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The role of micronutrients in the diet of HIV-1-infected individuals

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1. ABSTRACT

Vitamins, zinc and selenium are important micronutrients that play crucial functions at the cellular and molecular level. Immune response of several different cell types can be modulated by these micronutrients. Deficiency in micronutrients has been extensively reported in HIV-1-infected individuals and further correlated with CD4+ T-cell count, HIV-1 plasma viral load, disease progression and mortality. Supplementation by micronutrients has had controversial effects. Thorough future investigations and trials are certainly needed to strategically plan evidence-based interventions. Here, we review the available data on use of micronutrients during the course of HIV-1 infection.

2. INTRODUCTION

Highly active antiretroviral therapy (HAART) has led to viral suppression, to better immune function and ultimately to a consistent decrease in AIDS-related mortality and morbidity (1). HAART has significantly reduced HIV-RNA levels in blood, seminal plasma and vaginal secretions, even though it does not eradicate human immunodeficiency virus-1 (HIV-1) (2-6). Different therapeutic strategies, including a combined antiretroviral-immune approach, have been attempted to stimulate HIV out of latency, in order to reach HIV eradication, with no conclusive results (7, 8).

Because of its influence on both humoral and cell-mediated immune function, nutritional status has been extensively studied in HIV-1-infected subjects. A deficient micronutrient profile has been reported in HIV-1 positive individuals even in HAART era (9). Micronutrients, such as vitamins, selenium and zinc, exert important physiological functions at several levels. In fact, micronutrients interfere with immunologic functions, oxidative stress and many other metabolic processes, that are necessary to achieve a good health status (9).

Vitamins, as well as zinc and selenium deficiency, have been detected in HIV-1-infected individuals and have been associated with advanced disease progression and mortality. Based on this evidence, the effect of micronutrient supplementation has been investigated, especially, but not only, in developing countries, where HIV-1-infection is often accompanied by undernutrition, diarrhoea and other infections (9).

This review focuses on the role of micronutrients in HIV-1-infection and disease progression, from basic research data to clinical investigations and trials.

3. OXIDATIVE STRESS, SELENIUM AND ZINC IN HIV INFECTION

3.1. Oxidative stress

Oxidative stress is thought to play an important role in HIV disease progression. Reactive oxygen species (ROS), which include superoxide, hydrogen peroxide (H_20_2), and hydroxyl free radical, lead to oxidative stress (OS) and are normally counterbalanced by antioxidants. The alteration of this fine balance is responsible for OS and it ultimately causes cell damage (10).

HIV infection is characterized by high levels of oxidative stress; ROS stimulate HIV replication through the induction of oxygen-responsive transcription factors NF-kB and AP-1, which, in turn, activate HIV replication at a transcription level (10-15). Furthermore, chronically infected human T cells show decreased levels of thiol, as well as antioxidant enzymes, such as thioredoxin, superoxide dismutase and catalase (10-14).

Importantly, HIV activation can be inhibited by the use of antioxidants, such as glutathione (GSH), glutathione ester, N-acetylcysteine (NAC), catalase and glutathione peroxidase (GSHPx-1). GSHPx-1 is a selenium-dependent enzyme, capable of detoxifying both hydrogen and lipid peroxides, representing a major cellular defense against ROS (14). GSH has been noted to decrease HIV replication by the selective inhibition of HIV envelope glycoproteins (16). Furthermore, GSH and NAC inhibit HIV-1 replication in chronically infected cells, such as human primary monocytes and macrophages (17-21). Of importance, lower levels of GSH were found in the plasma and lung epithelial lining fluid (ELF) of symptom-free HIV-1-seropositive subjects, compared with healthy individuals (22), as well as in CD4+ T cells, CD8+ T cells and PBMCs (peripheral blood mononuclear cells) of HIV-1-infected individuals (19,23). Moreover, GSH deficiency in CD4+ T cells was associated with decreased survival (24); on the contrary, GSH intracellular levels positively correlated with the absolute CD4+ lymphocyte count and were lower in patients with more advanced immunodeficiency (25).

Look *et al* determined the levels of plasma thiol (-SH), GSH and erythrocyte glutathione peroxidase (GSH-Px) activity in HIV-1-infected patients and HIV-negative controls. GSH-Px activity was significantly lower in AIDS patients as compared with stage-I patients and healthy subjects. GSH-Px activity was positively correlated with serum selenium, showing the selenium-dependent activity of GSH-Px. Plasma SH and GSH levels were also lower in HIV-1-positive patients in comparison with healthy controls (14).

In a recent article, Suresh *et al* evaluated OS and antioxidants levels in 50 HIV-1-infected individuals and 50 healthy controls. The levels of vitamin C, vitamin E, superoxide dismutase (SOD), serum malondialdehyde (MDA) and total antioxidant capacity (TAC) were measured. MDA was used to determine lipid peroxidation, SOD to evaluate the dismutation of superoxide anion radical, TAC (measured by ferric reducing antioxidant assay) to establish the total effect of the antioxidants present in body fluids. The authors found that MDA levels were higher in HIV-1-positive patients, compared to healthy controls, while the levels of vitamin C, E, TAC and SOD were lower in HIV-1-infected patients *versus* HIV-1-negative controls. TAC levels were inversely correlated with MDA. They concluded that OS does occur in HIV-1-positive patients and that it increases with the progression of the disease; moreover, TAC was proposed as a possible marker of OS in HIV-1infected patients (26).

To evaluate the role of HAART in oxidative imbalance in HIV-1-infected individuals, Mandas *et al* measured the serum oxidant and antioxidant levels in 86 HAART-treated patients, 30 untreated patients and 46 healthy controls. The authors found higher serum oxidant levels and lower antioxidant levels in the HIV-1-treated population as compared to the other groups. They concluded that HAART had a synergistic effect with HIV on oxidative stress (27). On the other hand, Wanchu *et al* found no difference in terms of oxidative stress among HIV-1-infected patients on antiretroviral therapy and untreated controls (28).

Since GSH deficiency can be replenished by the administration of NAC, a prodrug used to supply cystein in acetaminophen overdose, De Rosa *et al* tested the administration of NAC in HIV-1-infected individuals with low GSH, CD4+ T cell count less than 500/mm^3 and no active opportunistic infections. NAC treatment for 8 weeks was shown to be safe and able to replenish whole blood and T cell GSH levels; moreover, oral NAC administration was associated with increased survival (2-3 years), suggesting the need for further trials (29). Similarly, Milazzo *et al* have recently assessed the protective effect of antioxidant supplementation on mitochondrial function in a cohort of HIV-1-infected patients with HAART-related lipoatrophy (30).

On the other hand, Sandstrom *et al* demonstrated that GSHPx-1 enhances HIV-1 replication *in vitro*, suggesting that a limited oxidative stress could control HIV spreading by predisposing T-cells to apoptosis (31).

3.2. Selenium

Selenium (SE) is an essential micronutrient that carries out its biological effects through its incorporation into selenoproteins; it is involved in the regulation of oxidative stress in almost all cells and tissues and it has been shown to play a key role in both cell-mediated and humoral immune responses. Several studies have remarked the antiviral effect of SE, whose protective role has been demonstrated against coxsackievirus in Keshan disease, influenza virus, poliovirus and in HIV infection (32).

In vitro, Makropoulos *et al* evaluated the effect of SE supplementation on the transcription factor NF-kB, whose activity is induced by several pro-oxidant factors. Considering that NF-kB has been described as a key regulator of HIV activation from a latent state, the authors investigated the possibility to interfere with NF-kB-controlled HIV replication, through the antioxidant properties of SE. In T-cell lymphocyte cultures, treatment with SE was able to strongly reduce NF-kB activation, suggesting that SE may be efficient to modulate the expression of NF-kB target genes, like tumor necrosis factor-alpha (TNF-alpha), and to inhibit HIV replication (33).

Kalantari *et al* demonstrated that SE inhibited HIV in a TNF-alpha-induced HIV replication system, in monocyte cultures. Furthermore, SE negatively influenced HIV-1 replication, through the selenoprotein TR1, by regulating Tat-dependent transcription independently of NF-kB (34). Another study demonstrated that HIV-1-infected human Jurkat cells had lower levels of selenoproteins compared to uninfected cells (35). These data were further confirmed in Rhesus monkeys by Xu *et al* (36).

Several trials have investigated the blood levels of SE in HIV-1-infected patients at different stages of HIV-1 infection and the effects of SE supplementation on HIV disease progression (37). In 1988, Dworkin *et al* had already reported SE deficiency in AIDS patients compared with healthy controls (38). These data were further confirmed by other investigations (39-46). A cross-sectional study conducted by Look *et al* on 104 HIV-1-infected patients at different stages of HIV disease, showed that serum SE levels were lower in HIV-1-infected subjects than in healthy controls. SE concentrations directly correlated with CD4+ T cell count and inversely correlated with serum levels of soluble TNF-alpha receptor II, neopterin and beta2-microglobulin. Opportunistic infections, neoplasms and hepatitis C coinfection were associated with lower SE levels (14). A subsequent study by Baum *et al* on 125 HIV-1-infected individuals found a direct association between SE levels and HIV-1-related mortality, independently of CD4+ T cell count (47). Moreover, in HIV-1-infected pregnant women, low plasma SE levels were associated with an increased risk of mortality and weakly related to CD4+ T cell count (48, 49). Sheehan *et al* have recently evaluated the prevalence of SE deficiency among drug users in Buenos Aires, Argentina. Out of 205 subjects, 82% were SE deficient; moreover, SE levels were lower in HIV- and/or HCV-infected drug users, compared with uninfected individuals (50).

Several studies have examined the association between SE status and HIV infection in children (45, 51). Campa *et al*, for instance, showed an increased risk of mortality in children with SE deficiency during a 5-year follow up on a pediatric cohort of HIV-1-positive subjects. A cross-sectional study conducted by Stephensen *et al* on 365 HIV-positive adolescents and young adults showed that HIV-1-infected individuals had normal SE levels compared with healthy controls. Despite the normal SE status, multiple regression analysis showed that immune activation was associated with lower SE levels (52).

On this basis, Hurwitz *et al* evaluated the association between SE supplementation and HIV viral load in a randomized, double-blind, placebo-controlled trial on 262 HIV-1-infected patients. They found that a nine-month SE supplementation period led to increased SE levels in the treated group, compared to the placebo group. Moreover, higher levels of SE were able to predict HIV viral load suppression and, indirectly, CD4+ T cell recovery (53). However, these findings were highly debated. Ross *et al* underlined that the authors never reported a direct comparison between the SE group and the placebo group in terms of CD4+ T cell count and viral load (54). Moreover, the baseline characteristics of the SE-treated responders and non-responders were not reported (55). Furthermore, Dillon *et al* wondered why non-responders had increased SE levels compared to the placebo group, without any effect on CD4+ count or HIV viral load (56).

SE supplementation has been shown to prevent some morbidities among people with HIV, reducing, for instance, the rate of hospital admission (57, 58) and decreasing the risk of diarrhoea among HIV-1-infected subjects (48).

3.3. Zinc

Zinc is an essential trace element which plays a critical role for immune functions. In fact, its deficiency leads to increased susceptibility to infections (59). Zinc deficiency has been associated with decreased serum levels of interleukin-2 (IL-2) and thymulin, a zinc-dependent thymic peptide whose activity is important for the maturation and differentiation of thymocytes (60); zinc is also needed for T cell proliferation and cytolytic activity (61). Moreover, in acrodermatitis enteropathy enteric zinc uptake is impaired, leading to thymic atrophy, low lymphocyte count, immunodeficiency and death from infections. Zinc stimulates water and electrolytes absorption from the intestinal mucosa and it increases the concentrations of enterocyte brush-border enzymes (62); it also exerts anti-inflammatory activities, through the inhibition of NF-kB activation and the consequential production of inflammatory cytokines (63).

Soon after the discovery of HIV, some studies had already reported zinc deficiency in HIV-1-infected patients (64). These data were confirmed by subsequent studies, showing not only low plasma zinc levels in patients with HIV, but also that zinc deficiency was independently associated with faster HIV disease progression (64, 65) and mortality (66).

Low plasma zinc levels were found in up to 50% of subjects, in several cohorts of HIV-1-infected patients (66-68); plasma zinc levels, routinely used to detect zinc deficiency, are considered a controversial biomarker of zinc status, since zinc is an acute-phase reactant (69) and its plasma concentration is depressed by inflammation (70, 71). For this reason, some authors measured hsCRP (high-sensitivity C-reactive protein) to distinguish between low plasma zinc levels due to zinc deficiency and low plasma zinc levels due to an acute phase reaction (72).

Lai *et al* found that zinc levels positively correlated with CD4+ T cell count and inversely correlated with HIV viral load in a cohort of homosexual men in Florida (73). Baum *et al* also showed a positive correlation between zinc levels and CD4+ Tcell count; low plasma zinc levels predicted a 3-fold increase in HIV-1-related mortality, whereas normalization was associated with significantly slower disease progression and a decrease in the rate of opportunistic infections. Other studies evaluated the impact of HAART on zinc levels (67, 74, 75): Wellinghausen *et al* (74), for instance, reported that 23% of patients were zinc deficient but there was no difference in zinc serum levels between patients on antiretroviral therapy and untreated subjects.

The potential benefits of zinc supplementation in HIV-1-infected individuals have been evaluated by several authors. The initial studies of Mocchegiani *et al* showed that zinc supplementation delayed HIV disease progression and decreased the rate of opportunistic infections (76). On the other hand, faster disease progression and increased mortality have been associated with zinc supplementation when exceeding the recommended daily allowance (77).

In a recent randomized, double-blind, placebo-controlled study, Baum *et al* evaluated the effect of a eighteen-month zinc supplementation protocol on immunological, virological and clinical parameters, in a cohort of 231 HIV-1-infected individuals with low plasma zinc levels. Zinc supplementation was associated with a 4-fold reduction in the likelihood of immunological failure, defined as CD4+ T-cell count less than 200 cells/mm^3, compared with placebo. Viral load, respiratory diseases and HIV-1-related mortality were not affected. Zinc supplementation was associated with delayed disease progression and with a lower rate of opportunistic infections (72). This study supports the use of zinc in those individuals with low plasma zinc levels, especially because supplementation is simple, safe and cost effective.

Zinc administration has also been associated with beneficial effects on diarrhoea in pediatric HIV-1-infected patients: zinc supplementation was shown to reduce the duration of diarrheal episodes, especially with the addition of vitamin A, because of their synergistic effect on reducing diarrhoea. A recent randomized controlled trial examined the effect of short-term multi-micronutrient supplementation, containing vitamins A, B complex, C, D, E, folic acid, copper, iron and zinc, on the duration of pneumonia and acute diarrhoea in 119 hospitalized HIV-1-infected children who were not on HAART; the duration of hospitalization was shorter among children admitted with pneumonia or diarrhoea receiving supplements than in those receiving placebo (78). On the contrary, other studies reported opposing results, showing no effect of zinc supplementation on the duration of diarrheal episodes (79, 80) and respiratory infections (81, 82). Similarly, equivocal results have been reported in HIV-1-infected adults: Baum *et al*, for instance, did not observe any difference in upper and lower respiratory infections between the group receiving zinc supplementation and the placebo group, although other studies reported a reduction in the rate of tuberculosis and pneumonia in zinc-supplemented HIV-1-infected subjects (83, 84).

4. VITAMINS

Vitamins have several functions at a cellular and molecular level, ultimately influencing the immune system (85). Vitamin A has been shown to have important immunoregulatory properties on monocyte differentiation and function, to increase the lymphocyte count, especially the CD4+ subset, to improve natural killer cell toxicity and to promote the maintenance of epithelia integrity. Vitamin B6 also leads to increased lymphocyte and antibody production, cell-mediated toxicity and delayed-type hypersensitivity responses. Folic acid improves neutrophil phagocytosis, while vitamin B12 promotes humoral responses. Vitamin E exerts its functions inducing IL-2 production, lymphocyte proliferation, neutrophil phagocytosis and natural killer cell toxicity and reducing the production of inflammatory cytokines, such as TNF-alpha and interleukin-6 (IL-6). T and B cell proliferative responses are also affected by vitamin C (85). Furthermore, cells and tissues are protected against damage caused by reactive oxygen and nitrogen species by vitamin C and E, which act as antioxidants. As reported above, HIV infection is a chronic disease associated with a long-term OS status which may damage immune cells and increase the severity of the disease (86).

Clinical studies have extensively reported low levels of vitamin A in HIV-1-infected individuals (87). In a cross-sectional study on 132 HIV-1-positive patients in South Africa, vitamin A levels were low in 39% of patients with early stages of HIV-1 disease and in 48% and 79% of patients with WHO stage III and IV, respectively. Serum retinol levels were weakly associated with CD4+ T cell count (88). Another study conducted in the United States on 108 HIV-1-infected individuals found low plasma vitamin A and vitamin B12 levels and a positive correlation with a significant decline of CD4+ T cell count. Of interest, normalization of vitamin A and vitamin B12 levels led to an increase of CD4+ T cells. Vitamin B12 levels were also associated with a faster HIV-1-disease progression (89)..

Semba *et al* analyzed blood vitamin A levels in 179 HIV-1-infected intravenous drug users (IVDUs). A positive correlation between CD4+ T cell count and vitamin A levels was found. Furthermore, low vitamin A levels also correlated with increased mortality (90-92). The San Francisco Men's Health Study (SFMHS) evaluated nutrient intake and HIV disease progression in 296 HIV-1-infected patients (93). Vitamin A intake was positively associated with baseline CD4+ T cell count but not with progression. In the Multicenter AIDS Cohort study (MACS) no association between serum vitamin A levels and progression to AIDS was noticed, although just 2% of the participants had vitamin A deficiency (94).

Some observational studies have examined the role of vitamin B12 on HIV-1 infection. In a substudy of the MACS cohort, HIV-1-positive patients with low vitamin B12 levels (less than 120 pmol/L) had a much faster progression to AIDS compared with patients with higher vitamin B12 concentrations (95). Moreover, B vitamins consumption was associated with an increased survival by more than 2 years and a delayed progression to AIDS (96). A high intake of vitamin B1, B3, C, E and niacin was also associated with a decreased risk of progression of HIV infection to AIDS (93-95).

As for vitamin C and E, several studies reported lower levels of these vitamins in HIV-1-patients than in healthy controls (68, 87, 89, 94, 97, 98). Pacht *et al* examined the prevalence of vitamin E deficiency in a cohort of 121 HIV-1-positive subjects; they found low vitamin E levels in 22.3% of patients and a significant decrease in vitamin E levels after 12 months, in comparison with baseline values (99). On the contrary, normal levels of vitamin E were reported by Stephensen *et al* (100). It is thought that ascorbate depletion could be related to oxidative stress; in fact, although oxidized ascorbate can be recycled, increased oxidation is presumed to fasten its depletion (86). Higher vitamin C intake in HIV infection may be of some help in maintaining a normal immune function by decreasing oxidative stress.

Several clinical interventions have evaluated vitamin supplementation in HIV-1-infected patients. Beta-carotene has been used in some studies because of its antioxidant properties and effect on vitamin A body stores. Coodley *et al* designed a double-blind placebo-controlled study comparing beta-carotene with placebo for 4 weeks, followed by a cross-over. In the beta-carotene group a significant increase in total leukocyte count, percentage change of CD4+ T cells and percentage change of CD4+/CD8+ ratio was reported (101). On the other hand, other studies did not find any difference in CD4+ or CD8+ T cell count when beta-carotene or vitamin A was administered to patients (92, 102-106).

A randomized, double-blind placebo-controlled study in Thailand assessed the role of micronutrient supplementation for 48 weeks (vitamins A, D, E, K, B1, B6, B12, folic acid, iron, zinc, selenium and beta-carotene) on 481 HIV-1-infected asymptomatic individuals. Micronutrient supplementation was associated with a reduction in mortality only when the baseline CD4+ T cell count was below 100 cell/mm^3, but no effect on CD4+ T cell count or viral load was found (107). On the other hand, McClelland *et al* reported that patients receiving selenium and multivitamin supplementation had higher CD4+ and CD8+ T cell count than patients on placebo. No effect on viral load was noticed (108). A trend toward a reduced viral load was reported by Allard *et al* in a randomized controlled trial in Canada, in which HIV-1-positive patients were randomized to receive vitamin C and E or matched placebo for three months. The authors also reported lower levels of oxidative stress in the supplemented group (109).

Since HAART has changed the mortality and morbidity of HIV-1 infection, several authors have investigated the role of HAART on micronutrients and micronutrient supplementation. Toma *et al* measured the levels of retinol in 6 HIV-1-infected individuals on HAART and in 5 HIV-1-infected patients not receiving HAART. Retinol levels were lower in patients on HAART compared with controls, while the levels of retinol-binding proteins were increased. Furthermore, the levels of retinol-dehydrogenase (RALDH), an enzyme involved in retinoic acid synthesis, were increased by several HIV-1-inhibitors (saquinavir, zalcitabine, ritonavir, nelfinavir, delavirdine, indinavir) (110). No differences in vitamin A and E levels between patients on HAART and those not receiving therapy were found by Rousseau *et al* (75).

Serum antioxidants, retinol, alpha and gamma-tocopherol, alpha and beta-carotene, lycopene, lutein/zeaxanthin and beta-cryptoxanthin were evaluated by Tang *et al* in a cohort of 175 HIV-1-positive IVDUs, 30 receiving HAART, 65 receiving dual or monotherapy, 80 not receiving any HIV medication. The levels of alpha-tocopherol, beta-carotene and beta-cryptoxanthin were higher in patients on protease inhibitors (PIs), showing that PIs were effective in reducing oxidative stress (111). Vitamin B12 concentration was also found to be higher among patients on HAART compared with untreated controls (112, 113).

Two non randomized studies on micronutrient supplementation in HIV-1-positive patients receiving HAART tested the effects of vitamin C, E and NAC for 24 weeks and vitamin A, C, E and selenium, respectively. No effects on CD4+ T cell count or HIV viral load were found (114, 115). A randomized trial on 29 HIV-1-infected patients on HAART in Brazil reported that daily vitamin E assumption for 6 months had no effects on CD4+ T cell count or HIV viral load (116). As well, no significant immunologic or virologic variations were found after daily supplementation with vitamin A, C and E for 6 months (117). On the contrary, a significant effect on CD4+ T cell count was reported in a micronutrient supplementation randomized, placebo-controlled trial on 40 HIV-1-infected subjects on HAART, even though no significant effects were found on viral load, fasting glucose, insulin and lipids (118).

4.1. Vitamin D

Vitamin D is synthesized in the skin from 7-dehydrocholesterol, after exposure to ultraviolet B (UVB) radiation. Vitamin D is first converted into 25-

hydroxyvitamin D (25OHD) in the liver by 25-alpha-hydroxylase, then it is metabolized in the kidney into 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), the active form of vitamin D, by 1-alpha-hydroxylase, or CYP27B1 (119,120,121). CYP27B1 is expressed also by extrarenal tissues: immune cells, like activated macrophages and dendritic cells, are able to locally synthesize $1,25(OH)_2D$ (122); the production of vitamin D is not regulated by Ca^{2+} homeostatic signals, but it is under the control of immune stimuli, mainly gamma-interferon (IFN) and agonists of toll-like receptors (TLRs) (123-125).

Vitamin D plays a major role in bone health, even though it is known to have pleiotropic functions, dealing with cell proliferation and differentiation, innate and adaptative immunity. Vitamin D has immunomodulatory effects, in fact its receptor (VDR) is expressed on T and B cells, dendritic cells and macrophages (126-129) and its signaling pathway is associated with anti-inflammatory and antimicrobial effects: vitamin D down regulates the production of pro-inflammatory cytokines, like TNF-alpha and IFN-gamma, and induces the production of antimicrobial peptides, like cathelicidin and defensins (130-133).

$1,25(OH)_2D$ was shown to have an inhibitory effect on HIV replication *in vitro*, on primary human monocyte/macrophages, although it enhanced HIV replication on promonocytic cell line U937 (134-137).

A high prevalence of vitamin D deficiency has been reported in several HIV-1-infected cohorts: 25OHD deficiency was 81% in a group of heavily pretreated Italian HIV-1-positive patients; it was 86% in a Spanish group of treated and untreated HIV-1-infected subjects (138, 139). In other studies, the prevalence of vitamin D deficiency was lower, 10.5% in Boston (140), 12% in Sidney (141) and 29% in the Netherland (142). In any case, it is difficult to compare these results since not all the authors used the same cut off to define vitamin D deficiency/insufficiency.

Comparisons of vitamin D levels between HIV-1-positive subjects and healthy controls have been presented in several studies. Teichmann *et al* found that HIV-1-infected patients had lower levels of $1,25(OH)_2D$, but not 25OHD, in comparison with controls (143). Furthermore, Haug *et al* reported lower levels of $1,25(OH)_2D$, compared with healthy controls, and a significant negative correlation with CD4+ T cell count and survival time, while 25OHD and vitamin D binding protein (VDBP) levels were normal (144). 25OHD levels depend on vitamin D assumption in the diet and production via the skin. Since the values of 25OHD were within normal limits, the authors hypothesized that $1,25(OH)_2D$ deficiency was probably due to a defect in 1-alpha-hydroxylation in the kidney. The authors speculated that it could be related to TNF-alpha hyperproduction, which is able to block the stimulatory effect of parathyroid hormone on 1-alpha-hydroxylase, or to an excessive consumption of vitamin D for T lymphocyte maturation and proliferation (144). In a study conducted on 115 HIV-1-infected individuals between November and December 2004, Bang *et al* reported that 36% of males in their cohort had insufficient levels (less than 50 nmol/L) of 25OHD, while deficient (less than 25 nmol/L) and severely deficient (less than 12.5 nmol/L) levels were found in 20% and 4% of them, respectively (145). In line with other studies, vitamin D levels were not associated with age, years of HIV infection, CD4+ T cell count or HAART (142, 143, 146). Unfortunately, the lack of a control group did not allow the authors to conclude about the direct effects of HIV on vitamin D levels. In contrast with most published studies, Dao *et al* found a higher prevalence of hypovitaminosis D (25OHD less than 30 ng/mL or 75 nmol/L) in the general US population (79.1%), in comparison with a cohort of HIV-1-positive subjects which had been enrolled for the SUN study (70.3%) (147).

The association between HAART and hypovitaminosis D remains controversial: a recent study on 211 HIV-1-infected individuals measured vitamin D levels before and after starting HAART. The samples were collected either between August and October or between February and April. Vitamin D deficiency was more prevalent in spring (42-52%), than in fall (14-18%), but remained unchanged after HAART (148). For some drugs, like protease inhibitors (PIs) and efavirenz (EFV), a causative role in vitamin D deficiency has been suggested, with no conclusive results. Welz *et al* found a correlation between the use of EFV and vitamin D deficiency (25OHD less than 10 ng/mL) (149), Brown *et al* demonstrated a significant decline in 25OHD levels after initiation of a HAART regimen including EFV, in comparison with HAART without EFV (150). In the SUN study, EFV administration was associated with low vitamin D levels, while tenofovir (TDF) exposure seemed to exert a protective effect (147). EFV may possibly reduce circulating levels of 25OHD by inducing 24-hydroxylase, a cytochrome P450 enzyme, which converts 25OHD to an inactive compound, $24,25(OH)_2D$ (151, 152). On the other hand, some studies reported a higher prevalence of hypovitaminosis D in patients receiving PIs. Madeddu *et al* found significantly lower $1,25(OH)_2D$ levels within HIV-1-infected patients compared to controls, with the lowest levels among PI-treated subjects (153). Furthermore, ritonavir has been shown *in vitro* to block the hydroxylation of 25OHD to $1,25(OH)_2D$ (154).

The effects of oral supplementation with vitamin D in the setting of HIV infection have been examined: in a small study Childs *et al* found that only 40% of HIV-1 patients receiving vitamin D supplementation were able to reach and maintain normal levels of vitamin D (25OHD more than 30 ng/mL) after a median of 16 weeks' follow up (155). Havens *et al* showed a 46% reduction in hypovitaminosis D (25OHD less than 30 ng/mL) after a 12-week supplementation regimen, in a cohort of young HIV-1 patients on HAART (156).

In synthesis, vitamin D deficiency is very common in HIV-1-positive patients, even though the reasons remain unknown: a defect in 25OHD hydroxylation, insufficient exposure to sunlight, inadequate dietary intake, HAART medications may all represent

concurrent causative factors. Further randomized, placebo-controlled, intervention trials are certainly needed to better establish the association with HAART and the benefits of vitamin D supplementation.

5. MICRONUTRIENTS, PREGNANCY AND CHILD GROWTH

Several studies have evaluated the levels and effects of vitamins on pregnancy, pregnancy outcome and HIV transmission. Mother-to-child-transmission (MTCT) can take place during pregnancy (intrauterine), intrapartum or during breast-feeding. In developing countries HIV is transmitted from mother to child in 25% to 48% of the cases if preventive measures, such as antiretroviral therapy, caesarean section or breastfeeding avoidance, are not taken (85).

The effect of vitamins has been investigated in several clinical trials. Semba *et al* conducted a study in Malawi to measure vitamin A levels in 338 HIV-1-infected women and assess the HIV-1 seropositivity in the newborns. The rates of MTCT were higher in women with lower vitamin A levels. In a subsequent study vitamin a levels were also predictive of infant mortality (157). Burns *et al* reported that the incidence of low birth weight was significantly higher in mothers with low vitamin A levels, although no association with HIV transmission was found (158). Increased vertical transmission was observed by Greenberg *et al* when comparing women with vitamin A levels below 0.70 micromole/L with women whose levels were greater or equal to 1.05 micromole/L (159). In Ethiopia, Mulu *et al* have recently determined the serum vitamin A levels among pregnant women with and without HIV infection, showing a significant difference between the two groups. Irrespective of pregnancy, HIV-1-positive women had lower serum concentration of vitamin A than uninfected women. In this study the overall prevalence of vitamin A deficiency among pregnant women was 18.4% (25% in the HIV-1-positive group) (160), a lower rate if compared with the 40-69% rates reported by other authors (161-163).

On the basis of these observational studies, randomized placebo-controlled studies were started. Kumwenda *et al* evaluated the effect of iron and folate supplementation, either alone or with vitamin A, in 697 pregnant women. MTCT and prematurity were not affected by vitamin A supplementation, although a lower incidence of anaemia and a higher birth weight were reported in the vitamin A group (164). In another study, 728 HIV-1-infected pregnant women were randomized to receive either vitamin A plus beta-carotene during the third trimester of pregnancy, plus 200.000 IU of vitamin A at delivery or placebo. Vitamin supplementation had no effect on MTCT, low birth weight or fetal/infant mortality. However, the vitamin A group was less likely to have preterm delivery (165). In the Trial of Vitamins (TOV) in Tanzania, Fawzi *et al* randomized 1078 HIV-1-infected pregnant women to receive vitamin A, multivitamins, both or neither. Vitamin A or multivitamin supplementation had no effect on MTCT or infant survival. Nevertheless, multivitamins were shown to decrease the risk of fetal death by 39%, to reduce the risk of low birth weight, severe preterm birth and small size for gestational age at birth. Multivitamins significantly augmented CD4+, CD8+ and CD3+ T cell count (166).

Of interest, vitamin A/beta-carotene supplementation resulted in an increased rate of breast-feeding vertical transmission (167, 168), while multivitamins had no effect. Furthermore, vitamin A/beta-carotene supplementation also increased viral shedding in the lower genital tract (108, 169).

Deficiency in micronutrients in HIV-1-infected children is particularly evident in developing countries with a higher incidence of weight loss, failure to thrive, severe pneumonia and persistent diarrhoea. Some studies have shown that micronutrient supplementation decreased diarrhoea-associated morbidity (78, 170), improved child growth (171), lowered the risk of respiratory infections (172), and reduced all cause of mortality (173, 174).

6. CONCLUSIONS

There is enough evidence to emphasize the importance of micronutrients in the diet of HIV-1-infected individuals. Deficiency in vitamins, zinc and selenium in HIV-1-positive patients has been extensively reported before and after the introduction of HAART. The association with immunological and virological parameters, disease progression and mortality further highlights the key role of micronutrients during the course of HIV-1.

The impact of HAART on micronutrient levels has not been definitively established, since conflicting data are available on the benefits of providing micronutrient supplements to HIV-1-positive persons receiving antiretroviral therapy. For this reason, we believe that further strategically planned studies and interventions are needed in order to better evaluate the binomial HAART-micronutrients and to optimize and standardize micronutrient supplementation in HIV-1-positive subjects.

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Abbreviations: $1,25(OH)_2D$: 1,25-dihydroxyvitamin D, 25OHD: 25-hydroxyvitamin D, EFV: efavirenz, ELF: epithelial lining fluid, GSH: glutathione, GSHPx-1: glutathione peroxidase, HAART: highly active antiretroviral therapy, HIV-1: human immunodeficiency virus-1, hsCRP: high-sensitivity C-reactive protein, IFN: interferon, IVDUs: intravenous drug users, IL-2: interleukin-2, IL-6: interleukin-6, MDA: malondialdehyde, MTCT: mother-to-child-transmission, MACS: Multicenter AIDS Cohort study, NAC: N-acetylcysteine, OS: oxidative stress, PBMCs: peripheral blood mononuclear cells, PIs: protease inhibitors, RALDH: retinol-dehydrogenase, ROS: reactive oxygen species, SFMHS: San Francisco Men's Health Study, SE: selenium, SOD: superoxide dismutase, TAC: total antioxidant capacity, TDF: tenofovir, TLRs: toll-like receptors, TNF-alpha: tumor necrosis factor-alpha, TOV: Trial of vitamins, UVB: ultraviolet B, VDR: vitamin D receptor, VDBP: vitamin D binding protein

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