ORIGINAL RESEARCH

Literature Review on the Top 10 CAM Substances Used by HIV-Positive Participants in the AMCOA Study

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Abstract

This literature review summarizes the scientific evidence for the 10 most commonly noted substances reportedly used by the first 1,016 participants of the Alternative Medical Care Outcomes in AIDS (AMCOA) study at Bastyr University: vitamin C (65%), vitamin E (56%), garlic (54%), beta-carotene (48%), vitamin B-12 (46%), acidophilus (44%), multi-vitamins (44%), vitamin B-6 (43%), zinc (42%), and selenium (39%). This review is restricted to articles published in peer-reviewed journals indexed in MedLine. Due to the paucity of clinical data available for the reviewed substances in HIV-positive populations, additional randomized, controlled clinical trials are recommended to assess their safety and efficacy. (J Naturopathic Med 2000; 9:20-31)

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Introduction

Research published in 1997 and 1998 has shown that between 42% and 50% of all Americans currently use some form of complementary or alternative medicine (CAM) (1, 2). Among human immunodeficiency virus (HIV) positive individuals the proportion is higher; a number of studies have indicated that over half of all HIV-positive homosexual/bisexual men are using alternative therapies (3, 4) and some estimates claim that the rate is as high as 78% (5). A treatment or therapy is usually considered CAM if it meets one or more of the following criteria: it is not approved by the Food and Drug Administration (FDA); it is not routinely taught in conventional medicine schools; it is taken in a dosage differing from FDA recommendations; it is used for a condition differing from the FDA approval; or it is not covered by most insurance policies. Despite widespread use, most of the CAM therapies that HIV-positive individuals are using have not undergone substantial clinical evaluation.

The Bastyr University AIDS Research Center (BUARC) was established in October 1994 through a cooperative agreement between the National Institute of Allergy and Infectious Diseases (NIAID) and the National Institutes of Health Office of Alternative Medicine (OCAM), now known as the National Center for Complementary and Alternative Medicine (NCCAM). BUARC's mission has been to describe the patterns of use of CAM and to screen these practices for efficacy in treatment of patients with

HIV disease. BUARC has implemented a longitudinal, observational study to broadly address the positive and negative outcomes associated with the use of CAM in the treatment of HIV/AIDS as a hypothesis generating tool for further clinical studies. The Alternative Medical Care Outcomes in AIDS (AMCOA) study, described in detail elsewhere (6), is designed to allow for the comparison of the outcomes associated with the use of different CAM treatments in HIV/AIDS. The Center has recruited 1675 eligible HIV-positive individuals to participate in the study. Each subject has received an extensive 27-page questionnaire every six months and has the option to release laboratory records that are obtained from the normal course of care. Outcome variables include CD4 counts, viral load, quality of life, body-mass index, disease progression, and mortality. Presented here is a review of the peer-reviewed literature indexed in MedLine for the ten most frequently cited substances by the first 1016 participants of the AMCOA study at the baseline data point.

METHOD

The AMCOA questionnaire divided CAM therapies into providers, substances and activities used for HIV/AIDS. The section of the questionnaire used to gather the information for this article is titled: Part1. "Alternative treatments for HIV," section 1-B "Alternative Medicine Substances." For each substance, subjects had to answer six questions:

1. "Have you ever used it?"
2. "Did you use it in the past 6 months?"
3. "How much did you use per day in the past six months?"
4. "How many days a week did you use it in the past six months?"
5. "How many weeks out of the past six months did you use it? (1-26 weeks)"
6. "Was it prescribed or did you take it on your own?"

Eighty-six substances were listed and additional blank rows were available so subjects could add substances that were not included in the questionnaire.

The frequency of use for the ten substances reviewed here was determined from the baseline questionnaire by tallying the number of times subjects checked "yes" next to question #2 regarding use of a given substance in the previous six months. The literature search for this review was restricted to published, peer-reviewed articles indexed in MedLine and Pre-MedLine as available in PubMed (7). The search was concluded in August 1998.

The studies covered in this paper pertain to HIV-positive adult cohorts or *in vitro* experiments performed in the presence of the HIV-1 virus. However, when this type of published research is lacking for a given treatment, brief citations are presented regarding immunomodulation studies or those dealing with conditions that commonly occur in HIV-positive populations. When citing articles of studies performed on HIV-positive subjects, emphasis has been placed on clinical trials whenever available. Longitudinal, animal, laboratory and qualitative studies have also been presented, especially when clinical studies are scarce or absent. Noted in the reviews of clinical studies are type of cohort, number of subjects, number and type of control subjects, type and length of the intervention and outcomes measured that are most relevant to both HIV-positive populations and the AMCOA study. Occasionally, similar parameters have been presented in selected observational studies, particularly when outcome variables are longitudinally measured over time. Citations of *in vitro* studies report the type of cell or tissue culture used, the measured outcomes and/or the mechanism of action under evaluation.

RESULTS

Besides the 86 substances specifically listed on the AMCOA questionnaire, subjects reported on using 854 additional substances. Following are the 10 most commonly noted substances by the first 1,016 AMCOA participants along with percentage figures in parentheses indicating the percent of the cohort using the substance at baseline: vitamin C (65%), vitamin E (56%), garlic (54%), beta-carotene (48%), vitamin B-12 (46%), acidophilus (44%), multi-vitamins (44%), vitamin B-6 (43%), zinc (42%), and selenium (39%).

VITAMIN C

Research has shown that vitamin C, or ascorbic acid, exhibits antiviral properties against a broad spectrum of viruses *in vitro* and *in vivo*, acts as an extracellular and intracellular antioxidant, and enhances both cell-mediated and humoral immune response (8). Some anecdotal reports have claimed that megadoses of oral and intravenous vitamin C ameliorate the symptoms of AIDS and reduce the severity of opportunistic infections, despite lack of change in $CD4^+/CD8^+$ lymphocyte ratios (9). However, no reports of controlled clinical studies on vitamin C specifically conducted in HIV-positive populations have been published to date.

Based upon a longitudinal study in which 281 HIV-positive men were followed over 6.8 years, Tang et al. (10) found that vitamin C was significant in slowing progression to AIDS when intake was higher than 720 mg/day or 12 times the Recommended Daily Allowance (RDA). However, this result became marginally significant when simultaneously adjusting for zinc, niacin and vitamin A consumption.

Additionally, a series of *in vitro* studies have been performed to test the inhibitory effect of ascorbic acid on HIV-1 replication. Harakeh et al. (11) found that ascorbic acid reduced extracellular HIV-1 reverse transcriptase (RT) activity and p24 antigen production in chronically infected lymphocytic cell lines. Ascorbic acid treatment also inhibited syncytia formation, another measure of antiviral effect, in acutely infected lymphocytes. Harakeh et al. have published additional studies in several lymphocytic cell lines. They found that ascorbic acid had an eight-fold synergistic effect with N-acetylcysteine in inhibiting HIV RT (12). The same authors determined that the HIV-1 inhibitory mechanism of ascorbic acid may be post-translational by impairment of enzyme activity (13). In the presence of tumor necrosis factor alpha (TNF-alpha), ascorbic acid was still able to suppress extracellular RT (14). However, ascorbic acid did not suppress the secretion of cellular transcription factor NF-kappa B in ACH-2 lymphocytic cell lines stimulated with a tumor promoter, TNF-alpha or hydrogen peroxide, suggesting that the molecular mechanism of HIV-1 inhibition by ascorbic acid is not mediated via NF-kappa B (15). Additionally, in a dose escalation study, Rawal & Vyas (16) determined that 500 mcg/ml of ascorbic

acid could inactivate cell-free HIV-1. However, the antiviral effect of ascorbic acid was reversed with the addition of magnesium chloride (17). The investigators caution about the need to include extra magnesium ions as a control in designing *in vitro* protocols for measuring anti-HIV-1 activity.

Vitamin E

There is a comprehensive body of scientific evidence in human and animal populations on the immune-enhancing and antioxidant properties of vitamin E. Supplementation has shown to modulate the immune response by increasing $CD4^+/CD8^+$ lymphocyte ratio, lymphocyte count, antibody response, natural killer (NK) cell activity, phagocytosis, and mitogen responsiveness (18). *In vitro* and *in vivo* studies in HIV-negative cohorts have also revealed that vitamin E can compensate for cellular and serum glutathione deficiency and decrease lipid peroxidation. These parameters are associated with progression to AIDS in HIV-positive populations (18-20). In the murine model of AIDS (MAIDS), which studies murine LP-BM5 leukemia retrovirus (MuLV) infection in mice and the resulting progression to MAIDS, research has suggested vitamin E supplementation enhances immune responses in MuLV-infected mice, such as restoring immune-induced splenic T and B cell proliferation, stimulating NK cell activity, and alleviating hypergammaglobulinemia (21-29).

While no clinical studies have been published on vitamin E therapy in HIV-positive populations, some longitudinal studies have detected abnormalities in vitamin E levels in HIV-positive individuals. In a study of 121 asymptomatic HIV-positive subjects, Pacht et al. (30) found that 22.3% of the subjects had a deficient serum vitamin E level early in the course of the disease. When 42 subjects were followed longitudinally, the investigators detected a significant decrease in serum vitamin E over 12 months. No significant correlation was observed between the decrease in serum vitamin E and the change in CD4 count, body mass index or serum albumin levels. In a comparative cross-sectional study of 38 intravenous drug users (21 HIV-positive and 16 HIV-negative) and 45 homosexual men (25 HIV-positive and 20 HIV-negative), Miguez-Burbano et al. (31) found that low plasma vitamin E levels were associated with higher IgE levels, a sensitive marker in the progression of HIV disease (32). In another study, researchers prospectively measured serum levels of vitamin E in 311 HIV-positive homosexual/bisexual men over nine years. They found a 34% decrease in risk of progression to AIDS and mortality in men in the highest quartile of serum vitamin E level versus men in the lowest quartile. Men who reported vitamin E supplementation also had significantly higher serum tocopherol levels (33).

Some preliminary *in vitro* studies in a chronically HIV-1 infected promonocytic U1 cell line suggest that a vitamin E derivative, 0-tocopheryl succinyl, significantly inhibits HIV-1 replication. Additionally, 0-tocopheryl succinyl increases cellular antioxidant status by inhibiting cell lipid peroxidation and preventing cellular glutathione consumption, thus decreasing oxidative stress, a marker associated with HIV disease progression (34, 35).

Vitamin E has also been studied *in vitro* in association with zidovudine (ZDV) therapy. Gogu et al. (36) found that at varying concentrations of ZDV, the addition of alpha-D-tocopherol acid succinate showed a dose-dependent increase in anti-HIV-1 activity in an MT4 lymphocytic cell line. Geissler et al. (37) showed that alpha-D-tocopherol increased the hematopoietic colony-forming capacity of bone marrow cells from HIV-positive patients both in the presence or absence of ZDV. No such increase was observed in cells from healthy controls.

Garlic

There is increasing evidence in the scientific literature regarding the therapeutic role of garlic (*Allium sativum*) in numerous diseases caused by fungi, protozoa, bacteria and viruses. However, only a few *in vitro* studies have been published pertaining to HIV-1. Two studies tested the antiviral activity of ajoene, a sulfur-containing compound obtained from the oxidation of the garlic component diallyl thiosulfinate or allicin. Tatarintsev (38, 39) showed that ajoene inhibited fusion of chronically HIV-infected with uninfected H9 lymphocyte cells as well as exhibited a moderate degree of antiviral activity in CEM13 lymphocyte cells. In another *in vitro* study by Walder et al. (40), ajoene protected acutely infected Molt-4 lymphocyte cells against HIV-1 and blocked further destruction of $CD4^+$ lymphocytes. The investigators suggest that the mode of anti-HIV-1 action of ajoene may be due to inhibition in early events of viral replication. Also, Shoji et al. (41) found diallyl disulfide, an active compound extracted from garlic, to strongly depress the proliferation of HIV-1 infected CEM/LAV-1 lymphocyte cells. No cytopathic effect was observed in uninfected CEM lymphocytes treated with diallyl disulfide.

Finally, preliminary evidence indicates that garlic may be effective against pathogens commonly seen in AIDS patients. Specifically, some *in vitro* studies suggest garlic extract or some of its constituents inhibit herpes simplex type 1 and type 2 (42), human cytomegalovirus (43), *Candida albicans* (44-46), *Cryptococcus neoformans* (47, 48), *Mycobacterium avium* (49) and *Mycobacterium tuberculosis* (50). However, no clinical studies in HIV-positive populations are cited in MedLine on the use of garlic to treat conditions caused by these pathogens.

Beta-carotene

Beta-carotene, a carotenoid with provitamin A activity, has been clinically shown to enhance cell-mediated immune response in HIV-negative subjects by increasing interleukin (IL)-1 and IL-2 secretion, NK cell activation, $CD4^+$ lymphocyte levels, and $CD4^+/CD8^+$ lymphocyte ratio (18).

Since several longitudinal studies have indicated a significant beta-carotene deficiency in HIV-positive populations (51-54), beta-carotene supplementation has been studied in clinical trials with HIV-positive individuals. In a single-blind study of 11 HIV-positive subjects who took a beta-carotene dose of 60mg/day for at least 6 and up to 12 months, Bianchi et al. (55) reported apparent recovery from AIDS related complex (ARC) symptoms with the exception of generalized lymphoadenopathy. Pontiggia et al. (56) performed a pilot study on whole body hyperthermia (WBH) and beta-carotene supplementation of 120 mg/day in 10 HIV-positive subjects with reference to two control groups: 31 AIDS subjects receiving only WBH and 64 ARC subjects receiving only beta-carotene

supplementation. The investigators found a better and longer-lasting response in the treatment group receiving the combination therapy as measured by viral burden diminution, clinical improvement, amelioration of laboratory data and a subjective improvement of quality of life.

Several studies have measured the effect of beta-carotene supplementation on immunologic indices. Fryburg et al. (57) completed a single-blind non-randomized clinical trial on seven subjects with AIDS in stable condition. Subjects were given two divided doses of 60 mg/day for four weeks followed by no therapy for six weeks. During the intervention, total lymphocyte numbers rose significantly and $CD4^+$ lymphocyte levels displayed a slight rise that was not significant. $CD4^+$ lymphocyte levels returned to baseline after 6 weeks off beta-carotene treatment. Garewal et al. (58) followed 11 HIV-positive patients who were receiving a beta-carotene dose of 60 mg/day over four months. The investigators observed increases in percentage of NK cell number, Ia antigen and transferrin receptor. However, no changes were seen in total lymphocyte counts, nor in $CD11^+$, $CD4^+$ and $CD8^+$ lymphocyte levels. Coodley et al. (59) conducted a randomized, double-blind, placebo-controlled trial on 21 HIV-positive patients who received either 180 mg/day of beta-carotene (treatment) or placebo (control) for four weeks and then crossed over to receive the alternate treatment for four weeks. Beta-carotene supplementation generated significant increases in total white blood cell count, percentage change of $CD4^+$ lymphocyte levels and $CD4^+/CD8^+$ lymphocyte ratio compared to placebo.

Given the promising results of these short-term and small size intervention studies, Coodley et al. (60) conducted a prospective, double-blind, placebo controlled trial on 72 HIV-positive subjects randomly assigned to either receive 60 mg of oral beta-carotene or a placebo three times daily. Participants in both groups were also given a multivitamin supplement containing 5000 IU of vitamin A. Study subjects were evaluated at baseline, one month and three months for T-lymphocyte quantitative subsets, NK cells, p24 antigen, beta-carotene levels, complete blood counts and chemistry, body weights and Karnofsky scores. With the exception of serum beta-carotene levels, there were no significant differences between the treatment and the placebo groups. The investigators postulated that the addition of a multivitamin supplement to both arms of the study may have masked any difference between the two groups and may account for the differences in the results seen between this trial and the earlier study by the same authors (59). On the basis of their findings, however, they do not recommend high doses of beta-carotene supplementation in HIV-positive individuals.

Beta-carotene supplementation has also been studied in the management of some HIV-related conditions. Silverman et al. (61) found no improvement in chronic oral candidiasis in 11 HIV-positive subjects who received 60 to 120 mg of beta-carotene daily for three to seven months. Delmas-Beauvieux et al. (62) studied the effect over one year of 60 mg/day beta-carotene supplementation on the enzymatic antioxidant system in blood and glutathione (GSH) status in 13 HIV-positive subjects. The control group consisted of 18 HIV-positive subjects with no supplementation. There was no significant change in superoxide dismutase (SOD) activity. However, a slight increase in glutathione peroxidase (GPX) and a significant increase in GSH values were observed after the 12-month intervention. This improvement in GSH status has the potential to slow down progression to AIDS (63).

VITAMIN B12

Low serum levels of vitamin B12 (cobalamin) have frequently been detected in HIV-positive subjects at all stages of infection (64-67). However, a study by Dowling et al. (68) with 35 HIV-positive subjects did not find low serum B12, although dietary intake of vitamin B12 was well above RDA (2 ug) for all patients. In a cross-sectional survey of 200 HIV-positive subjects, Paltiel et al. (69) determined that B12 deficient HIV-positive subjects were more likely to be taking Zidovudine (ZDV). In an observational study of 10 individuals with an AIDS diagnosis, Herzlich and Schiano (70) found that HIV-related gastric secretory failure was associated with B12 malabsorption. Thus, the investigators suggest vitamin B12 supplementation is necessary for individuals with advanced HIV infection.

Given the common occurrence of dementia, neuropathy and other neurological disorders in HIV disease, some preliminary cross-sectional studies have detected a potential association between vitamin B-12 deficiency in HIV-positive individuals and cognitive impairment and neurological dysfunction (71, 72). Also, the results of a four year longitudinal study with 88 HIV-positive homosexual men suggest that normalization of plasma cobalamin inadequacy may significantly improve the speed of retrieving overlearned information from long-term memory (73). However, one cross-sectional study detected no significant correlation between vitamin B-12 deficiency and neurophysiological parameters (74).

Additional longitudinal studies have examined vitamin B12 in relation to HIV outcomes and progression. In a study with 108 HIV-positive homosexual men over an 18-month follow-up, Baum et al. (75) observed vitamin B12 deficiency was significantly associated with decline in $CD4^+$ lymphocyte levels whereas normalization of vitamin B12 levels was associated with higher $CD4^+$ lymphocyte levels. A nine-year study of 310 HIV-positive men showed that low serum vitamin B12 is significantly associated with faster progression to AIDS (76).

Only one published *in vitro* study by Weinberg et al. (77) has examined the effect of vitamin B12 on HIV-1 infection. The investigators reported that different types of cobalamins significantly inhibited HIV-1 infection of normal human blood monocytes and lymphocytes. The mechanisms of action appeared to be pretranscriptional and not related to the early stages in the viral life cycle.

Vitamin B12 supplementation has also been clinically studied for specific HIV-related conditions. Herzlich and Schiano (78) reported a case of reversal in apparent HIV-related dementia complex following treatment with vitamin B12. Falguera et al. (79) conducted a prospective, randomized study on prevention of ZDV-induced bone marrow suppression by supplementation with vitamin B12 and folic acid. Sixty HIV-positive subjects received ZDV alone (n=31) or in combination with folic acid (15 mg daily) and intramuscular vitamin B12 (1 g monthly) (n=29). No correlation was found between vitamin B12 or folate

levels and development of ZDV-related myelosuppression.

ACIDOPHILUS

To date, no clinical trials have been reported on the use of *Lactobacillus acidophilus* as an intervention in HIV-positive populations. However, results from a number of clinical studies suggest that *L. acidophilus* treatment may be potentially therapeutic for certain HIV-related conditions. *L acidophilus* has been reported to help digest lactose in lactase deficient subjects (80), prevent the establishment of fungal, bacterial, and protozoan pathogens in the gastrointestinal tract (81-84), reduce recurrent vaginosis and lower urinary tract infections in women (84), and ameliorate antibiotic-induced diarrhea (85). Also, *L. acidophilus* has been shown to enhance immune response in gut cells by increasing their production of interferon (86, 87).

An *in vitro* study by Klebanoff & Coombs (88) indicated that *L. acidophilus* at high concentration is viricidal to HIV-1 in HIV-1 infected CEM lymphocyte cells by causing the generation of hydrogen peroxide. This effect is enhanced by the addition of a peroxidase and a halide. The authors suggest that the activity of the hydrogen peroxide-peroxidase-halide system in the female genital tract may be detrimental to the survival of HIV-1 and may thus reduce the likelihood of HIV-1 transmission. In this regard, a cross-sectional study of 4,718 Ugandan women aged 15-59 years was inconclusive in determining whether low levels of *Lactobacillus spp.* in the vagina correlated with an increase in susceptibility to HIV-1 infection (89). However, in view of the *in vitro* evidence presented by Klebanoff & Coombs (88), it may be pertinent to clinically assess the potential antiviral effect *L. acidophilus* may have on HIV-1 present in the gastrointestinal tract.

MULTI-VITAMINS

Results of several observational studies have been published regarding the micronutrient status in HIV-positive populations and the role vitamins and minerals may play as co-factors in immune dysfunction and disease progression. Table 1 presents the most significant findings of five observational studies that have examined micronutrient status in HIV-positive populations. Table 2 shows the most relevant conclusions from five observational studies on micronutrient supplementation in HIV-positive populations.

In a randomized double-blind, controlled trial, Fawzi et al. (90) studied the effects of vitamin supplements on pregnancy outcomes and T-lymphocyte counts in 1,075 HIV-positive pregnant women in Tanzania. The subjects were randomly assigned to placebo (n=267), vitamin A (30 mg beta carotene plus 5,000 IU of preformed vitamin A, n=269), multi-vitamins excluding vitamin A (n=269) or multi-vitamins including vitamin A (n=270). The two multi-vitamin groups experienced a significant increase in $CD4^+$, $CD8^+$, and $CD3^+$ lymphocyte levels. Also, the use of multi-vitamins significantly decreased the number of fetal deaths, the risk of low birthweight, severe preterm birth, and small size for gestational age at birth. Vitamin A supplementation had no significant effect on any of the variables in either the vitamin A supplemented group or the multi-vitamins plus vitamin A supplemented group.

Another aspect of micronutrient deficiency in HIV-positive individuals is the chronic oxidative stress generated by increased free radical production and by abnormal levels of antioxidants such as vitamin C,

TABLE 1. OBSERVATIONAL STUDIES ON *MICRONUTRIENT STATUS* IN HIV+ ADULT POPULATIONS (STUDIES GROUPED BY STUDY DESIGN)

STUDY DESIGN	AUTHORS	POPULATION	CONCLUSIONS
Cross-sectional	Beach et al. (65)	100 asymptomatic HIV+ homosexual men 42 HIV- homosexual men (controls)	HIV-related deficiencies in vitamins A, E, B6, B12, riboflavin, copper and zinc.
Cross-sectional	Coodley et al. (126)	45 HIV+ men 2 HIV+ women	Significantly lower levels of vitamin A, beta-carotene and folate in subjects with wasting syndrome (n=15)
Cross-sectional	Baum et al. (127)*	125 HIV+ I.V. drug users (82 men, 43 women)	Significantly poorer micronutrient status in women. Significantly greater vitamin A & E deficiencies in women.
Longitudinal 18 months	Baum et al. (75)	108 HIV+ homosexual men	Development of deficiencies in vitamin A, B12, and zinc associated with decline in CD4 counts. Normalization of these nutrients associated with increased CD4 counts.
Longitudinal 3.5 years	Baum et al. (117)	125 HIV+ I.V. drug users (82 men, 43 women)	Low prealbumin levels, deficiencies in vitamin A, vitamin B12, zinc, and selenium significantly associated with increased mortality, independent of CD4 counts at baseline and over time. Selenium deficiency is an independent predictor of mortality.

* This is a sub-study done with the same cohort as Baum et al. (117)

TABLE 2. OBSERVATIONAL STUDIES ON *MICRONUTRIENT SUPPLEMENTATION* IN HIV+ ADULT POPULATIONS (STUDIES GROUPED BY STUDY DESIGN)

STUDY DESIGN	AUTHORS	POPULATION	CONCLUSIONS
Cross-sectional	Baum et al. (95)	108 HIV+ homosexual men 38 HIV- matched controls	HIV+ subjects needed multiple RDA doses of vitamins A, E, B6, B12 and zinc to achieve normal plasma nutrient values.
Cross-sectional	Skurnick et al. (129)	64 HIV+ heterosexual men/women 33 HIV- controls (mixed cohort)	HIV+ subjects had lower plasma concentrations of magnesium, total carotenes, choline, and glutathione and higher concentrations of niacin than the controls. Participants who took supplements had fewer deficiencies.
Longitudinal 6 years	Abrams et al. (128)	296 HIV+ men	Daily multi vitamin supplement associated with reduced hazard of progression to AIDS and with significantly reduced risk for low CD4 counts at baseline.
Longitudinal 6.8 years	Tang et al. (10)	281 HIV+ homosexual/bisexual men	Highest quartile for vitamin C, B1 and niacin intake associated with significantly decreased progression to AIDS. Relation between vitamin A intake and progression was U-shaped. Lowest and highest quartile associated with faster progression. Middle two quartiles associated with slower progression Zinc intake monotonically associated with increased progression to AIDS.
Longitudinal 8 years	Tang et al. (98)*	281 HIV+ homosexual/bisexual men	Highest quartile for vitamin B1, B2,B6 and niacin intake were independently associated with improved survival. Third quartile for beta-carotene intake associated with improved survival. Zinc supplements at all quartiles associated with poorer survival.

* This article presents results from the same cohort as Tang et al. (10) after following the population for 1.2 additional years.

vitamin E, beta-carotene, selenium, zinc, SOD, and GSH (51, 91). Oxidative stress has been associated with several markers of HIV disease pathogenesis such as viral replication, inflammatory response, decreased immune cell proliferation, lipid peroxidation, loss of immune function, apoptosis, wasting, and increased sensitivity to drug toxicities (91, 92). Some observational studies link deficiency of antioxidant vitamins and trace minerals with oxidative stress in HIV-positive populations. In a study with 25 asymptomatic HIV-positive CDC stage II men, 18 HIV-positive CDC stage IV men, and 16 HIV-negative controls, Sappey et al. (52) investigated whether antioxidant status and lipid peroxidation were associated with disease stage. Decreases in antioxidant vitamins E, A, and beta-carotene, and in the antioxidant trace elements selenium and zinc levels were associated with severity of disease. The most dramatic decrease was seen in carotenoids, as stage II levels of beta-carotene were only half of normal value. Allard et al. (93) compared lipid peroxidation indices and plasma antioxidant micronutrients between 49 non-smoking HIV-positive subjects and 15 age-matched HIV-negative controls. Vitamin C, alpha-tocopherol, selenium and beta-carotene concentrations were significantly lower in the HIV-positive group. Lipid peroxides, breath pentane and ethane output, were significantly higher in HIV-positive subjects. These results demonstrate an increase in oxidative stress and a weakened antioxidant defense system in HIV-positive individuals, thus suggesting that antioxidant supplements may provide therapeutic benefit in HIV-positive individuals.

VITAMIN B-6

Results of animal and human studies suggest that vitamin B6 is involved in both humoral and cell-mediated immune response. Vitamin B6 deficiency alters lymphocyte differentiation and maturation, reduces delayed-type hypersensitive responses and diminishes antibody production (94). Only observational studies have been performed to evaluate vitamin B6 in HIV-positive populations; no clinical intervention trials have been reported.

Vitamin B6 deficiency has been observed in HIV-positive subjects (66, 95). It also appears that HIV-1 men require dietary vitamin B6

consumption equivalent to several times the RDA (2 mg/day) to achieve normal plasma vitamin B6 values (95). Vitamin B6 status in HIV-positive subjects has been shown to be significantly associated with functional parameters of immunity such as lymphocyte responsiveness to mitogens and reduced NK cell toxicity, yet it seems unrelated to levels of immune cell subpopulations such as $CD4^+$ and $CD8^+$ lymphocytes and levels of serum immunoglobulins (96).

In an 18-month longitudinal study with 88 HIV-positive subjects, Shor-Posner et al. (97) demonstrated a significant decline of psychological distress, as measured by the Profile of Mood States (POMS) survey, upon normalization of vitamin B6 status. In a 6.8 year study with 281 HIV-positive patients, intakes of B6 supplement at more than twice the RDA were associated with improved survival (98). However, the authors observing the same cohort over nine years, found that vitamin B6 deficiency was not associated with either progression to AIDS or decline in CD4 count (76).

Zinc

Zinc has been shown to have an important role in immune function as an antioxidant and through other immunostimulating mechanisms such as IL-2 secretion, thymulin activity and apoptosis prevention (51). Several observational studies have detected serum zinc deficiencies in HIV-positive populations (51, 52, 66, 99). However, Walter et al. (100) found no significant differences in serum zinc levels in HIV-positive individuals as compared to HIV-negative controls.

Results from longitudinal studies have illustrated the multifactorial complexity of the role of zinc in HIV infection. Table 3 presents five studies that link zinc deficiency to negative outcomes in HIV-positive populations. However, in a 6.8 year study with 281 HIV-positive participants, Tang et al. (10) found that zinc supplementation was monotonically associated with progression to AIDS. After eight years of follow-up in the same cohort, the investigators showed that any intake of zinc supplements was associated with poorer survival (98).

Such seemingly contradictory conclusions may be explained by results from *in vitro* studies that suggest zinc may have opposing effects on the HIV-1 replication cycle. Zinc may decrease replication by inhibiting the protease enzyme of the virus (101). Zinc binds to the active site of the viral protease at the catalytic aspartate residues (102). Other studies suggests the presence of zinc may be necessary in HIV-1 replication due to the presence of several zinc-finger proteins in the structure of HIV-1 (51, 103).

Additionally, Neves et al. (104) studied one of the immunomodulatory properties of zinc by testing the mitogenic effect of zinc supplementation *in vitro* on lymphocyte proliferative response in peripheral blood mononuclear cells (PBMC) of 48 HIV-positive individuals and 10 HIV-negative controls. The results showed similar increases in zinc-stimulated lymphocyte proliferation in PBMC cultures from both asymptomatic HIV-positive and HIV-negative controls, whereas a very low rate of zinc-stimulated proliferation was observed in PBMC cultures from

Table 3. Observational studies that associate zinc deficiency with negative outcomes in HIV+ populations (studies grouped by study design)

STUDY DESIGN	AUTHORS	POPULATION	CONCLUSIONS
Cross-sectional	Baum et al. (95)	108 HIV+ homosexual men 38 HIV- homosexual men	In HIV+ subjects, a relatively high number of biochemical deficiencies was associated with consuming zinc at the RDA level (15 mg).
Cross-sectional	Allavena et al. (132)	61 HIV+ men 19 HIV+ women CDC stage IV /Taking ZDV	Moderate zinc deficiency. Zinc levels negatively correlated with beta2 microglobulin.
Nested Case Control 2.5 years	Graham et al. (130)	54 HIV+ progressors 54 HIV+ non-progressors 54 HIV- subjects	Zinc levels lower in HIV+ progressors. Lower serum zinc predicted progression to AIDS independently of baseline CD4 count and calorie adjusted dietary zinc intake.
Longitudinal 8-12 months	Baum et al. (131)	15 HIV+ men treated with ZDV 22 HIV+ men non-treated	ZDV-treated subjects exhibited lower serum zinc levels. Plasma zinc was significantly related to immune function in ZDV-treated subjects.
Longitudinal 18 months	Baum et al. (75)	90 HIV+ homosexual men	Normalization of zinc levels was associated with a rise in CD4 counts.
Longitudinal 3.5 years	Baum et al. (117)	125 HIV+ drug users (82 men, 43 women)	Zinc deficiency associated with increased mortality.
Retrospective	Koch et al. (133, 134)	228 Hospitalized AIDS patients	Low zinc levels associated with increased incidence of systemic bacterial infections, yet not associated with hypoalbuminemia.

AIDS patients. However, in phytohaemagglutinin (PHA)-stimulated PBMC cultures, the addition of zinc had a synergistic effect on lymphocyte proliferative response for both asymptomatic HIV-positive and AIDS subjects. Also, there was a decreased percentage of apoptotic cells present in PBMC cultures from HIV-positive individuals that were treated with PHA plus zinc versus cultures treated with PHA only. Given the mitogenic effect zinc exerts on lymphocyte proliferation and the delaying effect on lymphocyte apoptosis as reported by Neves et al. (104), zinc may have the potential of increasing cell-mediated immunity in HIV-positive subjects. Hence, the investigators propose future clinical studies on the role of zinc supplementation associated with antiretroviral therapy in the improvement of immunological functions in HIV-positive individuals.

There have been only a few small clinical studies on zinc supplementation in HIV-positive individuals. Forty-two subjects who had cancer in remission (n=39) or AIDS (n=3) were supplemented with zinc gluconate and experienced increases in the $CD4^+/CD8^+$ lymphocyte ratio (105). In a controlled trial, Isa et al. (106) treated 11 HIV-positive male drug abusers with a zinc dose of one mg/kg/day for 10 weeks. Serum zinc increased significantly after treatment, yet zinc levels in red and white blood cells remained the same. All subjects showed progressive weight gain and a non-significant elevation in $CD4^+$ lymphocyte count. Mocchegiani et al. (107) administered 200 mg of zinc sulfate for 30 days to 25 HIV-positive patients treated with ZDV. The control group consisted of ten patients treated with ZDV only. Zinc supplementation increased or stabilized body weight, increased $CD4^+$ lymphocyte count, increased plasma level of active zinc-bound thymulin, and reduced frequency of opportunistic infections due to *Pneumocystis carinii* and *Candida albicans*. No variations were observed in the frequency of cytomegalovirus and *Toxoplasma gondii* infections.

SELENIUM

Selenium appears to play a major role in cell-mediated immunity. Selenium deficiency can cause reduced lymphocyte counts as well as impairing lymphocyte proliferation and responsiveness. Selenium is also critical in humans for the maintenance of glutathione-dependent antioxidant status (108).

Several observational studies have detected low plasma and cellular selenium levels in HIV-positive populations. Selenium deficiency has been correlated with decreased serum albumin (109) and glutathione peroxidase levels (110). In a cross-sectional study, selenium levels were positively correlated to $CD4^+$ lymphocyte levels, $CD4^+/CD8^+$ lymphocyte ratio, hematocrit and serum albumin, and inversely correlated with serum thymidine kinase and beta 2-microglobulin levels (111). In a separate study, the same authors found that mean selenium levels and erythrocyte GPX activity were significantly lower in hospitalized AIDS patients as compared to asymptomatic HIV-positive and HIV-negative subjects. Plasma thiol (-SH) and GSH levels were lower in both asymptomatic HIV-positive and AIDS subjects than in the HIV-negative controls (112). Selenium deficiency has also been linked to various HIV-related skeletal and cardiac muscle myopathies (113-116).

In a 3.5-year longitudinal study with 125 HIV-positive drug users, selenium deficiency was significantly associated with mortality even after factoring in all the variables that could affect survival, including baseline $CD4^+$ lymphocyte levels, $CD4^+$ lymphocyte levels over time, and nutrient deficiencies. Hence, the authors conclude that selenium deficiency is an independent predictor of survival for those with HIV infection (117).

In vitro studies have given emphasis to the antioxidant role of selenium as an integral component of GPX. Sappey et al. (118) and Makropoulos et al. (119) found that selenium supplementation in latently HIV-1 infected T-lymphocytes increased GPX activity and concomitantly decreased NF-kappa B activation in the presence of either hydrogen peroxide or TNF-alpha. Hori et al. (120) showed that in both chronically and acutely HIV-1 infected T-lymphocyte cells as well as in monocytic cell lines, selenium supplementation for three days partially suppressed induction of HIV-1 replication by exposure to TNF-alpha. This suppressive effect was not observed in acute HIV-1 infection or in the absence of exogenous TNF-alpha. Additional *in vitro* studies have shown that a number of genes in the HIV-1 virus encode selenoproteins which may partly explain the progressive decline in plasma selenium observed in HIV-1 infected populations (121, 122).

Several small trials have examined the effects of selenium supplementation. Nineteen selenium deficient HIV-positive males with AIDS or ARC were given selenium in doses of 400 mcg/day for up to 70 days to establish whether intestinal absorption of dietary selenium was impaired. Serum selenium levels increased significantly in all patients regardless of disease stage (123). Another study with 12 symptomatic HIV-positive patients found that selenium in the dose of 80 mcg/day increased serum concentrations and improved HIV-related symptoms. However, no changes were observed in the immunological parameters (124). One arm of an open-label controlled trial by Delmas-Beauvieux et al. (62) studied selenium supplementation of 100 mcg/day during a year in 14 HIV-positive subjects. The control group consisted of 18 HIV-positive subjects with no supplementation. The investigators detected significant increases in GSH levels and in GPX activity in the selenium supplementation group, which indicated that selenium could be useful in protecting cells against oxidative stress in HIV-positive infected individuals.

DISCUSSION

The ten substances reviewed here were selected because they were the most frequently used CAM substances as reported by the first 1,016 AMCOA participants in their baseline questionnaire. This paper reviews the results of studies on these CAM substances that have been published in peer-reviewed journals and indexed in the MedLine database. It excludes research published in approximately 100 CAM journals that have not been indexed in MedLine, many of which are peer-reviewed. Since MedLine is the most comprehensive medical database that is freely obtainable through the Internet, there is an inherent limitation in the accessibility to some of the published CAM research for HIV/AIDS.

Most of the scientific evidence presented here for the potential benefit of the cited substances in HIV-positive populations comes from either observational studies that establish statistically significant

associations and/or correlations with medical outcomes or *in vitro* assays that suggest basic antiviral and immunological effects. However, clinical evidence on cause and effect in HIV-positive populations is preliminary for beta-carotene, vitamin B12, multi-vitamins, selenium and zinc treatments, and is still absent for vitamin C, E, B6, garlic and acidophilus treatments. In this vein, we have only found two randomized, controlled clinical trials for beta-carotene, one for vitamin B12 and one for multi-vitamins. Additional clinical studies cited are often open label trials with a small cohort and no control group. Moreover, some of the clinical studies on micronutrients have not quantified dietary intake nor have they included a controlled diet as part of their protocol. In the case of garlic and acidophilus, only preliminary *in vitro* evidence was found on their potential as treatments against HIV disease.

The AMCOA data suggest that these ten substances may be used by a significant percentage of the HIV-positive individuals, regardless of the insufficient clinical data for HIV-positive populations. Possibly, HIV-positive individuals may be using these substances based on clinical and immunological information obtained from HIV-negative populations with other health conditions, especially chronic conditions that challenge the immune system. Conversely, patients may often accept anecdotes or sophisticated marketing as sufficient grounds to try new CAM therapies (125). In view of the paucity of the clinical evidence in HIV-positive populations for the substances reviewed in this paper and their widespread use among the first 1016 AMCOA HIV-positive participants, there appears to be a need to conduct additional controlled clinical trials with these substances in order to assess their safety and efficacy in HIV-positive populations.

Acknowledgements

The authors wish to acknowledge the efforts of a number of collaborators who have been instrumental in gathering articles and reviewing the contents of this paper. Our thanks to Annie Moffat, Michael Doff, Michelle Michaels, Maria Cartwright, Roberta CM Wines, Suzanne Myer MS RD, and Cindy Wenner PhD for their work. This research was supported by the National Institutes of Health Office of Alternative and Complementary Medicine (OCAM) and the National Institute of Allergy and Infectious Diseases (NIAID), grant number 3 U24 AI37628-01.

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