serum was isolated following centrifugation (EBA 20 S Hettich) and stored at -20°C
138 until shipment to VitMin Lab in Willstaedt, Germany for analysis. Serum C-reactive protein
139 (CRP), α 1-acid glycoprotein (AGP), serum soluble transferrin receptor (sTfR) and serum
140 ferritin (SF) were determined using a combined sandwich enzyme-linked immunosorbent
141 assay (18). All samples were measured in duplicate and the intra- and interassay coefficients
142 of variation were <10%. Samples were frozen and thawed only once prior to analysis.
143 The thresholds used for defining abnormal values were as follows: Hb <110 g/L (19), SF <12
144 μ g/L (19), sTfR >8.3 mg/L (18), CRP >10mg/l (20), AGP >1 g/L (21). Since SF is affected
145 by inflammation and therefore does not reliably reflect iron status in populations where
146 inflammation is common, SF was adjusted for inflammation prior to analysis using regression
147 models as previously described (15) and is referred to as SF adjusted for inflammation
148 (SFAI). Iron deficiency anemia (IDA) was defined as Hb < 110g/L and SFAI < 12 μ g/L.
149

150 ***Statistical analyses***

151 To be able to detect a 0.6 SD difference between any 2 combinations of the 3 factors with
152 80% power and a 5% significance level, while allowing for 20% loss to follow-up the
153 required sample size was 134 children per arm or 1608 in total (16). A 0.6 SD difference
154 approximately translates to a 10g/L difference for Hb, a 1.6µg/L difference for SFAI, a
155 0.9mg/L difference for sTfR, a 3.4 mg/L difference for CRP and a 0.95 g/L difference for
156 AGP. The outcomes reported here, namely Hb, sTfR, SF, CRP and AGP were secondary
157 outcomes of the trial. The primary outcome which was an increment in fat-free tissue has
158 been reported elsewhere (16).

159

160 Data were double entered into Epidata 3.1. software (Epidata Association, Odense, Denmark)
161 and double entry checks were carried out on a daily basis. All statistical analyses were carried
162 out using STATA 12. Characteristics of the study population were summarized as percentage,
163 mean ± SD or, if not normally distributed, as median (interquartile range). Chi² tests were
164 used to test for differences in proportions. T-tests and one-way ANOVAs were used to test
165 for differences in means for 2 or more groups, respectively. Changes in concentrations of Hb,
166 and of biomarkers of iron status and inflammation before and after the intervention were
167 assessed using t-tests. McNemar's Chi² was used to test for differences in proportions over
168 time.

169

170 The main analysis was based on the intention-to-treat (ITT) principle using available-case
171 data. A per protocol (PP) analysis was also carried out. Linear mixed models were used to
172 evaluate the effect of matrix, soy quality and amount of DSM on Hb, SF, SFAI, sTfR, CRP
173 and AGP and logistic mixed models for the effect on malaria. Site was included in the model
174 as a random effect. As a first step, all 3-way interactions between the 3 factors (matrix,

175 quality of soy and quantity of milk) were tested for using likelihood ratio tests and, where
176 possible, reduced to 2-way interactions or main effects. Pairwise comparisons of means were
177 then performed using model-based post-hoc tests in the reduced models. Where it was not
178 possible to reduce models, multiplicity was taken into account by adjusting all pairwise
179 comparisons using the Bonferroni method. Results were presented in terms of estimated
180 means with 95% confidence intervals. Analyses were done based on two models: model 1
181 was adjusted only for baseline measure of the outcome and site, and model 2 included
182 adjustment for baseline measure of the outcome, age, sex, MUAC, WHZ and month of
183 admission. Log transformations were applied to achieve normally distributed variables if
184 needed and estimates were subsequently back-transformed (22). Effect modification was
185 assessed for the factors and if present, investigated through sub-group analysis. Effect
186 modification was assessed for the following variables: admission criteria (MUAC only, WHZ
187 and MUAC, WHZ only), season, elevated CRP, elevated AGP, anemia, malaria and stunting
188 at baseline. Model checking was based on residual and normal probability plots.

189

190 *Ethical considerations*

191 All children in need received treatment free of charge according to an adapted version of the
192 Integrated Management of Childhood Illnesses guidelines (23,24) and the national protocol.
193 Children who developed SAM during the intervention period were treated with ready-to-use
194 therapeutic food (RUTF; Plumpy’Nut®, Nutriset, Malaunay, France). Children who did not
195 recover from MAM during the trial subsequently received treatment with RUTF. If they did
196 still not recover at the end of 4 weeks of supplementation with RUTF, they were referred to
197 the hospital for medical investigation. Children who had an Hb <110 g/L at the end of the
198 intervention period received iron and folic acid supplements; children who at any point had
199 an Hb <50 g/L were referred to the hospital. The study was carried out in accordance with the

200 declaration of Helsinki. Consent was obtained from caregivers, prior to inclusion, verbally
201 and in writing (signature or fingerprints). Data were kept confidential and in a locked facility.
202 The study was approved by the Ethics Committee for Health Research of the government of
203 Burkina Faso (2012-8-059) and consultative approval was obtained from the Danish National
204 Committee on Biomedical Research Ethics (1208204). The trial was registered in the
205 International Standard Randomized Controlled Trial Number registry under the number
206 ISRCTN42569496.

207

208 **Results**

209 As previously described (16), of the 3398 children assessed, 1613 were randomized
210 according to the 2x2x3 factorial design and four were later excluded as ineligible. A total of
211 1609 children were enrolled in the study (**Figure 1**). Baseline equivalence was achieved with
212 regard to key potential confounders (**Table 2**). Furthermore, there were no differences
213 between treatment groups in proportion of children who consumed foods from any of 7 food
214 groups, i.e. grains, legumes and nuts, dairy foods, eggs, flesh foods, vitamin A rich foods and
215 other fruit and vegetables.

216

217 Among the 1609 children who were randomized, 61 (3.8%) were lost to follow-up. Among
218 the 1548 children who completed the intervention, 1546 (96.1%) children had baseline and
219 end-line data for hemoglobin, 1523 (94.7%) for malaria and 1480 (92%) for iron status and
220 inflammation biomarkers and were included in the ITT analysis. Children who developed
221 SAM and were switched to RUTF (n=102), children who received ready-to-use
222 supplementary foods (Plumpy'Sup®, Nutriset, Malaunay, France) because of an unconfirmed
223 suspicion of *Salmonella* in one of the CSB products (n=17), and children who received iron

224 and folic acid supplements by error (n=69) or a combination were excluded from per protocol
225 analysis (**Figure 1**).

226

227 As previously described, mean baseline Hb was 100 ± 16 g/L, median SFAI was 16 (8,30)
228 $\mu\text{g/L}$ and median sTfR was 12.6 (9.1,17.3) mg/L (15). Baseline Hb differed by admission
229 criteria after adjusting for age and sex ($p=0.001$): it was 4 g/L (95% CI: 2, 7) higher in
230 children admitted based on only low WHZ compared to those admitted based on low MUAC
231 only and 3 g/L (95% CI: 1, 4) higher in children admitted based on low MUAC and WHZ
232 compared to MUAC only. There were no differences in baseline SFAI and sTfR according to
233 admission criteria. Hb increased by 7 g/L (95% CI: 6, 7) during the intervention ($p<0.001$),
234 which corresponded to 16.9 percentage points drop in prevalence of anemia (**Table 3**). SFAI
235 increased and sTfR, CRP and AGP decreased during the intervention period ($p<0.001$).
236 Prevalence of low SFAI, elevated sTfR and IDA decreased 8.7, 12.6 and 10.5 percentage
237 points, respectively. Prevalence of elevated CRP, elevated AGP and malaria decreased by
238 5.9, 19.9 and 9.2 percentage points, respectively (**Table 3**).

239

240 *Impact of supplements on Hb and biomarkers of iron status*

241 Compared to CSB, LNS resulted in higher Hb (2 g/L, 1, 4), higher SFAI (4.2 $\mu\text{g/L}$, 2.9, 5.5)
242 and lower sTfR (-0.9 mg/L, -1.3, -0.6) after adjustment for baseline measure, MUAC, WHZ,
243 age, sex, month of admission and site (**Table 4**). Results were similar if adjusted only for
244 baseline measure and site (**Table 4**) and in PP analysis (**Supplemental Table 1**). After the
245 intervention, the prevalence of anemia was 8 percentage points lower ($p=0.001$), of low SFAI
246 10 percentage points lower ($P<0.001$), and of elevated sTfR 5 percentage points lower
247 ($p=0.004$) in children who had received LNS compared to children who received CSB
248 supplements (**Table 4**). Similarly, the prevalence of IDA was 24.2% (n=183) in the CSB

249 group and 14.3% (n=110) in the LNS group after the intervention ($p<0.001$). There was no
250 effect of soy quality and milk protein content in ITT (**Supplemental Table 2 and 3**) and PP
251 analysis (**Supplemental Table 1**).

252

253 Season modified the effect of LNS vs CSB on SFAI (interaction, $p=0.02$) and sTfR
254 (interaction, $p=0.007$): SFAI was 5.3 $\mu\text{g/L}$ (95% CI: 3.7, 6.9) higher and sTfR 1.3 mg/L
255 (95% CI: -1.7, -0.9) lower in children who had received LNS compared to CSB during the
256 dry season but there was no difference during the rainy season. The effect of LNS vs CSB on
257 Hb was modified by baseline AGP (interaction, $p=0.03$), whereby the effect of LNS was
258 greater in children with elevated AGP at baseline (0.36 g/L, 95% CI: 0.2, 0.53) and was not
259 significant if AGP was <1 g/L (0.04 g/L, 95% CI: -0.18, 0.27). The effect of LNS vs CSB on
260 SFAI was modified by CRP (interaction, $p=0.045$) and malaria (interaction, $p=0.02$) at
261 baseline, i.e. it was greater in children who had elevated CRP at baseline (6 $\mu\text{g/L}$, 95% CI:
262 3.8, 8.2) than those who did not (3.2 $\mu\text{g/L}$, 95% CI: 1.5, 4.8) and in children with malaria
263 (6.1, 95% CI: 4.1, 8.2) than those without (2.9 $\mu\text{g/L}$, 95%CI: 1.2, 4.6). We did not find any
264 effect modification of admission criteria (MUAC only, WHZ and MUAC, WHZ only),
265 anemia, low SFAI or elevated sTfR or stunting at baseline on Hb or biomarkers of iron status.

266

267 *Impact of supplements on acute phase proteins*

268 After the intervention, children who received LNS supplements had a 0.8 mg/L (95% CI: 0.4,
269 1.2) higher mean CRP than those who received CSB (**Table 4**) after adjustment for baseline
270 measure, MUAC, WHZ, age, sex, month of admission and site (**Table 4**). Results were
271 similar if adjusted only for baseline measure and site (**Table 4**) and in PP analysis
272 (**Supplemental Table 1**). The prevalence of elevated CRP was 4.5 percentage points higher
273 among children who had received LNS compared to those who received CSB supplements

274 (Table 4). There was no effect of soy quality and milk protein content in ITT (Supplemental
275 Table 2 and 3) and PP analysis (Supplemental table 1). We found an interaction between
276 stunting and matrix, whereby the effect of LNS compared to CSB on CRP was greater in
277 children who were stunted (1.2 mg/L, 95% CI: 0.7, 1.8 mg/L) than in those who were not (0.4
278 mg/L, 95% CI: -0.12, 0.86). We did not find any effect modification between admission
279 criteria, elevated acute phase proteins, anemia, low SFAI or elevated sTfR and malaria at
280 baseline with any of the factors (interaction, $p > 0.05$).

281

282 Similarly to CRP, AGP was also higher in children who received LNS compared to those
283 who received CSB. However, there was a significant 3-way interaction between the factors
284 ($p=0.03$ in model 1, $p=0.045$ in model 2) whereby LNS-DS-50M led to higher AGP than
285 LNS-SI-50M (0.2 g/L, 95% CI: 0.1, 0.4). Results were similar in PP analysis (Supplemental
286 Table 1) but in the latter the interaction was not significant. There were no effects of soy
287 quality or milk protein content in ITT analysis (Supplemental Table 2 and 3) or PP analysis
288 (Supplemental Table 1). The effect of LNS vs CSB was modified by season (interaction,
289 $p=0.01$), whereby AGP was higher in children who received LNS vs CSB if they were
290 admitted during the rainy season (0.17g/L, 95% CI: 0.08, 0.26) compared to the dry season
291 (0.03 g/L, 95%CI: -0.04, 0.09).

292

293 *Impact on malaria prevalence*

294 There was no effect of matrix (Table 4), quality of soy (Supplemental Table 2) and quantity
295 of milk (Supplemental Table 3) on prevalence of malaria. Results were similar in the PP
296 analysis. There were no interactions between any of the factors and season, admission
297 criteria, anemia, low SFAI or elevated sTfR, CRP or AGP at baseline.

298

299 **Discussion**

300 In this randomized trial we have shown that LNS was more effective in improving Hb and
301 iron status than CSB but that concentrations of inflammatory markers were higher in children
302 who received LNS. There was no impact of quality of soy and quantity of milk.

303 Studies in children with MAM have previously reported better outcomes from LNS compared
304 to CSB or CSB++ (also known as supercereal+) in terms of weight gain (6,9,25), MUAC
305 gain and time to recovery (6). Better recovery rates have been found if LNS were compared
306 to standard CSB (5,9,25) but not if compared to CSB++ (6,26). Furthermore, based on data
307 from the same trial, we have recently shown that gain of fat-free tissue and rates of
308 anthropometric recovery were higher in children who received LNS compared to those who
309 received CSBs (16). Data on the impact of supplements for treatment with MAM on Hb and
310 iron status is, however, limited. One study carried out in Mali by Ackatia-Armah et al found
311 lower concentrations of sTfR in children who received LNS compared to CSB++ or locally
312 blended flours (26). In the same study, Ackatia-Armah et al also found higher Hb and SFAI
313 in children who received LNS compared to a locally produced blended flour, but there was no
314 difference between those who received LNS and CSB++ (26).

315

316 Several possible mechanisms could explain the greater impact of LNS: better absorption of
317 iron, better acceptability, or less sharing of the products. Absorption of iron from food
318 depends on the type of iron, content of iron enhancers (e.g. vitamin C) or iron inhibitors (e.g.
319 phytate), as well as iron stores of the individual and presence of infection (27). While
320 products contained the same type of iron the amount of vitamin C or phytate may play a role.
321 It has been estimated that 50% of vitamin C in CSB is lost during cooking (28). Double the
322 amount of vitamin C was therefore added to CSB compared to LNS; while this should be
323 sufficient, it is unclear how much vitamin C was present at the time of consumption since

324 losses depend on cooking time and temperature. Furthermore, the amount of soy products per
325 daily serving of CSB, and thus phytate from soy, was on average double than that of the LNS.
326 However, the total amount of phytate in the products is unknown since other ingredients, i.e.
327 corn and peanuts, are also sources of phytate and no analysis of phytate content was carried
328 out after production. We have previously shown that in this population, children and
329 caregivers preferred LNS and that more leftovers were reported in CSB groups (17). The
330 preference for LNS as well as the finding that appreciation of foods was greater and leftovers
331 less during the rainy season when food availability is reduced (17) may also explain why the
332 impact of LNS vs CSB on iron status was more pronounced during the dry season when a
333 better availability of family foods may have led to more leftovers of the less preferred
334 product. Furthermore, the effect of LNS vs CSB on Hb was greater in children who had
335 elevated AGP. In line with this, the effect of LNS vs CSB on SFAI was greater in children
336 with malaria or elevated CRP. While the latter could be an artefact since SFAI was adjusted
337 for inflammation, the general trend of LNS having greater effect in children with infection or
338 inflammation suggests that this may also have other reasons, such as the impact of infection
339 on appetite. Lastly, while previous studies have shown that CSBs are more likely to be shared
340 than LNS (29,30), this did not seem to be a problem in our study population (17).

341

342 We did not find an effect of soy quality. However, while SI contains less phytate than DS,
343 soy isolates do contain phytate, even small amounts of phytate have been shown to affect
344 absorption, (31,32) and soy is not the only phytate-containing ingredient in the products. We
345 did not find an effect of DSM quantity or an interaction between milk and soy. This means
346 that reducing milk and replacing it with soy did not negatively impact iron status and this was
347 not different if SI or DS was used. The lack of impact of milk may also be linked to the high
348 breastfeeding rate, which was 95% at baseline.

349 While the prevalence of inflammation reduced throughout the intervention period, at the end
350 of the intervention 20% of children had elevated CRP and 45% elevated AGP. This is not
351 surprising considering the high burden of diseases in the study location (14). Higher
352 concentrations of acute phase proteins in children who received LNS may be related to the
353 higher linoleic acid content in LNS which can be converted to inflammatory metabolites via
354 arachidonic acid (33) or the amount of absorbed iron. Iron status was better in children who
355 received LNS at the end of the intervention indicating that more iron was absorbed. While
356 iron is an essential nutrient, the safety of iron supplementation particularly in malaria-
357 endemic areas has been questioned (34,35). Even though a recent systematic review on this
358 issue concluded that iron supplements can be given to children if services to treat and prevent
359 malaria are provided (36), it is not clear whether iron supplementation would also be safe in
360 malnourished children, where iron withholding mechanisms may be impaired. In addition to
361 iron supplementation, studies have also found higher morbidity among children who received
362 micronutrient fortified complementary foods (37–39). We did not find an impact on malaria,
363 which is not unexpected since participants received regular treatment for malaria and we only
364 had data from rapid tests and not parasitemia. Nevertheless, the impact on inflammation
365 reported here, which occurred despite regular medical follow-up and treatment for all
366 identified infections, deserves further attention as both causes and implications are unclear.

455

456 In this population of children with MAM, anemia was very common. The lower Hb in
457 children admitted based on low MUAC only compared to those with low WHZ only at
458 baseline may be linked to a higher prevalence of malaria in this group as previously reported
459 (14). While the prevalence of anemia decreased during the intervention period, by 13% points
460 in the CSB and 21% points in LNS group, the prevalence in both groups remained high after
461 supplementation. Similar results have previously been reported (26). It is important to note

462 that, the iron content of the supplements was lower than therapeutic doses (40) and may have
463 been insufficient particularly for children with a Hb <110 g/L. However, considering the
464 large burden of infection and inflammation in this setting (14) it is unclear whether further
465 supplementation would have been beneficial. It is also worth mentioning that doubts about
466 the validity of current cut-offs for definition of anemia have been raised (41–44) and the
467 110g/L cut-off may be too high in young children living in Burkina Faso.

468

469 This study had a number of strengths and limitations. First it is one of few studies
470 investigating the effects of supplements on hemoglobin, iron status and inflammation in
471 children with MAM. The use of a factorial design enabled us to assess the effect of three key
472 factors in foods supplements and testing for interactions between the factors enabled us to
473 investigate the potential impact of different combinations of these factors, e.g. whether
474 removing DSM and adding more soy of different qualities and thus different amounts of anti-
475 nutrients affect iron status. However, a limitation of this design is that the ingredients differed
476 somewhat between products, e.g. since SI contains more protein than DS, content of other
477 ingredients had to be adapted to keep the overall energy and protein content constant. Other
478 limitations include the lack of an unsupplemented control group, the lack of data on malaria
479 parasitaemia and that we did not carry out a nutrient composition analysis to determine the
480 total phytate content in the products and vitamin C content in CSB after cooking.

481

482 In conclusion, we have shown that children supplemented with LNS had significantly better
483 Hb and iron status at the end of the supplementation period than those who received CSB
484 products but overall prevalence of anemia remained high. The higher concentrations of
485 inflammation biomarkers reported in children who received LNS requires further
486 investigation.

487

488 **Acknowledgements**

489 The authors declare no conflict of interest. HF, KFM, VBC, AB and SF conceived the study.

490 AI, BC, CF and CWY planned and conducted the study. BC, CF and CR did the statistical

491 analyses; BC wrote the first version of manuscript. All authors contributed to revisions of the

492 paper. BC had primary responsibility for final content.

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Matrix	Soy quality	Milk protein %		
		0	20	50
Corn-soy blend	Dehulled	a	b	c
	Isolate	d	e	f
Lipid-based nutrient supplement	Dehulled	g	h	i
	Isolate	j	k	l

Table 1. The experimental food supplements based on corn-soy blend or lipid-based nutrient supplement, with either dehulled soy or soy isolate, and with 0, 20 or 50% of total protein from milk. Product "a" is similar to CSB+ (also known as Supercereal) and product "b" to CSB++ (also known as Supercereal+). Product "i" is similar to Plumpy'Sup®, (Nutraset, Malaunay, France), but Plumpy'Sup® contains whey instead of dry skimmed milk.

Table 2. Baseline characteristics of 1609 6-23 months old children enrolled in the study by factorial design^{1,2,3}

	Matrix		Soy quality			Milk protein %	
	CSB (n=800)	LNS (n=809)	Dehulled (n=800)	Isolate (n=809)	0% (n=541)	20% (n=528)	50% (n=540)
Sociodemographic data							
Sex, male	356 (45)	374 (46)	373 (47)	357 (44)	246 (46)	241 (46)	243 (45)
Age, months, median (IQR)	11 (8-16)	12 (8-16)	11 (8-16)	11 (8-16)	11 (8-16)	11 (8-16)	11 (8-16)
Anthropometry							
MUAC, mm, mean (SD)	123 (4.0)	123 (4)	123 (4)	123 (4)	122 (4)	123 (4)	123 (4)
WHZ, mean (SD)	-2.2 (0.5)	-2.2 (0.5)	-2.2 (0.5)	-2.2 (0.5)	-2.2 (0.5)	-2.2 (0.5)	-2.2 (0.5)
Admission by:							
Low MUAC only	225 (28)	243 (30)	226 (28)	242 (29)	154 (29)	143 (27)	171 (32)
Low WHZ and low MUAC	404 (50)	400 (50)	406 (51)	398 (49)	276 (51)	275 (52)	253 (47)
Low WHZ only	171 (21)	166 (21)	168 (21)	169 (20)	111 (21)	110 (21)	116 (22)
Morbidity							
Ill in the previous two weeks	303 (38)	305 (38)	318 (40)	290 (36)	207 (39)	206 (39)	195 (36)
Positive malaria rapid test	324 (41)	320 (40)	322 (41)	322 (40)	216 (40)	207 (39)	221 (41)
Breastfed	755 (95)	766 (95)	755 (95)	766 (95)	515 (95)	93 (93)	513 (95)

¹Data are n (% of non-missing data) unless otherwise stated; ² Data missing: ill in the previous two weeks (n=9), malaria rapid test (n=8), breastfeeding (n=2).

³ Abbreviations: CSB= corn-soy blend, IQR=interquartile range; LNS=lipid nutrient supplements; MUAC=mid upper arm circumference; SD= standard deviation

Table 3. Changes in hemoglobin, biomarkers of iron status, inflammation and malaria prevalence in the full cohort during the 12-week supplementation period^{1,2}

	Baseline		After supplementation		Difference ⁴		p
	n		n		n		
Hemoglobin, g/L	1608	100 ± 16	1546	107 ± 14	1546	+7 (+6, +7)	<0.001
% (n) < 110 g/L	1608	70.2 (1129)	1546	53.2 (821)	1546	-16.9 (-56.5, -12.6)	<0.001
SFAI, µg/L	1564	16 (8-30)	1511	18.1 (11.0-28.8)	1462	+2 (+1.2, +2.6)	<0.001
% (n) < 12 µg/L	1564	38.3 (595)	1511	29.3 (443)	1462	-8.7 (-14.5, -3.0)	0.004
sTfR, mg/L	1564	12.6 (9.1-17.3)	1520	10.2 (8-13.5)	1480	-2.2 (-2.5, -2.0)	<0.001
% (n) > 8.3 mg/L	1564	82.9 (1296)	1520	70.1 (1065)	1480	-12.6 (-16.0, -9.1)	<0.001
Iron deficiency anemia ³ , % (n)	1555	30.0 (469)	1511	19.4 (293)	1462	-10.5 (-16.8, -4.2)	0.001
C-reactive protein, mg/L	1564	2.3 (0.8-9.3)	1520	1.7 (0.6-6.2)	1480	-0.6 (-0.8, -0.4)	<0.001
% (n) >10 mg/L	1564	25.4 (398)	1520	19.9 (302)	1480	-5.9 (-12.2, 0.2)	0.06
α1-acid glycoprotein, g/L	1564	1.2 (0.9-1.6)	1520	1 (0.7-1.4)	1480	-0.21 (-0.24, -0.17)	<0.001
% (n) >1 g/L	1564	66.4 (1039)	1520	45.7 (695)	1480	-19.9 (-24.6, -15.4)	<0.001
Rapid malaria test, % positive	1601	40.2 (644)	1531	31.3 (479)	1523	-9.2 (-14.8, -3.5)	0.002

¹ Data are mean ± SD for hemoglobin, median (IQR) SFAI, sTfR, C-reactive protein, α1-acid glycoprotein or mean (95%CI) for the differences unless otherwise stated; ² Abbreviations: SFAI, serum ferritin adjusted for inflammation; sTfR, serum soluble transferrin receptor; IQR, interquartile range; Iron deficiency anemia (IDA) was defined as hemoglobin < 110g/L and SFAI < 12 µg/L; ⁴Changes in concentrations before and after the intervention were assessed using t-tests. McNemar's Chi² was used to test for differences in proportions over time.

Table 4. Hemoglobin, markers of iron status and inflammation and malaria prevalence after 12 weeks of supplementation with CSB compared to LNS in the intention-to-treat population^{1,2}

	CSB	LNS	Model 1 ³ Mean difference	p	Model 2 ⁴ Mean difference	p
Hemoglobin, g/L	105 ±14	108 ± 13	3 (1, 4)	<0.001	2 (1, 4)	<0.001
% (n) < 110 g/L	57.4 (445)	49 (382)				
Serum ferritin, µg/L	23 [13-48.4]	30.6 [18-58.7]	9.5 (6.6, 12.3)	<0.001	9.8 (7.02, 12.6)	<0.001
% (n) < 12µg/L	22.1 (168)	11.3 (87)				
SFAI, µg/L	16.3 [9.5-25.5]	19.6 [12.2-30.9]	4.3 (2.9, 5.6)	<0.001	4.2 (2.9, 5.5)	<0.001
% (n) < 12µg/L	34.3 (257)	24.4 (186)				
sTfR, mg/L	10.6 [8.2-14.2]	9.8 [7.8-12.8]	-1 (-1.4, -0.6)	<0.001	-0.9 (-1.3, -0.6)	<0.001
% (n) > 8.3mg/L	72.8 (549)	67.4 (516)				
C-reactive protein, mg/L	1.4 (0.5-5.1)	2.2 [0.7-8.1]	0.7 (0.3, 1.1)	<0.001	0.8 (0.4, 1.2)	<0.001
% (n)>10 mg/L	17.6 (133)	22.1 (169)				
α1-acid glycoprotein, g/L	0.9 [0.7-1.4]	1 [0.7-1.5]	0.07 (0.02, 0.12) ⁵	0.02	0.08 (0.02, 0.13) ⁶	0.004
% (n)>1g/L	44.8 (344)	49.9 (389)				
Rapid malaria test, % (n) positive	29.7 (225)	32.9 (254)	3.1 (-1.6, 7.7)	0.19	2.7 (-1.0, 10.7)	0.16

¹ Data are mean ± SD for hemoglobin, median (IQR) for serum ferritin, SFAI, sTfR, C-reactive protein, α1-acid glycoprotein or mean difference (95% CI) unless otherwise stated.

² Abbreviations: CSB, Corn-soy blend; DS, dehulled soy; IQR, interquartile range; LNS, lipid nutrient supplement; SFAI, serum ferritin adjusted for inflammation; SI, soy isolate; sTfR, serum soluble transferrin receptor.

³ Results are based on linear mixed models for continuous outcomes and logistic mixed models for malaria adjusted only for baseline measure and site.

⁴ Results are based on linear mixed models for continuous outcomes and logistic mixed models for malaria adjusted for baseline measure, mid-upper arm circumference, weight-for-height z-score, age, sex, month of admission and site.

⁵ Interaction between matrix, soy quality and milk (p=0.03): LNS-DS-50% milk vs LNS-SI-50% milk= 0.22 (0.1; 0.44)

⁶ Interaction between matrix, soy quality and milk (p=0.045): LNS-DS-50% milk vs LNS-SI-50% milk= 0.21(0.06; 0.42)

Figure 1. Trial Profile