RESEARCH

Effects of nutritional supplementation for HIV patients starting antiretroviral treatment: randomised controlled trial in Ethiopia

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Abstract

Objectives To determine the effects of lipid based nutritional supplements with either whey or soy protein in patients with HIV during the first three months of antiretroviral treatment (ART) and to explore effects of timing by comparing supplementation at the start of ART and after three months delay.

Design Randomised controlled trial.

Setting Three public ART facilities in Jimma, Oromia region, Ethiopia.

Participants Adults with HIV eligible for ART with body mass index (BMI) >16.

Intervention Daily supplementation with 200 g (4600 kJ) of supplement containing whey or soy during either the first three or the subsequent three months of ART.

Outcome measures Primary: lean body mass assessed with deuterium dilution, grip strength measured with dynamometers, and physical activity measured with accelerometer and heart rate monitors. Secondary: viral load and CD4 counts. Auxiliary: weight and CD3 and CD8 counts.

Results Of 318 patients enrolled, 210 (66%) were women, mean age was 33 (SD 9), and mean BMI was 19.5 (SD 2.4). At three months, participants receiving the supplements containing whey or soy had increased their lean body mass by 0.85 kg (95% confidence interval 0.16 kg to 1.53 kg) and 0.97 kg (0.29 kg to 1.64 kg), respectively, more than controls. This was accompanied by an increased gain of grip strength of 0.68 kg (−0.11 kg to 1.46 kg) for the whey supplement group and 0.93 kg (0.16 kg to 1.70 kg) for the soy supplement group. There

were no effects on physical activity. Total weight gain increased by 2.05 kg (1.12 kg to 2.99 kg) and 2.06 kg (1.14 kg to 2.97 kg) for the whey and soy groups, respectively. In addition, in the whey supplement group overall CD3 counts improved by 150 cells/µL (24 to 275 cells/µL), of which 112 cells/µL (15 to 209 cells/µL) were CD8 and 25 cells/µL (−2 to 53 cells/µL) were CD4. Effects of the soy containing supplement on immune recovery were not significant. The effects of the two supplements, however, were not significantly different in direct comparison. Exploratory analysis showed that relatively more lean body mass was gained by patients with undetectable viral load at three months. Patients receiving delayed supplementation had higher weight gain but lower gains in functional outcomes.

Conclusions Lipid based nutritional supplements improved gain of weight, lean body mass, and grip strength in patients with HIV starting ART. Supplements containing whey were associated with improved immune recovery.

Trial registration Controlled-trials.com ISRCTN32453477.

Introduction

Access to antiretroviral treatment (ART) has improved substantially in sub Saharan Africa over the past decade, but high rates of mortality are still reported, especially in the first few months of treatment.[1] Poor nutritional status at start of ART has been identified as a predictor of mortality independent of immune status,[2-4] while patients who gain weight in the early phase of treatment have improved prognosis.[5-9] Nutritional

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support is becoming an integral part of ART programmes in African countries, and various supplements are now widely distributed.[10 11] At present, however, there is little evidence on the effects of supplementation.[12] Information is urgently needed to guide supplementation programmes towards optimal composition and timing as well as identification of individuals most likely to benefit.

Loss of lean body mass, in particular, has been associated with mortality in patients with HIV.[13-17] In addition, low lean body mass results in functional limitations,[18-20] which can have devastating consequences for patients and their families. Maintenance of work capacity and an adequate level of activity is essential to sustain everyday functioning and protect patients' livelihoods.[21] It has been suggested that whey protein can improve the recovery of lean body mass in patients with HIV,[22] but this has not yet been confirmed by empirical research.[23 24] Furthermore, whey might also have an immune modulating effect as it increases plasma glutathione, an important antioxidant often deficient in those with HIV.[25-27] This was supported by a recent study showing that supplementation with 40 g of whey a day was associated with increased CD4 counts.[28]

We conducted a randomised controlled trial among patients with HIV who were starting ART in Ethiopia to investigate the effects of a lipid based nutrient supplement containing whey or soy protein in the first three months of treatment compared with a control group of patients who started ART without nutrient supplements. Primary outcomes were lean body mass, grip strength, and expenditure of energy on physical activity. Secondary outcomes were HIV viral load and CD4 counts, while auxiliary outcomes included weight and CD8 and CD3 counts. The control group received delayed supplementation during the subsequent three months of ART, which allowed us to explore potential effects of timing in supplementation.

Methods

Study design

The ART food study was a randomised controlled trial, with randomisation stratified by body mass index (BMI). Participants with BMI >17 were allocated (1:1:1) to early supplementation with a supplement containing whey (group 1), early supplementation with a supplement containing soy (group 2), or delayed supplementation with either the whey or soy supplement (group 3a+b). Participants with BMI 16-17 were allocated (1:1) to early supplementation with whey (group 4) or early supplementation with soy (group 5) (fig 1⇓).

The study was designed to assess three main comparisons at three months' follow-up: whey containing supplement versus no supplement, soy containing supplement versus no supplement, and whey versus soy containing supplements. The first two comparisons were conducted in participants with BMI >17, as the delayed supplementation groups served as controls. The third comparison was conducted among all participants with BMI >16, as groups with BMI 16-17 and BMI >17 were merged for this analysis (group 1+4 *v* group 2+5). Secondly, we compared early and delayed supplementation in participants with BMI >17 to investigate potential effects of timing in supplementation (group 1+2 *v* group 3a+3b).

Participants

Patients eligible for ART at Jimma University Specialized Hospital, Jimma Health Centre, and Agaro Health Centre were invited to participate if they were aged ≥18, had a BMI ≥16, and lived within 50 km of the recruitment site. Exclusion criteria

were pregnancy, lactation, known diabetes, or current use of nutritional supplements. ART was initiated if CD4 counts were ≤200 cells/μL irrespective of clinical symptoms, if CD4 counts were ≤350 cells/μL in those classed as WHO clinical stage III, or if patients were classed as WHO clinical stage IV irrespective of CD4 count.[29] The preferred ART regimen consisted of lamivudine with tenofovir or zidovudine, and efavirenz or nevirapine. Patients were given co-trimoxazole prophylaxis and nutritional counselling according to national guidelines.[29 30]

Nutritional intervention

Participants received 200 g supplement (about 4600 kJ) a day for three months during either the first three months (early) or the subsequent three months of ART (delayed). The supplements were developed by Nutriset (Malaunay, France), based on Plumpy'Sup, with 32 g of either whey protein concentrate 80% or soy protein isolate. In both supplements, energy distribution was 60% fat, 24% carbohydrates, and 16% protein. The daily ration contained two recommended nutrient intakes of vitamins, zinc, selenium, and copper, and one intake of other minerals[31] (table 1⇓). Patients were instructed in using the supplement and allowed to try it for two days before study enrolment. Supplements were provided monthly in 100 g sachets, and adherence was assessed from returned sachets and self reported consumption. Furthermore, a qualitative study was conducted to investigate the use, perceptions, and acceptability of the supplement (reported in detail elsewhere[32]).

Data collection

Trained study staff collected data on all outcomes in local languages at baseline and at three and six months' follow-up. In addition, weight and CD4 counts were measured at 12 months' follow-up to assess post-trial differences between early and delayed intervention. Demographic data were collected at baseline with a structured questionnaire. Height and weight were measured with calibrated scales and stadiometers, respectively. Energy intake was assessed from a 24 hour food recall, with a pre-coded chart and a recall kit with household utensils and models of Ethiopian dishes, standard recipes, and local food composition tables.[33 34] Information regarding WHO clinical stage of HIV was extracted from routine patient records and checked by a study clinician.

Primary outcomes

The primary outcomes were lean body mass, grip strength, and expenditure of energy on physical activity. Body composition was assessed with 30 g deuterium oxide (99.8% ^{2}H, Sercon, Crewe, UK) weighed with 0.01 g precision and given orally after collection of pre-dose saliva samples.[35] Post-dose saliva samples were collected after four hours' equilibration. Saliva enrichment of deuterium was determined by Fourier Transform Infrared Spectrometer (IRAffinity-1, Shimadzu, Kyoto, Japan). Total body water was calculated from post-dose deuterium enrichment with adjustment for pre-dose enrichment, using a factor of 1.041 to adjust for proton exchange. Lean body mass was calculated based on an assumed hydration factor of 73.2%.[35] Grip strength was determined twice each for the right and left hand alternately with a digital dynamometer (Takei Scientific Instruments Co, Niigata, Japan). The mean of the highest value for the right and left hand was recorded. This procedure was then repeated and the highest value used for analyses. Expenditure of on energy physical activity was measured with a combined uniaxial accelerometer and heart rate sensor (Actiheart, CamNtech, Cambridge, UK), as described

elsewhere.[36] For logistical reasons we assessed physical activity only in participants from Jimma University Specialized Hospital and Jimma Health Centre. Heart rate was calibrated with a 250 m self paced walk test followed by a two minute recovery period. The monitor was worn continuously over four days, with data collected every 15 seconds. Physical activity energy expenditure was modelled with branched equations.[37] The estimation of intensity of activity was determined with group calibration equations for both accelerometry and heart rate.[38] We included in the analyses those individual recordings with ≥24 hours of valid data.

Secondary and auxiliary outcomes

Secondary outcomes included viral load and CD4 counts, and auxiliary outcomes included weight and CD3 and CD8 counts. Viral load (HIV-1 RNA) was determined from plasma, which was kept at −80°C, with a commercial real time PCR (polymerase chain reaction) assay with automated extraction of RNA (RealTime HIV-1, Abbott Molecular, Abbott Laboratories, IL). CD3, CD4, and CD8 counts were determined with flow cytometry (Fascount, Becton Dickinson, San José, CA).

Randomisation and blinding

The two supplement types were labelled with three codes each, which were kept in sealed envelopes until trial completion. Two stratified blocked randomisation sequences were prepared by HF for BMI 16-17 and BMI <17 in blocks of 18 with www. randomization.com. The study was fully blinded regarding comparison of the two supplements, which were similar in taste and texture, while it was blinded only for outcome assessors and data analysts regarding the comparisons of early and delayed supplementation groups. A designated person, not involved in recruitment or data collection, had sole access to the random sequence lists and was responsible for supplement allocation.

Sample size calculation

The study's sample size was calculated to detect differences in lean body mass. A previous study had found that lipid based nutrient supplementation resulted in an increase of lean body mass of 0.5 SD of the baseline distribution among HIV patients.[39] As we expected only a modest gain in lean body mass in the unsupplemented control group, we expected the difference between groups to be around 0.4 SD. We calculated that 100 participants in each group (that is, early whey supplement, early soy supplement, and delayed supplement) would allow us to detect a 0.4 SD difference in lean body mass with 80% power and 5% significance level. To allow for 15% loss to follow-up, we planned to recruit 350 patients. We used data on the BMI distribution of patients starting ART at the hospital in Jimma during the previous year to estimate the expected relative group sizes. Based on this, we expected that from 400 patients eligible for ART, 300 would have BMI >17, 50 would have BMI 16-17, and 50 would have BMI <16. To recruit this number of patients, we anticipated a 16 month inclusion period at Jimma University Specialized Hospital. Because of an increasing number of ART facilities in the study area, however, the number of eligible patients at the hospital declined. Consequently, we decided to include health centres in Jimma and Agaro as recruitment sites and extend the inclusion period. After 26 months (August 2012), we had included a total of 318 participants and as attrition was lower than anticipated, we estimated that we had reached an adequate sample size.

Data analyses

All data were double entered and validated with EpiData (EpiData Association, Odense, Denmark) and analysed with STATA/IC version 11.2 (StataCorp LP, College Station, TX). All analyses were done by the intention to treat principle. Associations with P values <0.05 were considered significant. We considered both unadjusted models, which included only the supplementation groups and baseline adjustment, and adjusted models, which included age, sex, and education level for all outcomes, and height for lean body mass and weight. The comparisons between supplementation groups at three months' follow-up were carried out with analysis of covariance models. The comparison of early and delayed supplementation at six and 12 months' follow-up was carried out with linear mixed models based on all available repeated measurements. These models included interactions between supplementation and time and participant specific random effects to capture the serial correlation in the repeated measurements within each participant. Outcomes were log transformed when appropriate. We also carried out exploratory analyses to investigate interactions between supplementation groups and selected variables, including sex, age group (<40 or ≥40), BMI (<18.5 or ≥18.5), CD4 counts at baseline (<200 or ≥200 cells/μL), and viral load at three month' follow-up (0 copies/mL or >0 copies/ml), to explore potential differential effects of supplementation between subgroups of participants.

Results

A total of 453 patients were screened between July 2010 and August 2012 (fig 2⇓). Of these, 318 (70%) were enrolled and 135 were not eligible because their BMI was <16 (7%) or they declined (23%). Of the enrolled participants, 281 (88%) completed three months' follow-up and 273 (86%) completed six months' follow-up. Table 2⇓ presents the baseline characteristics of participants with BMI >17 (n=282). We found no major differences between the three intervention groups. Furthermore, the two early supplementation groups remained similar when we included participants with BMI 16-17 (n=36, data not shown).

Of 318 enrolled participants, 210 (66%) were women, the mean age was 33 (SD 9), and the mean BMI was 19.5 (SD 2.4). The median adherence to the supplement was 88% (interquartile range 73-96%). There was no difference in adherence to the two supplements, but adherence was lower during early supplementation than delayed supplementation (86% (interquartile range 71-95%) *v* 93% (76-97%), P=0.01). Eighty one (28%) had "poor adherence," which was defined as intake <75%. This was supported by self reported consumption data (not shown).

Early supplementation

Table 3⇓ presents differences between groups at three months' follow-up among participants with BMI >17 (n=282). Participants receiving the whey and soy supplements had gained 0.85 kg (95% confidence interval 0.16 kg to 1.53 kg) and 0.97 kg (0.29 kg to 1.64 kg) lean body mass, respectively, more than controls. This was accompanied by a borderline significant effect on grip strength of 0.68 kg (−0.11 kg to 1.46 kg) for the whey supplement and an effect of 0.93 kg (0.16 kg to 1.70 kg) for the soy supplement. Differences in increases of energy expenditure on physical activity were not significant at 6% (−13 to 29%) and 10% (−9 to 33%) for whey and soy supplements, respectively (total n=98). The total weight gain in participants receiving whey and soy supplements was 2.05 kg (1.12 kg to

2.99 kg) and 2.06 kg (1.14 kg to 2.97 kg) higher than unsupplemented controls. In comparison, participants who started ART without nutritional supplementation had a total weight gain of only 0.87 kg (0.22 kg to 1.52 kg) kg, with no gain in lean body mass (0.06 kg, −0.43 kg to 0.55 kg) and no significant improvement in grip strength (0.47 kg, −0.09 kg to 1.04 kg).

Though we found no differences in suppression of viral load between groups, overall CD3 counts increased by 150 cells/µL (95% confidence interval 24 to 275 cells/µL) in the whey supplement group compared with controls. The increase was 112 cells/µL (15 to 209 cells/µL) for CD8 counts, 25 cells/µL (−2 to 53 cells/µL) for CD4 counts, which was of borderline significance. Effects on immune recovery were not significant for the soy supplement. The effects of the two supplements, however, did not differ significantly, when we compared them directly among participants with BMI >17 (data not shown).

There were no differences in ART regimens or adherence to supplementation between groups. The main ART regimen was tenofovir/lamivudine/efavirenz, which was received by 65 (70%, early whey supplement), 73 (80%, early soy supplement), and 63 (68%, delayed supplement). Self reported adherence to ART was >95%, with no difference between groups. Furthermore, the effects of supplementation were not modified by baseline BMI, sex, age, or CD4 count for any of the outcomes. At three months' follow-up, viral load had become undetectable in 81 (33%) participants with baseline BMI >17. Among these participants, supplementation was associated with an increase in lean body mass of 1.76 kg (95% confidence interval 0.79 kg to 2.74 kg). There was no significant gain in lean body mass among participants who still had detectable virus after three months of ART (0.42 kg, −0.37 kg to 1.20 kg; interaction P=0.03).

Table 4⇓ shows that there were no significant differences between effects of the two supplements during early supplementation in patients with BMI >16 when we combined the BMI groups (n=225).

Timing of supplementation

Table 5⇓ shows differences at six months' follow-up between groups allocated to early and delayed supplementation (BMI >17, n=282). There was no difference in gain in lean body mass, but participants who received delayed supplementation had a smaller increase in grip strength than those receiving early supplementation (−0.84 kg, 95% confidence interval −1.53 kg to −0.04 kg). Similarly, the comparison showed that increase in physical activity was lower in participants with delayed supplementation (−22%, −35% to −5%). Those receiving delayed supplementation, however, had a 1.20 kg (0.32 kg to 2.09 kg) higher gain of total body weight from baseline to six months than those receiving early supplementation. Adjustment for differences in adherence only partially explained this difference in weight gain (adjusted estimate 0.99 kg, 0.08 kg to 1.89 kg). We found no differences in energy intake from usual diet between groups at either three or six months' follow-up (P>0.69). Finally, we found that changes in viral load and immune recovery did not depend on timing.

Figure 3⇓ shows unadjusted mean differences in primary and secondary outcomes at three and six months' follow-up in intervention groups. Participants who received early supplementation subsequently lost some of the weight gained during supplementation. The weight loss consisted of fat mass only, however, as there was a continued gain of lean body mass after supplementation had ended. Furthermore, the figure shows

that differences in grip strength at three months were sustained at six months, while differences in CD4 counts were not.

Post-trial data from 12 months' follow-up (BMI >17, n=230) showed that the differences in weight gain between the early and delayed supplementation groups were not sustained. Furthermore, there was no difference between increases in CD4 counts in early and delayed supplementation groups (data not shown).

Discussion

Principal findings

Provision of lipid based supplements in the first three months of ART improved gains in weight, lean body mass, and grip strength among patients with HIV and BMI >17. The effects of supplementation were considerable, resulting in a more than threefold weight gain compared with the effects of starting ART alone and with substantially more lean body mass gained. Supplementation improved grip strength but had no significant effects on physical activity. Complete data on physical activity, however, were obtained for only a small number of participants because assessment was possible at only two of the three centres, and many participants declined monitoring of physical activity because of the additional study visit it involved. As physical activity is a behavioural outcome with high variability, we did not have sufficient power to detect potential differences between groups. Furthermore, the whey supplement had effects on immune recovery. The changes in CD8, CD3, and CD4 counts were not associated with differences in viral load suppression, which was similar in all groups. We found no differences between the two supplements in participants with BMI >17 or all participants with BMI >16.

Strengths and limitations of study

The main strength of this study is its randomised controlled design, making it the first study to investigate the effects of lipid based nutrient supplements and ART compared with ART alone. The study was not powered to detect differences in mortality, but detailed data on body composition, functional outcomes, viral load, and immune recovery strengthened the evaluation of supplementation effects. The study was limited by its exclusion of patients with BMI <16 and by including only patients with BMI >17 in the comparison between supplemented and unsupplemented groups. The associations between weight gain and improved clinical outcomes, however, have previously been observed across all BMI groups,[7 8] and, in line with this, we found that the effect of supplementation was not modified by baseline BMI. Finally, the validity of the deuterium dilution technique hinges on an assumption about lean tissue hydration, which has been questioned in patients with HIV.[40 41] As both HIV and malnutrition are associated with expansion of the extracellular water compartment, a reduction in extracellular water towards normal during ART and nutritional supplementation would potentially cause an underestimation of gains in lean body mass. We expect these changes to be of minor importance in the patients with BMI >17 included in the present analysis.

Comparison with other studies

The clinical relevance of weight gain after initiation of ART is well described but mainly in observational studies,[7-9] when it might just be a marker of individual clinical response. There are few data describing weight gain induced by nutritional supplementation. A recent Cochrane review[12] included data

from an unpublished trial in Kenya, showing that three months of fortified blended food compared with no supplement resulted in a weight gain of 1.12 kg (95% confidence interval 0.29 kg to 1.95 kg) among malnourished patients with HIV receiving ART, which is notably less than the effect seen in the present study. Although the study populations are not directly comparable, the smaller effect could be related to differences in supplement use, energy density, or nutrient quality. Lipid based nutrient supplements have previously been associated with higher weight gains than fortified blended foods.[39]

Effects on immune recovery were seen only for the whey containing supplement in comparisons with unsupplemented participants. We found no significant difference in the direct comparison of the two supplements. Both of the protein sources used in this study were of high quality. Soy isolate contains >90% protein, no anti-nutrients, and a high amount of glutamine, which could have positive effects similar to those suggested for whey.[42-44] Additional studies are needed to determine whether soy proteins can be used with similar effects to dairy based proteins in supplementation of HIV patients, as this could reduce programme costs.

Our inclusion of a delayed intervention group allowed us to explore effects of timing as part of post hoc analyses. Little is known about the optimal timing of nutritional supplementation. A study in Zambia suggested that metabolic derangements similar to the re-feeding syndrome might explain some of the increased mortality among malnourished patients with HIV starting ART.[45 46] Thus, it might be beneficial to postpone nutritional supplementation until inflammation has faded and the patient has been stabilised. We have previously reported that participants' consumption of the supplement was reduced in the first few weeks after ART initiation.[32] This study confirmed that adherence was lower during early supplementation than during delayed supplementation, but the difference in adherence only partially explained a higher weight gain observed for delayed supplementation. The comparison of outcomes at six months, however, should be interpreted with caution as some of the weight gained during supplementation might be lost subsequently, as seen after the end of early supplementation. In fact, data at 12 months showed that the weight difference was not sustained six months after the trial had ended. We also found that patients who had undetectable viral load three months after ART initiation had gained more lean body mass than patients with a poorer response to treatment. In line with this, a previous study found that therapeutic re-feeding resulted in gain of fat mass rather than lean body mass if patients had systemic infection.[47] Although causality cannot be determined from these findings, they indicate that patients might better use nutrients for synthesis of lean tissue after inflammation has declined, which could be an argument for delaying supplementation. Functional outcomes, however, improved mainly during early supplementation. Inclusion of groups initiating supplementation at various time points in relation to ART, measurement of inflammatory markers, and patients' appetite would enable further investigation of the optimal timing of supplementation.

Conclusions and implications

We tested the effect of a rather short duration (three months) of nutritional supplementation, while many of the current supplementation programmes provide similar supplements for up to six months. After this study ended, Ethiopia implemented guidelines for nutritional supplementation of all patients with HIV with BMI <18.5 for up to six months. A recent evaluation report showed that the programme is costly and challenging,

with more than 70% of patients defaulting from supplementation.[48] In addition, lipid based nutrient supplements are energy dense and high fat, and, while this is beneficial for maintaining an adequate energy intake, long term supplementation could increase the risk of metabolic complications in general and in particular in patients receiving ART.[49] Providing time limited supplementation involves less risk of metabolic complications and is less resource demanding.

We have shown that three months' lipid based nutrient supplementation at the start of ART improved weight, lean body mass, and grip strength. In addition, the whey containing supplement supported immune recovery. The role of timing and type of protein source in the supplement merits further investigation. We have previously shown that supplementation was feasible and acceptable in the study setting.[32] It is now important to investigate how barriers to large scale supplementation can be overcome to achieve effectiveness of nutritional supplementation for patients with HIV.

1   Braitstein P, Brinkhof MWG, Dabis F, Schechter M, Boulle A, Miotti P, et al. Mortality of HIV-1-infected patients in the first year of antiretroviral therapy: comparison between low-income and high-income countries. *Lancet* 2006;367:817-24.

2   Lawn SD, Harries AD, Anglaret X, Myer L, Wood R. Early mortality among adults accessing antiretroviral treatment programmes in sub-Saharan Africa. *AIDS* 2008;22:1897-908.

3   May M, Boulle A, Phiri S, Messou E, Myer L, Wood R, et al. Prognosis of patients with HIV-1 infection starting antiretroviral therapy in sub-Saharan Africa: a collaborative analysis of scale-up programmes. *Lancet* 2010;376:449-57.

What is already known on this topic

In patients with HIV, poor nutritional status in terms of low BMI at the start of antiretroviral treatment (ART) is associated with poor treatment outcomes

Various nutritional supplements are widely distributed as part of ART programmes in sub Saharan Africa, but little is known about the effects of these resource demanding programmes

What this study adds

Three months' supplementation with a lipid based nutrient supplement containing either whey or soy protein resulted in a more than threefold weight gain compared with ART alone. Substantially more lean body mass was gained and grip strength was improved by supplementation

Supplements containing whey protein were also associated with improved immune recovery

Differences in effects relating to protein type and timing of supplementation merit further investigation

4   Gupta A, Nadkarni G, Yang W-T, Chandrasekhar A, Gupte N, Bisson GP, et al. Early mortality in adults initiating antiretroviral therapy (ART) in low- and middle-income countries (LMIC): a systematic review and meta-analysis. *PLoS One* 2011;6:e28691.

5   Malvy D, Thiébaut R, Marimoutou C, Dabis F. Weight loss and body mass index as predictors of hiv disease progression to AIDS in adults. Aquitaine Cohort, France, 1985-1997. *J Am Coll Nutr* 2001;20:609-15.

6   Tang AM, Jacobson DL, Spiegelman D, Knox TA, Wanke C. Increasing risk of 5% or greater unintentional weight loss in a cohort of HIV-infected patients, 1995 to 2003. *J Acquir Immune Defic Syndr* 2005;40:70-6.

7   Madec Y, Szumilin E, Genevier C, Ferradini L, Balkan S, Pujades M, et al. Weight gain at 3 months of antiretroviral therapy is strongly associated with survival: evidence from two developing countries. *AIDS* 2009;23:853-61.

8   Koethe JR, Lukusa A, Giganti MJ, Chi BH, Nyirenda CK, Limbada MI, et al. Association between weight gain and clinical outcomes among malnourished adults initiating antiretroviral therapy in Lusaka, Zambia. *J Acquir Immune Defic Syndr* 2010;53:507-13.

9   Sudfeld CR, Isanaka S, Mugusi FM, Aboud S, Wang M, Chalamilla GE, et al. Weight change at 1 mo of antiretroviral therapy and its association with subsequent mortality, morbidity, and CD4 T cell reconstitution in a Tanzanian HIV-infected adult cohort. *Am J Clin Nutr* 2013;97:1278-87.

10 Lamb MR, El-Sadr WM, Geng E, Nash D. Association of adherence support and outreach services with total attrition, loss to follow-up, and death among ART patients in sub-Saharan Africa. *PLoS One* 2012;7:e38443.

11 Anema A, Zhang W, Wu Y, Elul B, Weiser SD, Hogg RS, et al. Availability of nutritional support services in HIV care and treatment sites in sub-Saharan African countries. *Public Health Nutr* 2012;15:938-47

12 Grobler L, Siegfried N, Visser ME, Mahlungulu SSN, Volmink J. Nutritional interventions for reducing morbidity and mortality in people with HIV. *Cochrane Database Syst Rev* 2013;2:CD004536.

13 Melchior JC, Niyongabo T, Henzel D, Durack-Bown I, Henri SC, Boulier A. Malnutrition and wasting, immunodepression, and chronic inflammation as independent predictors of survival in HIV-infected patients. *Nutrition* 1999;15:865-9.

14 Kotler DP, Tierney AR, Wang J, Pierson RN Jr. Magnitude of body-cell-mass depletion and the timing of death from wasting in AIDS. *Am J Clin Nutr* 1989;50:444-7.

15 Süttmann U, Ockenga J, Selberg O, Hoogestraat L, Deicher H, Müller MJ. Incidence and prognostic value of malnutrition and wasting in human immunodeficiency virus-infected outpatients. *J Acquir Immune Defic Syndr Hum Retrovirol* 1995;8:239-46.

16 Ott M, Fischer H, Polat H, Helm EB, Frenz M, Caspary WF, et al. Bioelectrical impedance analysis as a predictor of survival in patients with human immunodeficiency virus infection. *J Acquir Immune Defic Syndr Hum Retrovirol* 1995;9:20-5.

17 Schwenk A, Beisenherz A, Römer K, Kremer G, Salzberger B, Elia M. Phase angle from bioelectrical impedance analysis remains an independent predictive marker in HIV-infected patients in the era of highly active antiretroviral treatment. *Am J Clin Nutr* 2000;72:496-501.

18 Kusko RL, Banerjee C, Long KK, Darcy A, Otis J, Sebastiani P, et al. Premature expression of a muscle fibrosis axis in chronic HIV infection. *Skeletal Muscle* 2012;2:10.

19 Desquilbet L, Jacobson LP, Fried LP, Phair JP, Jamieson BD, Holloway M, et al. HIV-1 infection is associated with an earlier occurrence of a phenotype related to frailty. *J Gerontol A Biol Sci Med Sci* 2007;62:1279-86.

20 Raso V, Shephard RJ, do Rosário Casseb JS, da Silva Duarte AJ, D□Andréa Greve JM. Handgrip force offers a measure of physical function in individuals living with HIV/AIDS. *J Acquir Immune Defic Syndr* 2013;63:e30-2.

21 Gillespie SR. AIDS, poverty, and hunger. International Food Policy Res Inst (IFPRI), 2006. http://dx.doi.org/10.2499/0896297586BK

22 Hoppe C, Andersen GS, Jacobsen S, Mølgaard C, Friis H, Sangild PT, et al. The use of whey or skimmed milk powder in fortified blended foods for vulnerable groups. *J Nutr* 2008;138:145-61S.

23 Agin D, Kotler DP, Papandreou D, Liss M, Wang J, Thornton J, et al. Effects of whey protein and resistance exercise on body composition and muscle strength in women with HIV infection. *Ann N Y Acad Sci* 2000;904:607-9.

24 Agin D, Gallagher D, Wang J, Heymsfield SB, Pierson RN, Kotler DP. Effects of whey protein and resistance exercise on body cell mass, muscle strength, and quality of life in women with HIV. *AIDS* 2001;15:2431-40.

25 Bounous G, Baruchel S, Falutz J, Gold P. Whey proteins as a food supplement in HIV-seropositive individuals. *Clin Invest Med* 1993;16:204-9.

26 Micke P, Beeh KM, Schlaak JF, Buhl R. Oral supplementation with whey proteins increases plasma glutathione levels of HIV-infected patients. *Eur J Clin Invest* 2001;31:171-8.

27 Micke P, Beeh KM, Buhl R. Effects of long-term supplementation with whey proteins on plasma glutathione levels of HIV-infected patients. *Eur J Nutr* 2002;41:12-8.

28 Sattler FR, Rajicic N, Mulligan K, Yarasheski KE, Koletar SL, Zolopa A, et al. Evaluation of high-protein supplementation in weight-stable HIV-positive subjects with a history of weight loss: a randomized, double-blind, multicenter trial. *Am J Clin Nutr* 2008;88:1313-21.

29 Federal HIV/AIDS Prevention and Control Office. Guidelines for management of opportunistic infections and anti retroviral treatment in adolescents and adults in Ethiopia. Federal Ministry of Health, Ethiopia, 2008.

30 Federal Ministry of Health. National guideline for nutritional care and support for PLHIV. Federal Democratic Republic of Ethiopia Ministry of Health, 2011. www.ilo.org/wcmsp5/groups/public/---ed_protect/---protrav/---ilo_aids/documents/legaldocument/wcms_125388.pdf

31 FAO/WHO. Human vitamin and mineral requirements. Report of a joint FAO/WHO expert consultation, Bangkok, Thailand. Food and Nutrition Division FAO, 2001.

32 Olsen M, Tesfaye, Kæstel, Friis, Holm. Use, perceptions, and acceptability of a ready-to-use supplementary food among adult HIV patients initiating antiretroviral treatment: a qualitative study in Ethiopia. *Patient Prefer Adherence* 2013;7:481-8.

33 EHNRI, FAO. Food composition table for use in Ethiopia. Ethiopian Health and Nutrition Research Institute, 1998.

34 Ethiopian Nutrition Institute. Ethiopian traditional recipes. Ministry of Health, 1980.

35 IAEA human health series. Introduction to body composition assessment using the deuterium dilution technique with analysis of saliva samples by Fourier transform infrared spectrometry. International Atomic Energy Agency, 2010.

36 Brage S, Brage N, Franks PW, Ekelund U, Wareham NJ. Reliability and validity of the combined heart rate and movement sensor Actiheart. *Eur J Clin Nutr* 2005;59:561-70.

37 Brage S, Brage N, Franks PW, Ekelund U, Wong M-Y, Andersen LB, et al. Branched equation modeling of simultaneous accelerometry and heart rate monitoring improves estimate of directly measured physical activity energy expenditure. *J Appl Physiol* 2004;96:343-51.

38 Christensen DL, Faurholt-Jepsen D, Boit MK, Mwaniki DL, Kilonzo B, Tetens I, et al. Cardiorespiratory fitness and physical activity in Luo, Kamba, and Maasai of rural Kenya. *Am J Hum Biol* 2012;24:723-9.

39 Ndekha MJ, van Oosterhout JJG, Zijlstra EE, Manary M, Saloojee H, Manary MJ. Supplementary feeding with either ready-to-use fortified spread or corn-soy blend in wasted adults starting antiretroviral therapy in Malawi: randomised, investigator blinded, controlled trial. *BMJ* 2009;338:b1867.

40 Paton NI, Macallan DC, Jebb SA, Noble C, Baldwin C, Pazianas M, et al. Longitudinal changes in body composition measured with a variety of methods in patients with AIDS. *J Acquir Immune Defic Syndr Hum Retrovirol* 1997;14:119-27.

41 Wanke C, Polsky B, Kotler D. Guidelines for using body composition measurement in patients with human immunodeficiency virus infection. *AIDS Patient Care STDS* 2002;16:375-88.

42 Welbourne TC. Increased plasma bicarbonate and growth hormone after an oral glutamine load. *Am J Clin Nutr* 1995;61:1058-61.

43 Shabert JK, Winslow C, Lacey JM, Wilmore DW. Glutamine-antioxidant supplementation increases body cell mass in AIDS patients with weight loss: a randomized, double-blind controlled trial. *Nutrition* 1999;15:860-4.

44 Shabert JK, Wilmore DW. Glutamine deficiency as a cause of human immunodeficiency virus wasting. *Med Hypotheses* 1996;46:252-6.

45 Heimburger DC, Koethe JR, Nyirenda C, Bosire C, Chiasera JM, Blevins M, et al. Serum phosphate predicts early mortality in adults starting antiretroviral therapy in Lusaka, Zambia: a prospective cohort study. *PLoS One* 2010;5:e10687.

46 Koethe JR, Heimburger DC. Nutritional aspects of HIV-associated wasting in sub-Saharan Africa. *Am J Clin Nutr* April 2010;91:1138-42S.

47 Kotler DP, Tierney AR, Culpepper-Morgan JA, Wang J, Pierson RN Jr. Effect of home total parenteral nutrition on body composition in patients with acquired immunodeficiency syndrome. *JPEN J Parenter Enteral Nutr* 1990;14:454-8.

48 Sadler K, Bontrager E, Rogers B, Coates S, Ghosh S, Kidane Y, et al. Food by prescription: measuring the impact and cost-effectiveness of prescribed food on recovery from malnutrition and HIV disease progression among HIV+ adult clients in Ethiopia. Feinstein International Center, Friedman School of Nutrition Science and Policy, Tufts University, 2012.

49 Lugassy DM, Farmer BM, Nelson LS. Metabolic and hepatobiliary side effects of antiretroviral therapy (ART). *Emerg Med Clin North Am* 2010;28:409-19.

Tables

Table 1| Nutrient content in 200 g lipid based nutrient supplement given to patients with HIV starting antiretroviral treatment

Nutrient	Amount
Energy	4600 kJ
Fat (% of energy)	60%
Carbohydrate (% of energy)	24%
Total protein (% of energy)	16%
Total protein	45 g
WPC80* or soy protein isolate†	32 g
Vitamins:	
A‡	1000 µg
D‡	10 µg
E‡	15 mg
K‡	110 µg
C‡	100 mg
B1‡	2.2 mg
B2‡	2.2 mg
B6‡	2.6 mg
B12‡	4.8 µg
Biotine‡	60 µg
Folic acid‡	800 µg
Panthothenic acid‡	10 mg
Niacin‡	28 mg
Minerals:	
Calcium§	1000 mg¶
Magnesium§	220 mg
Zinc‡	6 mg
Iron§	9 mg
Iodine§	110 µg
Selenium‡	52 mg
Copper‡	3 mg
Manganese§	1 mg
Phosphorous**:	
Whey	519 mg
Soy	728 mg

*Whey protein concentrate 80% (Davisco Foods, Le Sueur, MN).

†Unisol Soy Isolate Protein Non GMO (Barentz (Hoofddorp, Netherlands).

‡Twice recommended nutrient intake (FAO/WHO 2001[31]).

§Recommended nutrient intake (FAO/WHO 2001[31]).

¶Total amount correcting for differences in calcium content from ingredients (whey: 222 mg, soy: 77 mg).

**Total amount correcting for differences in bioavailability in products.

Table 2| Baseline characteristics of participants with HIV and BMI >17 according to lipid based nutritional supplementation given at start of antiretroviral treatment (n=282). Figures are means (SD) unless stated otherwise

	Early LNS*		Delayed LNS* (n=93)
	Whey (n=94)	Soy (n=95)	
Demographic characteristics:			
No (%) of women	60 (63.8)	61 (64.2)	65 (69.9)
Mean (SD) age (years)	32.2 (8.0)	34.5 (10.3)	31.7 (8.5)
No (%) by education:			
No formal schooling	24 (25.5)	28 (29.5)	32 (34.4)
Primary school†	48 (51.1)	49 (51.6)	43 (46.2)
Secondary school or higher†	22 (23.4)	18 (19.0)	18 (19.4)
Nutritional status and energy intake:			
Weight (kg)	52.1 (7.2)	50.9 (7.2)	50.5 (7.6)
Height (cm)	161.2 (8.1)	160.7 (9.0)	160.0 (9.2)
Body mass index (BMI)	20.0 (2.2)	19.8 (2.5)	19.8 (2.1)
Total energy intake (MJ/day)	6.47 (3.20)	6.06 (2.55)	6.17 (2.66)
Body composition:			
Lean body mass (kg)	41.9 (6.3)	40.9 (6.2)	39.9 (6.8)
Fat mass (kg)	10.3 (4.6)	9.9 (5.5)	10.6 (4.8)
Functional characteristics:			
Grip strength (kg)	24.8 (6.3)	24.9 (6.8)	24.2 (6.5)
PAEE‡ (kJ/kg/day)	28.6 (19.1-42.7)	30.0 (16.3-40.4)	26.8 (18.6-41.0)
HIV characteristics:			
No (%) WHO stage I/II	62 (67.4)	60 (63.2)	52 (58.4)
No (%) WHO stage III/IV	30 (32.6)	35 (36.8)	37 (41.6)
Viral load (log(1+ copies/mL))	4.77 (0.95)	4.65 (1.05)	4.67 (0.78)
CD3 (cells/μL)	1215 (966)	1134 (557)	1138 (487)
CD4 (cells/μL)	181 (94)	191 (110)	191 (106)
CD8 (cells/μL)	876 (418)	875 (444)	880 (414)

LNS=lipid based nutrient supplement; PAEE=physical activity energy expenditure.

*Early LNS groups received supplement during first three months. Delayed LNS group received supplement during subsequent three months.

†Includes all with some/complete education level.

‡Median (interquartile range). Data available for 71 in early LNS/whey group, 60 in early LNS/soy group, and 64 in delayed LNS group.

Table 3| Differences in outcomes at three months between participants with HIV and BMI >17 (n=282) allocated to early or delayed lipid based nutrient supplement with whey or soy at start of antiretroviral treatment. Effect estimates are presented with 95% CI and adjusted for sex, age, and education

	Early LNS*		Delayed LNS* (n=93)
	Whey (n=94), P value	Soy (n=95), P value	
Primary outcomes:			
Lean body mass† (kg)	0.85 (0.16 to 1.53), 0.018	0.97 (0.29 to 1.64), 0.005	Reference
Grip strength, kg)	0.68 (−0.11 to 1.46), 0.090	0.93 (0.16 to 1.70), 0.019	Reference
PAEE‡ (kJ/kg/day)	1.06 (0.87 to 1.29), 0.56	1.10 (0.91 to 1.33), 0.31	Reference
Secondary outcomes:			
Viral load (log(1+copies/mL))	−0.02 (−0.38 to 0.34), 0.91	−0.01 (−0.37 to 0.34), 0.94	Reference
CD4 (cells/µL)	25 (−2 to 53), 0.073	15 (−12 to 42), 0.28	Reference
Auxiliary outcomes:			
CD3 (cells/µL)	150 (24 to 275), 0.020	79 (−44 to 202), 0.21	Reference
CD8 (cells/µL)	112 (15 to 209), 0.023	60 (−35 to 154), 0.22	Reference
Weight† (kg)	2.05 (1.12 to 2.99), <0.001	2.06 (1.14 to 2.97), <0.001	Reference

LNS=lipid based nutrient supplement; PAEE=physical activity energy expenditure.

*Early LNS groups received supplement during first three months. Delayed LNS group received supplement during subsequent three months.

†Lean body mass and weight adjusted for height.

‡Effect estimates back transformed from log scale. Data available for 33 in early LNS/whey group, 34 in early LNS/soy group, and 31 in delayed LNS group.

Table 4| Differences in outcomes at three months between participants with HIV and BMI >16 (n=225) allocated to lipid based nutrient supplement with whey or soy during first three months of antiretroviral treatment. Effect estimates are presented with 95% CI and adjusted for sex, age, and education

	Whey (n=112)	Soy (n=113)	P value
Primary outcomes:			
Lean body mass* (kg)	−0.18 (−0.82 to 0.45)	Reference	0.58
Grip strength (kg)	−0.50 (−1.21 to 0.21)	Reference	0.16
PAEE† (kJ/kg/day)	0.96 (0.82 to 1.14)	Reference	0.66
Secondary outcomes:			
Viral load (log(1+copies/mL))	−0.04 (−0.35 to 0.27)	Reference	0.80
CD4 (cells/µL)	8 (−21 to 37)	Reference	0.58
Auxiliary outcomes:			
CD3 (cells/µL)	37 (−87 to 160)	Reference	0.56
CD8 (cells/µL)	31 (−57 to 119)	Reference	0.49
Weight* (kg)	−0.41 (−1.27 to 0.46)	Reference	0.36

LNS=lipid-based nutrient supplement; PAEE=physical activity energy expenditure.

*Lean body mass and weight adjusted for height.

†Effect estimates back transformed from log scale. Data available for 39 in whey group and 43 in soy group.

Table 5| Differences in outcomes at six months between participants with HIV and BMI >17 (n=282) allocated to early or delayed lipid based nutrient supplement (whey and soy) at start of antiretroviral treatment. Effect estimates are presented with 95% CI and adjusted for sex, age, and education

|	Early LNS* (n=189)	Delayed LNS* (n=93)	P value
Primary outcomes:			
Lean body mass* (kg)	Reference	0.36 (−0.23 to 0.94)	0.23
Grip strength (kg)	Reference	−0.84 (−1.53 to −0.04)	0.018
PAEE† (kJ/kg/day)	Reference	0.78 (0.65 to 0.95)	0.012
Secondary outcomes:			
Viral load (log(1+copies/mL))	Reference	0.06 (−0.24 to 0.37)	0.68
CD4 (cells/µL)	Reference	−6 (−31 to 18)	0.62
Auxiliary outcomes:			
CD3 (cells/µL)	Reference	−26 (−132 to 79)	0.63
CD8 (cells/µL)	Reference	−24 (−107 to 60)	0.58
Weight* (kg)	Reference	1.20 (0.32 to 2.09)	0.008

LNS=lipid based nutrient supplement; PAEE=physical activity energy expenditure.

*Early LNS groups received supplement during first three months of antiretroviral treatment. Delayed LNS group received supplement during subsequent three months.
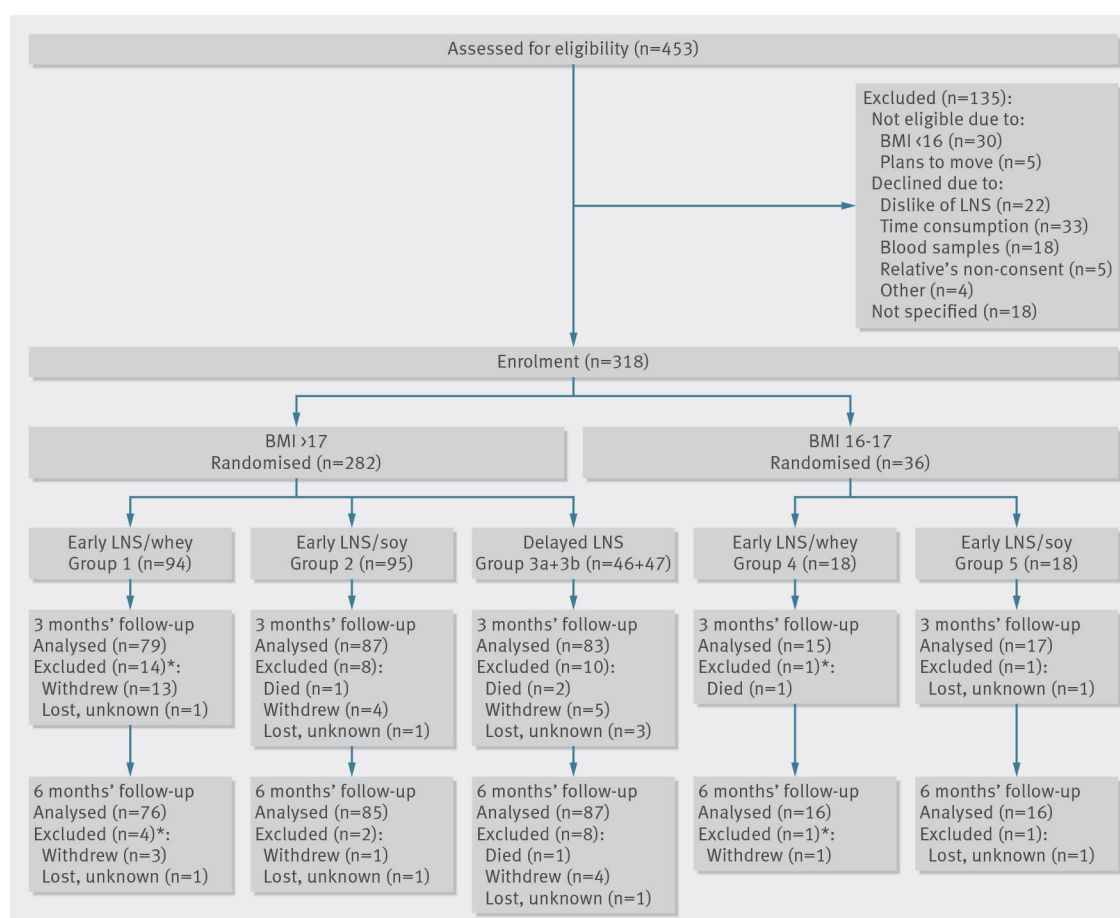
†Lean body mass and weight adjusted for height

‡Effect estimates back transformed from log scale. Data available for 77 in early LNS group and 36 in delayed LNS group.
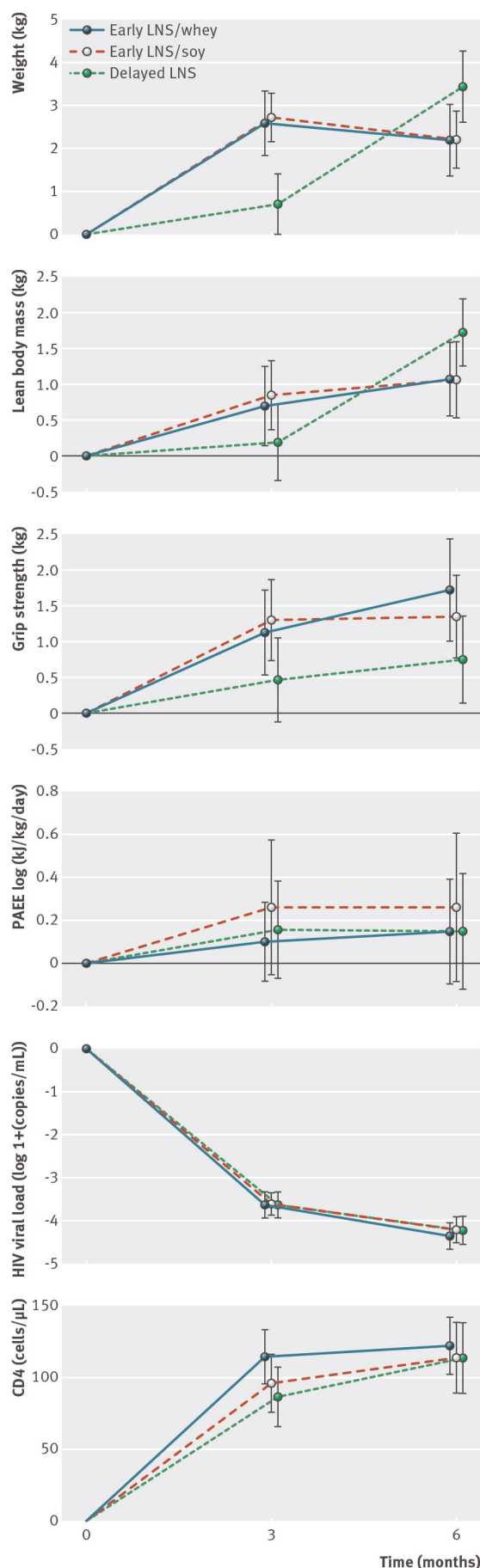
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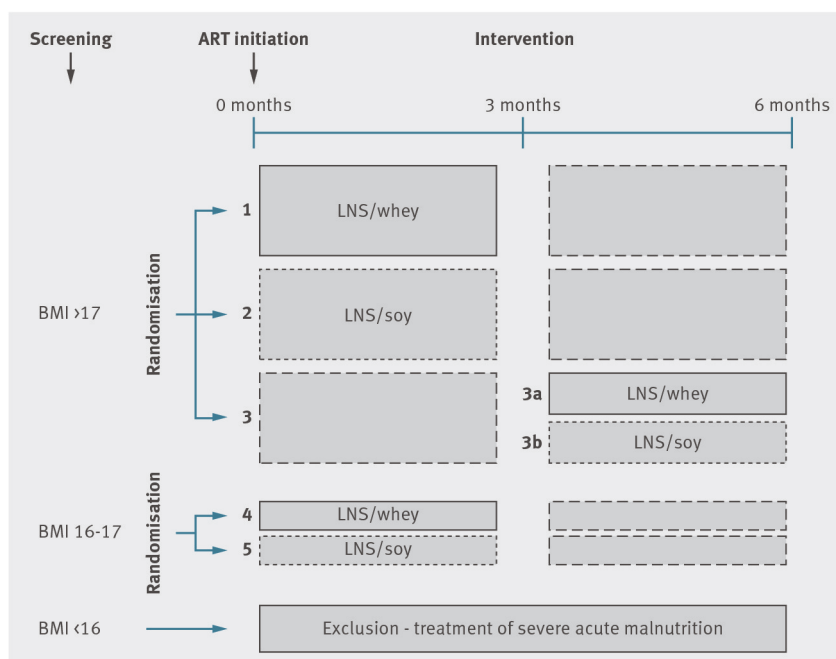
Fig 1 Study design with randomisation of participants stratified by BMI (LNS=lipid based nutrient supplement)

Fig 2 Flow chart of enrolment of participants and follow-up (LNS=lipid based nutrient supplement). *Numbers do not add up because of missed visits

Fig 3 Changes in weight, lean body mass, grip strength, physical activity energy expenditure (PAEE), HIV viral load, and CD4 count by intervention group. Graphs present unadjusted means with indication of 95% confidence interval for participants with complete data and BMI >17 (LNS=lipid based nutrient supplement)