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Effect of high-dose multivitamin supplements on alanine aminotransferase elevations among adults living with HIV on antiretroviral therapy in Tanzania

Sabina F. Mugusi[1,2*], David M. Sando[2], Ferdinand M. Mugusi[3], Claudia A. Hawkins[4], Said S. Aboud[5], Wafaie W. Fawzi[2] and Christopher R. Sudfeld[2]

Abstract

Background HIV infection can cause malabsorption and rapid utilization of nutrients. A randomized trial of multivitamin supplementation among people living with HIV/AIDS (PLWHA) initiating antiretroviral therapy (ART) in Tanzania was stopped early due to increased alanine aminotransferase (ALT) concentrations in the multiple recommended dietary allowances (RDA) multivitamin group. We conducted detailed analysis to assess the effect of multivitamins on ALT elevations and evaluate whether subgroups of PLWHA have greater hepatotoxicity risks associated with the use of high-dose multivitamins.

Methods We utilized data from a randomized, double-blind trial conducted in 2006–2009 that assessed the effect of high-dose multivitamins that contained vitamin B complex, vitamin C, and vitamin E at multiple RDA as compared to standard-dose multivitamins containing single RDAs among adults initiating ART in Tanzania. We evaluated the effect of high-dose multivitamins on incident mild/moderate ALT elevations > 40 IU/L, persistent ALT elevations > 40 IU/L (2 + clinic visits), and severe ALT elevations > 200IU/L using Cox proportional hazard models. We then evaluated effect modification by patient characteristics to determine if subgroups of PLWHA experienced different magnitudes of risk for ALT elevations associated with high-dose multivitamins.

Results High-dose multivitamins increased the risk of incident mild/moderate ALT elevations > 40 IU/mL as compared to standard-dose multivitamins (hazard ratio (HR): 1.41; 95%CI: 1.26,1.58) as well as incident sustained mild/moderate ALT elevations (HR: 1.19; 95%CI: 1.04,1.36), but there was no overall effect on severe ALT elevations (HR: 1.44; 95% CI: 0.91,2.28). There was no evidence that the effect of high-dose multivitamins on any or sustained mild/moderate ALT elevations was modified by any patient characteristic. However, CD4 T-cell count was found to modify the effect of high-dose multivitamins on severe ALT elevations (*p*-value for interaction:0.01). Among participants with a baseline CD4 T-cell count ≤ 100 cells/μL, individuals receiving high-dose multivitamins had 3.74 times (95%CI: 1.52–9.17) the risk of incident severe ALT elevations compared to standard-dose multivitamins, while participants

*Correspondence:
Sabina F. Mugusi
sabina.mugusi@gmail.com; smugusi@muhas.ac.tz

Full list of author information is available at the end of the article

with CD4 T-cell counts > 100 cells/μL, appeared to have no effect of high-dose multivitamins on severe ALT elevations (HR:0.92; 95% CI: 0.50,1.67).

Conclusions High-dose RDA multivitamin supplementation increased the incidence of any mild to moderate ALT elevations among adults starting ART in Tanzania and the magnitude of the risk does not appear to differ by patient characteristics. However, immunocompromised PLWHA with CD4 T-cell counts < 100 cells/μL may experience greater risk of severe ALT elevations associated with the use of high-dose multivitamins. Although the study findings offer significant insights, it is essential to take into account limitations imposed by newer cART regimes.

Keywords HIV, Antiretroviral, Multivitamins, ALT, Liver, Hepatotoxicity

Introduction

Universal access to combination antiretroviral therapy (cART) has revolutionized the management of human immunodeficiency virus (HIV) making it a chronic, yet manageable disease [1]. Unfortunately, with increased access and rapid scale-up of cART, there has been a rise in the number of people living with HIV/AIDS (PLWHA) experiencing adverse drug reactions (ADRs) [2, 3]. Adverse drug reactions, if not identified and managed early, may result in non-adherence or discontinuation of treatment, disease progression, or treatment failure [4, 5]. Hepatotoxicity has been identified as a safety concern for some cART regimens [6, 7]. The prominent aminotransferases, including alanine aminotransferase (ALT), are considered surrogate markers of hepatic stress or injury [8]. In addition to cART, opportunistic infections, co-infections with hepatitis B or C virus, alcohol abuse, non-alcoholic fatty liver disease (NAFLD), and toxicity related to co-medications are some of the factors accounting for liver enzyme abnormalities in people infected with HIV [9–12].

Micronutrient deficiencies are common in low- and middle-income countries (LMICs) but are particularly prominent among PLWHA [13–15]. HIV infection can cause malabsorption and rapid utilization of nutrients, increased basal metabolic rates, and protein catabolism leading to effects on micronutrient concentrations [13, 16]. Oxidative stress during HIV infection may also impair immune function due to deficiencies of vitamins B6, B12, and E [17]. Several randomized trials and studies have been conducted indicating that multivitamin supplementation may be beneficial for clinical outcomes among people living with HIV; however, other studies have found no effect of multivitamins on clinical outcomes [18–20]. There is currently no global recommendation for the provision of multivitamins for PLWHA on cART.

A double-blind, randomized controlled trial of vitamins and cART on HIV disease progression in Dar es Salaam Tanzania was done to evaluate the effect of high-dose as compared to standard-dose multivitamin supplements among people living with HIV that were initiating cART [21]. The trial was unexpectedly stopped early due to increased levels of alanine transaminase (ALT) among PLWHA receiving the high-dose multivitamin supplement. In terms of efficacy, there was no effect of high-dose supplements on the risk of HIV disease progression or death as compared to standard-dose multivitamin supplements among PLWHA before stopping the trial. In this study, we conducted a detailed analysis to characterize the effect of high-dose multivitamins on ALT elevations and evaluate whether subgroups of study participants had greater hepatotoxicity risks associated with the use of high-dose supplements. These results are intended to inform safety monitoring in future research as well as among patient populations of people living with HIV that may be choosing to take high-dose multivitamin supplements concurrently with cART.

Methods

This study utilized data from a randomized, double-blind trial that assessed the effect of daily oral supplements of vitamins B-complex, C, and E in multiple versus single recommended dietary allowances (RDA) on HIV disease progression that was conducted in Dar es Salaam, Tanzania between November 2006 to March 2009 (clinicaltrials.gov Identifier: NCT00383669) [21]. Inclusion criteria for trial participants were adults aged ≥ 18 years, confirmed HIV infection, and ready to initiate cART at enrolment. Pregnant and lactating women were excluded from the study. During the time of the study (2006–2010) the World Health Organization (WHO) and Tanzanian HIV/AIDS treatment guidelines recommended PLWHA to be initiated on cART when they had a WHO clinical stage IV disease or CD4 count of less than 200cells/μL, or with WHO clinical stage III disease together with a CD4 count of less than 350cells/μL [22]. The first-line cART combination during the study period included stavudine (d4T), lamivudine (3TC), nevirapine (NVP), zidovudine (AZT), and efavirenz (EFV). Based on these recommendations the possible ART regimen combinations used were either d4T + 3TC + NVP/EFV or AZT + 3TC + NVP/EFV. Enrolled study participants were randomized 1:1 to receive either the standard dose multivitamins (single RDA) or high-dose multivitamins (multiple RDAs; 2 to 21 times the RDA for the B vitamins, 2 times the RDA

for vitamin E, and 6 times the RDA for vitamin C) [21]. Allocation was concealed using multivitamin bottles that were prelabelled with participant identification numbers. Trial participants, research staff, and investigators were blinded since the two multivitamin supplement regimens were identical in appearance and taste. The study was approved by the institutional review boards of the Harvard School of Public Health, Muhimbili University of Health and Allied Sciences, Tanzania Food and Drugs Authority, and National Institute of Medical Research. The study was stopped early in March 2009 on the recommendation of the Data and Safety Monitoring Board due to increased ALT concentrations in the high-dose multivitamin supplement.

At enrollment, study nurses collected socio-demographic and clinical data together with participants past medical history using a standardized questionnaire. Study nurses also collected participant height and weight using standardized procedures. A full laboratory workup at baseline before cART initiation was done to quantify absolute CD4 T-cell count (FACSCalibur flow cytometer, Becton Dickinson, San Jose, CA), serum ALT concentration (Cobas Integra 400 plus analyzer by Roche Diagnostics System), complete blood count (CBC) (AcT5 Diff AL analyzer, Beckman Coulter, Miami, FL), and HIV viral loads (Cobas Amplicor HIV-1 Monitor test version 1.5, Roche Diagnostics Systems). Serum ALT, CD4, CBC, and viral load tests were repeatedly assessed at 4 monthly intervals during the study duration after the randomization. Elevated serum ALT levels were defined based on the Division of AIDS criteria for Grading the Severity of Adult Adverse Events [23]. Elevated ALT levels were defined as >40 IU/L or >1x the upper limit of normal (ULN), persistent mild/moderate ALT elevations were defined as ALT>40 IU/L at two or more consecutive ALT assessments and severe ALT elevations were defined as patients having ALT levels>200IU/L or >5x ULN [21].

Statistical analysis

All analyses were conducted based on the intention to treat principle. The analytic study population included all participants with at least 1 post-baseline ALT measurement to assess the incidence of ALT outcomes. Time-to-event analyses were conducted since there were different amounts of follow-up time for each participant due to the trial being stopped early. We analyzed the effect of randomized multivitamin supplementation on the time to first mild/moderate ALT elevation>40 IU/L, persistent mild/moderate elevation of ALT>40 IU/L, and time to first ALT>200 IU/L. Individuals with the outcome of interest at baseline, either ALT>40 IU/L or >200 IU/L, were excluded from analyses of incident mild/moderate and severe ALT elevation analyses, respectively.

We then examined baseline effect modifiers that may alter the relative impact of multivitamin supplements on ALT elevations. Effect modifiers to define subgroups of interest were selected based on a literature review of demographic and biological factors that may alter the risk of hepatotoxicity including sex (male, female), age (<35 and ≥35 years), WHO clinical stage (I/II, and III/IV), CD4 T-cell count (<100 and ≥100 cells/μL), antiretroviral regimen (d4T- or NVP-based), body mass index (BMI) (<18.5 vs.≥18.5 kg/m^2), hemoglobin level (<8.5 and ≥8.5 g/dL), cholesterol concentrations (<200 vs. ≥200 mg/dL), and triglyceride concentrations (<150 vs. ≥150 mg/dL). The likelihood ratio test (LRT) was used to assess the statistical significance of interaction terms. All p-values were 2-sided with p-values<0.05 considered statistically significant. Statistical analyses were performed using Stata version 15 (StataCorp, College Station, TX).

Results

The participant flow chart is presented in Fig. 1. A total of 3,418 PLWHA were randomized between November 2006 and November 2008 to either the high-dose or standard-dose multivitamin groups. The study was stopped early in March 2009 when the median length of follow-up was 15 months (interquartile range (IQR), 6–19). Follow-up ALT assessment data was available for 3,023 (88.4%) participants and these participants were considered the analytic study population.

Table 1 summarizes the baseline socio-demographic, clinical and laboratory characteristics of the study population. Two thirds of the population were females (68.3%) and the median age was 37 years (IQR: 31.9–42.9). The median CD4 T-cell count at baseline cART initiation was 123 cells/μL (IQR:58–190) with over a third of the participants (41%) being severely immunocompromised with CD4 T-cell counts ≤100cells/μL. The majority of the participants were on stavudine as compared to zidovudine-based ART (73.9% vs. 26.1%), and similarly majority were on nevirapine compared to efavirenz (72.2% vs. 27.8%). At trial baseline, 2,667 (88.2%) participants had normal ALT concentrations≤40 IU/L, 353 (11.7%) had mild/moderate ALT elevation>40IU/L and <200 IU/L, and 3 (0.1%) participants had a severe ALT elevation>200 IU/L at randomization.

During follow-up, 1195/2667 (44.8%) participants experienced incident mild/moderate ALT elevations>40IU/L among those with normal ALT (<40IU/L) concentrations at baseline. A total of 75/3020 (2.5%) participants experienced incident severe ALT elevations among those with ALT concentrations<200 IU/L at baseline. Individuals randomized to the multiple RDA multivitamins had significantly increased hazard of incident mild/moderate ALT elevations as compared to single RDA multivitamins (HR: 1.41; 95% CI: 1.26, 1.58). Figure 2 presents

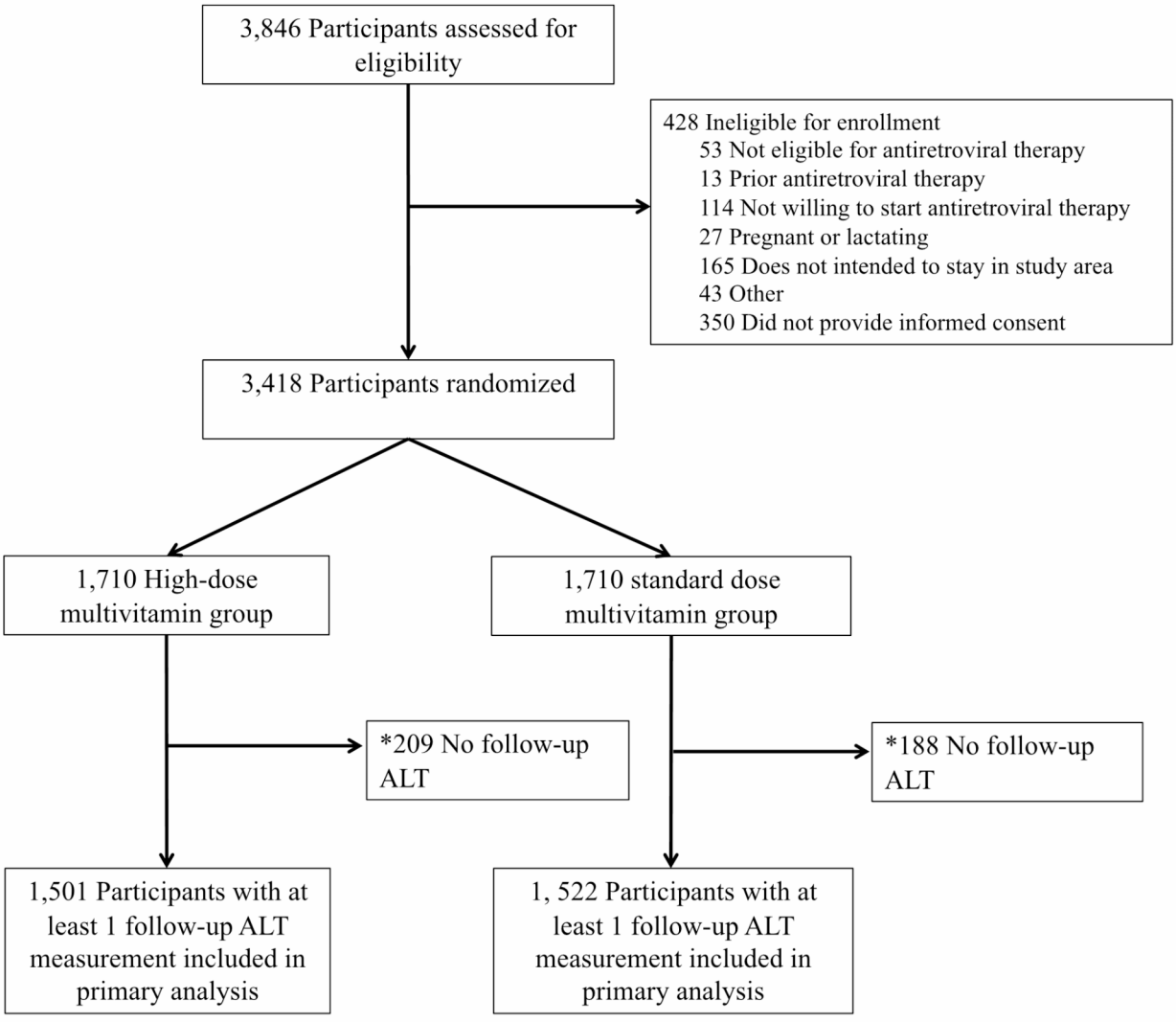

Fig. 1 Flow chart of participants
* Participants excluded due to missing follow-up serum ALT levels.

the Kaplan-Meir survival curve for time to incident mild/moderate ALT elevations; those randomized to multiple RDA multivitamins had a median time to mild/moderate ALT elevation of 365 days compared to 573 days for those randomized to single RDA (log-rank $p<0.001$). Individuals randomized to multiple RDA multivitamins also had significantly increased risk of sustained mild/moderate ALT elevations (HR: 1.19; 95% CI: 1.04, 1.36; *p*-value: 0.01); however, there was no statistically significant overall effect on incidence of severe ALT elevations (HR: 1.44; 0.91, 2.28; *p*-value: 0.12).

We then evaluated effect modifiers or subgroups of individuals that may experience greater risks of ALT elevations associated with use of multiple RDA multivitamins (Table 2). There was no evidence that any baseline factor including sex, age, BMI, WHO HIV disease stage, CD4 T-cell count, cART regimen, alcohol intake, TB co-infection, hepatitis coinfection, hemoglobin concentrations, total cholesterol concentrations, or triglyceride concentration modified the effect of multiple RDA multivitamins on any or persistent incident mild/moderate ALT elevations (p-values for interaction>0.05). Although there was no statistically significant interaction

Table 1 Baseline, sociodemographic and clinical characteristics by randomized single or multiple RDA multivitamin group (n=3,023)

		Standard-dose multivitamin (*N* = 1,501)	High-dose multivitamin (*N* = 1,522)
Sex	Females	1005 (66.9%)	1061 (69.7%)
	Males	496 (33.1%)	461 (30.3%)
Age, years	≤35	622 (41.6%)	584 (38.5%)
	> 35	874 (58.4%)	935 (61.5%)
WHO HIV disease stage	I or II	333 (24.3%)	318 (22.4%)
	III &IV	1041 (75.7%)	1101 (77.6%)
CD4 T-cell count, cells/μL	≤100	622 (42.7%)	591 (39.9%)
	> 100	833 (57.3%)	888 (60.1%)
Body mass index, kg/m^2	≤18.5	352 (25.3%)	354 (24.8%)
	> 18.5	1037 (74.7%)	1072 (75.2%)
Hemoglobin, g/dL	≥8.5	1142 (79.1%)	1137 (77.2%)
	< 8.5 Severe anaemia	302 (20.9%)	336 (22.8%)
d4T based cART regimen	No	378 (27.1%)	362 (25.2%)
	Yes	1018 (72.9%)	1075 (74.8%)
NVP based cART regimen	No	390 (27.9%)	398 (27.7%)
	Yes	1006 (72.1%)	1039 (72.3%)
Self-reported alcohol use	No	1347 (89.8%)	1397 (91.8%)
	Yes	33 (10.2%)	19 (8.2%)
Tuberculosis co-infection	No	1483 (98.8%)	1508 (99.1%)
	Yes	18 (1.2%)	14 (0.9%)
Hepatitis B	Negative	942 (93.9%)	985 (93.2%)
	Positive	61 (6.1%)	66 (6.8%)
Hepatitis C	Negative	767 (98.2%)	826 (98.5%)
	Positive	14 (1.8%)	13 (1.5%)
Total cholesterol, mg/dL	< 200	994 (88.3%)	1056 (88.1%)
	≥200	131(11.6%)	142 (11.8%)
Triglycerides, mg/dL	< 150	897 (80.0%)	916 (78.6%)
	≥150	224 (20.0%)	249 (21.4%)
Baseline ALT concentration (IU/L)	≤40	1330 (88.6%)	1337 (87.8%)
	41–200	169 (11.3%)	184 (12.1%)
	> 200	2 (0.1%)	1 (0.1%)

Abbreviations: WHO – World Health Organization; cART – combination antiretroviral therapy; d4T – Stavudine, NVP – Nevirapine; RDA – recommended dietary allowances, ALT – alanine amino transferase, IQR – Interquartile Range;

(*p*-value for interaction: 0.07), there was some indication individuals with a baseline BMI < 18.5 kg/m^2 may experience greater risk of persistent mild/moderate ALT elevations associated with use of multiple RDA multivitamins (HR: 1.51; 95% CI: 1.06, 2.15) as compared to those with a baseline BMI ≥ 18.5 kg/m^2 (HR: 1.05; 95% CI: 1.06, 2.15)

As for severe elevations, CD4 T-cell count was found to modify the effect of high-dose multivitamins on severe ALT elevations (*p*-value for effect modification: 0.01). Among participants with a baseline CD4 T-cell count ≤ 100 cells/μL, high-dose multivitamins had 3.74 times (95% CI: 1.52–9.17) the risk of incident severe ALT elevations as compared to standard-dose multivitamins, while among participants with a CD4 T-cell count > 100 cells/μL, there appeared to be no increased risk of severe elevations associated with use of multiple RDA multivitamins (HR:0.92; 95% CI: 0.50–1.67). No other factor was found to significantly modify the effect of multiple RDA multivitamins on the risk of severe ALT elevations (*p*-values > 0.05)

Discussion

In this secondary analysis of a randomized clinical trial of multivitamins for adults initiating ART in Tanzania we confirmed that multiple RDA multivitamins were associated with increased risk of mild/moderate ALT elevations. In addition, we add that multiple RDA multivitamins also increased the risk of persistent mild to moderate ALT elevations but there was no statistically significant overall effect on severe ALT elevations. We also examined whether subgroups of participants experience greater risk for ALT elevations associated with use of multiple RDA multivitamins. There was no significant evidence that the effect of multiple RDA multivitamins on incidence of any or persistent mild/moderate ALT elevations was modified by any patient characteristic.

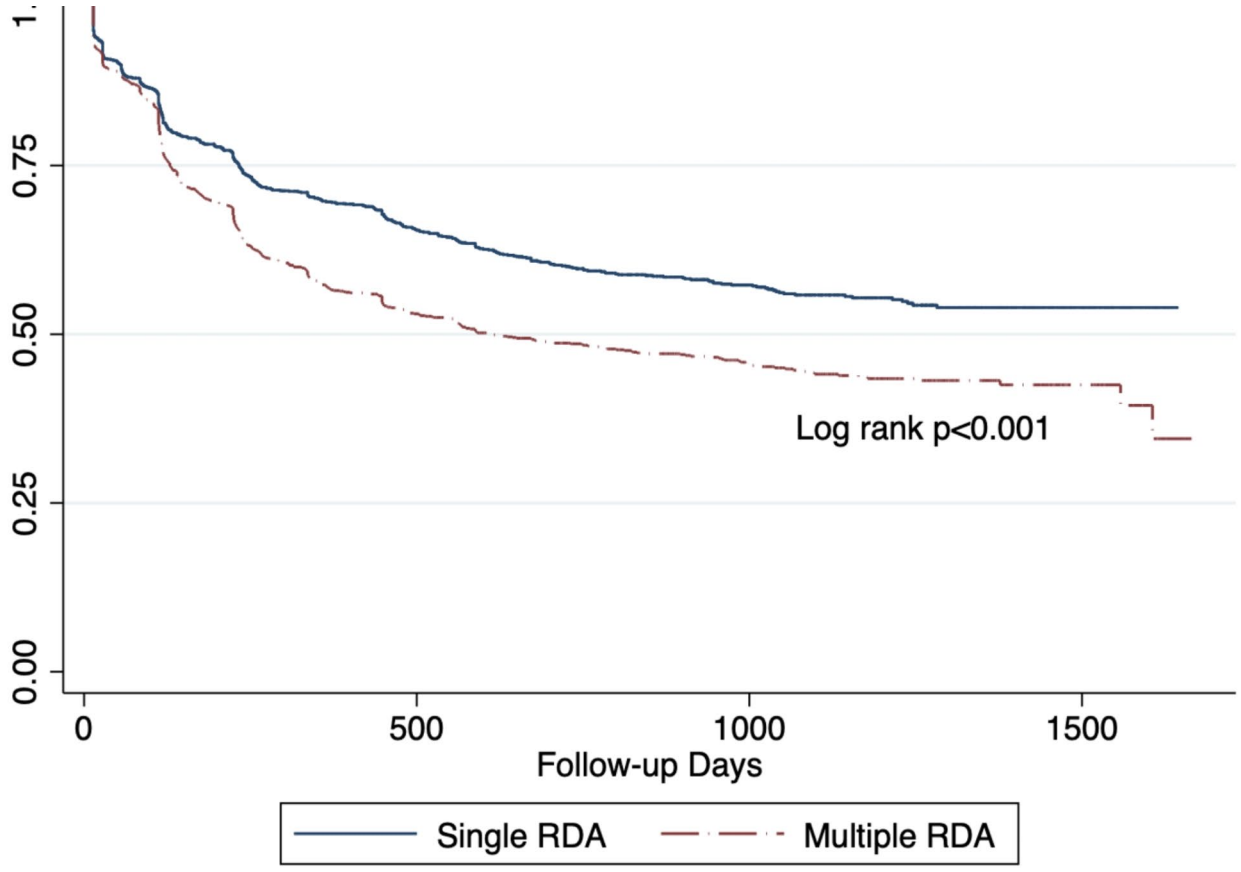

Fig. 2 Kaplan-Meier survival curve for mild or moderate incident ALT > 40IU/L stratified by randomization to single RDA and multiple RDA multivitamin regimen

However, we found that severely immunocompromised individuals with a CD4 T-cell count < 100 cells had greater risks of severe ALT elevations associated with multiple RDA multivitamins as compared to standard dose multivitamins

The study found that the effect of multiple RDA varied within different strata of CD4 cell count category (< 100cells/μL and > 100cells/μL). Other studies among African populations found a similar association of low CD4 cell counts and risk of having elevated ALT [24–26]. Mechanisms underlying this association are not clear, although direct immune activation and pro-apoptotic effects of HIV on hepatocytes as explained by a correlation with higher viral loads has been hypothesized [27]. Contradicting data were reported by different investigators of elevated liver enzymes being associated with higher CD4 counts of > 250 cells/μL particularly among female PLWHA [28, 29]. This is thought to be related to enhanced immunity postulated to decrease opportunistic infections during HIV infection thus contributing to the reduction of liver enzymes

The results suggests that the estimated effect of multiple RDA on PLWHA with CD4 cells < 100cells/μL was larger than the estimated effect of multiple RDA on PLWHA with CD4 cells > 100cells/μL in relation to severe ALT > 200IU/L. Among the participants with low CD4 cell counts, the addition of multiple RDA multivitamin to their cART regimen significantly increases the risk of having elevated ALT > 200IU/L. Mechanisms of multiple RDA in causing liver injury and hence elevated ALT still remains unclear, and potential drug-drug interactions of the high doses cannot be ruled out. We also postulate the use of thymidine analogues (either d4T or AZT) which were the common cART used by our study participants could also explain elevated ALT levels which causes long term mitochondrial toxicity [30]. Apoptosis of liver cells in HIV-infected CD4 cells has been believed to result from HIV infection itself and from concomitant antioxidant imbalances in host cells [31]. The possible mechanism postulated by Dusingize et al., was that the pathogenesis of liver damage in advanced HIV infection with low CD4 cell counts the body protein turnover is increased, resulting in low serum albumin levels and higher liver transaminases [32]. Shiferaw et al., also reported significant association of low CD4 cell counts (< 200) and elevated liver enzyme [26]

Table 2 Effect modification on incident mild or moderate ALT elevation (> 40 IU/L), sustained mild or moderate ALT elevation (> 40 IU/L at two or more consecutive visits), and severe ALT elevation (> 200 IU/mL)

	Incident mild or moderate ALT elevation (ALT > 40IU/L) (*n* = 2,667)		**Incident sustained mild or moderate ALT elevation (ALT > 40IU/L at 2 + consecutive visits) (*n* = 1,239)**		**Incident severe ALT elevation (ALT > 200IU/L) (*n* = 3,023)**	
	Hazard Ratio (95% CI)	***p*-value for interaction**	**Hazard Ratio (95% CI)**	***p*-value for interaction**	**Hazard Ratio (95% CI)**	***p*-value for interaction**
Overall effect high-dose vs. standard-dose multivitamins	1.41 (1.26–1.58)	-	1.19 (1.04–1.36)	-	1.44 (0.91–2.28)	-
Modifiers of interest						
Sex						
Females	1.39 (1.21–1.60)	0.87	1.16 (0.95–1.42)	0.98	1.78 (0.96–3.30)	0.28
Males	1.42 (1.17–1.73)		1.17 (0.90–1.52)		2.24 (1.11–4.54)	
Age, years						
≤35	1.32 (1.09–1.59)	0.33	1.23 (0.95–1.60)	0.57	1.48 (0.67–3.26)	0.96
> 35	1.48 (1.28–1.72)		1.12 (0.92–1.37)		1.45 (0.82–2.55)	
BMI (kg/m^2)						
≤ 18.5	1.53 (1.20–1.95)	0.44	1.51 (1.06–2.15)	0.07	2.07 (0.84–5.13)	0.49
> 18.5	1.37 (1.20–1.57)		1.05 (0.87–1.26)		1.42 (0.81–2.49)	
WHO HIV disease stage						
Stage I or II	1.37 (1.08–1.73)	0.83	1.25 (0.93–1.70)	0.46	3.28 (1.08–9.96)	0.11
Stage III or IV	1.42 (1.23–1.62)		1.09 (0.89–1.33)		1.24 (0.72–2.15)	
CD4 groups						
≤ 100cells/μL	1.47 (1.23–1.76)	0.62	1.04 (0.82–1.33)	0.29	3.74 (1.52–9.17)	0.01
> 100cells/μL	1.38 (1.19–1.61)		1.24 (0.99–1.54)		0.92 (0.50–1.67)	
d4T based cART						
No	1.19 (0.93–1.52)	0.12	1.18 (0.83–1.67)	0.86	1.54 (0.64–3.72)	0.94
Yes	1.49 (1.30–1.70)		1.14 (0.94–1.37)		1.60 (0.91–2.82)	
NVP based cART						
No	1.35 (1.06–1.72)	0.72	1.32 (0.94–1.85)	0.33	2.24 (0.85–5.91)	0.41
Yes	1.42 (1.24–1.62)		1.09 (0.90–1.32)		1.41 (0.81–2.44)	
Self-reported Alcohol Intake						
No	1.38 (1.22–1.55)	0.37	1.17 (0.99–1.37)	0.60	1.42 (0.88–2.29)	0.24
Yes	2.38 (0.95–5.97)		0.69 (0.23–2.12)		0.82 (0.13–4.89)	
TB co-infection						
No	1.41 (1.26–1.58)	0.83	1.17 (0.99–1.37)	0.63	1.44 (0.91–2.29)	Not estimable
Yes	1.24 (0.39–3.91)		0.74 (0.12–4.44)		No events	
Hepatitis B						
Negative	1.47 (1.28–1.70)	0.49	1.16 (0.95–1.42)	0.71	1.42 (0.80–2.54)	0.56
Positive	1.12 (0.64–1.98)		0.86 (0.40–1.81)		3.01 (0.61–14.9)	
Hepatitis C						
Negative	1.57 (1.33–1.84)	0.15	1.16 (0.92–1.46)	0.74	1.48 (0.74–2.95)	0.91
Positive	1.39 (0.52–3.75)		2.14 (0.42–11.07)		0.79 (0.05–12.6)	
Haemoglobin (g/dL)						
≥8.5 g/dL	1.40 (1.23–1.59)	0.17	1.17 (0.97–1.39)	0.55	1.56 (0.93–2.65)	0.12
< 8.5 g/dL	1.57 (1.21–2.03)		1.04 (0.72–1.51)		1.49 (0.52–4.31)	
Total Cholesterol (mg/dL)						
≤ 200 mg/dL	1.42 (1.23–1.62)	0.39	1.26 (1.03–1.53)	0.30	1.59 (0.89–2.85)	0.78
> 200 mg/dL	1.12 (0.78–1.62)		0.86 (0.54–1.38)		1.52 (0.25–9.11)	
Triglycerides (mg/dL)						
≤ 150 mg/dL	1.35 (1.17–1.57)	0.54	1.18 (0.96–1.45)	0.97	1.36 (0.71–2.62)	0.77
> 150 mg/dL	1.39 (1.05–1.85)		1.17 (0.79–1.73)		2.03 (0.68–6.07)	

Abbreviations: WHO – World Health Organization; cART – combination antiretroviral therapy; BMI – body mass index; TB – Tuberculosis; AZT – Zidovudine; 3TC – Lamivudine; d4T – Stavudine, NVP – Nevirapine; EFV – Efavirenz; RDA – recommended dietary allowances

Meta-analysis has demonstrated how micronutrient supplementations have improved the clinical picture of PLWHA by decreasing disease progression [33]. A systematic review of randomized clinical trials with selenium supplementation showed evidence of delayed CD4 decline among PLWHA [34]. A study by Sudfeld et al., showed that the national program provision of multivitamins was associated with improved clinical benefits, reduced TB incidence and improved survival among Tanzanian PLWHA [20]. Similarly, another study by Kaiser et al., showed that improvement in CD4 cell count reconstitution with micronutrient supplementation among cART naïve PLWHA [35]. Multivitamin supplementation in this cohort has shown to cause elevations of ALT, with a low prevalence of severe ALT elevation > 200IU/L (2.5%) and most elevations were transient, mild-moderate with none presenting with clinical features needing intervention. Results from our previous analysis showed higher risk of elevated liver enzyme > 200IU/L among PLWHA with hepatitis B co-infection (HBV and HCV) as well as a 3 times higher risk of elevated ALT > 200IU/L among PLWHA with self-reported alcohol consumption [36]. Larger studies are required in order to determine the efficacy and safety of multiple micronutrient supplements among PLWHA on cART to determine their short-term and long-term benefits

Limitations of the study

Some of the limitations in our study is that some study participants did not have complete laboratory data. However, analysis showed that missing ALT measurements was non-differential between the two groups and thus was unlikely to cause bias. Over time, cART treatment guidelines have changed, and our analysis was done on regimens that are not currently being used such as stavudine and nevirapine. Therefor this may not be generalizable to current PLWHA who are using newer regimens. The study participants received multiple micronutrient supplementations; hence our analysis was unable to determine which micronutrient component was related to the increased risk of altered liver enzymes. We performed interaction analysis however, this could therefore be a disadvantage in that these analyses compare smaller subsets of study subjects and thus have less precision than the primary study analysis. However, it is important that statistical interaction (effect-measure modification) should not be confused with biologic interaction

Conclusion

We confirm an increased risk of persistent and severe ALT elevations among PLWHA receiving cART and multiple RDA and its effects on CD4 cell counts. The PLWHA with low CD4 cell counts of < 100cells receiving multiple RDA have almost four times higher risk of having elevated ALT > 200IU/L. The overall effect of multivitamin supplementation on elevated ALT seems to be mild-moderate and transient in nature. Further studies looking into interactions of cART and multiple multivitamin supplementations are required to determine the optimal micronutrient doses

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Author contributions
Conceptualization, WWF and FMM; Data curation, SFM, DMS and CRS; Formal analysis, SFM, DMS and CRS; Funding acquisition, WWF, FMM, SSA, CAH and CRS; Investigations, WWF, FMM, SSA, CAH and CRS; Methodology, SFM, DMS and CRS; Supervision, WWF, SSA and FMM; Visualization, SFM, DMS and CRS; Writing original draft, SFM; Writing – review & editing, DMS, WWF, FMM, SSA, CAH and CRS.

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Data availability
The datasets generated during and/or analyzed during the current analysis are available from the corresponding author on reasonable request.

Declarations

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Institutional Review Board Statement
The protocol was approved by the institutional review boards of the Harvard School of Public Health (IRB12981), Muhimbili University of Health and Allied Sciences (MU/DRP/AEC/Vol.XVI/164), Tanzania Food and Drugs Authority (CD/TFDA.226/6), and the National Health Research Ethics Sub-Committee (NIMR/HQ/R.8a/Vol. IX/432).

Informed consent
All study participants provided a written informed consent to participate in the study.

Author details
[1]Department of Clinical Pharmacology, School of Medicine, Muhimbili University of Health and Allied Sciences, United Nations Road, P.O.Box 65001, Dar es Salaam, Tanzania
[2]Department of Global Health and Population, Harvard T.H. Chan School of Public Health, Boston, United States of America
[3]Department of Internal Medicine, School of Medicine, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania
[4]Center for Global Health, Division of Infectious Diseases, Northwestern University Feinberg School of Medicine, Chicago, United States of America
[5]Department of Microbiology and Immunology, School of Medicine, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania

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