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Original Article

Effects of "5 a day" fruit and vegetable intake on micronutrient level and oxidative stress markers in HIV positive patients: a cluster randomized trial

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Key Words
Counseling; HIV; Malondialdehyde

Abstract

Although evidence of the beneficial impact of antioxidant supplements in human immunodeficiency virus (HIV) patients exists, the effectiveness of dietary antioxidants is not certain for resource limited settings. The hypothesis that nutritional education and lifestyle modification can improve both dietary habits and antioxidant level, and reduce oxidative stress was tested in a cluster randomized trial, involving 5 health facilities, randomized either to intervention or the control group. The intervention consisted of two counselling lessons, each lasting 1,5 h/week during 6 months, on: "nutrition", "hygiene" and "coping with stigma and discrimination". Lessons were embedded in practical activities such as shopping tours at local markets, cookery seminars on regional food and workshops on healthy lifestyle. 201 participants were enrolled and followed-up for 24 months. After 6 months, mean malondialdehyde (MDA) increased by 6% in both groups. Fruit and vegetable intake increased from less than two servings a day to two or three in the intervention group compared to the control group. Zinc, iron, vitamin A, C and E, β-carotene and calcium intake significantly increased in the intervention group, compared to the control group. Positive correlation was observed between fruit intake and vitamin A and β-carotene; and between vegetable intake and vitamin A and vitamin C. Negative correlation was observed between fruit intake and MDA, and between CD4 cell count and MDA. The intervention provides an effective low-cost alternative improving dietary intake of fruits and vegetables, antioxidant intake, thus improving health outcomes in HIV infected patients.

INTRODUCTION

The human immunodeficiency virus (HIV) infection is characterized by a numerical and functional decline in CD4 cells, which progressively leads to the acquired immunodeficiency syndrome (AIDS) [1, 2]. Several factors including oxidative stress seem to be responsible for the pathogenesis of HIV [3]. The presence of the HIV in the body provokes the activation of phagocytic cells (macrophages and neutrophils), to destroy the micro-organisms, through the generation of reactive oxygen species (ROS). Excess production of ROS can attack double bonds in polyunsaturated fatty acids, inducing the production of lipid peroxidation products such as malondialdehyde (MDA) and 4-hydroxynonenal, which may result in more oxidative stress. Oxidative stress occurs when the balance between pro-oxidants and antioxidants is upset, and there is overproduction of ROS and resulting pathological effect [4].

Oxidative stress promotes the replication of the virus by up-regulating the activation of nuclear factor-kappa B (NF-κB), a transcriptional promoter of proteins which is involved in the inflammatory and acute-phase response. NF-κB is bound to I-κB (inhibitor of NF-κB) in the cytoplasm in its inactive form but tumor necrosis factor-α (TNF-α) and ROS can cause the activation of

NF-κB from I-κB. NF-κB then translocates to the nucleus and binds to the DNA, promoting transcription of HIV-1 [5, 6].

Studies have shown that other exogenous factors such as stress, induced by stigma and discrimination in HIV patients, excess alcohol and smoking could significantly increase the production of ROS in the body and influence the quality of life [6-8]. Previous investigations by Wu and Cederbaum [9] showed that alcohol could become a pro-oxidant where oxygen is needed to metabolize alcohol. Changes in the NAD^+/NADH ratio in the cell as a result of alcohol metabolism leads to the conversion of alcohol by the enzyme alcohol dehydrogenase to acetaldehyde (a toxic and reactive molecule) and then later on to acetate, thus enhancing the activity of the oxidative chain and production of ROS. Meanwhile the generation of ROS in cigarette smoke has long been established [10].

To prevent such damages, several enzymatic endogenous antioxidants exist, that can protect against ROS toxicity. Catalase for example, can convert ROS into less active molecules and prevent the transformation of these less active species to a more deleterious form. Besides, non-enzymatic exogenous antioxidants (ascorbate, tocopherol, carotenes, retinol, and polyphenols *etc*) available in food and especially in fruits and vegetables can neutralize free radicals by either acting on their own or in conjunction with the enzymatic system [11-13].

Although previous studies have ascertained the beneficial effects of micronutrient supplements in reducing the index of immune biomarkers in HIV patients, they are expensive for HIV patients in resource limited settings and often associated with side effects. Studies show that the supplementation of vitamin E for example increases the expression of C-C chemokine receptor type 5 (CCR5, also known as CD195) by preventing the production of its natural ligands, hence promote an increased infection of CD4 cells and increased viral load [14, 15].

In this study we took advantage of the abundant cheap and seasonally availability of fruits and vegetables in Cameroon [16], to evaluate the effect of fruits and vegetable intake on antioxidant level and oxidative stress markers in HIV positive patients in Yaounde, Cameroon.

METHODS

Study population and recruitment

The intervention was conducted between June 2010 and December 2012 in Yaounde. Considering the nature of the study, a cluster randomization by health facility was preferred to minimize the risk of contamination through exchange of information between the intervention and the control group during routine visits.

Health facilities (HF) offering HIV care and/or treatment and a minimum of 100 HIV patients registered, were eligible for inclusion. The recruitment of study participants was conducted in the HF in 3 stages:

1) Identifying HIV infected patients aged between 20 and 72, CD4 > 350 cells/µl, viral load < 100.000 copies of HIV in a milliliter of blood, and who were not receiving antiretroviral (ARV) drugs.

2) Counselling individual patients during an information meeting to explain both the study aim and procedure and to provide a chance to ask questions.

3) Giving patients a possibility to return their written informed consent.

Ethical approval was obtained from the national ethics committee of Cameroon (Authorisation N°106/CNE/ DNM/08), the Institutional Review Board of the Cameroon Baptist Health Unit (IRB2010-02), and the Ministry of Public Health in Cameroon (Division de la Recherche Operationnelle en Sante) (Authorisation Administrative de Recherche: N°631-0211).

Intervention phase

Participants in the intervention group (HIV-Care Programme Cameroon/HCP-Cam) received:

-***Individual counselling:*** here, the participants' nutritional status, nutritional need and nutritional knowledge were assessed using a 3-day dietary protocol, a food frequency questionnaire (FFQ) and self-administered questionnaires. Individual counselling took place during the first 2 weeks of the intervention phase and counselling duration was 30 min per participant. Subsequently, the intervention group of 100 participants were divided into 6 groups (16-20 participants/group) for group counselling.

-***Group counselling:*** group counselling included the following lessons:

-*HIV and nutrition:* effect of HIV on immune cells, effects of HIV on nutritional status, nutritional needs of HIV positive patients, composition of a balanced diet (emphasis was laid on the consumption of "5 a day" intake of fruits and vegetables, high intake of carbohydrates, high intake of protein of plant origin, *e.g.* kidney beans, soy bean *etc*, high intake of dairy products and water, low intake of fat), "one dollar shopping" (aimed to help participants buy the right food even with less financial resources), malnutrition (causes of malnutrition, use of nutrition to reduce effects of malnutrition), nutrition and ARV (interaction between food and ARV) and food preservation (adequate methods for food preservation, consequences of food preservation methods on nutritional content of

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food). Lessons were based on WHO guidelines [17-19].

HIV and Hygiene: personal hygiene, food and water hygiene and hygiene of the home (kitchen, toilet, home) [20].

-*Coping with stigma and discrimination:* the following questions were discussed; how to reconcile ones' situation with ones' self, reconciling one's self with others, and reconciling with the society. Coping strategies included; problem focused (*e.g.* joining a support group, getting counselling, *etc*) and emotion-focused (avoidance of problem, optimism, believing in God) [21, 22].

-*Physical activity:* participants were advised to practice physical activity (PA) at a moderate rate. Moderate PA was defined as 25-30 min walk per day, also equivalent to 2,500-3,000 steps in 30 min/day (*i.e.* 100 steps/min on level land) [23]. Lessons were accompanied by practical activities such as shopping tours in local market (participants were advised on when to go for food shopping, quality of good food, *e.g.* fruits and vegetables), cookery seminars on regional high quality food (participants were provided with practical tips for effective washing of fruits and vegetables before preparation, minimizing nutrient loss when cooking vegetables, preparation of soy milk, *etc*) and workshops on healthy lifestyle (role play on the use of condoms, reducing cigarette and alcohol intake). Food for cookery seminars was provided.

Transport cost was refunded for participants who attended the counselling meetings. Group counselling took place once a week over six months and meeting duration was 3 h per group. During this phase, facilitators were trained according to a standardized curriculum, to conduct the refresher sessions and support groups.

Participants in the control group (Usual-Care-Treatment Cameroon (UCT-Cam)) were subjected to the general practitioner's choice of therapy. In Cameroon, the usual care treatment for HIV/AIDS patients consists of periodic CD4 cell count and viral load check-up, and provision of family planning accessories such as condoms.

Follow-up phase

This phase continued for 24 months. Participants received refresher sessions, lasting 3 every 2 weeks for 12 months, and subsequently 3 h every month for 12 months. All refresher sessions were carried out by trained facilitators, assisted by the study dietician and coordinator.

Sample size considerations

With 6 practices, recruiting an average of 60 patients each, the study would have 80% power to detect a difference between the two groups of 20%, with a between cluster variance of 0.005 [24]. A difference of 20% was chosen as an estimate of clinically relevant change. To yield an average of 60 patients per HF and a 10% drop out rate, a minimum of 100 patients per health facility were contacted. After 4 months of recruitment, only about 100 participants were enrolled in each group. For these reasons and others related to funding, the study management decided to begin the intervention with a sample sized of 100/101 study participants per group.

Randomization and masking of treatment allocation

Prior to study initiation, potential HF were assessed to determine size number of HIV patients available, then randomly assigned to the intervention or control group, using a computer generated random list. This was done by an investigator not involved in the study, and stratification was done by HF size, allowing a high degree of matching (Fig.1). The code was held only by the study coordinator and data bank manager during the trial. Also, to preserve blinding, staff responsible for measuring and collecting health and socio-demographic outcomes, were unaware of group allocation.

Data collection and outcome measurements

Clinical and biochemical parameters: the main outcomes were changes in initial MDA concentration, changes in dietary habits (fruit and vegetable intake) and antioxidant level after 6 months. MDA was measured using the thiobarbituric acid (TBA) test. CD4 cell count was measured using the flow cytometry (FacsCalibur; Becton Dickinson Immunocytometry system (BDIS), San Jose, CA, USA). Plasma HIV viral load was measured with real time Abbot (Abbot Molecular Diagnostics, Wiesbaden, Germany),

Nutritional assessment: dietary intake was assessed with a 3-day dietary record; including all food and beverages consumed, portion size and method of preparation. Participants who could not read or write presented a relative who assisted them to record their dietary intake over 3 days, after which the participant were interviewed by a trained study staff to make sure the information recorded was coherent. Nutrient intakes were analysed using a Nutrition Database, EBIS (Ernährungsanamnese Beratungs Informations System; version 2011, University of Hohenheim, Stuttgart, Germany). The complete dietary analysis contains 46 nutrients, including vitamins A, C, E, β-carotene, zinc and iron. A food frequency questionnaire was used to assess the frequency of food intake, grouped in 9 major categories (meat, fish, vegetables, fruits, starchy food, dairy products, fats and oils, local Cameroonian dishes, miscellaneous).

Anthropometric measurements: height was measured to the nearest centimeter with a standiometer and weight to the nearest 0.1 kg with a standard scale (Seca 216 and 792, Hamburg, Germany).

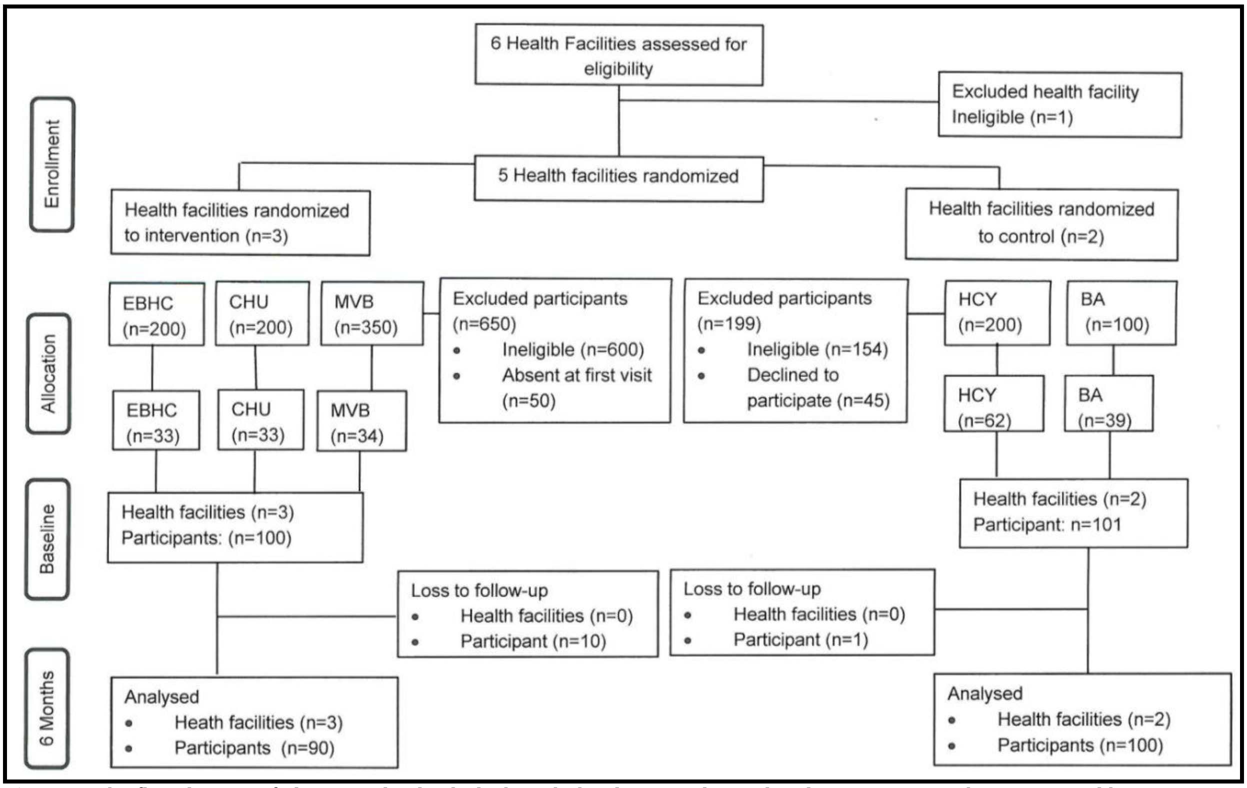

Figure 1. The flow diagram of clusters and individuals through the cluster randomized trial. EBHC, Etoug-Ebe Baptist Health Center; MVB, Mvog-Besi Hospital; CHU, University Teaching Hospital; HCY, Yaounde Central Hospital; BA, Biyem-Assi Hospital.

Questionnaire: a prior to study start validated self-administered questionnaire included the following items;

1) demographic information: age, region of origin, educational level, occupation, marital status and socioeconomic status

2) medical history and medication intake

3) quality of life

4) physical education

5) feeding habits

6) alcohol intake and smoking

7) food and personal hygiene

8) coping with stigma and discrimination

9) personal judgement on program relevance

Assessments of all parameters and collection of data were conducted at baseline, after 3, 6, 12, 18, 24 and 30 months in the intervention group, and at baseline, after 6, 18 and 30 months in the control group.

Compliance

At the end of each weekly meeting, participants were asked to sign a register. Based on the number of weekly meetings attended, compliance was good with about 65% participation at each meeting, over the 6 months intervention period.

Data analysis

Statistical analysis was carried out using SPSS 20 (IBM Corporation, 2011). Baseline characteristics in the two groups were compared using an analysis of covariance (ANCOVA) for continous variables while the chi-square test was used for categorial variables. After 6 months, ANCOVA was used to compare both groups, adjusting for baseline variables and HF.

Finally, we also examined whether changes in fruit and vegetable intake, antioxidant and MDA were associated with baseline parameters. Data that was not normally distributed were log transformed before analysis. All analysis were conducted according to the intention-to-treat population and values were considered significant at $P < 0.05$.

RESULTS

Study population

The trial profile is shown in Fig.1. All five HF completed the trial. Of the 201 participants enrolled in the study, 190 were evaluated for outcomes after 6 months. The number of participants lost to follow-up was 10 (10%) in the intervention and 1 (1%) in the control group. The program was well attended with more than 90% of the participants judging the intervention as being relevant and useful for their health.

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Table 1 and 2 presents the baseline characteristics of health facilities and participants in both groups. The differences between the intervention and control group were not statistically significant at baseline with respect to their age, sex, marital status and; weight, BMI, CD4 count, duration of HIV and MDA. The difference in macro- and micronutrient intake between the groups was statistically significant, and somehow higher than the required daily allowance (Table 2). The highest level of education in both groups was secondary education and most patients belonged to the lowest socioeconomic strata with an average monthly income of 100,000 frs CFA (about 165 €).

Fruit and vegetable intake

After 6 months, data from the FFQ show that the frequency of fruit and vegetable intake increased from less than two servings a day to two or three servings a day and above in the intervention group compared to high frequencies for less than two servings a day in the control group The difference between the groups was not statistically significant in regards to fruit intake ($P = 0.197$), while the difference in vegetable intake was statistically significant ($P = 0.006$) (Figs.2-5). A small negative correlation was observed between fruit intakes and MDA concentration ($\rho = -0.189$, $P = 0.015$). After adjusting for clusters, no correlation was observed. No correlation was observed between vegetable intake and MDA. There was no correlation between BMI, CD4 and fruit and vegetable intake.

Micronutrient intake

Nutrient analysis from the 3 day dietary record after 6 months showed an increase of 3% in vitamin A intake in the intervention group compared to a 3% decrease in the control group ($P = 0.017$). β-Carotene intake increased by 10% in the intervention group; compared to a 10% decrease in the control group ($P = 0.009$). Vitamin C intake increased in the intervention group by 9% compared to a 4.5% increase in the control group ($P = 0.037$). Vitamin E intake increased by 9% in the intervention group compared to no change in the control group ($P = 0.02$). Zinc concentration in the intervention group increased by 11% compared to 20% increase in the control group ($P < 0.001$). Iron concentration in the intervention group remained the same compared to a 8.3% increase in the control group. Calcium concentration increased by 27.8% in the intervention group compared to 14.1 % in the control group ($P < 0.001$). Meanwhile a small correlation was observed between fruits intakes and vitamin A and β-carotene ($\rho = 0.165$, $P = 0.041$ and $\rho = 0.163$, $P = 0.044$, respectively). After adjusting for clusters, no correlations were observed. Also, a small positive correlation between vegetable intakes and vitamin A ($\rho = 0.164$, $P = 0.042$) and vitamin C ($\rho = 0.206$, $P = 0.011$) was observed. After adjusting for clusters, there was no correlation between these parameters.

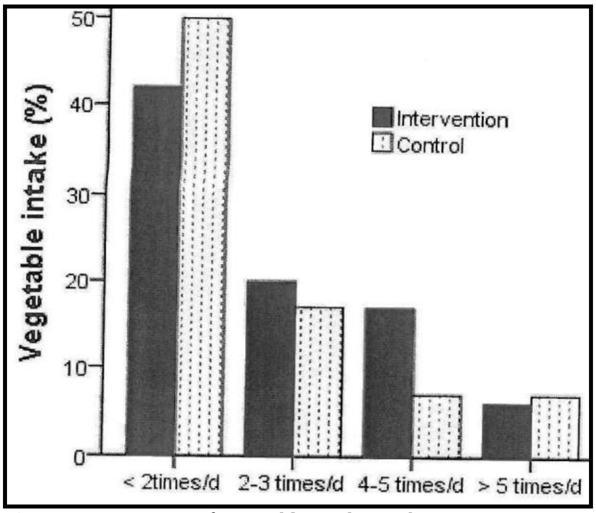

Figure 2. Frequency of vegetable intake in the intervention group compared to the control group at baseline.

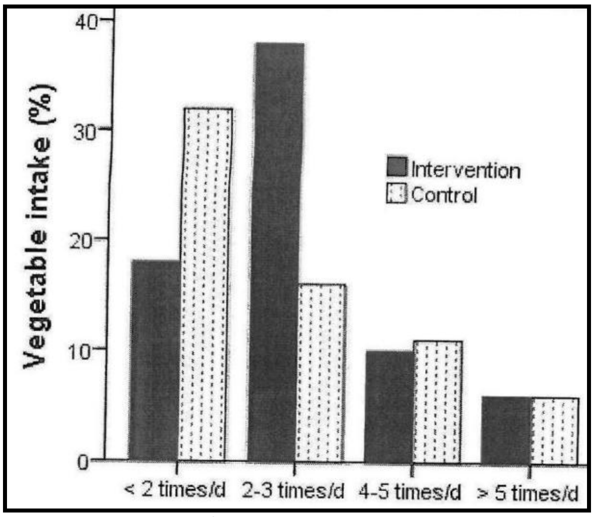

Figure 3. Frequency of vegetable intake in the intervention group compared to the control group after 6 month.

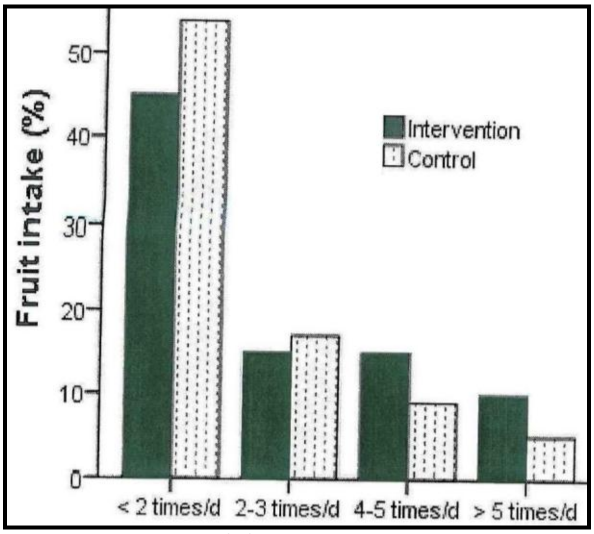

Figure 4. Frequency of fruit intake in the intervention group compared to the control group at baseline.

Table 1. Baseline characteristics of study participants: clusters, demographic, anthropometric and clinical parameters

Characteristics	Intervention group (n=100)	Control group (n=101)
Health facilities randomized (n (%))	3 (60)	2 (40)
Cluster size (n (%))		
Etoug-Ebe Baptist Hospital	33 (16.4)	0 (0)
University Teaching Hospital	33 (16.4)	0 (0)
Mvog-Besi Catholic Hospital	34 (16.9)	0 (0)
Yaounde Central Hospital	0 (0)	62 (30.8)
Biyem-Assi Hospital	0 (0)	39 (19.4)
Demographic parameters		
Age (years, mean ± SD)	33.0 ± 8.3	34.4 ± 10.0
Sex n (%)		
Male	31 (31)	35 (34.7)
Female	69 (69)	66 (65.3)
Region of origin (n (%))		
Centre, South, East	32 (32)	54 (53.5)
South West, Littoral	10 (10)	10 (9.9)
West, Northwest	53 (53)	30 (29.7)
Adamaoua, North, Far North	5 (5)	7 (6.9)
Marital status (n (%))		
Married	38 (38)	42 (41.6)
Unmarried	58 (58)	50 (49.5)
No response	4 (4)	9 (8.9)
Education (n (%))		
None	8 (8)	16 (15.8)
Primary	36 (36)	32 (31.7)
Secondary	37 (37)	38 (37.6)
University	19 (19)	14 (13.9)
No response	0	1 (1)
Employment (n (%))		
Yes	37 (37)	29 (28.7)
No	59 (59)	69 (68.3)
No response	4 (4)	3 (3)
Monthly income (frs CFA, n (%))*		
< 100.000	58 (58)	36 (35.6)
100.000 - 200.000	13 (13)	5 (5)
> 200.000	5 (5)	3 (3)
No response	24 (24)	57 (56.4)
Clinical and anthropometrical parameters (mean ± SD)		
Weight (kg)	70.1 ± 13	69.7 ± 14.7
Body mass index (kg/m²)	26.1 ± 4.2	25.9 ± 5.
CD4 (cells/µl)	603.8 ± 213.6	555.2 ± 198.2
Viral load (log)	4.5 ± 4.6	4.3 ± 4.4
Malondialdehyde (µmol/l)	3.3 ± 1.7	3.5 ± 2.7
Years since HIV disease was diagnosed	3.1 ± 2.1	3.7 ± 2.2

*100,000 frs CFA ~ 165 €; statistical estimates were based on an F-test and a chi-square test.

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Table 2. Baseline characteristics: lifestyle

Parameter	Intervention group (n=100)	Control group (n=101)	RDA
Nutrition			
Energy (Kcal)	2114.9 ± 496.9	2457.5 ± 966.1	2127.5[1]
Protein (g)	68.1 ± 22.2	84.8 ± 44.8	57.1
Fat (g)	81.7 ± 32.4	101.2 ± 49.9	65.6
Carbohydrate (g)	266.5 ± 70.8	288.8 ± 123.1	276.1
Vitamin A (µg)	3.3 ± 3.1	3.4 ± 3.6	800 (2.9)*
ß-Carotene (mg)	1.0 ± 0.8	1.0 ± 0.7	8 (0.9)*
Vitamin C(mg)	2.2 ± 2	2.2 ± 2.1	100 (2)*
Vitamin E (mg)	1.1 ± 0.7	1.3 ± 0.1	12 (1.1)*
Calcium (mg)	479.5 ± 253.5	482.2 ± 221.8	1000
Zinc (mg)	0.9 ± 0.5	1.0 ± 0.7	12 (1.1)*
Iron (mg)	1.1 ± 0.6	1.2 ± 0.8	15.0 (1.2)*
Alcohol intake (n (%))			
Yes	41 (41)	45 (44.6)	-
No	53 (53)	27 (26.7)	-
No response	6 (6)	29 (28.7)	-
Smoked in the past (n (%))			
Yes	9 (9)	7 (6.9)	-
No	79 (79)	69 (68.3)	-
No response	12 (12)	25 (24.8)	-
Physically active (n (%))			
Yes	68 (68)	61(60.4)	-
No	20 (20)	8 (7.9)	-
No response	12 (12)	32 (31.7)	-

RDA = required daily allowance. [1]Energy requirement for HIV asymptomatic patients increases by 10% compared to HIV negatives, thus (1934.1 + 193.4 = 2127.5 RDA). *log values for RDA.

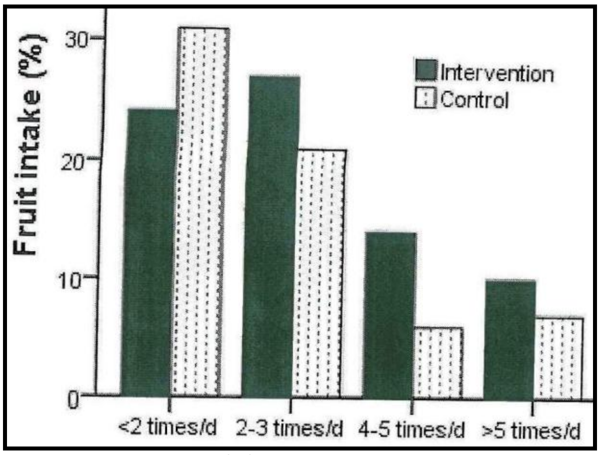

Figure 5. Frequency of fruit intake in the intervention group compared to the control group after 6 month.

Changes in MDA, BMI, CD4 cell counts 6

After 6 months, the mean MDA concentration increased by 6% in both groups compared to baseline (P = 0.934). Mean BMI increased by 1.9% in the intervention group compared with 3% decrease in the control group (P = 0.089). The mean absolute CD4 cell count dropped by 7% in the intervention group compared to 23% in the control group (P = 0.003). A negative Pearson correlation was observed for the comparison between CD4 cell count after 6 months and MDA (r = -0.216, P = 0.003), and a partial correlation after adjusting for clusters. (r = -0.209, P = 0.005)

DISCUSSION

Reactive oxygen species have been shown to play a vital role in cell signaling and control of the HIV infection. High levels of ROS can cause important damages on cell structure, affecting cell function, and leading to faster progression of the HIV infection [25]. There is increasing evidence that intake of antioxidants could reduce oxidative stress and improve immunological functions in HIV patients [13, 26]. Hence, in a cluster randomized trial, we investigated the effect of “5 a day” fruit and vegetable intakes on levels of antioxidants and MDA, an oxidative stress marker in ARV naive patients in comparison to ARV naive controls.

Results of this study show that the HIV-Care-Program was able to improve dietary habits (fruit and vegetable intake) and antioxidant intake in the intervention group compared to the control group (Figs.2-5), although the increase in the frequency of fruit and vegetable intake was relatively small compared to the enormous increase in antioxidant level observed in the dietary record. This could be due to size of fruit or vegetable intake at a given point of time. John *et al* [27], after administering 80 g of fruit and vegetable, observed a significant increase in plasma concentration of α- and β-carotene as well as ascorbic acid, with small changes in self-reported frequency of fruit and vegetable intake. Also, nutrient analysis from the 3 days dietary record showed that apart from calcium, zinc and iron with intakes below the required daily allowance (RDA), vitamins A, C, E and β-carotene intake for both groups were higher than RDA (Table 2). A possible explanation for this could be due to over-reporting usually observed in studies, when reporting dietary intake [28, 29]. The positive correlation between fruit intake and β-carotene, as well as vegetable intake and vitamin C, is similar to observations made by Winkler *et al* [26] after ingestion of fruit juice and vegetable concentrate. However, after adjusting for clusters, there was no correlation observed between fruit and vegetable intake and micronutrient intake.

The authors observed an increase of 3% in MDA concentration after 6 months in both the intervention and the control group despite an increase in dietary antioxidant intake in the intervention group. This increase was neither statistically nor clinically relevant. Similar observations have been made by other researchers supplementing with antioxidants in HIV patients [26, 30]. This could be an indication that the interaction between T-cell apoptosis and MDA metabolism is a complex process and does not depend solely on intake of antioxidants.

After 6 months, mean CD4 cell count decreased by 46 cells in the intervention group compared to 129 cells in the control group. The difference between the groups was statistically and clinically relevant; implying the rate of decline in CD4 cells was slower in the intervention group compared to the control group (Table 3). Similar observations were made by Muller *et al* [30], after antioxidant supplementation. A negative correlation was observed between CD4 and MDA. Ibeh and Emeka-Nwabunnia [31] made similar observations with HIV patients. Contrary to our study, these were already taking highly active antiretroviral therapy (HAART). Studies indicate that intake of HAART is associated with side effects including oxidative stress, which would further increase MDA concentration [3, 32].

A negative correlation was observed between fruit intakes and MDA, although MDA concentration did not decrease in our study. A potential reason could be the excessive intake of antioxidant through fruits and vegetables, while the quantity of antioxidant consumed through fruits and vegetable was not directly measured. Rolina and Lindi [33] suggest that excessive supplementation of antioxidants could cause fluctuating CD4 cell concentration, which could indirectly affect the rate of oxidative stress. Birringer and Ristow [15] indicate in a review, that high doses of antioxidant supplements may have adverse effect on health, while asserting that consumption of fruits and vegetable can delay the development of various diseases in the body. Another reason for the lack of awaited effect on MDA could be linked to the rate of physical activity practiced by our patients. Evidence exists that exercise increases ROS and oxidative stress [34]. Previous studies *in vitro* investigated the effects of exercise on MDA, a marker of oxidative stress, and found a 90% increase in MDA after a 20 meter run per min [35, 36]. Since our results are based on self-reporting, the possibility that a rate of underreported physical activity exists.

Table 3. Comparison of micronutrient intake and biological parameters after 6 months in both groups

Micronutrient	Intervention group (n=90)	Control Group (n=100)	P-value
Vitamin A (µg)	3.4 (3.4, 3.5)	3.3 (3, 3.4)	0.017
β-Carotene (mg)	1.1 (1.1, 1.2)	0.9 (0.6, 1.2)	0.009
Vitamin C (mg)	2.4 (2.3, 2.4)	2.3 (1.9, 2.5)	0.037
Vitamin E (mg)	1.2 (1.1, 1.2)	1.3 (1.1, 1.4)	0.02
Zinc (mg)	1.0 (0.9, 1)	1.2 (1.1,1.3)	< 0.001
Fe (mg)	1.1 (1.1, 1.2)	1.32 (1.2, 1.4)	< 0.001
Calcium (mg)	612.9 (533.7, 692.2)	440.1 (17, 707.2)	0.01
Biochemical and clinical parameter			
MDA (µmol/l)	3.56 (3.1, 4)	3.76 (2.3, 5.2)	0.934
BMI (kg/m^2)	26.6 (24.9, 29)	25.1 (23.7, 26.4)	0.089
CD4 (cells/µl)	557.3 (477.9, 636.8)	432.4 (370.6, 494.3)	0.003

Values shown are mean and confidence interval (CI) in brackets; log transformed for vitamin A, C, E, zinc and iron values are based on ANCOVA after 6 months, adjusted for baseline CD4 and clusters.

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Several limitations of our study should be taken into consideration. Cluster randomization generally reduces the units available for allocation of trial groups. This trial originally included a relatively small cluster of 6 HF, but due to the one year delay observed in starting the intervention, potential participants from one health facility where completely lost, since CD4 count decreased during this period to values below 350. Thus, this HF could no longer take part in the study. Also, the overall small number of HF is a result of the limited number of HF involved in the care and/or treatment of HIV/AIDS patients in Yaounde. A larger trial would have allowed more precise estimation of the intervention and detection of smaller differences between groups. Further, during the period between study design and implementation, changes in the global recommendations for initiation of HIV patients to ARV were made, changing the criteria for ARV initiation from 250 cell/µl to 350 cells/µl [37], limiting the number of HF as well as patients fulfilling our study criteria. The subjective approach (questionnaire) used to measure knowledge on nutrition and lifestyle was based on self-reporting [28]. Therefore, participants' response on delicate topics may be inaccurate, reflecting what participant feel the investigator may wish to hear or think about them [38]. Also, in this study, nutrient intake was estimated using a FFQ and a 3 day dietary record, which could be susceptible to bias. Also, the fact that portion-size of fruit and vegetable was not considered, greatly limits our study. For future studies, direct measurements of fruit and vegetable portion-size consumed at each point of time as well as antioxidants would provide more accurate results. While quantification of antioxidants, especially water soluble vitamins may be influenced by handling, storage, processing and preparation [12, 39].

This study demonstrates that an intervention based on nutritional education and lifestyle modification for ARV naive HIV infected patients in a sub-Saharan low/middle income country setting can significantly improve dietary habits and improve micronutrient level. Although there was no significant change in MDA concentration after 6 months, the negative Pearson association observed between MDA and CD4 is an indication that MDA might be used as an additional clinical factor in assessing HIV disease progression.

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COMPETING INTERESTS

The authors declare that they have no conflicts of interest.x

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