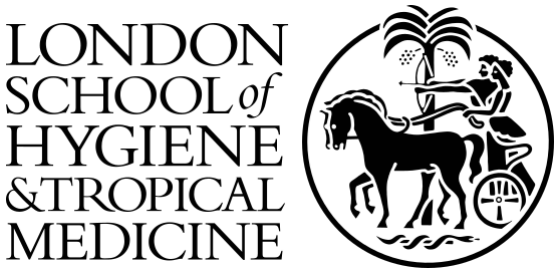

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RESEARCH ARTICLE

Effects on body composition and handgrip strength of a nutritional intervention for malnourished HIV-infected adults referred for antiretroviral therapy: a randomised controlled trial

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Abstract

Lipid-based nutrient supplements (LNS) may be beneficial for malnourished HIV-infected patients starting antiretroviral therapy (ART). We assessed the effect of adding vitamins and minerals to LNS on body composition and handgrip strength during ART initiation. ART-eligible HIV-infected patients with BMI <18·5 kg/m^2 were randomised to LNS or LNS with added high-dose vitamins and minerals (LNS-VM) from referral for ART to 6 weeks post-ART and followed up until 12 weeks. Body composition by bioelectrical impedance analysis (BIA), deuterium (^{2}H) diluted water (D_2O) and air displacement plethysmography (ADP), and handgrip strength were determined at baseline and at 6 and 12 weeks post-ART, and effects of LNS-VM *v.* LNS at 6 and 12 weeks investigated. BIA data were available for 1461, D_2O data for 479, ADP data for 498 and handgrip strength data for 1752 patients. Fat mass tended to be lower, and fat-free mass correspondingly higher, by BIA than by ADP or D_2O. At 6 weeks post-ART, LNS-VM led to a higher regain of BIA-assessed fat mass (0·4 (95 % CI 0·05, 0·8) kg), but not fat-free mass, and a borderline significant increase in handgrip strength (0·72 (95 % CI −0·03, 1·5) kg). These effects were not sustained at 12 weeks. Similar effects as for BIA were seen using ADP or D_2O but no differences reached statistical significance. In conclusion, LNS-VM led to a higher regain of fat mass at 6 weeks and to a borderline significant beneficial effect on handgrip strength. Further research is needed to determine appropriate timing and supplement composition to optimise nutritional interventions in malnourished HIV patients.

Key words: **Body composition: Handgrip strength: Vitamins: Minerals: Antiretroviral therapy: Malnutrition**

Abbreviations: ADP, air displacement plethysmography; ART, antiretroviral therapy; BIA, bioelectrical impedance analysis; CD4, cluster of differentiation 4; D_2O, deuterium (^{2}H) diluted water; FFMI, fat-free mass index; FMI, fat mass index; LNS, lipid-based nutrient supplement; LNS-VM, lipid-based nutrient supplement with added vitamins and minerals; NUSTART, Nutritional Support for African Adults Starting Antiretroviral Therapy.

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HIV infection continues to be a major public health problem in sub-Saharan Africa[1]. Despite increased access to antiretroviral therapy (ART) in countries in Africa, about 15–30 % of patients[2,3] attending ART clinics are malnourished due to nutritional problems associated with advanced HIV infection[4]. Food insecurity resulting from HIV infection may aggravate the problem, since HIV-infected patients and their families may have reduced capacity to produce or afford nutritionally adequate food[5].

Malnutrition, particularly wasting of lean mass (used here synonymously with fat-free mass), is associated with delayed clinical and functional recovery, and increased risk of mortality among patients starting ART[2,6,7]. With the goal of improving nutritional rehabilitation and survival, prior randomised controlled trials have investigated the role of macronutrient and micronutrient supplementation on health and mortality of HIV-infected patients[8,9]. In some but not all macronutrient trials, these interventions have been shown to have benefits on the health of HIV patients. For example, a trial in Ethiopia among malnourished HIV-infected patients found that both whey- and soya-based supplementary foods increased fat-free mass measured by the deuterium (^2H) diluted water (D_2O) technique among patients with viral suppression at 3 months following these interventions[10] and in Malawi malnourished HIV-infected patients receiving ready-to-use fortified spread had higher gain in fat-free mass compared with those on a corn–soya blend[11]. However, most of the micronutrient trials showed no, limited or only short-term benefits during HIV care. Part of the reasons for such limited effects may be that some studies were conducted in settings where malnutrition including micronutrient deficiency is not prevalent among the HIV-infected population[9].

Some discrepancies among results may also be due to different techniques used to assess body composition. The bioelectrical impedance analysis (BIA) method is simple and cheap to apply, and hence can be used in large sample sizes. Although it provides acceptable results, it has lower accuracy in individuals compared with reference methods such as air displacement plethysmography (ADP) or D_2O[12,13] which measure tissue masses directly; however, ADP and D_2O are expensive and may be difficult logistically to administer. Given contrasting strengths and limitations of these methods, using more than one method may help to better understand the effect of interventions on body composition. As potential differences between HIV-infected patients and normal healthy adults in lean tissue properties (e.g. hydration) may have an impact on direct body composition assessment, further information can be gained through assessment of grip strength, a functional indicator of fat-free mass which is reduced in malnutrition and which is inversely associated with survival[14,15].

In the Nutritional Support for African Adults Starting Antiretroviral Therapy (NUSTART) trial, we found that vitamins and minerals added to lipid-based nutrient supplements (LNS-VM) increased cluster of differentiation 4 (CD4) count and some anthropometric measures by 12 weeks of ART[16,17]. In the present paper we examine the effects of LNS-VM, compared with LNS, on grip strength and body composition. We hypothesised that the intervention would have beneficial effects on handgrip strength and fat-free mass since it contained nutrients including Zn, P and Mg which are essential for repletion of muscle and organs[18].

Methods

Study setting

The study was conducted from August 2011 to December 2013 at the National Institute for Medical Research (NIMR), Mwanza, Tanzania and the University Teaching Hospital (UTH), Lusaka, Zambia. In Mwanza HIV-infected patients were screened at six peripheral ART clinics and recruitment was conducted at a research clinic located at the Sekou-Toure Regional Hospital. In Lusaka patients were recruited from six peripheral ART clinics and were referred to UTH for enrolment. HIV diagnosis and treatment followed local national guidelines[19,20]. The trial was registered at the Pan African Clinical Trials Registry as PACTR201106000300631 (http://www.pactr.org).

Inclusion and exclusion criteria

HIV-infected patients who were being referred for ART in Mwanza and Lusaka were included in the trial if they met the following criteria: age 18 years and above, ART-naive (except for standard short-course regimens to prevent maternal-to-child HIV transmission), undernourished (BMI <18·5 kg/m^2), eligible for ART according to national criteria at the time (CD4 count <350 cells/μl or had WHO stage 3 or 4 disease), willing to undertake intensive ART follow-up in the study clinic, and provided written informed consent. Patients were not invited into the trial if they were participating in a similar study or were pregnant by self-report.

Study design and interventions

The NUSTART study was a phase III randomised controlled trial comparing in a two-stage protocol LNS-VM (intervention) *v.* LNS (control) given from recruitment at referral for ART until 6 weeks after starting ART[16]. In the first stage, from recruitment to 2 weeks after starting ART, participants in the control or treatment intervention were given 30 g/d LNS or LNS-VM, about 150 kcal (630 kJ)/d, and in the second stage, from 2–6 weeks after initiating ART, participants were given 250 g/d LNS or LNS-VM, about 1400 kcal (5860 kJ)/d. The LNS was manufactured for the trial by Nutriset (Malaunay, France) and came in ready-to-eat packets. Due to high nutrient requirements in HIV patients, the amounts of added vitamins and minerals in LNS-VM were three times the Recommended Nutrient Intake (RNI) for British women[21], but to avoid possible deleterious effects of Fe during severe infections[22], Fe was not included in the first stage, and in the second stage we provided one RNI only. We included bulk minerals, i.e. K, Mg and P, in both stages to address deficiencies of these minerals, correct electrolyte imbalance and promote tissue repletion. Further details of the intervention are in Supplementary Table S1 and published[16].

Outcomes

The primary trial outcome was mortality between recruitment and 12 weeks post-ART initiation[16]. Secondary outcomes presented here include effects on handgrip strength, fat mass and fat-free mass at 6 and 12 weeks post-ART initiation and during the follow-up period. BIA results were used as our main measure of body composition, both because we had results for the greatest proportion of participants and because it is a relatively cheap and feasible technique even in fairly poorly resourced settings. BIA produces results predicted from impedance, age, sex, weight and height and we wished to compare these results with the more direct measurements given by ADP and D_2O.

Randomisation, allocation concealment and blinding

Randomisation was conducted by the Data and Safety Monitoring Board (DSMB) statistician using computer-generated blocks of sixteen and stratified by country. Packages of LNS-VM and LNS, in both small- and large-dose formats, were delivered by the producer in lots designated by allocation code. Clinic pharmacists not involved in recruitment or provision of care to study participants labelled intervention packets with the study identity numbers at the time packets were dispensed. Participants were recruited by clinic nurses with no access to the code and assigned sequential identity numbers (within sites) after they were found to be eligible and had signed informed consent. Both participants and recruiting staff were not aware of the group of the dispensed supplements and intervention and control supplements packets were of equal size, colour, and similar taste. Adherence to study supplements was modest, with only 39 % of participants consuming at least 75 % of their expected number of sachets of supplement[16].

Sample size justification

As we reported earlier, by the end of recruitment, we had recruited 1815 patients[16]. This number was sufficient to detect, at 5 % significance, 90 % power and 25 % attrition by 12 weeks due to death or loss to follow-up, differences of 0·18 of a standard deviation in secondary continuous outcomes measured at 6 and 12 weeks.

Ethics

The study was conducted according to principles laid down in the Declaration of Helsinki. Ethics committees of the London School of Hygiene & Tropical Medicine, the University of Zambia Biomedical Research Ethics Committee, and the Medical Research Coordinating Committee of NIMR, Tanzania provided ethics clearances. Patients were enrolled after providing written or thumbprint informed consent and medical care of patients was provided according to national guidelines.

Data collection

Data on demographic and socio-economic status were collected at patient enrolment. Handgrip strength and body composition data were collected at enrolment (before ART initiation), and at the 6th and 12th week post-ART initiation. The time of starting ART was determined by factors outside the investigators' control and was a median of 21 (interquartile range 15–30) d after referral for ART[16]. Patients who did not attend study visits were reminded by telephone or those in Mwanza were also traced to their residences and encouraged to come to clinics for follow-up measurements. Patients were asked to fast overnight and were invited for anthropometry, handgrip strength and body composition measurements in the morning. While barefoot and with minimal clothing, weight was determined to the nearest 0·1 kg using a digital scale and height (baseline only) was measured to the nearest 0·1 cm using a stadiometer fixed to the office wall. Anthropometric measurements were taken in triplicate and medians were used during analysis. We assessed body composition using BIA using Tanita instrumentation (Tanita BC418) as the primary method. In addition, we used D_2O (Cortecnet) and ADP (BodPod Model 2007A; Life Measurement Instruments/COSMED) in subsamples, limited by logistic and financial constraints to Zambian participants, to supplement BIA findings. All the methods assessed fat mass (kg) and fat-free mass (kg) and, in addition, BIA also produced results on trunk and segmental fat and fat-free mass, all expressed in kg.

For the D_2O technique, patients were asked to provide a 4-ml saliva sample (pre-dose saliva sample), after which they were asked to take a previously prepared dose of 30 g D_2O using a straw from a 50-ml, screw-capped, leak-proof bottle. Then they drank 100 ml of drinking water from the same bottle to ensure all D_2O was consumed. We collected two post-dose saliva samples at 3 and 4 h. All samples were collected into tightly capped cryogenic tubes and kept away from direct sunlight. While waiting for post-dose sample collection, patients were asked to refrain from walking, eating or drinking. On the day of collection, samples were transported in cool boxes to research laboratories at UTH, where they were stored at −20°C pending transfer to the Zambian National Institute for Scientific and Industrial Research in Lusaka for analysis.

Enrichment of D_2O in saliva samples was determined by Fourier Transform Infrared Spectrophotometer (FTIR Model 8400s; Shimadzu). Post-dose enrichment used the mean of the 3- and 4-h samples except where the 4-h enrichment was appreciably higher than the 3-h, in which case only the 4-h sample was used. Using post-dose enrichment data, the dilution space and total body water (TBW) were calculated using conventional formulae[23]. Fat-free mass was calculated as TBW/0·723[24] and fat mass was calculated as body weight minus fat-free mass.

From BIA, D_2O and ADP fat and fat-free mass, and height measurements, fat mass index (FMI) was computed as fat mass (kg)/(height (m^2)), and fat-free mass index (FFMI) as fat-free mass (kg)/(height (m^2))[25]. We used FMI and FFMI in data management and fat and fat-free mass in evaluation of the effect of intervention.

Handgrip strength was determined to the nearest 0·1 kg using a digital dynamometer (Takei Scientific Instruments). Four measurements were taken, with the mean of the two

maximum measurements (one in each hand) reported. Venous blood samples were taken for CD4 count (baseline and week 12 only)[16].

Data management and statistics

Data were double entered into OpenClinica databases in Lusaka and into MySQL databases in Mwanza. Analyses were conducted in STATA version 13. The D_2O technique is susceptible to chance errors resulting from incomplete dosing, sample contamination, or unrecorded fluid intake during the equilibration period. Therefore, at baseline, we excluded those with implausibly low fatness (fat mass <0 kg). We further excluded those with implausibly high fatness, given that the entire population had BMI <18·5 kg/m^2; this resulted in excluding those with FMI >6 kg/m^2, or FMI >4 kg/m^2 if FFMI <11 kg/m^2. For 6-week samples, we excluded those with implausibly low fatness (fat mass<0 kg). We also excluded those with poor agreement between BIA and ADP (difference >±7 kg TBW). For 12-week samples, we excluded those with implausibly low fatness (fat mass <0 kg), and implausibly high fatness (FMI >8 kg/m^2). We also excluded those with poor agreement between BIA and ADP (difference >±7 kg TBW). Based on this we excluded fifty-six (10·5 %) at baseline, twenty (8 %) at 6 weeks and twenty (9 %) at 12 weeks.

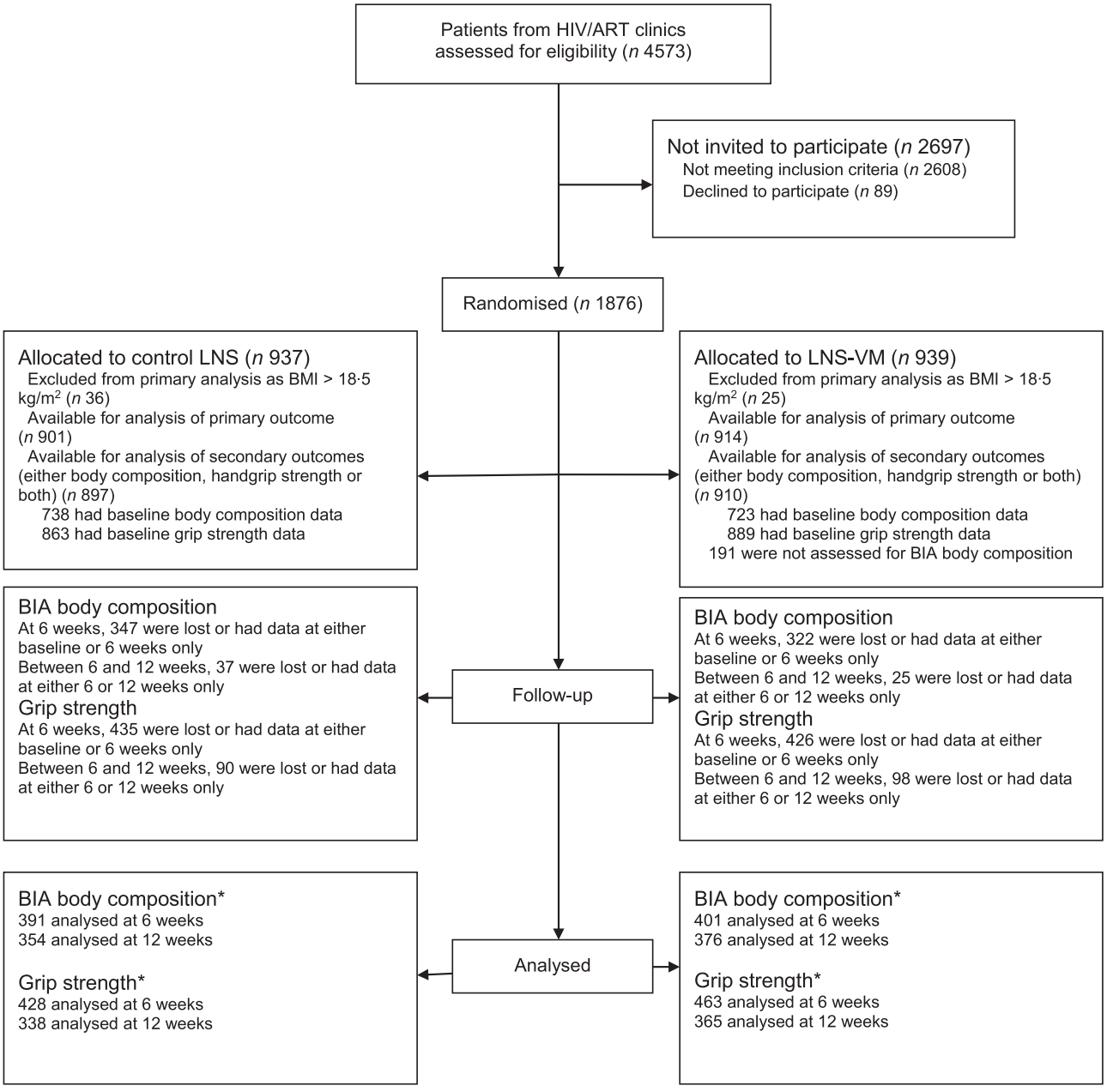

Fig. 1. Trial flow chart. * Number analysed for the effect of interventions. This may differ slightly with the actual number available at either 6 or 12 weeks because analysis of the effect of intervention included participants with data at two time points, i.e. baseline and 6 weeks and 6 weeks and 12 weeks. ART, antiretroviral therapy; LNS, lipid nutritional supplement; LNS-VM, lipid nutritional supplement with vitamins and minerals; BIA, bioelectrical impedance analysis (the main body composition assessment method for the present study).

Baseline characteristics were presented as means and standard deviations if continuous variables and percentages if categorical variables to assess comparability of treatment arms. Socio-economic status was derived using principal component analysis of a list of housing characteristics and durable assets[16,26]. Outcome measures (i.e. fat mass, fat-free mass and handgrip strength) at 6 and 12 weeks post-ART were compared using linear regression with final estimate found by adjusting for baseline values, sex, age, BMI, CD4 count and socio-economic status.

In addition, since results at 6 and 12 weeks could be analysed only for patients who survived and attended 6- and 12-week visits, we also investigated treatment effects using the detailed longitudinal data collected on all patients over the course of the study. We used piece-wise mixed-effects quadratic regression models to allow inclusion of data from patients who died or were lost to follow-up up prior to 12 weeks. The additional flexibility of cubic models and cubic splines was assessed but fit was considered adequate with quadratic models[27]. The time axis was split at the date of starting ART, allowing two lines with differing slopes to be fitted per person while restricting these lines to join at the date of ART initiation. For presentation, the marginal predictions after starting ART were based on the median time, 21 d, spent prior to starting ART; predictions pre-ART are not graphed because of the complexity of showing different lengths of time before ART.

To assess comparability of BIA against D_2O and ADP methods, we used the Bland–Altman method[28]. Based on this approach, we analysed differences (bias) in fat and fat-free mass between BIA and the other methods and their standard deviations (error) and calculated the limits of agreement (bias ± 1·96 error) to determine if the degree to which these methods differed was within a clinically acceptable range. We further assessed the dependency of bias on the mean of fat and fat-free mass for BIA and D_2O and BIA and ADP using linear regression. Among both sexes, the acceptable range of error for fat and fat-free mass is thought to be 2 to 4·5 kg for males and 1·5 to 3·6 kg for females[12].

Results

As shown in the trial flow chart (Fig. 1), of 4573 participants screened, 1876 were randomised, and 1815 included in the analysis of the primary outcome[16], but only 1807 (897 allocated to LNS and 910 to LNS-VM) were included in the present analysis because they had either body composition or grip strength data or both. Of these patients, 1752 had baseline data on handgrip strength and 1461, 479 and 498 had baseline body composition data based on BIA, D_2O and ADP, respectively. The mean participant age was 35·8 (range 18–78) years, 59·2 % had BMI <17 kg/m^2, and 49·6 % were females. All baseline characteristics were equally distributed between treatment arms (Table 1). From baseline to 6 weeks, and 6 and 12 weeks, proportions not followed up between LNS and LNS-VM groups on participants with body composition measurements based on the three methods (i.e. BIA, D_2O and ADP) were not different (47·0 *v.* 44·5 % ($P = 0·34$) and 9·5 *v.* 6·2 % ($P = 0·12$); 70·8 *v.* 70·8 % ($P = 0·99$) and 37·7 *v.* 32·4 % ($P = 0·65$; and 72·1 *v.* 74·1 % ($P = 0·62$) and 20·0 *v.* 17·2 % ($P = 0·73$), respectively). Similarly, proportions of participants with handgrip strength measurements not followed-up between baseline to 6 weeks and 6 weeks to 12 months were not different between LNS and LNS-VM groups (50·4 *v.* 47·9 % ($P = 0·30$) and 21·0 *v.* 21·2 % ($P = 0·97$), respectively). Overall, compared with patients remaining in the study, those not followed up for body composition and handgrip strength assessments at 6 and 12 weeks were more likely to be males, younger, thinner, more immunocompromised, and not on tuberculosis treatment, all factors associated with mortality in the cohort[29].

Table 1. Baseline characteristics of patients included in the evaluation of the effect on Nutritional Support for African Adults Starting Antiretroviral Therapy (NUSTART) intervention on secondary outcomes (i.e. handgrip strength and body composition)*
(Mean values and standard deviations; numbers and percentages)

	LNS (*n* 897)		LNS-VM (*n* 910)	
	n	%	*n*	%
Age (years)				
Mean	35·7		35·9	
SD	9·4		9·4	
Female	456	50·8	440	48·4
Tuberculosis treatment	197	22·0	249	27·4
CD4 count (cells/μl)				
Mean	135		140	
SD	97		103	
<100 cells/μl	390	43·5	395	43·4
BMI (kg/m^2)				
Mean	16·5		16·5	
SD	1·4		1·3	
<17 kg/m^2	530	59·1	539	59·2
Socio-economic status				
Lowest	197	22·0	166	18·2
Low	187	20·9	184	20·2
Middle	162	18·1	199	21·9
High	182	20·3	172	18·9
Highest	169	18·8	189	20·8
Marital status				
Married	433	48·3	417	45·8
Widow/widower	107	11·9	95	10·4
Divorced/separated	243	12·3	264	29·0
Single	110	11·4	132	14·5
Living with partner	3	0·33	2	0·22
Occupation				
Salaried	135	15·1	134	14·7
Self-employed	458	51·1	484	53·2
Housewife	97	10·8	86	9·5
Student	9	1·0	9	1·0
Unemployed	197	22·0	197	21·7
Education level				
None	173	19·3	168	18·5
Primary	516	57·6	526	57·8
Secondary	182	20·3	192	21·1
University/tertiary	25	2·8	24	2·6
Study site				
Lusaka	548	61·1	556	61·1
Mwanza	349	38·9	354	38·9

LNS, lipid nutritional supplement; LNS-VM, lipid nutritional supplement with added vitamins and minerals; CD4, cluster of differentiation 4.
* Of the participants, 1752 had baseline data on handgrip strength, and baseline body composition data were collected on 1461 using bioelectrical impedance analysis, 479 using the deuterium (^{2}H) diluted water method and 498 using air displacement plethysmography.

Table 2. Fat mass, fat-free mass and grip strength at baseline and at 6 and 12 weeks after starting antiretroviral therapy (Mean values and standard deviations)

	Baseline						Week 6						Week 12					
	LNS			LNS-VM			LNS			LNS-VM			LNS			LNS-VM		
	n	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD
Fat mass (kg)																		
Total by D_2O	236	5·9	2·8	243	6·0	2·7	115	8·1	3·2	113	8·6	3·4	96	8·9	4·6	105	9·7	4·6
Total by ADP	251	6·9	2·8	247	6·7	3·0	108	8·0	3·1	102	9·0	3·5	83	9·7	4·1	89	9·9	3·9
Total by BIA	738	5·0	2·8	723	4·9	2·8	436	6·8	3·3	461	6·9	3·3	399	7·6	3·5	437	7·6	3·5
Truncal	738	2·1	1·6	723	2·1	1·5	435*	3·0	1·9	461	3·1	1·9	399	3·5	2·1	437	3·5	2·1
Limb	738	3·0	1·8	723	3·0	1·9	436	3·9	2·0	461	3·9	2·0	399	4·2	2·1	437	4·2	2·1
Fat-free mass (kg)																		
Total by D_2O	236	39·8	5·9	243	39·3	6·0	115	39·7	5·4	113	40·6	6·2	96	40·6	6·0	105	40·7	6·4
Total by ADP	251	38·7	5·6	247	38·9	5·7	108	39·4	5·5	102	39·4	5·4	83	39·7	5·9	89	40·9	5·7
Total by BIA	738	39·6	6·5	723	40·0	6·2	436	41·4	6·7	461	41·6	6·6	399	41·8	6·6	437	42·6	7·1
Truncal	738	21·9	3·0	723	22·1	3·0	435*	23·1	3·4	461	23·2	3·3	399	23·3	3·3	437	23·8	3·6
Limb	738	17·5	3·3	723	17·7	3·3	436	18·2	3·5	461	18·3	3·5	399	18·4	3·5	437	18·7	3·7
Grip strength (kg)	863	19·4	6·8	889	19·9	7·0	450	21·1	6·8	476	22·3	7·0	371	22·8	7·1	406	23·6	7·0

LNS, lipid nutritional supplement; LNS-VM, lipid nutritional supplement with vitamins and minerals; D_2O, deuterium (^2H) diluted water method; ADP, air displacement plethysmography; BIA, bioelectrical impedance analysis.
* One observation is missing.

Table 2 presents body composition and handgrip strength data at baseline, and at 6 and 12 weeks by treatment arm. Supplementary Table S2 presents the same information among patients with data from all three body composition assessment methods. Effects of LNS-VM *v.* LNS on changes in body composition between baseline and 6 weeks of ART are shown in Table 3 and similar results for changes between 6 and 12 weeks of ART are shown in Table 4. At the end of 6 weeks of ART, patients given LNS-VM tended to have higher fat mass than those randomised to LNS (0·4 (95 % CI 0·05, 0·8) kg); although the point estimate was highest for fat mass assessed by D_2O, the effect was significant only for fat mass by BIA, possibly because of the larger number of participants assessed by this method. Treatment arm showed no association with change in fat-free mass. Between 6 and 12 weeks of ART there were no significant differences between treatment arms in fat mass and fat-free mass change although the point estimates suggested higher fat-free mass in the LNS-VM group. Similar results for trunk and limb composition by BIA were seen as for total body tissue masses by BIA. In a sensitivity analysis involving patients with body composition measurements by all three methods (twenty-two patients at 6 weeks and eighteen at 12 weeks), we found no effect of the intervention on fat and fat-free mass (Supplementary Tables S3 and S4).

At the end of 6 weeks post-ART, patients in the LNS-VM group had 0·72 (95 % CI −0·03, 1·5) kg greater handgrip

Table 3. Effects of intervention on the change in body composition and grip strength between baseline and 6 weeks of antiretroviral therapy (ART) (Mean values and 95 % confidence intervals)

	Change between baseline and 6 weeks of ART											
	LNS			LNS-VM			Difference between arms*			Adjusted difference between arms†		
	n	Mean	95 % CI	*n*	Mean	95 % CI	Mean	95 % CI	*P*	Mean	95 % CI	*P*
Fat mass (kg)												
Total by D_2O	69	1·7	0·9, 2·5	71	2·5	1·7, 3·2	0·7	−0·4, 1·8	0·22	0·7	−0·4, 1·9	0·21
Total by ADP	70	1·2	0·6, 1·9	64	1·6	1·0, 2·0	0·3	−0·6, 1·2	0·50	0·4	−0·5, 1·3	0·42
Total by BIA	391	1·5	1·3, 1·7	401	1·9	1·7–2·2	0·4	0·06, 0·8	0·02	0·4	0·05, 0·8	0·02
Truncal	390‡	0·8	0·6, 0·9	401	1·0	0·9, 1·2	0·2	0·02, 0·5	0·03	0·2	0·01, 0·4	0·04
Limb	391	0·7	0·6, 0·8	401	0·8	0·7, 0·9	0·1	−0·02, 0·3	0·09	0·1	−0·02, 0·3	0·09
Fat-free mass (kg)												
Total by D_2O	69	1·3	0·5, 2·2	71	1·1	0·2, 2·0	−0·2	−1·4, 1·0	0·77	0·2	−1·0, 1·5	0·76
Total by ADP	70	1·0	0·5, 1·6	64	0·9	0·2, 1·5	−0·1	−0·9, 0·7	0·74	−0·2	−1·0, 0·7	0·70
Total by BIA	391	1·5	1·1, 1·9	401	1·8	1·5, 2·1	0·2	−0·3, 0·7	0·42	0·2	−0·3, 0·7	0·51
Truncal	390‡	1·0	0·9, 1·3	401	1·1	0·9, 1·3	0·07	−0·2, 0·3	0·57	0·06	−0·2, 0·3	0·66
Limb	391	0·7	0·5, 0·8	401	0·7	0·6, 0·9	0·03	−0·2, 0·2	0·77	0·01	−0·2, 0·2	0·90
Grip strength (kg)	428	1·2	0·7, 1·7	463	1·9	1·4, 2·4	0·73	−0·02, 1·5	0·06	0·72	−0·03, 1·5	0·06

LNS, lipid nutritional supplement; LNS-VM, lipid nutritional supplement with vitamins and minerals; D_2O, deuterium (^2H) diluted water method; ADP, air displacement plethysmography; BIA, bioelectrical impedance analysis; CD4, cluster of differentiation 4.
* Difference between change over time, adjusted for age and sex.
† Difference between change over time, adjusted for age, sex, baseline CD4, baseline BMI and socio-economic status.
‡ One observation missing.

Table 4. Effects of intervention on the change in body composition and grip strength between 6 and 12 weeks of antiretroviral therapy (ART) (Mean values and 95 % confidence intervals)

	Change between 6 and 12 weeks of ART											
	LNS			LNS-VM			Difference between arms*			Adjusted difference between arms†		
	n	Mean	95 % CI	*n*	Mean	95 % CI	Mean	95 % CI	*P*	Mean	95 % CI	*P*
Fat mass (kg)												
Total by D_2O	43	1·1	0·04, 2·3	48	1·6	0·4, 2·7	0·4	−1·2, 1·9	0·64	0·8	−0·8, 2·4	0·32
Total by ADP	56	0·7	−0·1, 1·5	53	0·4	−0·1, 1·0	−0·3	−1·2, 0·7	0·58	−0·3	−1·3, 0·8	0·62
Total by BIA	354	0·9	0·7, 1·2	376	0·8	0·6, 1·0	−0·14	−0·4, 1·1	0·31	−0·2	−0·0·5, 0·1	0·20
Truncal	353‡	0·6	0·4, 0·7	376	0·4	0·3, 0·6	−0·1	−0·3, −0·1	0·19	−0·1	−0·3, 0·04	0·13
Limb	354	0·4	0·3, 0·4	376	0·4	0·3, 0·4	0·003	−0·1, 0·1	0·95	−0·01	−0·1, 0·09	0·84
Fat-free mass (kg)												
Total by D_2O	43	−0·2	−1·4, 0·9	48	0·4	−0·7, 1·5	0·6	−1·0, 2·0	0·48	0·4	−1·3, 2·0	0·67
Total by ADP	56	0·0	−0·6, 0·6	53	0·7	0·1, 1·2	0·6	−0·2, 1·4	0·12	0·7	−0·2, 1·6	0·12
Total by BIA	354	0·3	0·05, 0·6	376	0·4	0·1, 0·6	0·1	−0·3, 0·5	0·64	0·1	−0·3, 0·4	0·71
Truncal	353‡	0·2	0·1, 0·4	376	0·3	0·1, 0·4	0·06	−0·1, 0·3	0·54	0·05	−0·1, 0·3	0·63
Limb	354	0·1	−0·0, 0·3	376	0·15	0·03, 0·3	0·03	−0·1, 0·2	0·76	0·02	−0·1, 0·2	0·82
Grip strength (kg)	338	1·37	0·8, 1·9	365	1·1	0·5, 1·6	−0·29 9	−1·1, 0·5	0·47	−0·28	−1·1, 0·5	0·48

LNS, lipid nutritional supplement; LNS-VM, lipid nutritional supplement with vitamins and minerals; D_2O, deuterium (^2H) diluted water method; ADP, air displacement plethysmography; BIA, bioelectrical impedance analysis; CD4, cluster of differentiation 4.
* Difference between change over time, adjusted for age and sex.
† Difference between change over time, adjusted for age, sex, baseline CD4, baseline BMI and socio-economic status.
‡ One observation missing.

strength regain compared with patients in the LNS arm after adjusting for sex, age, baseline CD4, baseline handgrip strength, and socio-economic status, although this was marginally significant ($P = 0·06$) (Table 3). However, at 12 weeks, LNS-VM intervention did not have any effect on handgrip strength (−0·28 (95 % CI −1·1, 0·5) kg; $P = 0·48$) (Table 4).

As shown in Figs 2 and 3, in the longitudinal analysis including all patients in the course of the study we found no treatment effect of the intervention on handgrip grip strength ($P = 0·71$ overall, $P = 0·87$ pre-ART and $P = 0·65$ post-ART), fat mass ($P = 0·16$ overall, $P = 0·86$ pre-ART and $P = 0·39$ post-ART), or fat-free mass ($P = 0·66$ overall, $P = 0·43$ pre-ART and $P = 0·76$ post-ART).

Fat mass measured by BIA tended to be lower than when measured by ADP or D_2O and fat-free mass tended to be correspondingly higher and the limit of agreements were −8·0 to 4·6 kg and −7·8 to 2·4 kg for fat mass and −4·8 to 7·8 kg and −3·0 to 7·2 kg kg for fat-free mass, respectively (Fig. 4).

Discussion

In the NUSTART trial, we found benefits of the vitamin and mineral supplementation for CD4 count and some anthropometric measures: calf and mid-upper arm circumferences and triceps skinfold[16,17]. Here we investigated the effect of the vitamin and mineral intervention on body composition assessed by three different methods as well as handgrip strength. We found that the LNS-VM intervention led to an increase in BIA-assessed fat mass at 6 but not 12 weeks.

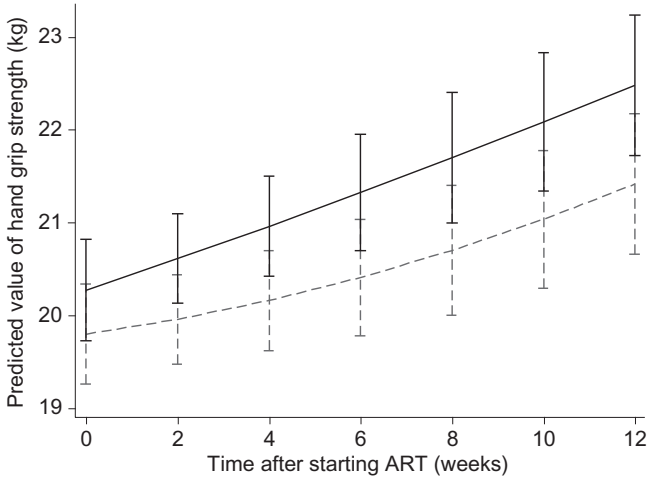

Fig. 2. Effects of the Nutritional Support for African Adults Starting Antiretroviral Therapy (NUSTART) intervention (lipid-based nutritional supplement (LNS; ----) or LNS with added vitamins and minerals (LNS-VM; ——) on handgrip strength during antiretroviral therapy (ART). Values are means, with standard deviations represented by vertical bars. Difference between LNS-VM and LNS: P=0·71.

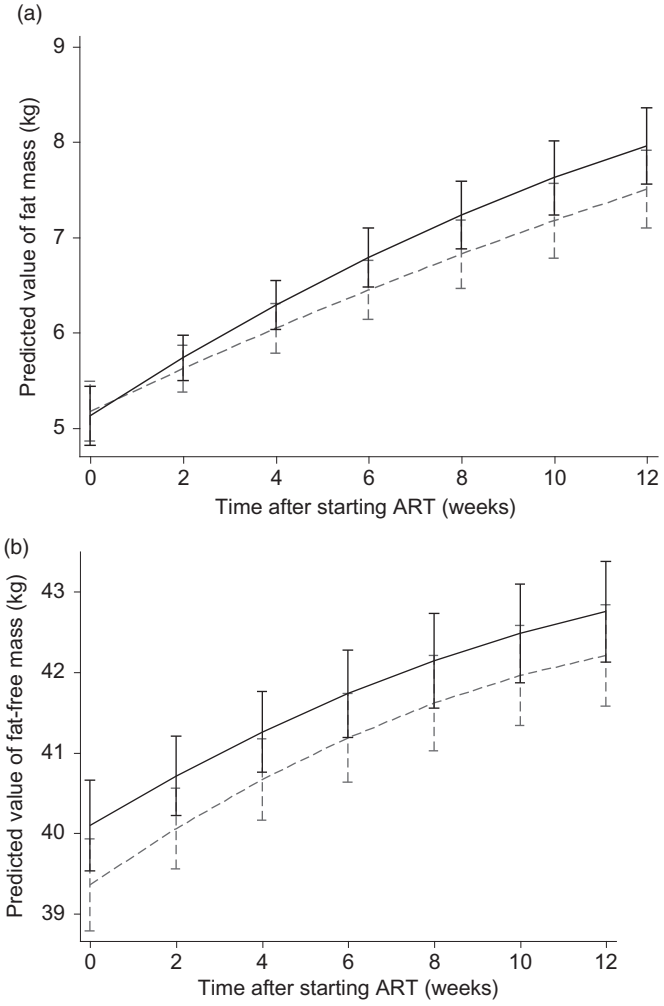

Fig. 3. Effects of the Nutritional Support for African Adults Starting Antiretroviral Therapy (NUSTART) intervention (lipid-based nutritional supplement (LNS; ----) or LNS with added vitamins and minerals (LNS-VM; ——) on body composition changes during antiretroviral therapy (ART). Values are means, with standard deviations represented by vertical bars. (a) Effects of NuSTART intervention on fat mass during ART. Difference between LNS-VM and LNS: P=0·16. (b) Effects of NUSTART intervention on fat-free mass during ART. Difference between LNS-VM and LNS: P=0·66.

ADP and D_2O measures of fat mass showed similar point estimates of the changes but were not statistically significant, possibly due to lower sample size. The intervention had no effect on fat-free mass, an outcome measure associated with improved physical functions[30] and reduced mortality[7]. The intervention had a borderline significant effect on handgrip strength at 6 weeks but this was not sustained at 12 weeks. Based on longitudinal analysis, the intervention did not alter any outcome measures throughout the follow-up period.

Several possible mechanisms could have mediated the effect on fat mass found with BIA, including treatment group differences in food intake or physical activity, neither of which was directly measured in our study. In theory, there could have been an increase in food intake in the LNS-VM group as a result of increase in appetite secondary to higher intake of vitamins and minerals as has been shown previously in HIV-infected South African children[31]. However, in NUSTART we found no effect of the intervention on appetite[17]. A higher intake of vitamins and minerals leading to improved muscle functions, as supported by our data on grip strength, should have resulted in increased rather than reduced energy expenditure, so effects on fat mediated by differential physical activity seem unlikely. As an additional potential mechanism, the persistence of inflammation could have favoured fat accretion over fat-free tissue accretion as seen following severe infections[32,33]. We have shown that patients in this cohort had severe inflammation which had not appreciably decreased by 6 weeks of ART and which may have limited fat-free mass but not fat mass regain[34].

The acute-phase response due to severe inflammation may be a major factor explaining the lack of tissue repletion during nutritional trials of HIV patients[35]. Despite high vitamin and mineral intake, the acute-phase response to infection may have depressed the serum or plasma concentrations of vitamins A, C and E and folate, and Zn and Se[36,37], possibly reducing their availability for tissue synthesis in the first weeks of ART initiation when the acute-phase response remains high. Results from previous nutritional trials provide support for this possible explanation. Among tuberculosis patients, a population with as severe inflammation as HIV patients, nutritional supplementation did not lead to full nutritional recovery due to impaired anabolism during treatment[32,33]. Among

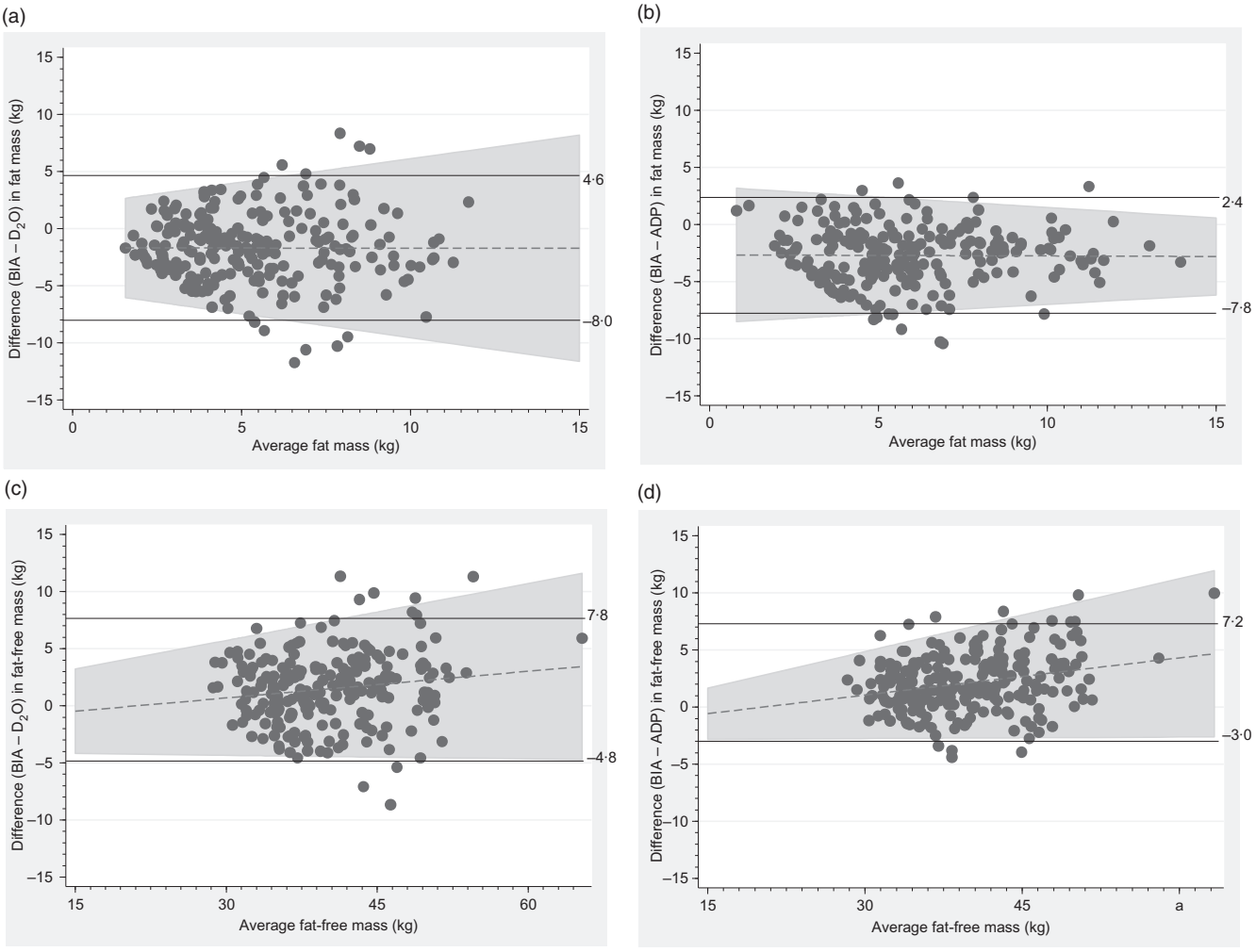

Fig. 4. Bland–Altman plots of fat mass and fat-free mass comparing bioelectrical impedance analysis (BIA) with the deuterium (^2H) diluted water (D_2O) method and air displacement plethysmography (ADP) technique. (a) Plot with regression line of difference *v.* mean fat mass measured by BIA and D_2O. The bias was –1·7 kg (±3·2) and the limit of agreement was between –8·0 and 4·6 kg. (b) Plot with regression line of difference *v.* mean fat mass measured by BIA and ADP. The bias was –2·7 kg (±2·5) and the limit of agreement was between –7·8 and 2·4 kg. (c) Plot with regression line of difference *v.* mean fat-free mass measured by BIA and D_2O. The bias was 1·5 kg (±3·2) and the limit of agreement was between –4·8 and 7·8 kg. (d) Plot with regression line of difference *v.* mean fat-free mass measured by BIA and ADP. The bias was 2·1 kg (±2·6) and the limit of agreement was between –3·0 and 7·2 kg.

apparently healthy HIV-infected individuals, a multi-micronutrient supplement given for 3 months did not result in increased plasma Fe and Zn among those who had inflammation, but led to increased levels among those who had no inflammation[38,39]. In a nutritional supplementation trial in Ethiopia, at 3 months of follow-up there was a higher increase in fat-free mass in a subgroup of patients with viral suppression[10], compared with those without viral suppression, suggesting that reduction of inflammation may have contributed to the accrual of fat-free mass in this subgroup.

Other factors limiting accrual of fat-free mass in the present study may include low compliance with interventions[16] and poor nutrient absorption. Although the supplement contained high amounts of growth nutrients including Zn, phosphate and Mg, bioavailability may have been limited since in the study area maize meal and legumes are the staple foods[40] and have high content of phytates which tend to chelate metals like Zn, Ca and Mg resulting in insoluble complexes which are poorly absorbed[41,42]. We did not study nutrient absorption properties in this study. Lastly, although the supplement contained many essential micronutrients in high amounts, the amounts of growth nutrients including Zn provided may have been inadequate to support additional synthesis of tissues at the time of very high demand.

In the NUSTART study we had hypothesised that the LNS-VM would have led to significant repletion of fat-free mass and help improve physical functions and work capacity[30] and reduce mortality[7]. In contrast, the intervention led to an increment in adipose tissue rather than fat-free mass and to no mortality reduction[16]. It is possible that at the time of ART initiation when the inflammation is severe, regaining fat mass rather than fat-free mass is a more appropriate short-term survival strategy in malnourished HIV patients starting ART since fat mass provides energy needed to sustain inflammation[43], a process aimed at modulating HIV infection. This is consistent with recent findings from a systematic review which found that patients starting ART with CD4 counts <350 cells/mm^3 have greater risk of developing obesity during treatment in comparison with patients starting ART with higher CD4 counts[44]. Adiposity is

characterised by a trade-off between short-term benefits and long-term risks[45]. Thus, in the long run, patients starting ART when malnourished may have increased risk for developing non-communicable diseases including diabetes and hypertension. In fact, several large cohorts have shown that HIV-infected patients have higher risk of diabetes and hypertension partly explained by nutritional recovery during ART[46–48].

The beneficial effect on handgrip strength of about 700 g, although only marginally significant, may be of clinical and survival importance[14,49]. The increase may reflect improvements in muscle functions rather than muscle size since we did not see any effect on fat-free mass. Irrespective of the mechanisms involved, this seemingly positive effect on grip strength in the present and other trials[10,50] suggests that nutritional interventions with added vitamins and minerals may help increase physical strength and work capacity of HIV-infected patients[14].

Our results for the different body composition methods showed that BIA tended to give lower values for fat mass than D_2O or ADP but that this did not appreciably affect the group differences over time. Although limits of agreement were wide, the biases and errors were within acceptable ranges[12]. In an Ethiopian study there was no overall difference between BIA and D_2O but BIA tended to overestimate fat-free mass at low fat-free mass[51]. Among Senegalese HIV-infected adults, the bias between BIA and D_2O methods was considered relatively small such that BIA, the simpler and cheaper method, was acceptable[52]. Similarly, BIA, when compared with D_2O, was found to be acceptable for HIV-infected and uninfected breastfeeding South African women[53]. Overall, it appears that, in spite of concerns, BIA is a useful tool for measuring body composition even in seriously ill, malnourished HIV-infected patients and particularly in low-income settings where resources may be a major limitation in using superior methods. The findings in the present analysis and previous studies[9] suggest that further research is required to understand what should be provided to HIV patients in order to achieve optimal nutritional recovery.

The strengths of the present trial were that it was randomised and double-blinded, had large sample size and body composition was assessed using BIA and other better methods rather than anthropometry alone. However, we did not obtain direct measures of food intake and energy expenditure and compliance with nutritional intervention was modest and assessed using reported information. This limited the scope of our data interpretation. In addition, the study was conducted 5 years ago and during this period changes in ART regimens and eligibility criteria have occurred which could limit the external validity of our study findings. Future studies should include mechanistic substudies to be able to better explain the findings.

Conclusions

Added high doses of vitamins and minerals led to a higher regain of fat mass but not fat-free mass and borderline significant effect on handgrip strength at 6 weeks post-ART. Taken together, these findings suggest the need for a well-considered approach in providing nutritional supplements high in vitamins and minerals to malnourished HIV-infected patients starting ART in sub-Saharan Africa. Further research on appropriate intervention timing, micronutrients requirements, optimal regimens and uptake monitoring is needed to guide the design of future nutritional intervention trials in HIV patients.

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/jns.2019.15

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S. F., H. F., D. C. H., J. C., L. K., J. C. K. W. and J. R. K. designed the study. G. P., M. C., J. S., S. W. and K. J. collected data. G. P. and A. M. R. analysed data. G. P. wrote the first draft and all authors critically reviewed subsequent drafts and approved the final version for submission.

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