Probiotics and prebiotics for severe acute malnutrition (PRONUT study): a double-blind efficacy randomised controlled trial in Malawi

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Summary

Background Severe acute malnutrition affects 13 million children worldwide and causes 1–2 million deaths every year. Our aim was to assess the clinical and nutritional efficacy of a probiotic and prebiotic functional food for the treatment of severe acute malnutrition in a HIV-prevalent setting.

Methods We recruited 795 Malawian children (age range 5 to 168 months [median 22, IQR 15 to 32]) from July 12, 2006, to March 7, 2007, into a double-blind, randomised, placebo-controlled efficacy trial. For generalisability, all admissions for severe acute malnutrition treatment were eligible for recruitment. After stabilisation with milk feeds, children were randomly assigned to ready-to-use therapeutic food either with (n=399) or without (n=396) Synbiotic2000 Forte. Average prescribed Synbiotic dose was 10^{10} colony-forming units or more of lactic acid bacteria per day for the duration of treatment (median 33 days). Primary outcome was nutritional cure (weight-for-height >80% of National Center for Health Statistics median on two consecutive outpatient visits). Secondary outcomes included death, weight gain, time to cure, and prevalence of clinical symptoms (diarrhoea, fever, and respiratory problems). Analysis was on an intention-to-treat basis. This trial is registered as an International Standard Randomised Controlled Trial, number ISRCTN19364765.

Findings Nutritional cure was similar in both Synbiotic and control groups (53·9% [215 of 399] and 51·3% [203 of 396]; p=0·40). Secondary outcomes were also similar between groups. HIV seropositivity was associated with worse outcomes overall, but did not modify or confound the negative results. Subgroup analyses showed possible trends towards reduced outpatient mortality in the Synbiotic group (p=0·06).

Interpretation In Malawi, Synbiotic2000 Forte did not improve severe acute malnutrition outcomes. The observation of reduced outpatient mortality might be caused by bias, confounding, or chance, but is biologically plausible, has potential for public health impact, and should be explored in future studies.

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Introduction

Severe acute malnutrition affects 13 million children globally, and causes 1 to 2 million child deaths every year.[1] Mortality in some settings is especially high. Programme case fatality rates of 20–30%, sometimes 50–60%, have been reported.[2] Advances have been made initially by improving treatment protocols,[3] and recently with community-focused programmes.[4] However, challenges persist, especially where HIV is prevalent,[5,6] and therapeutic innovations to improve severe acute malnutrition outcomes remain a public health priority.

Probiotic and prebiotic functional foods are claimed to provide health benefits beyond the provision of essential nutrients.[7] For some conditions such as diarrhoea, evidence of effect is good.[8,9] Most functional-food research, however, focuses on high-income countries—where product sales are high (ie, US$16 billion per year for probiotics alone)[10]—whereas only a few studies are done in low-income nations, where severe acute malnutrition and preventable mortality are high.[11]

In some patients, probiotics and prebiotics reduce diarrhoea, promote healthy gut flora, reduce pathogenic gut bacteria, and directly or indirectly modulate the immune system.[12] Therefore, they should also be beneficial in severe acute malnutrition when impaired gut function is a problem, manifested as diarrhoea and malabsorption,[13,14] small bowel overgrowth,[15] increased intestinal permeability,[16] enteropathy,[17] gram-negative (enteric) bacteraemia,[18,19] and suboptimal immune response.[20]

International efforts to deal with severe acute malnutrition are currently focused on community-based management of acute malnutrition,[21] which aims to mobilise communities, decentralise services, and facilitate early presentation to care. These measures ensure high programme coverage.[22] With community-based management of acute malnutrition programmes now treating many children, improved foods with even modest benefits to individual patients mean large beneficial effects for public health.

However, probiotics have a small risk of causing invasive infection;[23] therefore, caution is recommended in immune-compromised patients.[24] Improved risk–benefit data are vital: the most vulnerable, most

immunosupressed children with severe acute malnutrition[25] and HIV[26] are also those needing therapeutic improvements.

Ready-to-use therapeutic foods are high-energy, nutrient-dense paste, formulated to WHO standards for the treatment of severe acute malnutrition,[27] and are used in almost all programmes of community-based management of acute malnutrition. As the product is manufactured in food-processing factories and packaged in sealed containers, inclusion of functional additives to the standard ready-to-use therapeutic foods recipe can be readily achieved, and can be used for large-scale rollout in feeding programmes.

Here, we report the findings of a randomised controlled trial that assessed the effect of probiotic[28] and prebiotic[29] functional food to improve existing treatments for severe acute malnutrition (the PRONUT study).

Methods

Setting

The PRONUT study was done at MOYO nutrition rehabilitation unit, Queen Elizabeth Central Hospital, Malawi. Set within the paediatric department of a large urban teaching and referral hospital, the MOYO unit is responsible for all children admitted with severe acute malnutrition. Over 40% admissions are HIV seropositive, whereas the national average is 24%.[30]

Participants

Malawian children (age range 5 to 168 months [median 22, IQR 15 to 32]) were recruited between July 12, 2006, to March 7, 2007. We defined severe acute malnutrition as weight-for-height of less than 70% of the median (National Center for Health Statistics reference), nutritional oedema (kwashiorkor), or both, mid-upper arm circumference of less than 11 cm, or both.[31] Anthropometry protocols followed research standards.[32] Children were weighed on Tanita 1582 digital scales accurate to 20 g and calibrated daily. Lengths and mid-upper arm circumferences were measured to the nearest 1 mm using locally made height boards and mid-upper arm circumferences insertion tapes procured by UNICEF.

HIV counselling and testing was a routine part of standard ward protocols, and consisted of two ELISA rapid tests (Determine HIV-1/2 [Abbott Laboratories, USA] and Uni-Gold HIV [Trinity Biotech PLC, Ireland]), with a third (Hema Strip HIV 1/2 [Chembio Diagnostic System Inc, USA] or SD-Bioline HIV 1/2 [Standard Diagnostics Inc, Korea]) for discordant results. PCR for definitive diagnosis in children younger than 18 months of age was unavailable. HIV-positive patients received further care according to national protocols, which included referral for antiretroviral medication based on clinical-staging criteria (ie, failure to respond to nutritional treatment). An antiretroviral waiting list at the time of the study meant that additional inputs (ie, antiretroviral medication) rarely started during our nutritional treatment.

All children admitted to the nutrition rehabilitation unit were eligible for recruitment after written informed consent. The study was approved by the College of Medicine Research and Ethics Committee (Malawi) and the Institute of Child Health (UK).

Randomisation and masking

A random sequence for the two study groups was computer generated independently of the field team. Permuted blocks of 50 (25 group 1 and 25 group 2 per block) ensured balanced groups for interim safety analysis. An independent volunteer inserted one of two sticky labels (printed group 1 and group 2) into sealed, opaque, sequentially numbered envelopes.

When consenting and eligible patients received ready-to-use therapeutic food for the first time, study staff would open the next numbered envelope to reveal the label concealed inside, which assigned the patient to one of the two groups. To ensure the correct group was maintained for all subsequent distribution rounds, the label was stuck in the back page of the child's health passport, which the carer held at all times.

The study was double blind. Taste, colour, and texture of standard (control) and intervention (Synbiotic) food were indistinguishable, so patients were blind to their group allocation. A small printed label on the food bottle lid was the only way to identify the correct group. At the beginning of the study, the food factory manager decided independently and at random whether group 1 or 2 should contain Synbiotic. Project field staff were unaware of whether group 1 or 2 contained the Synbiotic. They were also blind to allocation when assessing or managing a patient. Group status 1 or 2 was recorded hidden inside the child health passport but nowhere else on patient or study files. All investigators except MK were unaware of study codes. MK was inadvertently unblinded part way into the study while coordinating ready-to-use therapeutic food quality control testing. However, we believe that the study design was robust enough to prevent this causing any biases: main outcomes were hard and objective (eg, weight, death); other staff remained fully blinded; group randomisation, concealment, and allocation were all independent of MK; and randomisation data were entered and stored independently from other databases and merged only before final data analysis.

Interventions

Treatment followed Malawi national guidelines for the management of acute malnutrition,[31] which are based on standard international WHO and community-based management of acute malnutrition guidelines.[33,34] All children were initially fed F75 therapeutic milk. This stabilisation phase usually lasted 2–4 days. PRONUT began when a child progressed to rehabilitation phase feeds. Criteria for progression were clinical improvement and return of active appetite (ie, easily finishing the prescribed volume of F75 milk). Ready-to-use therapeutic

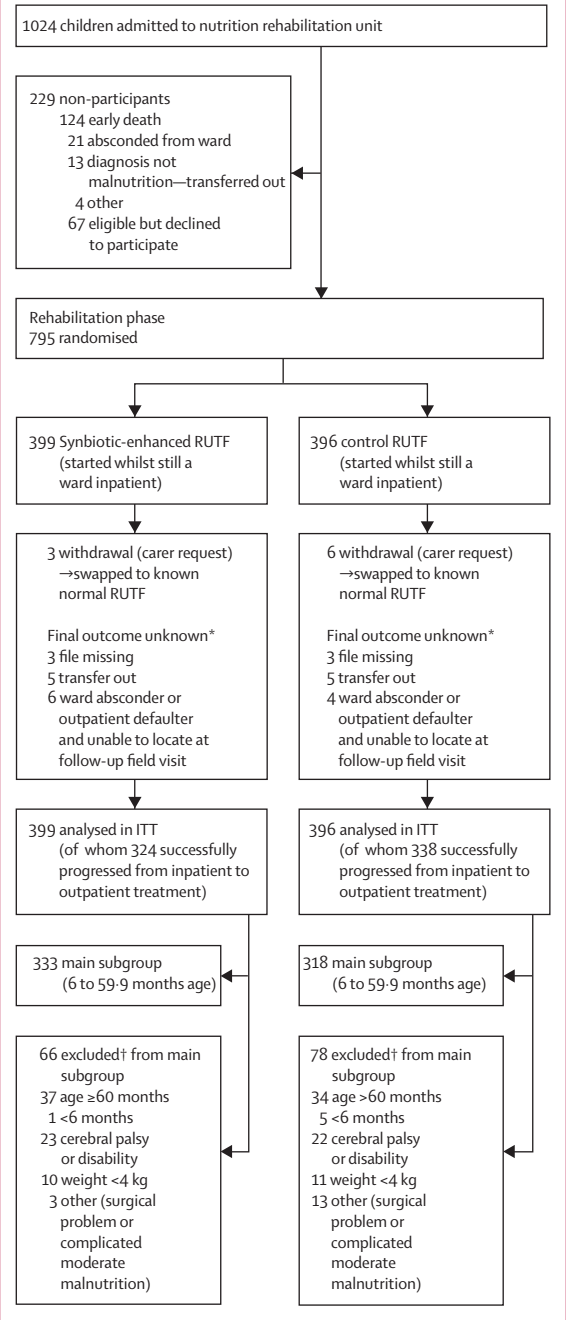


Figure 1: **Trial profile**
ITT=intention to treat. RUTF=ready-to-use therapeutic food. *Included in the analysis as far as possible because, even if study notes were missing, some outcome data were available from other sources (eg, from ward register, notes before transfer, or before default or absconding). †Same patients can have more than one reason for exclusion.

food[27] was added to the diet at this stage. Therapeutic food prescriptions provided 200 kcal per kg of bodyweight per day. For a 7 kg child (median nutrition rehabilitation unit admission weight), prescription was about 300 g of ready-to-use therapeutic food per day.

	Synbiotic (n=399)	Control (n=396)
Demographic profile		
Age in months	22 (15 to 32)	21 (15 to 31)
Boys	214/399 (53·6%)	216/393 (55·0%)
Nutritional diagnosis		
Wasting (<70% weight-for-height (NCHS) and/or MUAC <11 cm, no oedema)	142/399 (35·6%)	146/396 (36·9%)
Kwashiorkor (oedematous malnutrition)	238/399 (59·6%)	217/396 (54·8%)
Anthropometry		
Height-for-age	−3·19 (1·5)	−3·12 (1·4)
Weight-for-height	−2·19 (1·2)	−2·33 (1·3)
Weight-for-age	−3·50 (1·3)	−3·58 (1·3)
Child HIV status		
HIV seropositive	170/399 (42·6%)	192/396 (48·5%)
HIV seronegative	203/399 (51·0%)	190/396 (48·0%)
Not tested or unknown	26/399 (6·5%)	14/396 (3·5%)
Family and socioeconomic status		
Main carer is mother	329/387 (85·0%)	321/384 (83·6%)
Mother literate	246/378 (65·1%)	243/366 (66·4%)
Household water source		
Piped	208/386 (53·9%)	217/382 (56·8%)
Borehole or protected well	132/386 (34·2%)	117/382 (30·6%)
Household toilet		
Traditional pit latrine	373/386 (96·6%)	367/382 (96·1%)

Data are number (%), mean (SD), or median (IQR). MUAC=mid-upper arm circumference. NCHS=National Center for Health Statistics.

Table 1: **Baseline patient characteristics**

The control group received standard ready-to-use therapeutic food, whereas the intervention group received ready-to-use therapeutic food plus Synbiotic2000 Forte (Medipharm AB, Kågeröd, Sweden). Freeze-dried Synbiotic powder was factory-mixed into ready-to-use therapeutic food at a weight ratio of 1 to 50. Synbiotic constituents were: four different probiotic lactic acid bacteria (10^{11} colony-forming units of bacteria total) (*Pediococcus pentosaceus* 16:1 LMG P-20608, *Leuconostoc mesenteroides* 23-77:1 LMG P-20607, *Lactobacillus paracasei ssp paracasei* F-19 LMG P-17806, and *Lactobacillus plantarum* 2362 LMG P-20606) and four prebiotic fermentable bioactive fibres (2·5 g of each per 10^{11} bacteria) (oat bran [rich in β-glucans], inulin, pectin, and resistant starch).

Randomly selected samples of ready-to-use therapeutic food were regularly sent to Medipharm's laboratories in Sweden within a week of manufacture and again after 2 months storage at local ambient temperature in Malawi. Microbiological culture showed that intervention therapeutic food consistently contained more than 1×10^8 colony-forming units of lactic acid bacteria per gram of food, which equates to a prescribed average dose of more than 1×10^{10} colony-forming units of bacteria per patient per day.

	Synbiotic (n=399)	Control (n=396)	Relative risk (discrete variables) (95% CI)	p value
Primary outcome				
Nutritional cure (total)	215/399 (53·9%)	203/396 (51·3%)	1·06 (0·93 to 1·21)	0·40
HIV-seropositive cures	66/170 (38·8%)	71/192 (37·0%)	1·05 (0·81 to 1·37)	0·71
HIV-seronegative cures	145/203 (71·4%)	131/190 (68·9%)	1·04 (0·91 to 1·18)	0·59
Secondary outcomes				
Death (total)*	108/399 (27·1%)	119/396 (30·0%)	0·90 (0·72 to 1·12)	0·31
Outpatient defaulters or ward absconders	27/399 (6·8%)	36/396 (9·0%)	0·74 (0·46 to 1·20)	0·23
Failures of nutritional treatment	14/399 (3·5%)	14/396 (3·5%)	0·99 (0·48 to 2·05)	0·98
Readmissions	27/399 (6·8%)	16/396 (4·0%)	1·67 (0·92 to 3·06)	0·08
Other (transfers out; final outcome unknown)	8/399 (2·0%)	8/396 (2·0%)	1·12 (0·44 to 2·86)	0·81
Rate of weight gain (g/kg/day)	4·18 (4·0)	4·14 (4·1)	0·04 (–0·53 to 0·61)†	0·65
Length of stay in programme (days to cure)	37 (34 to 48)	38 (34 to 47)	..	0·42
Outcomes stratified by treatment phase‡				
Deaths (inpatient, during first admission)	61/399 (15·3%)	52/396 (13·1%)	1·16 (0·83 to 1·64)	0·38
Deaths (any time during remainder of study)	47/338 (13·9%)	67/344 (19·4%)	0·71 (0·51 to 1·00)	0·05
Total deaths during all inpatient treatment episodes (including readmissions)	78/486 (16·1%)	74/467 (15·9%)	1·01 (0·76 to 1·36)	0·93
Total deaths during all outpatient treatment episodes (including readmissions)	30/394 (7·6%)	45/387 (11·6%)	0·65 (0·42 to 1·02)	0·06

Data are number (%), mean (SD), or median (IQR). *Children who died during outpatient treatment, or at any time during subsequent admission episodes during the study, were classified under the total death category. †Mean difference (continuous variables). ‡See webappendix p 4 for further details.

Table 2: **Main outcomes**

Once clinically well and easily finishing at least three quarters of their daily food ration, children were discharged to complete nutritional rehabilitation at home. They continued original group allocations of either control or Synbiotic-enhanced food. Prescriptions remained at 200 kcal per kg of bodyweight per day. Every 14 days, children attended outpatient clinics for clinical review and to collect further portions of therapeutic food.

Safety and sepsis monitoring

To monitor trial safety, an interim analysis reviewed the main outcomes—cure and death rates. Early stopping criteria were group differences exceeding the Peto-Haybittle rule ($p<0·001$) or serious adverse events.

On admission, all children routinely started a 7-day course of co-trimoxazole. Dose was 120 mg twice a day for children weighing less than 10 kg; 240 mg twice a day for children weighing 10–25 kg; and 480 mg twice a day for those weighing more than 25 kg. HIV-seropositive children were continued on long-term daily prophylaxis (120 mg co-trimoxazole once a day for children weighing less than 10 kg; 240 mg twice a day for children weighing 10–25 kg; and 480 mg twice a day for those weighing more than 25 kg). According to clinical need, some also received parenteral second-line or third-line antibiotics. Standard second-line regimen was chloramphenicol and gentamicin, whereas third-line was ceftriaxone or ciprofloxacin plus gentamicin.

In cases of presumed sepsis in a sick child, a blood culture was taken. To identify possible probiotic-related sepsis, we developed additional microbiology protocols involving culture on MRS agar (Oxoid Ltd, Cambridge, UK) and subsequent bacterial subspecies identification. These were never needed because no suspicious organisms grew at the initial culture stage.

Outcomes

The primary outcome was the percentage of children achieving nutritional cure, defined as two consecutive outpatient visits, 14 days apart, with weight-for-height of 80% or more of the median (National Center for Health Statistics reference).

The main secondary outcomes were: routine nutrition programme performance indicators:[33–35] death rate; default rate (defined as missing two consecutive outpatient visits, about 4 weeks without contact); nutritional failure rate (defined as not achieving cure despite five visits, about 10 weeks of follow-up); readmission to hospital; weight gain (calculated using minimum, non-oedematous, inpatient weight as the baseline weight); and length of stay in programme (days). Failures, most commonly due to underlying HIV, were referred for further care. Defaulters were followed up by a mobile team. Deaths at home were classified as outpatient deaths .

Carer-reported clinical outcome indicators were also collected. Trained staff used prepiloted, standardised questionnaires. Symptoms were reported daily while the

	Synbiotic	Control	p value
Clinical symptoms (total patient days with symptom over 1000 days of patient observation)			
Total days of patient observation	12 909 (32 [16 to 41])	14 124 (33 [21 to 46])	0·04
Total days inpatient observation	2527 (5 [4 to 8])	2552 (6 [4 to 8])	0·35
Total days outpatient observation	10 408 (27 [10 to 33])	11 562 (28 [14 to 39])	0·03
Diarrhoea as inpatient	250	202	0·31
Severe diarrhoea as inpatient	80	51	0·01
Diarrhoea as outpatient	41	41	0·95
Severe diarrhoea as outpatient	11	16	0·07
Vomiting as inpatient	273	215	0·05
Vomiting as outpatient	12	12	0·64
Abdominal pain as inpatient	160	166	0·57
Abdominal pain as outpatient	19	15	0·43
Fever as inpatient	310	265	0·21
Fever as outpatient	42	47	0·26
Cough as inpatient	476	421	0·05
Cough as outpatient	124	106	0·69
Fast or difficult breathing as outpatient	5	7	0·15
Outpatient visits (total visits)	2 (0 to 2) (667)	2 (0 to 2) (702)	0·34
Use of non-routine drugs (prescribed elsewhere since last review or prescribed at nutrition outpatient clinic review)			
Total visits at which any drugs used over total outpatient visits	246/667 (36·9%)	258/702 (36·8%)	0·96*
Total visits at which antibiotics used over total outpatient visits	151/667 (22·6%)	169/702 (24·1%)	0·53*
Unscheduled outpatient consultations (visits per 1000 patient days)	9	8	0·93

Data are numbers or median (IQR). *χ^2 test.

Table 3: **Clinical outcomes (carer-reported patient symptoms)**

	Synbiotic	Control	Relative risk (discrete variables) (95% CI)	p value
Problem with RUTF				
As inpatient (patient not tolerating RUTF or clinically worsening, needing F100 milk instead)	6/396 (1·5%)	5/393 (1·3%)	1·19 (0·37 to 3·87)	0·77
As outpatient (number of visits with problem/total outpatient visits)	26/667 (3·9%)	48/702 (6·8%)	0·57 (0·36 to 0·91)	0·02
Bottles of RUTF remaining (if portion unfinished)	1·17 (2·4)	1·35 (2·3)	–0·18 (–0·57 to 0·20)*	0·35
Blood cultures and sepsis				
Blood culture taken	68/399 (170%)	69/396(17·4%)	0·98 (0·72 to 1·33)	0·89
Positive blood culture (of those taken)	17/68 (25·0%)	23/69 (33·3%)	0·75 (0·44 to 1·27)	0·28
Probiotic associated blood culture (of those taken)	0 (0%)	0 (0%)	..	..
Deaths by HIV status				
Total HIV-seropositive deaths	73/170(42·9%)	85/192(44·3%)	0·97 (0·77 to 1·23)	0·80
Total HIV-seronegative deaths	21/203 (10·3%)	25/190 (13·1%)	0·79 (0·46 to 1·36)	0·39
Total readmission episodes†	87/399(21·8%)	71/396(17·9%)	1·22 (0·92 to 1·61)	0·17
Readmitted patients who eventually died	29/87 (33·3%)	28/71 (39·4%)	0·85 (0·56 to 1·28)	0·43

Data are number (%) or mean (SD). RUTF=ready-to-use therapeutic food. *Mean difference (continuous variables). †Same patient can have more than one readmission episode. Maximum admission episodes was 4.

Table 4: **Indicators of Synbiotic safety**

child was an inpatient. At baseline, and at each outpatient clinic, carers were asked about symptoms in the preceding 2 weeks.

Statistical analysis

Data from 2003–04 were used to estimate baseline and control group outcomes.[36] We chose a 10% increase in cure as clinically relevant. Using α=0·05 and 80% power, 348 patients per group were needed to detect an improvement from 65% control group cure to 75% Synbiotic cure (StatCalc, EpiInfo version 3.3.2, Atlanta, USA). To account for follow-up losses and ensure adequate numbers for subgroup analyses, we aimed to recruit 800 patients.

Data were entered in EpiData version 3.1 (EpiData Association, Odense, Denmark). Simple macros (check files) helped to ensure high-quality data entry—eg, variables plausible, in-range, and consistent with other related variables. Key data (anthropometry, dates, final outcomes, and HIV status) were double entered. WHO Anthro 2005 version 1 (WHO, Geneva, Switzerland) was used to calculate anthropometric *Z* scores with the National Center of Health Statistics reference, still current in Malawi.

Main analyses were done with SPSS version 15. StatCalc and Stata Intercooled version 10.0 were used for additional analyses. χ^2 tables and approximate CIs for relative risk were used to examine categorical data. Normality of continuous variables was explored visually (histogram, Q-Q plots) and numerically (Kolomogorov-Smirnov and Shapiro-Wilk tests). Either independent *t* tests or Mann-Whitney *U* tests were done accordingly. To assess the role of HIV as a possible confounder or effect modifier, all major analyses included HIV serostatus (positive or negative) as a stratification level. Analysis was on an intention-to-treat basis.

For comparability with standard reporting of severe acute malnutrition outcomes and to detect a possible subgroup effect, we made a-priori plans for a major secondary analysis. This subgroup excluded small numbers of children younger than 6 months or older than 60 months;[35] those of very low weight (<4 kg); those with cerebral palsy, an obvious dysmorphic syndrome, severe acute malnutrition secondary to major surgical problems, or moderate acute malnutrition with complications. Another secondary analysis explored a post-hoc hypothesis that Synbiotic benefits would be greatest in children during the outpatient phase of severe acute malnutrition treatment.

This trial is registered as an International Standard Randomised Controlled Trial, number ISRCTN19364765.

Role of the funding source

The sponsor of the study did not have any role in study design, data collection, data analysis, interpretation of results, or writing of the report. The corresponding author had full access to all the data and coordinated the decision to submit for publication.

Results

From July 12, 2006, to March 7, 2007, 1024 Malawian children were admitted to the child malnutrition ward (Queen Elizabeth Central Hospital, Blantyre, Malawi) (figure 1). 399 were randomly assigned to Synbiotic-enriched ready-to-use therapeutic food and 396 to control ready-to-use therapeutic food. The most common reason for non-enrolment was early death while still on stabilisation phase.

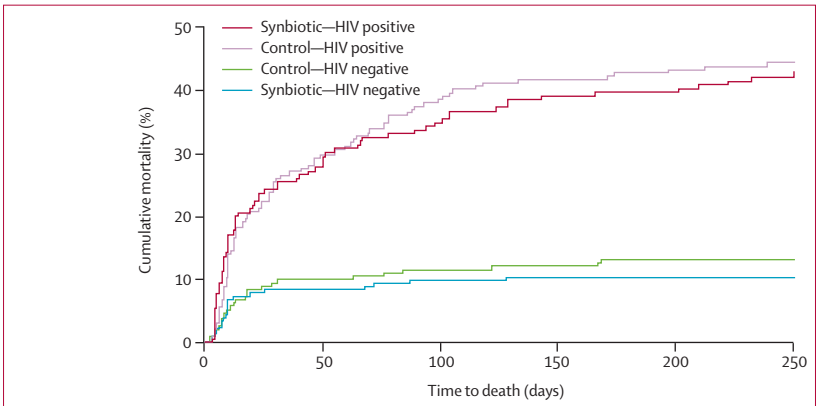
Patient characteristics were similar in both groups at baseline (table 1). Severe acute malnutrition subtypes (kwashiorkor and wasting) were balanced between groups and did not affect any subsequent results. HIV serology was known for 755 of 795 (95%) of all children. Seropositivity was similar in the two groups. HIV status was included as a stratification level in all major subsequent analyses. Detailed baseline profiles are shown in the webappendix (pp 1,2).

Table 2 shows main study outcomes. Total deaths during the study period were similar between groups. Other secondary outcomes were also similar. Less than 10% of patients defaulted. When followed-up in the community after first default episode, 19/32 (59·4%) Synbiotic defaulters and 23/36 (63·9%) controls were seen or reported to be alive and well (p=0·70). Information was unavailable for only 6/32 (18·8%) Synbiotic defaulters and 4/36 (11·1%) controls (p=0·37) who could not be located.

Table 2 shows also main outcomes stratified by treatment phase. There were no group differences in initial inpatient deaths. Deaths at any time during the remainder of the study period showed a possible trend in favour of the Synbiotic group. Details of deaths during each subsequent readmission episode are shown in the webappendix p 4. Overall, there were 486 patient admission episodes in the Synbiotic group and 467 in the control group. Total deaths during inpatient care did not differ between groups, but total deaths during outpatient care showed a trend towards being lower in the Synbiotic group than in the control group (table 2). The observed group differences were greatest during the outpatient phase of the first admission episode (18/324 Synbiotic deaths *vs* 39/338 control deaths, p=0·006) and in HIV-seronegative outpatients (3/202 Synbiotic deaths *vs* 11/194 control deaths, p=0·02). Weight gain during outpatient treatment did not differ between the Synbiotic group and the control group (p=0·48).

Webappendix p 3 shows baseline characteristics at point of entry to outpatient treatment. HIV prevalence was marginally lower in the Synbiotic outpatient group than in the control group (135 of 331 [41%] *vs* 157 of 341 [46%], p=0·17). Weight-for-height *Z* score was also different at point of entry to outpatient treatment (–2·24±1·1 in the Synbiotic group *vs* –2·44±1·1 in the control group, p=0·02).

Table 3 shows carer-reported post-randomisation clinical symptoms. Total days of outpatient observation were less in the Synbiotic group than in the control group, which was partly because mean time to death was shorter in Synbiotic outpatients who died than in control outpatients who died. 16 Synbiotic group outpatient deaths contributed a total 617 days of outpatient observation (mean 38·6 days observation per patient who died), whereas 39 control outpatient deaths contributed 1726 days (mean 44·3 days).



Number at risk (dead)

Synbiotic—HIV positive	169	(47)	121	(12)	109	(7)	102	(1)	101	(5)	97
Control—HIV positive	192	(57)	135	(17)	117	(6)	111	(3)	108	(2)	106
Control—HIV negative	190	(19)	171	(3)	168	(1)	167	(2)	165	(0)	165
Synbiotic—HIV negative	203	(17)	186	(3)	183	(1)	182	(0)	182	(0)	182

***Figure 2*: Kaplan-Meier time-to-death plot, by HIV status**
All deaths in the study are shown.

See Online for webappendix

Inpatients consuming Synbiotic had cough and vomiting for longer, and severe diarrhoea (≥6 abnormally loose or watery stools) for longer (table 3). Synbiotic patients reported more days of inpatient severe diarrhoea (73 days/1000) than control patients (65 days/1000). Outpatient symptoms were similar between groups. Overall outpatient diarrhoea did not differ, but there was a trend to less severe diarrhoea in the Synbiotic group. Consistent with symptom reports, unscheduled outpatient visits and use of non-routine outpatient medication were similar between groups.

Table 4 shows indicators of Synbiotic safety. Ready-to-use therapeutic food was tolerated equally well in both groups during inpatient care. During outpatient care, Synbiotic patients reported less problems eating this food than did control patients. This difference did not affect the total amount consumed: if the food was left unfinished, the amount was small (just over one pot unfinished was about one day's portion every 14 days) and similar in both groups.

Rate of sepsis did not differ between groups. Similar numbers of patients needed blood cultures and similar numbers of blood cultures were positive. No cases of probiotic-associated sepsis were noted. Table 4 also shows deaths stratified by HIV serostatus. There was no excess mortality in HIV-seropositive Synbiotic patients.

The adverse effects of HIV were quickly evident in both Synbiotic and control patients (figure 2). In both

HIV-positive and HIV-negative children, early deaths were more frequent in the Synbiotic than in the control group, although these early inpatient deaths were not significantly different between groups (table 2 and webappendix p 4). Median time to death at first admission episode was 8 (IQR 5 to 13) days in the Synbiotic patients who died, and 11 (6 to 28) days in control patients (p=0·004). A log-rank test for overall time-to-death curves was non-significant (p=0·36).

All analyses have been repeated for the prespecified subgroup of children who we thought might benefit most from Synbiotic addition (secondary analyses). Overall trends and patterns of difference were similar to those obtained in the primary analyses.

Discussion

In our setting, Synbiotic2000 Forte, prescribed in ready-to-use therapeutic food at an average dose of at least 10^{10} lactic acid bacteria per day, did not improve prespecified nutritional or clinical outcomes from severe acute malnutrition. This is an important negative finding. PRONUT is a very large randomised trial on the effect of probiotics and prebiotics. It is also based in a low-income, high-mortality country.

A 2004 Cochrane review[8] of probiotics for the treatment of diarrhoea included 1917 patients from 23 studies, only two of which were set in high child and adult mortality countries. A meta-analysis[9] of probiotics in diarrhoea prevention consisted of 4844 patients from 34 trials, only one of which was in a developing country and community based.

A post-hoc observation of reduced outpatient mortality in children receiving Synbiotic is interesting. If true, it could be clinically relevant. We emphasise that this observation should not be overestimated, but the results justify further research.

Lack of Synbiotic efficacy for inpatients with severe acute malnutrition might be because the children in the study are the most vulnerable.[20] Some causes of early death are unlikely to be affected by known Synbiotic actions (eg, re-feeding syndrome and electrolyte imbalance).[37,38]

Possible benefits for children surviving inpatient care are consistent with known Synbiotic effects, such as immune stimulation and improved gut environment and integrity. These effects take time and cannot occur until organ, cellular, and immune functions have begun to recover. Such recovery is greatest in outpatients after initial discharge, as was observed. Repeated admissions (ie, repeated episodes of severe illness) dilute any intergroup differences; indeed, outpatient differences are most pronounced during the first episode of outpatient care but are attenuated when all subsequent admission episodes are taken into account.

Antibiotics might also modulate observed inpatient and outpatient effects. In-vitro testing showed co-trimoxazole sensitivity in two of four Synbiotic organisms. Following standard protocols, all our inpatients received co-trimoxazole, and 50% also had parenteral antibiotics. Children who were HIV seropositive continued on long-term co-trimoxazole prophylaxis after ward discharge, during and after outpatient treatment. Antibiotics might reduce gut colonisation and hence efficacy of Synbiotic. By contrast, HIV-negative outpatients, in whom the possible outpatient Synbiotic effect was strongest, received no antibiotics.

The study presents several strengths. Nutritional cure and death are hard outcomes in functional food studies.[9,39] The results of this trial are directly relevant to nutrition policy makers; programme managers,[35] and families and communities whose children die because of severe acute malnutrition.[40]

For maximal generalisability to real-world nutrition programmes, we did the trial in a government hospital providing routine service work. We also enrolled typical rather than selected patients.[41] Children with disabilities and other conditions whose growth patterns are abnormal were included.[42] PRONUT was powered to explore both whole-group and subgroup effects. Final outcome was obtained for all but 26 of 795 (3·2%) of randomised patients.

We noted no excess sepsis and no excess mortality, as was discussed in one recent study on probiotics and prebiotics in severe pancreatitis.[43] Increased inpatient vomiting, severe diarrhoea, and cough, and a non-significant increase in inpatient mortality were noted in the Synbiotic group compared with the control group. One explanation for these observations is chance. Known probiotic effects do not offer a clear explanation for these findings. Increased severe diarrhoea might be due to the osmotic effect of prebiotics. Therefore, future studies should distinguish prebiotic and probiotic effects.

Some limitations of the study might explain our negative results. Although we discouraged intergroup ready-to-use therapeutic food sharing, we cannot be certain it did not happen. Faeco-oral probiotic cross-contamination was also possible,[44] which would result in dilution of true group differences. Such sharing and contamination would be maximal while patients were living closely together during inpatient care, whereas it would be minimal during home-based treatment because close neighbours on different groups are unlikely. If contamination had occurred, it would also explain our observed trends towards group differences during outpatient treatment alone.

Probiotics and prebiotics are large and diverse groups, each with specific effects in specific patients. This heterogeneity, combined with a paucity of data for their use in the treatment of severe acute malnutrition, makes the comparison of our findings with others difficult. Research using *Bifidobacterium bifidum* and *Streptococcus thermophilus* probiotics found an effect increasing mean CD4+ cell count in HIV-positive patients,[45] whereas another study[46] using *Lactobacillus GG* have found no

effect in healthy Malawian children. We chose a probiotic and a prebiotic with a proven record in some patient groups.[47–49] Another formulation might have shown different results.

Probiotics have almost linear dose–response effects, at least on diarrhoea outcomes. The commonly accepted efficacy threshold is more than 10^8 bacteria per day.[39] Regular quality control checks showed that we prescribed more than 10^{10} colony-forming units per day, well above this lower limit. Furthermore, a suboptimal consumed dose of ready-to-use therapeutic food (and thus of Synbiotic) might have contributed to the negative results. This might have happened at home if carers shared ready-to-use therapeutic food with other children. We had only carer reports rather than direct observation to confirm non-sharing and compliance. Steady weight gain was, however, consistent with the food being eaten rather than shared.

Probiotics are often ingested as a single large bolus dose. Our patients consumed ready-to-use therapeutic food (and thus Synbiotic) in divided amounts spread throughout the day. Probiotic effects depend on successful transit through the upper gastrointestinal tract to the small and large intestines. Some probiotic organisms die during transit. Larger single doses (perhaps timed away from antibiotic administration) might have colonised the gut better and be more effective. We were unable to confirm the success of our protocol directly and assumed that colonisation occurred on the basis of previous research[44] and the fact that gastric acidity, normally a barrier to live probiotic transit, is reduced in severe acute malnutrition.

The four prebiotics in Synbiotic might have caused non-specific overgrowth of enteric flora that reduced the effectiveness of the probiotic component. Unfortunately, we do not have stool cultures to test this possibility.

One isolated outpatient finding was reduced severe diarrhoea, but patient numbers were small. Benefits can be subtle, at immune system level for example. True symptom differences might have been missed, obscured by the noise inherent to any self-reported variable. Group imbalances in days of outpatient observation might also have had a role. To address such limitations, future work might add laboratory-measured clinical response indicators.[45]

At randomisation, groups seemed to be well balanced. Minor differences at point of entry to outpatient care (lower HIV rate and less malnourished according to weight-for-height *Z* score in the Synbiotic group) raise the possibility of confounding or bias at this point.

Longitudinal data obtained during the PRONUT study emphasise the need for effective, evidence-based interventions. Control group cure rates were low (51·3%) and death rates high (30·0%). Such statistics are unfortunately not unusual.[2,5,50] HIV and late presentation to care, with complications of severe acute malnutrition already present, are major factors underlying these poor outcomes. In this setting, Synbiotic2000 Forte, prescribed in the food at an average dose of at least 10^{10} bacteria per day did not improve outcomes from current therapies.

An observation of reduced mortality in Synbiotic outpatients is important to explore in future studies. Since PRONUT was not designed to look at outpatients alone, we cannot rule out bias, confounding, or chance. An effect is however biologically plausible. Enteropathy associated with severe acute malnutrition is an especially important problem that might be addressed by probiotics, which makes them also useful to other less severe but more prevalent forms of acute malnutrition.[51] Future studies with probiotics in malnutrition should focus on severe acute malnutrition outpatients, and possibly even children with moderate malnutrition, whose treatment is receiving increasing interest worldwide. Such research would nest well in community-based management of acute malnutrition programmes, which focus on early identification and outpatient treatment of children with malnutrition.

Acute malnutrition and especially outpatient-focused community-based management of acute malnutrition strategies are currently high on the international child health and nutrition policy agenda.[21] Opportunities for future outpatient-focused severe acute malnutrition research should be taken. The potential to make a difference to 13 million children affected by severe acute malnutrition and over 1 million deaths related to severe acute malnutrition has never been better.

Contributors

SC conceived the study and, together with PB and KS, wrote the original study protocol. AS, JB, and MK updated and further developed the protocol when the originally planned study site was changed from central to southern Malawi. MK, JB, and MT led the study in the field. MK and JB did data analysis, and MK wrote the first draft of the report. All authors contributed to data interpretation and writing.

Conflicts of interest

SC is an unpaid director of Valid Nutrition, a charity that produces ready-to-use therapeutic food in developing countries. The other authors declare that they have no conflicts of interest.

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