Lipid-based nutrient supplements containing vitamins and minerals attenuate renal electrolyte loss in HIV/AIDS patients starting antiretroviral therapy: A randomized controlled trial in Zambia

D. Munkombwea,\*, T.L. Muungoa, C. Micheloa, P. Kellya,b, S. Chirwac, S. Filteaud,

a School of Medicine, University of Zambia, Lusaka, Zambia.

b Barts and London School of Medicine and Dentistry, Queen Mary University of London, UK.

c Neuroscience & Pharmacology, Meharry Medical College, Nashville TN, USA.

d London School of Hygiene and Tropical Medicine, London, UK.

\* **Corresponding Author**:

Derick Munkombwe. Department of Pharmacy, School of Medicine, University of Zambia. P. O. Box 50110, Lusaka, Zambia. Phone +260 977 704144. Email: [hachuuludm@yahoo.co.uk](mailto:hachuuludm@yahoo.co.uk)

**Abbreviations**: ART, antiretroviral therapy; estimated creatinine clearance, eCcr; FEK, fractional excretion of potassium; FEMg, fractional excretion of magnesium; GFR, glomerular filtration rate; LNS, lipid-based nutrient supplements; LNS-VM, lipid-based nutrient supplements with vitamins and minerals; NUSTART, nutritional support for Africans starting antiretroviral therapy; TRP, tubular reabsorption of phosphate.

**Trial registration**: [www.pactr.org](http://www.pactr.org) ID number: PACTR201106000300631.

**SUMMARY**

**Background & Aims**: Advanced HIV infection combined with undernutrition and antiretroviral therapy (ART) places HIV/AIDS patients at high risk of electrolyte abnormalities and increased morbidity and mortality. Here, in a sub-study of a large published randomized trial, we evaluated if nutritional supplements will help curtail renal electrolyte loss in HIV/AIDS patients starting ART.

**Methods:** 130 malnourished HIV-positive patients referred for ART received lipid-based nutrient supplements alone (LNS, n=63) or together with vitamins and minerals (LNS-VM, n=67). Serum and spot urine samples were collected and assayed for creatinine, potassium, magnesium and phosphate concentrations at baseline and after 12 weeks of ART, and fractional excretion and absorption were calculated using standard equations.

**Results:** Eighteen (28.6%) patients from the LNS and 16 (23.9%) from LNS-VM groups died, most during the referral interval before starting ART. Phosphate excretion at baseline, was high in both LNS (mean ± SD: 1.2 ± 0.6 mg/mg creatinine) and LNS-VM (1.1 ± 0.8 mg/mg creatinine) groups relative to normal physiological ranges. Phosphate excretion remained high in the LNS group (1.1 ± 0.41 mg/mg creatinine) but significantly decreased in the LNS-VM group (0.6 ± 0.28 mg/mg creatinine; p < 0.001) after 12 weeks of ART. This difference is probably explained by increased renal tubular reabsorption of phosphate in the LNS-VM group (88.3 ± 5.7%) compared to the LNS group (76.6 ± 8.9%). The fractional excretion of potassium (FEK) was not significantly different at baseline between the two groups (p=0.69) but the values were above normal physiological ranges (i.e. >6.4%) reflecting renal potassium wasting. However, FEK was significantly lowered in the LNS-VM group (6.2 ± 3.4%) but not in the LNS group (12.8 ± 4.7%) after 12 weeks of ART (p < 0.001). Finally, the fractional excretion of magnesium was not significantly different between the two groups at baseline (p=0.68) and remained unchanged within normal physiological ranges at 12 weeks of ART (p = 0.82) in both groups.

**Conclusions**: The LNS-VM regimen appeared to offer protection against phosphate and potassium loss during HIV/AIDS treatment. This offers potential opportunities to improve care and support of poorly nourished HIV-infected patients in resource-limited settings.

Trial registration: [www.pactr.org](http://www.pactr.org) ID number: PACTR201106000300631.

**Key Words**

Randomized-Trial; HIV/AIDS; Nutritional supplements; Electrolytes; Renal excretion; Zambia.

**1. Introduction**

With the massive scale-up of anti-retroviral therapy (ART), HIV infection has been transformed from an incurable disease to a chronic disease; however, this is not without challenges [1, 2]. In sub-Saharan Africa, a high number of HIV/AIDS patients starting ART die but the causes remain unclear [3]. A retrospective analysis of ~28,000 HIV-1 infected patient cohort in Zambia showed that mortality in the first 3 months of starting ART was significantly elevated among patients with body-mass index (BMI) less than 16.0 kg/m2 compared to other BMI strata [4]. A number of factors including genetic influences, life style, malnutrition and ART have been attributed to the increased mortality. HIV infection, malnutrition and ART are known to contribute to renal tubular dysfunction resulting in life-threatening electrolyte loss [1-2, 5-6].For instance, malnutrition is linked to depletion or altered metabolism of vitamins and minerals [7]. Similarly, ART can exacerbate bone loss leading to electrolyte wasting [8]. Furthermore, clinical cases of renal proximal tubular dysfunction [6, 9] including development of life-threatening renal Fanconi syndrome have been well documented with tenofovir-based ART use [2, 10-11]. Fanconi’s syndrome is the generalized dysfunction of proximal tubules in the kidney resulting in excessive loss of substances (e.g. phosphate, bicarbonate, amino acids, glucose, and low molecular weight proteins) in the absence of high plasma concentrations [12, 13]. Consequently, the pathophysiological overlap of malnutrition, HIV infection and/or ART affect renal function that could drive clinically significant electrolyte losses. Hence, there is an urgent need for evidence-based interventions that may be employed to prevent or manage renal dysfunction linked to malnutrition and/or ART in HIV-infected patients.

Malnutrition is linked to depletion or altered metabolism of vitamins and minerals [7]. Published reports suggest that low serum phosphate concentrations in severely malnourished HIV-infected patients predicted early mortality [3]. Thus, in an effort to improve treatment outcomes a significant proportion of malnourished HIV-1 infected patients are now recommended to concurrently initiate ART with ‘structured’ nutritional supplementation [14]. Recent findings indicate that nutritional supplements coupled with vitamins and minerals significantly improve anthropometric measures [15]. However, there is a paucity of studies that have directly assessed whether supplements improve the homeostatic functions of the kidneys, for example, and curtail electrolyte loss. This was examined in the present study. The main objective was to measure the effects of nutrient supplements on kidney functions during the early phases of starting ART. Within this context, it was theorized that lipid-based nutrition supplements (LNS) with vitamins and minerals would curtail loss of electrolytes in HIV/AIDS patients initiating ART. For instance, normal phosphate homeostasis is balanced by daily phosphate intake and body excretion in the urine and feces. Both phosphate intake and excretion are modulated by the active metabolite of vitamin D (i.e. 1,25(OH)2D3; also termed ‘calcitrol’) [16, 17]. Specifically, calcitrol enhances phosphate uptake from the intestines and also fosters phosphate reabsorption in renal proximal tubules. Thus, nutritional supplements containing significant compliments of both phosphorous and vitamin D would be expected to curb phosphate excretion, in part, by promoting renal proximal tubular reabsorption of phosphates in the body. This notion and similar ideas pertaining to potassium and magnesium homeostasis were tested in the present study.

**2. Materials and Methods**

*2.1. Subject Selection*

One hundred and thirty HIV/AIDS patients aged between 18-49 years were enrolled in the study which was embedded in the Nutritional Support for Africans Starting Antiretroviral Therapy (NUSTART) trial [15, 18]. Inclusion criteria were, ART-naive, BMI <18.5 kg/m2, CD4 count <350 cells/µL or stage 3 or 4 disease, and not pregnant. The NUSTART study was conducted in Zambia and Tanzania. Patients for this sub-study were drawn from the Lusaka NUSTART site in Zambia based on recruitment between May and November 2013 but in all other respects were entirely representative of the whole trial population [18]. Participants were recruited to sequential identification (ID) numbers by clinic nurses who had no access to the study treatment codes. The randomization of subjects was done by the Data Safety and Monitoring Board statistician using a computer generated blocks of 16 randomization table stratified [18]. Figure 1 shows the final subject distribution for the sub-study. The project was conducted towards the end of the parent NUSTART randomized clinical trial.

*2.2. Intervention*

The control research arm received lipid-based nutrient supplements (LNS), whereas the experimental arm was given LNS with minerals and vitamins (LNS-VM). The products were made for the trial by Nutriset (Malaunay, France) and they both contained 60% calories as fat and 10% calories as protein available in ready-to-eat packets [18]. LNS-VM also contained micronutrients mostly at 3 times the United Kingdom recommended nutrient intake for adult women [19], except iron that was at 1x recommended nutrient intake only in the second stage. By contrast, LNS contained vehicle and flavorings similar to LNS-VM but without added vitamins or minerals (see Table 1 for composition of supplements).

*2.3. Study Design*

Patients received a stepped regimen of LNS or LNS-VM as described elsewhere [18]. Packages of LNS and LNS-VM were labeled with the study ID numbers by the clinic pharmacists at the time the packets were dispensed. Briefly, the supplementation started with small daily doses containing limited calories (30 g, 150 kcal), from time of referral for ART through the pre-ART preparation phase and until 2 weeks after start of ART. This was followed by larger daily doses containing greater calorie provisions (250 g, 1400 kcal) for four weeks. All patients were started on a single fixed-dose ART tablet that contained 300 mg tenofovir disoproxil fumarate, 200 mg emtricitabine, and 600 mg efavirenz at a median interval of 3 weeks after referral. In addition, daily prophylactic doses of 960 mg co-trimoxazole to prevent pneumocystis development were given to HIV-infected patients as part of their standard clinical care. Venipuncture was performed to collect 5-10 mL of blood at baseline and 12-weeks after ART. Half of each drawn blood sample was transferred into a sterile collection tube, allowed to clot then centrifuged and the supernatant serum fraction was carefully extracted and stored under refrigeration. The other half of the blood sample was stored in an EDTA-stabilized collection tube and preserved for blood work including determination of CD4 cell counts. In addition, spot urine samples (20-30 mL) were obtained from each patient and processed for storage as follows. The urine samples were divided into several aliquots of 750 μL shortly after collection and transferred into sterile microfuge tubes containing 150 μL of 10% nitric acid that was used as a preservative and for acidification to keep all metals solubilized in urine during storage under refrigeration [20]. In each case, samples were collected between 9:00 AM and 10:00 AM approximately 14 hours after the patient most recent ARV dose and stored under refrigeration for subsequent analyses. Creatinine, potassium, magnesium and phosphate were measured in blood and urine samples using standard clinical assays as described elsewhere [15].

*2.4. Sample size determination*

Based on projected renal fractional extraction rates, a sample size of 130 patients with an anticipated 20% loss to follow-up (i.e. minimum of 54 patients per group), at α=0.05 and two-tailed, has a statistical power greater than 95% and this is sufficient to detect a 31.6% difference between the experimental (LNS-VM) group and control (LNS) group means with a standard deviation of ±61.3%. The power analysis calculation was done using GraphPad StatMate 2.0 for Windows GraphPad Software, San Diego, California, USA, [www.graphpad.com](http://www.graphpad.com).

*2.5. Data analyses*

A measure of estimated glomerular filtration rate (GFR) was determined by calculating the estimated creatinine clearance (eCcr) using the Cockroft-Gault formula for men (15% less for women) [16, 21].

The urine phosphate-creatinine ratio (concentrations measured in molar units) was used to correct for and estimate urinary phosphate excretion; renal tubular reabsorption of phosphate (TRP) was calculated as follows:

TRP values >96% were taken to be normal [16, 22]. Lastly, renal fractional excretion of magnesium (FEMg) and potassium (FEK) percentages were calculated using the following equations, respectively:

FEMg is a marker of an intact tubulointerstitial structure [16, 23] and values <4% reflect intact tubular function for reabsorption of filtered magnesium. FEK is a useful marker of potassium excretion. Unlike most electrolytes, urinary potassium excretion is governed by tubular secretion rather than reabsorption [16, 24]. In patients with normal glomerular filtration rate, FEK <6.4% is consistent with appropriate potassium conservation.

Data were expressed as mean ± SD or percentage. Within group and between groups comparisons were performed using unpaired t-test and/or one-way ANOVA with Tukeys for multiple comparisons as the posthoc test (α = 0.05, two-tailed tests).

*2.6. Ethical Consideration*

The study complied with guidelines of the Declaration of Helsinki and was approved by the University of Zambia Biomedical Research Ethics Committee (research study approval number 014-10-13). The main NUSTART trial was also approved by the ethics committee of the London School of Hygiene and Tropical Medicine. All patients gave written informed consent.

**3. Results**

Table 2 shows patient characteristics in the LNS and LNS-VM research arms. Baseline data were similar between the two groups in terms of social and demographic profiles, as well as biomarkers for renal homeostatic functions (i.e. estimated GFR, FEK, FEMg, TRP) and HIV treatment outcomes (i.e. CD4 cell counts, BMI). Estimated GFR from calculated creatinine clearance was within a normal range of 88 to 137 ml/min. Subsequently, 18 patients from the LNS arm and 16 from the LNS-VM arm died before 12 weeks ART, most during the interval between referral for ART and starting the drugs. Overall, the loss to follow up was 32.3% by week 12 of ART and this exceeded the projected loss of 20% used to determine sample sizes with >90% statistical power. Thus, a new power analysis was calculated based on standard deviation values from the obtained data to ensure the study did not miss a small effect due to the higher than anticipated number of patients lost to follow-up. It was found that using the renal fractional excretion data, group sample sizes of at least 42 (i.e. LNS group size) still had >90% power to detect a difference between means of at least 25% with α=0.05 (two-tailed).

The group averages for serum concentrations of phosphate, magnesium and potassium in the HIV-1 infected patients were within the normal physiological ranges at baseline (Table 2), but there was wide variability in individual values as shown in Figure 2. Briefly, at baseline 25 patients (19.2%) had low serum phosphate concentrations (LNS 20.6%, LNS-VM 17.9%) and 18 patients (13.8%) had high phosphate (LNS 12.7%, LNS-VM 14.9%) relative to normal physiological ranges. Similarly, 13 patients (10.0%) had low serum potassium concentrations (LNS 7.9%, LNS-VM 11.9%) and 11 patients (8.5%) had high potassium (LNS 9.5%, LNS-VM 7.5%). [NB: The low and high cut-off serum values in mmol/L were as follows: phosphate, 0.8 - 1.5; potassium, 3.5 - 5.5; and magnesium, 0.7 - 1.1; see ref. 15-16, 25].

Phosphate excretion was high in both the LNS (mean ± SD in this and subsequent entries: 1.2 ± 0.6 mg/mg creatinine; n = 67) and LNS-VM (1.1 ± 0.8 mg/mg creatinine; n = 63) groups (Table 2). Phosphate excretion remained high in the LNS group (1.1 ± 0.41 mg/mg creatinine; n = 42), whereas it was significantly decreased in the LNS-VM group (0.6 ± 0.28 mg/mg creatinine; n = 46; p < 0.001) by 12 weeks of ART. This difference is probably explained by increased renal tubular reabsorption of phosphate in the LNS-VM group (88.3 ± 5.7%; n = 46) compared to the LNS group (76.6 ± 8.9%; n = 42; see Figure 3A). [NB: The decrease in sample size numbers at 12 weeks of ART in both study groups was due to loss to follow up as previously outlined in Figure 1]. Similarly, the fractional excretion of potassium values were high and exceeded normal physiological ranges (i.e. >6.4%) at baseline in both research groups and this indicated the presence of renal potassium wasting (data in Table 2). However, the fractional excretion of potassium significantly decreased to within normal physiological ranges in the LNS-VM group (6.2 ± 3.4%; n = 46) but not in the LNS group (12.8 ± 4.7%; n = 42) after 12 weeks of ART (p < 0.001; Figure 3B). Finally, the results also showed that fractional excretion of magnesium was unchanged and was within normal ranges at 12 weeks of ART in both groups (LNS, 3.3 ± 1.6; LNS-VM, 3.5 ± 1.3) relative to baseline values (LNS, 3.1 ± 1.9; LNS-VM, 3.2 ± 1.7; p = 0.82; Figure 3C), respectively.

**4. Discussion**

The present study has presented data that demonstrates deficits in renal phosphate reabsorption and potassium excretion in malnourished HIV/AIDS patients. The findings show for the first time that subsequent administration of lipid-based nutrient supplements containing vitamins and minerals significantly improved phosphate reabsorption and reduced potassium excretion. The group means for plasma concentrations of phosphates, potassium and magnesium were within normal physiological ranges and this was suggestive of ‘compensatory’ mechanisms at play. Briefly, plasma levels of phosphates, potassium, and magnesium are maintained within very narrow limits to sustain life [16, 25]. Thus, loss of body electrolytes via excretion is expected to be counter-balanced by dietary intake. If this is inadequate (as may occur in malnutrition) then bone resorption and/or cell destruction is likely to occur to maintain electrolyte homeostasis. The finding that there was primarily potassium and phosphate wasting, but not magnesium,is suggestive of cell loss rather than bone resorption. By contrast, a finding of magnesium with phosphate wasting would have been suggestive of bone re-sorption more than cell loss [16, 25]. Consequently, it is predicted that the improved renal electrolyte retention observed in the LNS-VM group will be associated with a reduced cell wasting that occurs to maintain electrolyte homeostasis and this idea is supported by our recently reported findings of more rapid tissue deposition in the LNS-VM group relative to the LNS group [15].

It was unclear what mechanisms accounted for the benefits in renal homeostatic function derived from LNS-VM co-administration with ART. However, it is feasible that the benefits could be attributed to the effects of anti-oxidant vitamins and mineral replacement leading to improved kidney homeostatic functions for the following reasons. Firstly, phosphate homeostasis is balanced by daily phosphate intake and renal excretion. Within this context, serum levels are altered by intestinal phosphate absorption mediated by type 2b sodium-phosphate (Npt2b) co-transporters. Npt2b co-transporters are regulated by dietary phosphorus intake as well as calcitrol derived from the metabolism of vitamin D [16-17, 27]. Calcitrol is a strong stimulant of both the rate and maximal capacity of intestinal absorption of phosphate. It is likely that LNS-VM exerted some of these effects because the product contained both vitamin D and a higher phosphorous content (see Table 1). Secondly, phosphate is freely filtered in the glomerulus but greater than 80% of the filtered load is reabsorbed in the renal proximal tubules mainly via type 2a and type 2c (Napt2a and Napt2c) co-transporters that move 3 sodium ions and 1 phosphate molecule [16, 25-26]. The abundance of Napt2a and Napt2c in the apical membranes of proximal tubular cells is regulated by parathyroid hormone (PTH) secreted from the parathyroid gland [26, 28]. PTH inhibits the insertion of Napt2a and Napt2c into the proximal tubular cell apical membranes and this causes phosphaturia [17]. However, PTH also acts to increase the plasma concentration of calcium by promoting active absorption from the small intestines and mediating calcium resorption from bone [17]. Raised plasma calcium subsequently inhibit PTH secretion and this secondarily curtails the PTH-linked inhibition of Napt2a and Napt2b insertions [16-17, 25]. This results in increased phosphate reabsorption from the kidney and probably contributed towards the improvements in tubular reabsorption of phosphate observed in the LNS-VM group after 12 weeks of ART. Thirdly, dietary potassium deficiency leads to changes in brush border membrane lipid composition that are thought to inhibit sodium-phosphate co-transport across the apical membranes of renal proximal tubular cells [29]. In the present study, LNS-VM had a higher compliment of dietary potassium (see Table 1) and this inclusion may have helped to augment renal tubular reabsorption of phosphate. Taken together, the presented findings suggest that nutritional supplements containing phosphates, vitamin D and potassium worked by increasing both phosphate absorption from the intestines and phosphate reabsorption in the kidneys.

In terms of potassium excretion, the most parsimonious explanation is that the ingredients in LNS-VM partly augmented energy metabolism leading to increased production of cellular ATP [30]. Increased ATP stores would favor the activation of sodium-potassium pumps (i.e. Na+/K+-ATPase) in various body cells. If this is valid then two important outcomes are predicted to occur as follows. Firstly, Na+/K+-ATPases are powered by cellular ATP and activated ionic pumps are responsible for maintaining the high potassium ions concentration and low sodium ions concentration in the cytoplasm, respectively [16, 25]. This is achieved via coupled transportation of 3 sodium ions from the cytoplasm and entry of 2 potassium ions into the cytoplasm. This ‘unbalanced’ ionic movements generates an electrical current that hyperpolarizes the cell membrane resulting in more negative resting membrane potentials and this contributes towards the retention of potassium in the cytoplasm. Secondly, plasma potassium is freely filtered in the glomerulus but 87-96% of the filtered load is reabsorbed primarily in the proximal tubule and ascending limbs of Henle [16, 25]. However, potassium is also secreted by the cells of the renal distal tubules and collecting duct system. In fact, potassium secretion from the blood into the tubular fluid by the cells of the distal tubule and collecting duct system is the major determinant of urinary potassium excretion [16, 24-25]. This follows a ‘two-step’ process starting with potassium uptake from the blood across the basolateral membrane of the distal tubule and collecting duct system by the ‘Na+/K+-ATPase’ [16] and then diffusion of potassium from the cells into the renal tubular fluids occurs via potassium channels driven by the high chemical gradient. In addition, the apical membrane contains sodium channels that increase permeability to sodium ions whose entry lowers the resting membrane potential (i.e. make less polar) thereby producing an electrical driving force that, in turn, can drive potassium secretion across the apical membrane into the tubule fluid. However, rapid removal of sodium ions by activated Na+/K+-ATPase polarizes the cells and this diminishes the tendency of potassium ions to flow across the apical membranes of the cells of the distal tubules and into the collecting ducts. Taken together, it seems that lipid-based nutrient supplements containing vitamins and minerals facilitated cellular energy production partly leading to greater activations of Na+/K+-ATPase that both helped to ‘restore’ the active maintenance of high intracellular potassium concentrations and reduced potassium efflux from cells in the kidney. These ideas will need further experimental validation.

In conclusion,controlled lipid-based nutrients with vitamins and minerals supplementation may be useful in curtailing electrolyte loss in resource-limited settings where malnutrition and HIV/AIDS are common. These observations suggest presence of nutrition-driven opportunities to improve care and support of poorly nourished HIV-infected patients in resource-limited settings. Furthermore, assessment of serum and spot urine electrolyte concentrations permit easy, rapid, and inexpensive estimation of kidney function and electrolyte loss in relation to HIV infection and ART regimen.

**Conflict of interest**

None.

**Authors’ Contributions**

All authors designed the research, DM conducted research; DM, SC and SF analyzed data; DM wrote the initial draft. All authors contributed towards, read and approved the final manuscript.

**Acknowledgements**

Authors wish to thank NUSTART research team especially Molly Chisenga and Joshua Siame who provided technical assistance. Study supported by European and Developing Countries Clinical Trials Partnership (EDCTP) grant to SF; DM received fellowships from EDCTP, Southern African Consortium for Research Excellence, and University of Zambia.

**References**

1. Ali MK, Magee MJ, Dave JA, Ofotokun I, Tungsiripat M, Jones TK, Levitt NS, Rimland D, Armstrong WS. HIV and metabolic, body, and bone disorders: What we know from low- and middle-income countries. J. Acquir. Immune Defic Syndr 2014; 67(suppl.1): S27-S39.

2. Kalyesubula R, Wearne N, Neph C, Semitala FC, Bowa K. HIV-associated renal and genitourinary comorbidities in Africa. J Acquir Immune Defic Syndr 2014; 67(suppl.1): S68-S78.

3. Heimburger DC, Koethe JR, Nyirenda C, Bosire C, Chiasera JM, Blevins M, Munoz AJ, Shepherd BE, Potter D, Zulu I, Chisembele-Taylor A, Chi BH, Stringer JS, Kabagambe EK. Serum Phosphate Predicts Early Mortality in Adults Starting Antiretroviral Therapy in Lusaka, Zambia. PLoS One 2010; 5(5):e10687. doi:2F10.1371/2Fj.pone.0010687.

4. Koethe JR, Lukusa A, Giganti MJ, Chi BH, Nyirenda CK, Limbada MI, Banda Y, Stringer JS. Association between weight gain and clinical outcomes among malnourished adults initiating antiretroviral therapy in Lusaka, Zambia. J Acquir Immune Defic Syndr 2010; 53: 505-515.

5. Maggi P, Bartolozzi D, Bonfanti P, Calza L, Cherubini C, Di Biagio A, Marcotullio S, Montella F, Montinaro V, Mussini C, Narcico P, Ruscon S, Vescini F. Renal complications in HIV disease: between present and future. AIDS 2012; 14: 37-53.

6. Horberg M, Tang B, Towner W, Silverberg M, Bersoff-Matcha S, Hurley L, Chang J, Blank J, Quesenberry C Jr, Klein D. Impact of tenofovir on renal function in HIV-Infected, antiretroviral-naive patients. J Acquir Immune Defic Syndr 2010; 53: 62-69.

7. Musoke PM, Fergusson P. Severe malnutrition and metabolic complications of HIV-infected children in the antiretroviral era: clinical care and management in resource-limited settings. Am J Clin Nutr 2011; 94(suppl): 1716S-1720S.

8. Cotter AG, Powderly WG. Endocrine complications of human immunodeficiency virus infection: hypogonadism, bone disease and tenofovir-related toxicity. Best Prac Res Clinical Endocrinol Metab 2011; 25: 501-515. doi:10.1016/j.beem.2010.11.003.

9. Laprise C, Baril J-G, Dufresne S, Trottier H. Association between tenofovir exposure and reduced kidney function in a cohort of HIV-positive patients: results from 10 years of follow-up. CID 2013; 56: 567-575. doi: 10.1093/cid/cis937.

10. Scherzer R, Estrella M, Li Y, Choi AI, Deeks SG, Grunfeld C, Shlipak MG. Association of tenofovir exposure with kidney disease risk in HIV infection. AIDS 2012; 26: 867-875.

11. Hall AM. Update on tenofovir toxicity in the kidney Pediatr Nephrol 2013; 28:1011-1023.

12. Izzedine H, Launay-Vacher V, Isnard-Bagnis C, Deray G. Drug-induced Fanconi’s syndrome. Am J Kidney Dis 2003; 41: 292-309.

13. Klootwijk ED, Reichold M, Unwin RJ, Kleta R, Warth R, Bockenhauer D. Renal Fanconi syndrome: taking a proximal look at the nephron. Nephrol Dial Transplant 2015; 30: 1456-1460.

14. Grobler L, Siegfried N, Visser ME, Mahlungulu SS, Volmink J. Nutritional interventions for reducing morbidity and mortality in people with HIV. Cochrane Database Syst Rev 2013 Feb 28; 2:CD004536. doi: 10.1002/14651858.CD004536.pub3.

15. Rehman AM, Woodd S, PrayGod G, Chisenga M, Siame J, Koethe JR, Heimburger DC, Kelly P, Friis H, Filteau S. 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 2015; 68: 405-412.

16. Cogan MG. Fluid and electrolytes. Physiology and pathology. 1st Edition, Appleton and Lange. 1991.

17. Blaine J, Chonchol M, Levi M. Renal calcium, phosphate, and magnesium. Clin J Am Soc Nephrol 2014. doi: 10.2215/CJN.09750913.

18. NUSTART (Nutritional Support for Africans Starting Antiretroviral Therapy) Study Team, Filteau S, PrayGod G, Kasonka L, Woodd S, Rehman AM, Chisenga M, Siame J, Koethe JR, Changalucha J, Michael D, Kidola J, Manno D, Larke N, Yilma D, Heimburger DC, Friis H, Kelly P. Effects on mortality of a nutritional intervention for malnourished HIV-infected adults referred for antiretroviral therapy: a randomized controlled trial. BMC Medicine (2015) 13:17 DOI 10.1186/s12916-014-0253-8.

19. UK Departments of Health. Dietary reference values for food energy and nutrients for the UK. London: Department of Health; 1991.

20. Iyengar GV, Subramanian KS, Woittiez JRW. Element analysis of biological samples: Principles and practices, Volume 2. 1997 CRC Press pp. 95-101.

21. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976; 61: 31-41.

22. Walton RJ, Bijvoet OLM. Nomogram for derivation of renal threshold phosphate concentration. Lancet 1975; 306: 3309-310 (originally published as volume 2, issue 7929).

23. Gheissari A, Andalib A, Labibsadeh N, Modarresi M, Azhir A, Merrikhi A. Fractional excretion of magnesium (FEMg), a marker for tubular dysfunction in children with clinically recovered ischemic acute tubular necrosis. Saudi J Kidney Dis Transpl 2011; 22: 476-481.

24. Elisaf M, Siamopoulos KC. Fractional excretion of potassium in normal subjects and in patients with hypokalemia. Postgrad Med J 1995; 71: 211-212.

25. Koeppen BM, Stanton BA. Renal physiology. 4th Edition, Mosby Elsevior. 2007.

26. Bellorin-Font E, Milanes CL, Urbina D, Pernalete N, Paz-Marinez V. The regulation of sodium phosphate cotransport in the kidney. In: Puschett J, Greenberg A, eds. Diuretics, vol. 11. London: Elsevior Science, 1990: pp 427-433.

27. Marks J, Debnam ES, Unwin RJ. Phosphate homeostasis and the renal-gastrointestinal axis. Am J Physiol Renal Physiol 2010; 299: F285-F296.

28. Forster IC, Hernando N, Biber J, Murer H. Proximal tubular handling of phosphate: a molecular perspective. Kidney Int 2006; 70: 1548-1559.

29. Breusegem SY, Takahashi H, Giral-Arnal H, Wang X, Jiang T, Verlander JW, Wilson P, Miyazaki-Anzai S, Sutherland E, Caldas Y, Blaine JT, Segawa H, Miyamoto K, Barry NP, Levi M. Differential regulation of the renal sodium phosphate co-transporters NaPi-IIa, NaPi-IIc, and PiT-2 in dietary potassium deficiency. Am J Physiol Renal Physiol. 2009; 297: F350-F361.

30. Huskisson E, Maggini S, Ruf M. The role of vitamins and minerals in energy metabolism and well-being. J Intern Med Res 2007; 35: 277-289.

**Figure Legend**

**Figure 1.** **Flow of the participants through the sub-study.** Screening was limited to the Lusaka site in Zambia and involved all HIV-infected patients referred for CD4 testing and also had BMI <18.5 kg/m2. Recruited patients were randomized in the parent NUSTART controlled trial. The sub-study was conducted towards the end of the parent NUSTART study. Abbreviations: LNS, lipid-based nutritional supplement; LNS-VM, lipid-based nutritional supplement with vitamins and minerals.

**Figure 2. Effect of nutrient supplements on serum concentrations of phosphate, magnesium and potassium**. The group means for phosphate, magnesium and potassium concentrations were within normal physiological ranges in the HIV infected patients at baseline and at 12 weeks of antiretroviral therapy. However, note the wide spread distribution of values particularly at baseline. The number of subjects are shown in brackets but the plotted symbols reflecting individual patients may be less in number because some subjects had similar scores. Furthermore, the difference in sample sizes after treatment relative to baseline is due to loss to patient follow up as explained in main text. Horizontal dotted lines in each graph reflect the low and high cut-off concentrations for phosphates (A), magnesium (B), and potassium (C) ions. The solid horizontal and vertical lines within the scatter points are mean ± SD, respectively. Abbreviations: ART, antiretroviral therapy; LNS, lipid-based nutritional supplement; LNS-VM, lipid-based nutritional supplement with vitamins and minerals.

**Figure 3. Effect of nutrient supplements on renal excretion of phosphate, magnesium and potassium**. The scatter plots show significant increases in tubular reabsorption of phosphate (A) and significant decreases in fractional excretion of potassium ions (B) following LNS-VM relative to LNS at 12-weeks on antiretroviral therapy as well as both LNS and LNS-VM at baseline. By contrast, neither LNS nor LNS-VM affected fractional excretion of magnesium ions (C). Dotted horizontal lines reflect cut-off points for normal physiological levels with the following interpretations: Tubular reabsorption of phosphate values >96% are considered to be normal; Fractional excretion of potassium <6.4% is consistent with appropriate potassium conservation; and Fractional excretion of magnesium <4% reflect intact tubular function for reabsorption of filtered magnesium. The number of subjects are shown in brackets but total number of plotted symbols reflecting individual patients may be less in number because a few subjects had similar scores. The difference in sample sizes at 12 weeks of antiretroviral therapy relative to baseline is due to loss to patient follow up. The solid horizontal and vertical lines within the scatter points are mean ± SD, respectively. Abbreviations: ART, antiretroviral therapy; LNS, lipid-based nutritional supplement; LNS-VM, lipid-based nutritional supplement with vitamins and minerals.

**Table 1. Nutritional composition of trial supplements – amounts per day\***

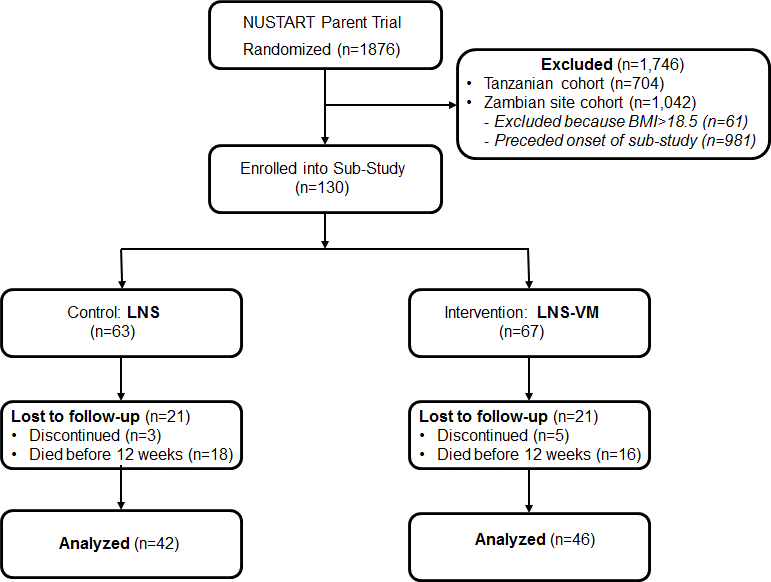
|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Nutrient | First phase supplement (from recruitment to 2 weeks of ART) | | Second phase of supplement (from 2 to 6 weeks of ART) | |
| **LNS-VM**  **(30 g)** | **LNS**  **(30 g)** | **LNS-VM**  **(250 g)** | **LNS**  **(250 g)** |
| Calories (kcal) | 139 | 168 | 1,397 | 1,416 |
| Protein (g) | 2.4 | 2.3 | 55 | 55 |
| Fat (g) | 11.0 | 10.9 | 97.5 | 97.5 |
| Potassium (mmol/L) | 30 | 0.9 | 32 | 15.8 |
| Phosphorus (mmol/L) | 47 | 0.4 | 38 | 9.3 |
| Magnesium (mmol/L) | 16 | 0.3 | 17 | 5.7 |
| Calcium (mg) | 29.8 | 5.0 | 140 | 115 |
| Iron (mg) | 0.4 | 0.4 | 14.7 | 8.4 |
| Zinc (mg) | 21 | 0.2 | 21 | 3.8 |
| Copper (mg) | 3.6 | 0.06 | 3.6 | 1.2 |
| Manganese | 4.2 | - | 4.2 | - |
| Iodine (μg) | 420 | - | 420 | - |
| Selenium (μg) | 180 | - | 180 | - |
| Chromium | 75 | - | 75 | - |
| Retinol (as palmitate) (μg) | 1,800 | - | 1,800 | - |
| Vitamin D (μg) | 10 | - | 10 | - |
| Vitamin E (mg) | 45 | - | 45 | - |
| Vitamin K (μg) | 95 | - | 95 | - |
| Vitamin C (mg) | 120 | - | 120 | - |
| Thiamin (mg) | 2.4 | - | 2.4 | - |
| Riboflavin | 3.3 | - | 3.3 | - |
| Niacin (mg) | 39 | - | 39 | - |
| Pyridoxine (mg) | 3.6 | - | 3.6 | - |
| Folate (μg) | 600 | - | 600 | - |
| Vitamin B12 (μg) | 4.5 | - | 4.5 | - |
| Pantothenic acid (mg) | 9 | - | 9 | - |

The listed nutrient contents for both LNS and LNS-VM are values from analysis by the manufacturer. Where values for only LNS-VM are given, these were not assessed in the prepared foods but refer to amounts added, that is they do not include those intrinsic to the LNS. Abbreviations: ART, antiretroviral therapy; LNS, lipid-based nutritional supplement; LNS-VM, LNS with added vitamins and minerals. [Table adapted from parent study reported in ref. 18].

**Table 2: Baseline Characteristics of 130 HIV/AIDS Patients in Randomized Clinical Trial**

|  |  |  |
| --- | --- | --- |
| **Variable** | **LNS-VM**  **(n = 67)** | **LNS**  **(n = 63)** |
| **A. Demographic and Background Data** |  |  |
| Age (years) | 38 ± 9 | 35 ± 8 |
| Male/Female numbers | 41/26 | 36/27 |
| Employed | 7 (10%) | 11 (18%) |
| Education, ˂High school | 43 (64%) | 41 (65%) |
| Taking Anti-tuberculosis drugs | 10 (15%) | 14 (22%) |
| Taking Cotrimoxazole | 62 (93%) | 59 (94%) |
| **B. Clinical and Biomarker Data** |  |  |
| CD4 count (cells/μL) | 119 ± 96 | 136 ± 104 |
| Body-Mass Index (Kg/m2) | 16.3 ± 1.8 | 16.8 ± 1.3 |
| Serum Potassium (mmol/L) | 4.5 ± 0.6 | 4.6 ± 0.6 |
| Serum Magnesium (mmol/L) | 0.8 ± 0.1 | 0.8 ± 0.1 |
| Serum Creatinine (μmol/L) | 63.7 ± 16.6 | 59.4 ± 16.7 |
| Serum Phosphate (mmol/L) | 1.1 ± 0.3 | 1.0 ± 0.4 |
| Urine Creatinine (mmol/L) | 7.9 ± 4.1 | 7.2 ± 3.3 |
| Estimated GFR (ml/min) | 92.0 ± 27.4 | 101.7 ± 30.0 |
| Urine Phosphate-Creatinine Ratio (mg/dL) | 1.1 ± 0.8 | 1.2 ± 0.6 |
| Tubular Reabsorption of Phosphate (%) | 72.3 ± 17.6 | 73.2 ± 16.3 |
| Fractional Excretion of Magnesium (%) | 3.2 ± 1.7 | 3.1 ± 1.9 |
| Fractional Excretion of Potassium (%) | 13.6 ± 2.7 | 13.5 ± 2.5 |

**Abbreviations**: LNS, lipid-based nutritional supplement; LNS-VM, lipid-based nutritional supplement with vitamins and minerals. GFR, glomerular filtration rate. Values are mean ± SD.



**Figure 1. Flow of the participants through the sub-study**

