

Minimal impact of an iron-fortified lipid-based nutrient supplement on Hb and iron status: a randomised controlled trial in malnourished HIV-positive African adults starting antiretroviral therapy

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Abstract

Anaemia, redistribution of Fe, malnutrition and heightened systemic inflammation during HIV infection confer an increased risk of morbidity and mortality in HIV patients. We analysed information on Fe status and inflammation from a randomised, double blind, controlled phase-III clinical trial in Lusaka, Zambia and Mwanza, Tanzania. Malnourished patients (n 1815) were recruited at referral to antiretroviral therapy (ART) into a two-stage nutritional rehabilitation programme, randomised to receive a lipid-based nutrient supplement with or without added micronutrients. Fe was included in the intervention arm during the second stage, given from 2 to 6 weeks post-ART. Hb, serum C-reactive protein (CRP), serum ferritin and soluble transferrin receptor (sTfR) were measured at recruitment and 6 weeks post-ART. Multivariable linear regression models were used to assess the impact of the intervention, and the effect of reducing inflammation from recruitment to week 6 on Hb and Fe status. There was no effect of the intervention on Hb, serum ferritin, sTfR or serum CRP. A one-log decrease of serum CRP from recruitment to week 6 was associated with a 1.81 g/l increase in Hb (95% CI 0.85, 2.76; $P < 0.001$), and a 0.11 log decrease in serum ferritin (95% CI -0.22, 0.03; $P = 0.012$) from recruitment to week 6. There was no association between the change in serum CRP and the change in sTfR over the same time period ($P = 0.78$). In malnourished, HIV-infected adults receiving dietary Fe, a reduction in inflammation in the early ART treatment period appears to be a precondition for recovery from anaemia.

Key words: Hb: Ferritin: Transferrin receptor: Lipid-based nutrient supplement: HIV: Inflammation: Antiretroviral therapy

Independent risk factors for mortality amongst African patients starting antiretroviral therapy (ART) include anaemia⁽¹⁾, a failure to increase Hb within the first few months of ART⁽²⁾, and malnutrition represented by a BMI of < 18.5 kg/m²^(3–5). Heightened systemic inflammation is a hallmark of both untreated and treated HIV infection⁽⁶⁾, and higher levels of persistent inflammation, despite treatment with ART⁽⁷⁾, confer an increased risk of morbidity and mortality in HIV patients^(8,9). Redistribution of Fe during HIV infection can

lead to increased Fe sequestration in macrophages with an accompanying decline in Fe available for tissue supply and erythropoiesis^(7,10). This disordered Fe metabolism has been associated with rapid progression of HIV^(11–13), exacerbation of co-infections⁽¹⁴⁾, especially tuberculosis^(15,16), and early death^(17,18). Although ART is increasingly available, initiation of ART is associated with a high mortality rate: 17% of patients starting ART in sub-Saharan Africa die within 1 year, and the majority within the first 3 months⁽¹⁹⁾. The detrimental effects

Abbreviations: ART, antiretroviral therapy; CRP, C-reactive protein; DSMB, Data Safety and Monitoring Board; LNS, lipid-based nutrient supplement; LNS-VM, lipid-based nutrient supplement with added vitamins and minerals; NUSTART, Nutritional Support for Africans Starting Antiretroviral Therapy; sTfR, soluble transferrin receptor.

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of anaemia, Fe redistribution, malnutrition, and inflammation on early ART mortality have been well-documented. In sub-Saharan Africa the HIV disease burden remains vast⁽²⁰⁾, with a third of adults starting ART being malnourished in some African countries^(3,21). The control of anaemia and normalisation of Fe metabolism within this population remains, therefore, a critical strategy for improving patient survival.

Markers other than Hb are required to assess Fe status. Serum ferritin can be used as a marker of body stores of Fe⁽²²⁾, and soluble transferrin receptor (sTfR) to estimate tissue Fe demand⁽²³⁾. The determination of Fe status in the presence of inflammation is notoriously challenging⁽²⁴⁾, with no internationally agreed methodology as yet⁽²⁵⁾. It is well known that inflammation alters many markers of Fe status, including increasing serum ferritin as an acute phase protein⁽²⁵⁾. sTfR is less affected by the inflammatory response, and can therefore be used to distinguish anaemia of inflammation (with elevated serum ferritin and normal-to-elevated sTfR) from Fe deficiency anaemia (with low serum ferritin and high sTfR)^(25,26). However, Fe deficiency and inflammation often co-exist, complicating assessment of Fe status and needs. Measurement of C-reactive protein (CRP) and α_1 -acid glycoprotein – acute phase proteins – can assist interpretation of the Fe biomarkers in order to separate those patients with inflammation-induced Fe sequestration from those who are both sequestering Fe and Fe-deficient⁽²⁷⁾. Hepcidin, a peptide hormone which regulates iron metabolism by controlling the absorption of dietary Fe, Fe transport, and Fe distribution among different cell types, can be measured to help elucidate the complex interplay between anaemia, Fe status and immunity⁽²⁸⁾.

There are currently many unanswered questions about: the range and characteristics of disordered Fe metabolism among malnourished, HIV-infected adults; the preferred Fe-related biomarkers for assessing health risks; the effect of oral Fe supplementation on Fe status and health. The common assumption that low Hb requires therapeutic correction through Fe supplementation may be erroneous in HIV, since supplementation can exacerbate the risk of co-infections, and hasten disease progression. Paradoxically it is high serum ferritin that predicts a worse outcome, despite the association of anaemia and mortality⁽¹⁸⁾. Controversy therefore remains as to what extent Fe supplementation amongst HIV patients affects infection rates and mortality⁽²⁹⁾. Some interventions supplementing Fe to HIV-positive adults have reduced anaemia without increasing viral load^(30,31); longer-term outcomes were not assessed. This creates a therapeutic dilemma for the clinician as to how HIV-infected patients with anaemia should be treated. Given the clear link between anaemia and early ART mortality, the existing knowledge gaps jeopardise the health and survival of thousands of malnourished HIV/AIDS patients.

The present study uses a clinical trial of a malnourished group of adults starting ART, to answer three main research questions. Firstly, what effect does a nutritional intervention including Fe have on Fe status? Secondly, does any impact depend on the baseline Fe status of patients? Thirdly, does inflammation have an independent effect on changes in Fe

status? We hypothesised that the nutritional intervention would improve Fe status, indicated by an increase in Hb accompanied by no change or a slight decrease in sTfR; effects on sTfR would depend on whether the anaemia was due primarily to chronic disease which has little effect on sTfR, or to Fe deficiency which results in increased sTfR⁽²⁵⁾. We expected overall serum ferritin results to be harder to predict: decreasing in the correction of anaemia of inflammation, but increasing in the correction of Fe deficiency. We speculated that failing to normalise systemic inflammation after starting ART would attenuate any improvements.

Subjects and methods

Study design

The study analyses information on Fe status and inflammation from a randomised, double blind, controlled phase-III clinical trial in Lusaka, Zambia and Mwanza, Tanzania – the Nutritional Support for Africans Starting Antiretroviral Therapy (NUSTART) trial (registered in the Pan-African Clinical Trials Register as PACTR201106000300631). Details of the trial are described in full elsewhere^(32,33). In brief, the NUSTART trial was conducted between August 2011 and December 2013 to assess the effect of a fortified lipid-based nutrient supplement (LNS; prepared by Nutriset) on survival of malnourished patients starting ART. The present paper focuses on two secondary outcomes: markers of Fe status and inflammation. A total of 1815 patients were recruited at the two sites, using the inclusion criteria of age >18 years, BMI <18.5 kg/m², CD4 count <350 cells/ μ l or stage 3 or 4 AIDS, ART-naïve apart from those who received ART during standard prevention of mother-to-child transmission regimens, and informed consent. Self-reported pregnancy was an exclusion criterion.

The trial intervention was based on established protocols for managing severe malnutrition in young children involving two phases aimed at stabilisation and then rehabilitation⁽³⁴⁾. Fig. 1 summarises the NUSTART design. The first phase took place between referral and 2 weeks post-ART initiation. Participants were randomised to receive vitamins and minerals, without Fe as is done for malnourished children, in low-energy (30 g containing approximately 628 kJ (150 kcal)/d) LNS (low-dose LNS with added vitamins and minerals (LNS-VM)) in the intervention group *v.* LNS without the vitamins and minerals (low-dose control LNS) in the control group. This phase aimed to stabilise metabolism before trying to promote weight gain during the second phase. The second phase involved a 4-week intervention, starting 2 weeks after ART initiation and continuing until 6 weeks post-ART. Participants in the intervention group received a higher-energy (250 g containing approximately 5858 kJ (1400 kcal)/d) LNS containing the same added vitamins and minerals as in phase 1 plus Fe as sulphate (high-dose LNS-VM). The control group received the high-dose LNS without the added vitamins, minerals, or Fe (high-dose control LNS). Vitamin and mineral levels in both the high- and low-dose LNS-VM were mostly set at three times the UK recommended nutrient intakes for adult women⁽³⁵⁾



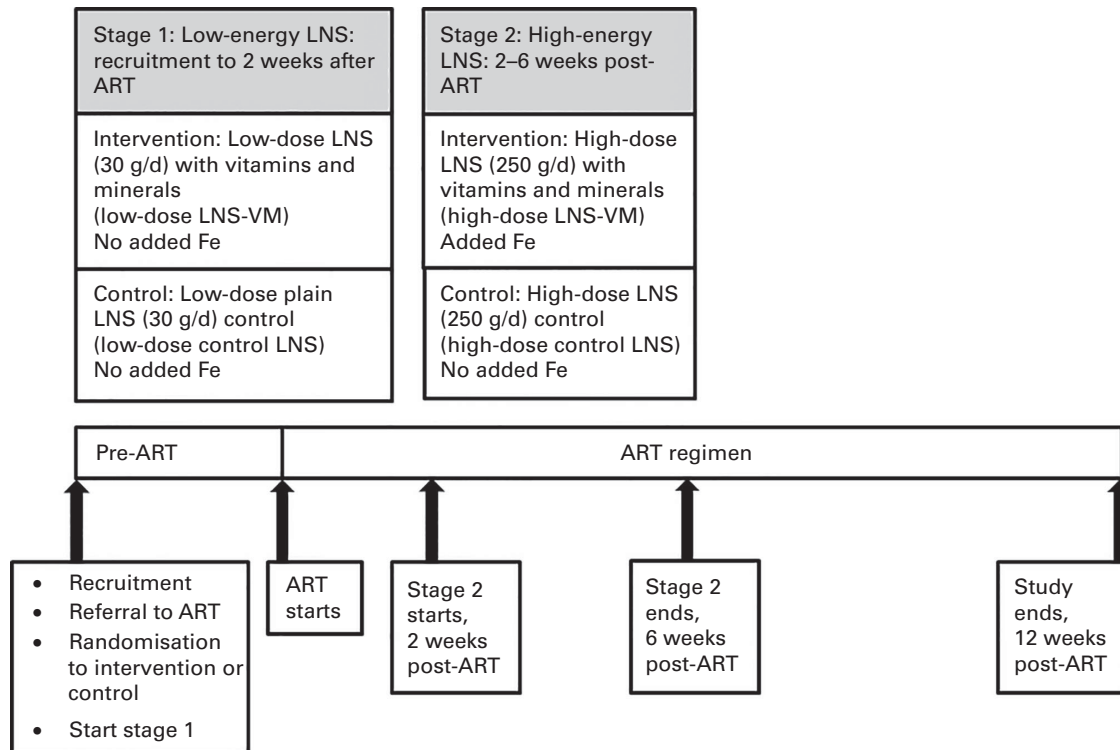


Fig. 1. Overview of the Nutritional Support for Africans Starting trial design. LNS, lipid-based nutrient supplement; ART, antiretroviral therapy; LNS-VM, LNS with added vitamin and mineral mix.

with the exception of Fe which was only in the second stage, high-dose LNS and only at one recommended nutrient intake (14.7 mg/d) (nutritional composition details in online Supplementary material Table S1).

The interval between referral for ART and starting ART was based on the individual patient's readiness to start life-long drug treatment and practices of the different clinics from which the study recruited patients for investigation. Study personnel were not involved in deciding when to initiate ART; the duration of phase 1 reflects routine practices in these clinics at the time. The median interval between referral for ART and starting ART for both arms was 21 d, (interquartile range 15–30). During this period, the routine medications that the study participants continued to take were: Tenofovir (TDF)/Emtricitabine (FTC)/Efavirenz (EVF) (55.4%); Zidovudine (AZT)/Lamivudine (3TC)/Nevirapine (NVP) (16.0%); AZT/3TC/EVF (9.2%); TDF/FTC/NVP (4.3%); another (3.6%); a small group of 11.5% of the population had no ART regimen information⁽³²⁾.

The Data Safety and Monitoring Board (DSMB) statistician conducted the randomisation using sixteen computer-generated blocks stratified by site. The contents of the LNS packets were assigned an allocation code (letters A to H), known only to the DSMB statistician and Nutriset, which were linked to study ID numbers using a randomisation code. This randomisation code was only known to the DSMB statistician and site-based pharmacists, none of whom had direct patient contact. The LNS and LNS-VM packets were delivered by Nutriset in lots assigned by allocation code. Clinic pharmacists labelled packets with study ID

numbers as packets were dispensed. Clinic nurses (with no access to the allocation or randomisation code) then recruited eligible participants to the study using sequential ID numbers.

Blood collection

Patients were seen weekly from referral for ART until the ART initiation visit, and then at 2, 6, 8, and 12 weeks after starting ART. They were asked at each follow-up visit whether they were taking Fe supplements in addition to the study supplement. Hb and serum CRP were measured for all patients, whilst serum ferritin and sTfR were analysed for a fifth of the patients, referred to as the Fe marker subsample, and chosen systematically for every patient ID divisible by 5. Hb was measured at recruitment and at 6 weeks post-ART. Fe markers and CRP were measured in serum from the recruitment and 6 weeks post-ART samples which were stored at –80°C until batch analysis. The flow of participants included in the Fe marker subsample from identification to analysis at baseline and week 6 is shown in Fig. 2.

Iron and inflammatory marker analysis

Fingerstick capillary blood samples from all patients were used for analysis of Hb levels by a portable haemoglobinometer (Hemocue®). Anaemia severity cut-offs of the present study followed standard WHO categorisations⁽³⁶⁾. Mild anaemia was defined as Hb <120 g/l for women and <130 g/l for men. Moderate and severe anaemia categories

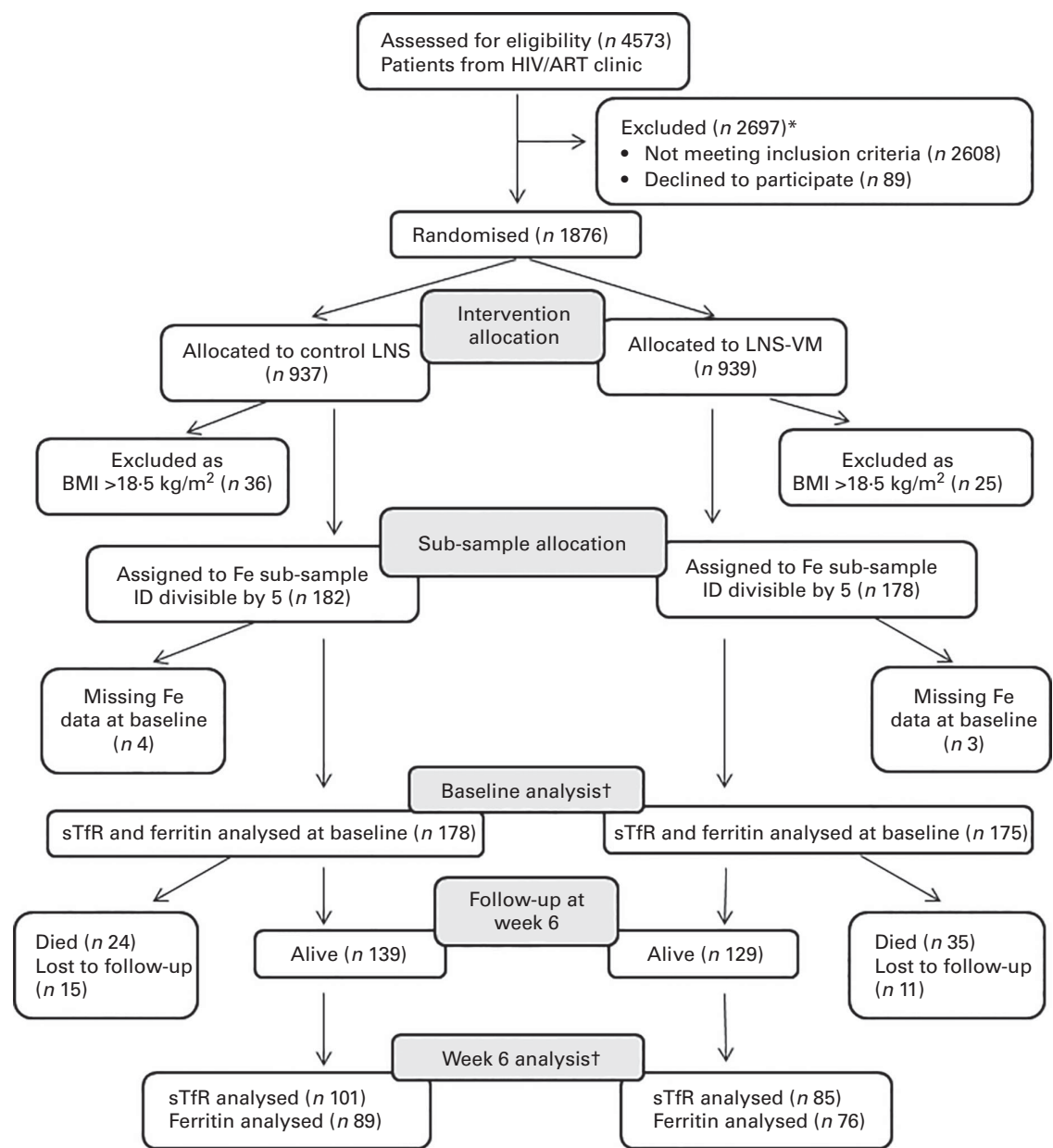


Fig. 2. Flow of subsample participants from identification to analysis of iron markers at baseline and week 6 post-antiretroviral therapy (ART). * Inclusion criteria: > 18 years, BMI < 18.5 kg/m², CD4 count < 350/μl or stage 3 or 4 AIDS, ART-naïve apart from those who received ART during standard prevention of mother-to-child transmission regimens, and informed consent. Self-reported pregnancy was an exclusion criterion. † Note Hb and C-reactive protein (CRP) were collected from all patients at recruitment and 6 weeks post-ART. The flow diagram for the whole sample is published elsewhere⁽³²⁾. Available samples at baseline: CRP, n 1762; Hb, n 1670. Available samples at week 6: CRP, n 863; Hb, n 826. ID, identification number; LNS, lipid-based nutrient supplement; LNS-VM, LNS with added vitamin and mineral mix; sTfR, soluble transferrin receptor.

used the same cut-offs for both sexes, defined as <110 and <80 g/l respectively.

Serum ferritin was measured by ELISA (AssayPro Human Ferritin ELISA Kit, catalogue no. EF2003-1). The intra-assay and inter-assay CV were 2 and 7% in Mwanza and 5 and 23% in Lusaka respectively. sTfR was measured by ELISA (Quantikine® IVD® Human sTfR Immunoassay, Ref DTFR1; R&D Systems, Inc.). The intra-assay and inter-assay CV were 2 and 4% in Mwanza and 3 and 13% in Lusaka respectively. Serum CRP was analysed by ELISA (AssayPro). The intra-assay and inter-assay CV were 3 and 37% in Mwanza and

6 and 32% in Lusaka respectively. For all analyses, values over the upper range of the standard curve were set to the top standard multiplied by the dilution factor. For all assays plates with poor precision were re-run.

Sample size

The original trial sample size was powered on the primary outcome (mortality). Our ‘one in five’ subsample for the Fe markers was sufficient to detect an inter-group difference of 0.35 standard deviation using 90% power.

Data analysis

Continuous variables were assessed for normality using normal probability plots and visual inspection of histograms. Hb and sTfR approximated a normal distribution and remained on the linear scale. Serum CRP and serum ferritin were skewed to the right and transformed to natural logs. We considered using correction factors for ferritin derived from the methodology suggested by Thurnham *et al.*⁽²⁷⁾; however, our population was extremely malnourished, and exhibited high levels of inflammation with very deranged Fe metabolism so it was unclear whether correction factors derived from populations less ill were appropriate. We decided instead to simply adjust for CRP in regression analyses, as has been done elsewhere⁽³⁷⁾.

We compared baseline characteristics of those in the smaller subsample containing data on sTfR and serum ferritin (*n* 353) with those not in the subsample (*n* 1462) to assess the generalisability applicable to the whole sample. The χ^2 test was used to compare proportions, independent *t* tests to compare means of normally distributed data, and the Wilcoxon–Mann–Whitney test to compare medians of non-parametric data. Sample sizes of all further analyses were set by the number of available samples at 6 weeks post-ART.

A variable was created to summarise the frequency of taking Fe supplements in addition to the study supplement over the follow-up period; this was categorised as ‘never consumed’ (62%), ‘reported consumed at one follow-up’ (19%), and ‘reported consumed at two or more follow-up visits’ (19%). We assessed the within-subject changes in markers of Fe

Table 1. Baseline characteristics of the iron marker subsample by trial arm and overall summaries of those included in and excluded from the iron marker subsample

(Number of participants and percentages; mean values and standard deviations; median values and interquartile ranges (IQR))

Variable	Level	Fe marker subsample (overall)		Fe marker subsample (LNS-VM)		Fe marker subsample (LNS control)		Not included in Fe marker subsample (overall)		P (subsample v. not in subsample*)
		<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	
Site (Lusaka)		353	100	175	49.6	178	50.4	1462	100	0.82
Age (years)		218	62	107	61	111	62	893	61	0.67
	Mean	36.0		36.4		35.7		35.8		
	SD	9.1		9.2		9.1		9.5		
Female		181	51	91	52	90	51	728	50	0.72
BMI (kg/m ²)	All									0.58
	Mean	16.4		16.5		16.4		16.5		
	SD	1.4		1.3		1.5		1.3		
	BMI < 16 kg/m ²	111	31	51	29	60	33	495	34	0.26
	BMI 16–16.9 kg/m ²	103	29	56	32	47	26	365	25	
	BMI 17–18.5 kg/m ²	139	39	68	39	71	40	602	41	
Oedema		12	3	6	3.4	6	3.3	54	4	0.79
CD4 count (cells/ μ l)	All									0.86
	Mean	138		134		141		137		
	SD	100		99		101		100		
Hb (g/l)	All									0.012
	Mean	93		93		93		96		
	SD	23		23		22		23		
Hb group†	Severe anaemia	98	28	51	29	47	26	300	21	0.011
	Moderate anaemia	141	40	68	39	73	41	669	46	
	Mild anaemia	54	15	23	13	31	17	231	16	
	Normal	26	7	15	9	11	6	151	10	
	Missing	34	10	18	10	16	9	111	7	
Serum CRP (mg/l)										0.014
	Median	71		69		83		57		
	IQR	18–160		20–160		16–160		13–155		
TB treatment pre-ART		99	28	49	28	50	28	352	24	0.12
Using Co-trimoxazole		294	84	141	81	153	86	1192	82	0.43
sTfR (nmol/l)										N/A
	Mean	44		43.2		43.7		N/A		
	SD	18		17.6		19.0				
Serum ferritin (μ g/l)										N/A
	Median	752		722		754		N/A		
	IQR	288–1246		325–1287		281–1200				

LNS-VM, lipid-based nutritional supplement with added vitamins and minerals; LNS, lipid-based nutritional supplement without added vitamins and minerals; CRP, C-reactive protein; TB, tuberculosis; ART, antiretroviral therapy; sTfR, soluble transferrin receptor; N/A, not applicable.

* χ^2 test to compare proportions, independent *t* tests to compare means of normally distributed data, and the Wilcoxon–Mann–Whitney test to compare medians of non-parametric data.

† Normal defined as ≥ 120 g/l for women and ≥ 130 g/l for men. Mild anaemia defined as Hb < 120 g/l for women and < 130 g/l for men. Moderate and severe anaemia categories defined as < 110 and < 80 g/l respectively for both sexes.

status and inflammation between baseline and week 6 by intervention arm using paired *t* tests.

For our first objective assessing the effect of the intervention on Fe marker status at week 6 we used multivariable linear regression. The first model adjusted only for the baseline value of the Fe marker being assessed, the second model additionally adjusted for serum CRP at week 6 given our hypothesis that inflammation would affect Fe markers, and the third model further adjusted for sex, site, age, baseline BMI and CD4 count, being on TB treatment at recruitment, taking Fe supplements in addition to the study supplement, and length of time taken from recruitment to starting ART. TB treatment at recruitment was coded yes or no and taking Fe supplements in addition to the study supplement was a categorical variable.

For our second objective, we repeated the third (fully adjusted) model analysis stratified by baseline values of the Fe markers to determine whether these modified the effect of the intervention. We used a binary Hb category: normal Hb and mild anaemia *v.* those with moderate and severe anaemia. Due to lack of internationally agreed cut-offs for serum ferritin and sTfR, and the specific context of our malnourished sample with heightened systemic inflammation, we divided these variables into two groups using the median value to create binary categories. We chose binary categories rather than continuous measures since we felt this would provide a more accessible way of interpreting overall trends that may have physiological significance. The test for interaction between the baseline Fe marker category and intervention arm used a likelihood ratio test between the multivariable linear regression models with and without the interaction term.

For our third objective, we assessed the extent to which inflammation was driving the changes in our Fe markers independent of the intervention. We investigated interrelations among the Fe markers and serum CRP using Pearson correlation matrices. We then created a multiple linear regression model exploring the association between change in Fe marker from baseline to week 6 with change in serum CRP over the same timeframe, adjusting for trial arm, sex, site and being on tuberculosis treatment at recruitment as binary variables; taking Fe supplements in addition to the

study supplement as a categorical variable; and baseline BMI, age, CD4 count and length of time taken from recruitment to starting ART as continuous variables.

Stata version 13.1 (StataCorp) was used for all analyses.

Ethical considerations

The present study was conducted according to the guidelines laid down in the Declaration of Helsinki. All NUSTART trial procedures, including the collection and analysis of the Fe markers, were approved by the ethics committee of the London School of Hygiene and Tropical Medicine, the University of Zambia Biomedical Research Ethics Committee (reference no. 009-01-11), and the National Institute for Medical Research, Tanzania. Written informed consent or thumbprint was obtained from all patients before enrolment.

Results

Detailed baseline characteristics of the 1815 patients recruited are published elsewhere⁽³²⁾. In summary, one-third had BMI < 16 kg/m² and mean age was 35.8 (SD 9.4) years. Only 10% of the patients were without anaemia at baseline, with two-thirds categorised as either moderately or severely anaemic. Table 1 shows the baseline characteristics for the subsample assessed for Fe markers (*n* 353). Mean baseline Hb was lower amongst those in the Fe marker subsample compared to those not included (93 (SD 23) *v.* 96 (SD 23) g/l, *P*=0.012). Median serum CRP was higher amongst those in the subsample compared to those not included: 71 (interquartile range 18–160) *v.* 57 (interquartile range 13–155) mg/l, *P*=0.004). Patient baseline characteristics in the Fe subsample were very similar in the two treatment arms (Table 1), as was the case for the whole sample⁽³²⁾.

In the control group from baseline to week 6 post-ART, patients gained a mean of 3 g/l Hb (*P*=0.029, *n* 369), decreased their serum ferritin by 100 µg/l (*P*=0.021, *n* 89), increased their sTfR by 4 nmol/l (*P*=0.045, *n* 101), but experienced no overall change in CRP levels (*P*=0.08, *n* 407) (Table 2). The intervention group displayed similar trends: patients gained a mean of 6 g/l

Table 2. Overview of changes in iron and inflammatory markers from baseline to week 6 by trial arm, unadjusted (Mean values, geometric mean values and 95% confidence intervals)

Variable	<i>n</i> *	LNS-VM (intervention)					<i>P</i> †	LNS control					<i>P</i> †
		Baseline		Week 6		Baseline		Week 6					
		Mean	95% CI	Mean	95% CI	Mean		95% CI	Mean	95% CI			
Hb (g/l)	383	98	96, 100	104	102, 106	<0.001	369	100	97, 102	103	100, 105	0.029	
Serum ferritin (µg/l)	76					0.004	89					0.021	
Geometric mean			425		284				466		366		
95% CI			329, 547		219, 368				373, 581		289, 463		
sTfR (nmol/l)	85	41	38, 45	46	43, 50	0.030	101	41	38, 45	45	42, 48	0.045	
Serum CRP (mg/l)	431					0.36	407					0.08	
Geometric mean			35		32				28		33		
95% CI			30, 40		28, 37				24, 33		28, 38		

LNS-VM, lipid-based nutrient supplement with added vitamins and minerals; LNS, lipid-based nutrient supplement; sTfR, soluble transferrin receptor; CRP, C-reactive protein.

* Only patients with week 6 data included; therefore sample size lower than that of Table 1.

† Paired *t* test.

Table 3. Linear regression showing the effect of the intervention on Hb, iron and inflammatory markers at week 6 with regression coefficients (*B*), 95% CI and the corresponding *P* values, using three models of adjustment

(Regression coefficients and 95% confidence intervals)

Variable (week 6)	<i>n</i> §	Model 1*			Model 2†			Model 3‡		
		<i>B</i>	95% CI	<i>P</i> ¶	<i>B</i>	95% CI	<i>P</i> ¶	<i>B</i>	95% CI	<i>P</i> ¶
Hb (g/l)	705	2.16	-0.91, 5.23	0.17	1.88	-1.13, 4.90	0.22	1.60	-1.30, 4.49	0.28
Log serum ferritin	165	-0.20	-0.50, 0.09	0.18	-0.20	-0.49, 0.09	0.18	-0.19	-0.46, 0.07	0.14
sTfR (nmol/l)	164	0.99	-3.57, 5.55	0.67	1.02	-3.55, 5.59	0.66	1.68	-2.95, 6.33	0.47
Log serum CRP	838	-0.10	-0.29, 0.10	0.33				-0.11	-0.29, 0.08	0.26

sTfR, soluble transferrin receptor; CRP, C-reactive protein.

* Adjusted for baseline value of the same dependent variable.

† Adjusted for the baseline value of the same dependent variable and log-CRP at week 6 for the Fe markers.

‡ Adjusted for the baseline value of the same dependent variable, log-CRP at week 6 for the Fe markers, sex, site, age, baseline CD4 count, being on tuberculosis medicine at recruitment, taking Fe supplements in addition to the study supplement, length of time from recruitment to antiretroviral therapy and baseline BMI.

§ Number restricted to the same sample as in the fully adjusted Model 3.

¶ The coefficient shows the effect associated with the intervention on week 6 outcomes in comparison to the control.

|| Two sample *t* test.

Hb ($P \leq 0.001$, n 383), decreased their serum ferritin by 141 $\mu\text{g/l}$ ($P = 0.004$, n 76), increased their sTfR by 4 nmol/l ($P = 0.030$, n 85), and also experienced no overall change in CRP levels ($P = 0.36$, n 431). There was no effect of the vitamins and minerals added to the intervention LNS on Hb, serum ferritin, sTfR or serum CRP in any of the three statistical models (Table 3). Note that sample sizes in Table 3, which used various adjusted models, were restricted to the patients who had no missing data in all the variables we adjusted for; they differ, therefore, from those seen in Table 2, which used unadjusted data.

Table 4 shows the extent to which the intervention effect differed for patients, based on their baseline Fe marker category. The coefficient shows the change in week 6 Fe marker associated with the intervention in comparison to the control within the baseline Fe marker category strata. There

was no evidence that the impact of the intervention on Hb at week 6 was affected by baseline Fe marker category ($P > 0.18$ for interaction tests). Amongst those with moderate and severe anaemia at baseline, the intervention was associated with a decrease in 0.40 of log serum ferritin at week 6 ($P = 0.023$). However, evidence for an overall interaction between the intervention and baseline Hb on log serum ferritin was weak ($P = 0.12$). There was no evidence of any interaction between the intervention and baseline Fe marker categories on sTfR at week 6 ($P > 0.52$ for interaction tests).

At both baseline and week 6, Hb was negatively correlated with serum CRP, serum ferritin was positively associated with serum CRP and there was no correlation between sTfR with serum CRP (Table 5). At both time points sTfR was negatively correlated with Hb and serum ferritin. Serum ferritin was not

Table 4. Linear regression models showing the effect of the intervention on iron markers at week 6, stratified by baseline iron marker category*

Dependent variable (week 6)	<i>n</i>	Baseline Fe marker category stratification†	Coefficient‡	95% CI	<i>P</i> §	<i>P</i> (test for interaction)
Hb (g/l)	705	Normal and mild anaemia	3.31	-1.82, 8.44	0.21	0.38
		Moderate and severe anaemia	0.58	-2.95, 4.10	0.75	
	138	Ferritin below median	0.70	-7.56, 8.97	0.87	0.18
		Ferritin above median	8.73	-0.83, 18.28	0.07	
Log serum ferritin	138	sTfR below median	3.59	-5.00, 12.19	0.41	0.61
		sTfR above median	6.64	-2.75, 16.04	0.16	
	148	Normal and mild anaemia	0.05	-0.43, 0.54	0.83	0.12
		Moderate and severe anaemia	-0.40	-0.74, -0.06	0.023	
sTfR (nmol/l)	165	Ferritin below median	-0.08	-0.42, 0.26	0.63	0.30
		Ferritin above median	-0.35	-0.76, -0.06	0.10	
	165	sTfR below median	-0.27	-0.63, 0.09	0.14	0.52
		sTfR above median	-0.11	-0.48, 0.26	0.55	
sTfR (nmol/l)	147	Normal and mild anaemia	1.77	-6.77, 10.31	0.68	0.60
		Moderate and severe anaemia	-0.91	-6.98, 5.17	0.77	
	164	Ferritin below median	2.77	-3.05, 8.59	0.35	0.52
		Ferritin above median	-0.06	-7.09, 6.97	0.99	
164	sTfR below median	2.74	-3.71, 9.19	0.40	0.59	
	sTfR above median	0.32	-6.24, 6.88	0.92		

sTfR, soluble transferrin receptor.

* Adjusted for the baseline value of the same dependent variable, log-C-reactive protein at week 6 for the Fe markers, sex, site, age, baseline CD4 count, being on tuberculosis medicine at recruitment, taking Fe supplements in addition to the study supplement, length of time from recruitment to antiretroviral therapy and baseline BMI.

† Hb categories defined as normal ≥ 120 g/l for women and ≥ 130 g/l for men, mild anaemia < 120 g/l for women and < 130 g/l for men, moderate and severe anaemia < 110 and < 80 g/l respectively for both sexes. Serum ferritin median = 752 $\mu\text{g/l}$. sTfR median = 45 nmol/l.

‡ The coefficient shows the change in week 6 Fe marker associated with the intervention in comparison to the control within the baseline Fe marker category strata.

§ Two sample *t* test

|| Likelihood ratio test comparing models with and without the interaction between trial arm and baseline Fe marker category.

Table 5. Pairwise correlation matrix between iron markers and C-reactive protein (CRP) at baseline and week 6, unadjusted††

	Log serum CRP		Hb		sTfR		Log serum ferritin		Log serum CRP		Hb		sTfR		Log serum ferritin		
	Baseline coefficient	Week 6 coefficient	Baseline coefficient	Week 6 coefficient	Baseline coefficient	Week 6 coefficient	Baseline coefficient	Week 6 coefficient	Baseline coefficient	Week 6 coefficient	Baseline coefficient	Week 6 coefficient	Baseline coefficient	Week 6 coefficient	Baseline coefficient	Week 6 coefficient	
Log serum CRP, baseline	1.00																
Hb, baseline	-0.31**		1.00														
sTfR, baseline	-0.10		-0.26**		1.00												
Log serum ferritin, baseline	0.34**		-0.02		-0.12*		1.00										
Log serum CRP, week 6	0.30**		-0.19**		-0.15*		0.24**		1.00								
Hb, week 6	-0.13**		0.51**		-0.10		0.11		-0.26**	1.00							
sTfR, week 6	-0.10		-0.14		0.32**		-0.31**		0.00	-0.27**	1.00						
Log serum ferritin, week 6	0.16*		0.20*		-0.07		0.54**		0.24**	0.16*	-0.22**	1.00					

sTfR, soluble transferrin receptor.

* $P < 0.05$.

** $P < 0.01$.

† Pearson's correlation.

†† n: serum CRP baseline (1762), Hb baseline (1670), sTfR baseline (353), serum ferritin baseline (353), serum CRP week 6 (863), Hb week 6 (826), sTfR week 6 (166), serum ferritin week 6 (165). Note that CRP and Hb were available for the whole trial sample, sTfR and ferritin only for the subsample, and sample sizes for the individual variables are determined by the availability of completed week 6 data.

correlated with Hb at baseline, but showed a weak positive correlation at week 6.

Table 6 shows the associations between changes in serum CRP and changes in Fe markers. A decrease in one-log of serum CRP from baseline to week 6 was associated with an increase of 1.81 g/l of Hb (95% CI 0.85, 2.76; $P < 0.001$) and a decrease of 0.11 log of serum ferritin (95% CI -0.20, 0.03; $P = 0.012$) from baseline to week 6. There was no association between the change in serum CRP and the change in sTfR over the same time period ($P = 0.78$).

Discussion

We hypothesised that the two-stage nutritional intervention involving a stabilisation phase followed by the provision of Fe together with other micronutrients would help reverse anaemia of chronic disease and improve Fe deficiency anaemia among malnourished, HIV-infected adults in sub-Saharan Africa. Contrary to expectations, the present results show that the intervention with fortified LNS-VM made no overall difference to Hb or any Fe markers. Furthermore, there was no obvious subgroup, defined by baseline anaemia, serum ferritin or sTfR, which demonstrated any clinically meaningful improvement from the intervention. Although there was weak evidence to suggest that the effect of the intervention on serum ferritin at week 6 was dependent upon baseline levels of Hb, the reduction in log serum ferritin was small and there was no concomitant improvement in Hb or reduction in sTfR in this subgroup, suggesting that the finding was of no clinical significance.

In unadjusted correlation analyses between the Fe markers and CRP, it was not surprising that serum ferritin, being a positive acute phase protein, was positively correlated with CRP at both time points. The negative correlation of sTfR with Hb and serum ferritin was also to be expected, due to sTfR being a marker of tissue Fe deficiency and, more specifically, the requirement of Fe for erythropoiesis⁽²³⁾. The linear regression model exploring the relationship between serum CRP and Hb suggested that reducing systemic inflammation between baseline and week 6 was associated with an increase of Hb over that time period. Fe metabolism involves a series of complex, tightly regulated mechanisms to ensure homeostasis, especially during infection or inflammation. Chief amongst these is the need to maintain Fe tightly protected in order to avoid oxidative damage and to limit its availability to pathogens⁽²⁸⁾. During HIV infection the chronic inflammation causes a hepcidin-mediated redistribution of Fe within the body, a process that becomes more pronounced as the HIV stage progresses^(1,38). Upregulated hepcidin inactivates ferroportin (the only Fe-efflux channel in cells) causing decreased intestinal Fe absorption as well as sequestration of Fe in macrophages⁽²⁸⁾, thus blocking erythropoiesis. This leads to anaemia, and possibly it creates a niche for intra-cellular pathogens such as mycobacteria^(14,39). The present results suggest that to reverse anaemia and normalise Fe redistribution, the source of the innate immune activation first needs to be identified and addressed, and only then, after systemic inflammation has

Table 6. Multivariable linear regression model showing the effect of a one-log decrease in C-reactive protein (CRP) on change in iron markers from baseline to week 6*

Variable (change from baseline to week 6)	<i>n</i>	Coefficient†	95% CI	<i>P</i> ‡
Hb (g/l)	687	1.81	0.85, 2.76	<0.001
Log serum ferritin	165	-0.11	-0.20, 0.03	0.012
sTfR (nmol/l)	164	-0.24	-1.89, 1.42	0.78

sTfR, soluble transferrin receptor.

* Adjusted for trial arm, sex, site, age, baseline CD4 count, being on tuberculosis medicine at recruitment, taking Fe supplements in addition to the study supplement, length of time from recruitment to antiretroviral therapy and baseline BMI.

† The coefficient represents the change in Fe marker from baseline to week 6 associated with a one-log decrease in CRP from baseline to week 6.

‡ Two sample *t* test score result.

been brought under control, will an Fe-containing nutritional intervention be likely to have an impact.

Irrespective of whether the LNS was fortified with vitamins and minerals or not, it appeared that ART plus LNS improved Hb levels and reduced serum ferritin. ART has been associated with a reduction in prevalence of anaemia in other studies^(40–42), although some ART drugs, e.g. zidovudine⁽⁴³⁾ which was prescribed to 26% of NUSTART patients⁽³³⁾, have increased anaemia in some patients. However, in the NUSTART context the overall mean improvement of Hb and reduction of serum ferritin was modest. For there to have been enough of a functional improvement in the distribution and use of Fe in the body, we would have expected sTfR to remain stable at least, if not drop, and yet in this context sTfR levels increased slightly. Irrespective of whether LNS was fortified with vitamins and minerals or not, the combination of LNS and ART for 6 weeks does not appear to improve sufficiently the Fe profile of our patients or reduce their systemic inflammation.

The present study has several limitations. Patients in the subsample had lower baseline Hb and were more inflamed compared to those not in the subsample. This suggests that the subsample patients were slightly sicker than those not included, and restricts the possibility of extending the results to the whole sample. Budget limitations precluded analysis of Fe markers in the full cohort and analysis of results at other time points, for example, at the end of phase 1, as well as assessment of other potentially interesting markers such as hepcidin or α_1 -acid glycoprotein. Since there was no control group not receiving LNS (for ethical reasons), we were unable to separate the overall impact of ART and LNS on our outcomes.

The level of Fe fortification of the LNS during stage two was modest (one recommended nutrient intake) in comparison to higher levels (usually three recommended nutrient intakes) of other micronutrients. This was a conservative approach to avoid potentially increasing the risks associated with higher serum ferritin stores. It would appear that the level of Fe included in the fortified LNS was safe in this regard, since there was no overall increase in serum ferritin from the intervention. That said, we would recommend that Fe dosage within fortified LNS not be increased in future research amongst similar populations before investigating the impact this modest fortification level has, once inflammation has

been successfully controlled. Further research is required: firstly, to determine whether non-nutritional interventions designed to reduce systemic inflammation are sufficient to correct anaemia of inflammation in HIV; secondly, to assess whether a product with a different nutrient composition may also assist this process; and thirdly, to quantify the level of improvement in inflammation necessary before a nutritional intervention will improve Fe deficiency anaemia.

Conclusion

Our large clinical trial of Fe supplementation as part of a nutritional intervention showed no appreciable effect on Hb and Fe metabolism, even when the majority of patients were anaemic at baseline. HIV-related inflammation resulting in disordered Fe metabolism appears to severely attenuate the potential impact of receiving dietary Fe in an intervention. Given the clear associations between anaemia, disordered Fe metabolism and mortality amongst HIV-positive patients starting ART, it is of critical importance that strategies to reduce the level of systemic inflammation (going beyond the provision of ART) are investigated. Without the ability to control inflammation, it would appear that the impact of a type of nutritional intervention similar to the one used in the present study would remain severely restricted.

Supplementary material

To view supplementary material for this article, please visit <http://dx.doi.org/10.1017/S0007114515001920>

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References

- O'Brien ME, Kupka R, Msamanga GI, *et al.* (2005) Anemia is an independent predictor of mortality and immunologic progression of disease among women with HIV in Tanzania. *J Acquir Immune Defic Syndr* **40**, 219–225.
- Giganti MJ, Limbada M, Mwango A, *et al.* (2012) Six-month hemoglobin concentration and its association with subsequent mortality among adults on antiretroviral therapy in Lusaka, Zambia. *J Acquir Immune Defic Syndr* **61**, 120–123.
- Koethe JR, Lukusa A, Giganti MJ, *et al.* (2010) Association between weight gain and clinical outcomes among malnourished adults initiating antiretroviral therapy in Lusaka, Zambia. *J Acquir Immune Defic Syndr* **53**, 507–513.
- Lawn SD, Harries AD, Anglaret X, *et al.* (2008) Early mortality among adults accessing antiretroviral treatment programmes in sub-Saharan Africa. *AIDS* **22**, 1897–1908.
- Paton NI, Sangeetha S, Earnest A, *et al.* (2006) The impact of malnutrition on survival and the CD4 count response in HIV-infected patients starting antiretroviral therapy. *HIV Med* **7**, 323–330.
- Nasi M, Pinti M, Mussini C, *et al.* (2014) Persistent inflammation in HIV infection: established concepts, new perspectives. *Immunol Lett* **161**, 184–188.
- Armitage AE, Stacey AR, Giannoulou E, *et al.* (2014) Distinct patterns of hepcidin and iron regulation during HIV-1, HBV, and HCV infections. *Proc Natl Acad Sci U S A* **111**, 12187–12192.
- Koethe JR, Blevins M, Nyirenda C, *et al.* (2011) Nutrition and inflammation serum biomarkers are associated with 12-week mortality among malnourished adults initiating antiretroviral therapy in Zambia. *J Int AIDS Soc* **14**, 19.
- Kuller LH, Tracy R, Belloso W, *et al.* (2008) Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. *PLoS Med* **5**, e203.
- Wisaksana R, Sumantri R, Indrati AR, *et al.* (2011) Anemia and iron homeostasis in a cohort of HIV-infected patients in Indonesia. *BMC Infect Dis* **11**, 213.
- Chang H-C, Bayeva M, Taiwo B, *et al.* (2015) High cellular iron levels are associated with increased HIV infection and replication. *AIDS Res Hum Retroviruses* **31**, 305–312.
- Salhi Y, Costagliola D, Rebutta P, *et al.* (1998) Serum ferritin, desferrioxamine, and evolution of HIV-1 infection in thalassaemic patients. *J Acquir Immune Defic Syndr Hum Retrovirol* **18**, 473–478.
- Gordeuk VR, Delanghe JR, Langlois MR, *et al.* (2001) Iron status and the outcome of HIV infection: an overview. *J Clin Virol* **20**, 111–115.
- De Monyé C, Karcher DS, Boelaert JR, *et al.* (1999) Bone marrow macrophage iron grade and survival of HIV-seropositive patients. *AIDS* **13**, 375–380.
- Minchella PA, Armitage AE, Darboe B, *et al.* (2014) Elevated hepcidin at HIV diagnosis is associated with incident tuberculosis in a retrospective cohort study. *Int J Tuberc Lung Dis* **18**, 1337–1339.
- McDermid JM, Hennig BJ, van der Sande M, *et al.* (2013) Host iron redistribution as a risk factor for incident tuberculosis in HIV infection: an 11-year retrospective cohort study. *BMC Infect Dis* **13**, 48.
- Gordeuk VR, Onojobi G, Schneider MF, *et al.* (2006) The association of serum ferritin and transferrin receptor concentrations with mortality in women with human immunodeficiency virus infection. *Haematologica* **91**, 739–743.
- McDermid JM, Loeff MFS, Van Der, Jaye A, *et al.* (2009) Mortality in HIV infection is independently predicted by host iron status and SLC11A1 and HP genotypes, with new evidence of a gene-nutrient. *Am J Clin Nutr* **90**, 225–233.
- Gupta A, Nadkarni G, Yang W-T, *et al.* (2011) Early mortality in adults initiating antiretroviral therapy (ART) in low- and middle-income countries (LMIC): a systematic review and meta-analysis. *PLoS One* **6**, e28691.
- Joint United Nations Programme on HIV/AIDS (UNAIDS) (2013) *Global Report: UNAIDS Report on the Global AIDS Epidemic 2013*. Geneva: UNAIDS.
- Liu E, Spiegelman D, Semu H, *et al.* (2011) Nutritional status and mortality among HIV-infected patients receiving antiretroviral therapy in Tanzania. *J Infect Dis* **204**, 282–290.
- Finch CA, Bellotti V, Stray S, *et al.* (1986) Plasma ferritin determination as a diagnostic tool. *West J Med* **145**, 657–663.
- Cook JD, Skikne BS & Baynes RD (1993) Serum transferrin receptor. *Annu Rev Med* **44**, 63–74.
- Jonker FAM, Boele van Hensbroek M, Leenstra Tet *et al.* (2014) Conventional and novel peripheral blood iron markers compared against bone marrow in Malawian children. *J Clin Pathol* **67**, 717–723.
- World Health Organization/Centers for Disease Control and Prevention (2007) *Assessing the Iron Status of Populations, Including Literature Reviews. Report of a Joint World Health Organization/Centers for Disease Control and Prevention Technical Consultation on the Assessment of Iron Status at the Population Level*, 2nd ed. Geneva: WHO.
- Gibson R (2005) *Principles of Nutritional Assessment*, 2nd ed. Oxford: Oxford University Press.





27. Thurnham DI, McCabe LD, Haldar S, *et al.* (2010) Adjusting plasma ferritin concentrations to remove the effects of sub-clinical inflammation in the assessment of iron deficiency. *Am J Clin Nutr* **92**, 546–555.
28. Drakesmith H & Prentice AM (2012) Hepcidin and the iron–infection axis. *Science* **338**, 768–772.
29. Esan MO, van Hensbroek MB, Nkhoma E, *et al.* (2013) Iron supplementation in HIV-infected Malawian children with anemia: a double-blind, randomized, controlled trial. *Clin Infect Dis* **57**, 1626–1634.
30. Olsen A, Mwaniki D, Krarup H, *et al.* (2004) Low-dose iron supplementation does not increase HIV-1 load. *J Acquir Immune Defic Syndr* **36**, 637–638.
31. Semba RD, Ricketts EP, Mehta S, *et al.* (2007) Effect of micronutrients and iron supplementation on hemoglobin, iron status, and plasma hepatitis C and HIV RNA levels in female injection drug users: a controlled clinical trial. *J Acquir Immune Defic Syndr* **45**, 298–303.
32. Filteau S, PrayGod G, Kasonka L, *et al.* (2015) Effects on mortality of a nutritional intervention for malnourished HIV-infected adults referred for antiretroviral therapy: a randomised controlled trial. *BMC Med* **13**, 17.
33. Rehman AM, Woodd S, PrayGod G, *et al.* (2015) Effects on anthropometry and appetite of vitamins and minerals given in lipid nutritional supplements for malnourished HIV-infected adults referred for antiretroviral therapy: results from the NUSTART randomized controlled trial. *J Acquir Immune Defic Syndr* **68**, 405–412.
34. World Health Organization, World Food Programme, United Nations System Standing Committee on Nutrition and United Nations Children's Fund (2007) *Community-based Management of Severe Acute Malnutrition: A Joint Statement by the World Health Organization, the World Food Programme, the United Nations System Standing Committee on Nutrition and the United Nations Children's Fund*. Geneva: WHO.
35. Department of Health (1991) *Dietary Reference Values for Food Energy and Nutrients for the United Kingdom: Report of the Panel on Dietary Reference Values of the Committee on Medical Aspects of Food Policy*. London: HMSO (Report on Health and Social Subjects 41).
36. World Health Organization (2011) *Haemoglobin Concentrations for the Diagnosis of Anaemia and Assessment of Severity*. Geneva: WHO.
37. Friis H, Range N, Braendgaard Kristensen C, *et al.* (2009) Acute-phase response and iron status markers among pulmonary tuberculosis patients: a cross-sectional study in Mwanza, Tanzania. *Br J Nutr* **102**, 310–317.
38. Savarino A, Pescarmona GP & Boelaert JR (1999) Iron metabolism and HIV infection: reciprocal interactions with potentially harmful consequences? *Cell Biochem Funct* **17**, 279–287.
39. al-Khafaji B, Kralovic S & Smith R (1997) Increased hepatic iron in the acquired immunodeficiency syndrome: an autopsy study. *Mod Pathol* **10**, 474–480.
40. Semba RD, Shah N, Klein RS, *et al.* (2001) Highly active antiretroviral therapy associated with improved anemia among HIV-infected women. *AIDS Patient Care STDS* **15**, 473–480.
41. Berhane K, Karim R, Cohen MH, *et al.* (2004) Impact of highly active antiretroviral therapy on anemia and relationship between anemia and survival in a large cohort of HIV-infected women: Women's Interagency HIV Study. *J Acquir Immune Defic Syndr* **37**, 1245–1252.
42. Moore RD & Forney D (2002) Anemia in HIV-infected patients receiving highly active antiretroviral therapy. *J Acquir Immune Defic Syndr* **29**, 54–57.
43. Kiragga AN, Castelnuovo B, Nakanjako D, *et al.* (2010) Baseline severe anaemia should not preclude use of zidovudine in antiretroviral-eligible patients in resource-limited settings. *J Int AIDS Soc* **13**, 42.