Micronutrients and HIV infection: a review of current evidence

Henrik Friis

Consultation on Nutrition and HIV/AIDS in Africa: Evidence, lessons and recommendations for action

Durban, South Africa
10–13 April 2005

World Health Organization

Department of Nutrition for Health and Development

Micronutrients and HIV infection: a review of current evidence

Henrik Friis

Consultation on Nutrition and HIV/AIDS in Africa: Evidence, lessons and recommendations for action

Durban, South Africa
10–13 April 2005

World Health Organization
Department of Nutrition for Health and Development

Contents

1. Introduction

With the raging global HIV epidemic, there is an urgent need to exploit all potential interventions to halt its continued spread and to enhance the health, quality of life and survival of those already infected. Nutritional interventions may play a major role. Current evidence on the role of micronutrients in childhood infections has led to the development and implementation of preventive and therapeutic interventions that reduce infectious disease morbidity and mortality among children in developing countries. Similarly, existing data and data emerging from ongoing and future research could result in interventions to improve micronutrient intake and status which could contribute to a reduction in the magnitude and impact of the global HIV epidemic. Such interventions are feasible and affordable, do not require HIV testing facilities and may even be beneficial to people without HIV infection.

2. The malnutrition-infection complex

It is well established that an infection may lead to micronutrient deficiencies and that micronutrient deficiencies may affect the risk of infectious disease morbidity (*1,2*). As seen from the conceptual framework presented in **Figure 1**, the effects of an infection are mediated via the acute phase response and localized lesions, leading to reduced intake and absorption and increased utilization and loss of micronutrients. A micronutrient deficiency may affect the risk of infection with a specific infectious agent as well as the severity of the infectious disease morbidity. These effects are mediated via pathogenicity of the infectious agent, host risk behaviour or the host defence (*3*) and may be either synergistic or antagonistic. A synergistic relationship exists when a specific micronutrient deficiency increases infectious disease morbidity, in which case either improved micronutrient intake or treatment of the infection will break the vicious circle. An antagonistic relationship exists when a specific micronutrient deficiency reduces—or increased intake increases—infectious disease morbidity (*1*). In fact, a micronutrient may act synergistically in moderate doses but antagonistically in high doses. For example, zinc, although essential to the immune system (*4*), is immunosuppressive in high doses (*5*).

Studies on the role of micronutrients in infectious diseases have resulted in an evidence base, which can be used to develop preventive and therapeutic micronutrient interventions which, if implemented, may save millions of lives (*6,7*). For example, vitamin A supplementation has been shown to reduce all-cause mortality in children under 5 years of age by 30% and fatality among children hospitalized with measles by 60–70% (*8,9*). On the basis of global prevalence data, vitamin A deficiency was estimated to be the underlying cause of up to 2.5 million child deaths annually (*10*). Vitamin A supplementation programmes targeting children have therefore become a key health intervention in developing countries (*11*). Similarly, zinc deficiency is widespread in developing countries (*12*), and data from randomized, controlled trials (RCTs) have documented that zinc supplementation reduces the risk of both diarrhoea and respiratory tract infections (*13*) and the severity and fatality of diarrhoea (*14*).

3. Effects of HIV infection on micronutrient status

The effect of an infection on nutritional status is determined by its natural history and actual course and is particularly detrimental if generalized, severe, long lasting or recurrent.

3.1. Natural history of HIV infection

HIV infection is characterized by an acute syndrome accompanying the primary infection, followed by a prolonged asymptomatic state eventually leading to advanced HIV disease (*15*). Around 3–6 weeks after being infected with HIV, most individuals experience a febrile illness lasting a couple of weeks with anorexia, nausea and diarrhoea followed by weight loss. During this acute HIV syndrome, the viral load peaks and is mirrored by a nadir in CD4+ count which occasionally results in opportunistic infections. The CD4+ count then returns to almost normal values while the viral load stabilizes around an individual set point (**Figure 2**). A long asymptomatic period then follows during which viral load slowly increases and CD4+ count declines. This period is not a true latency period because viral replication continues (*16*), resulting in a progressive decline in CD4+ counts of around 50 cells per year. After a number of years, opportunistic and other infections become increasingly frequent. The length of the asymptomatic period and the type, timing and frequency of the subsequent opportunistic infections may vary depending on general health and exposure to

pathogens. For example, in developing countries more than 90% of HIV-positive individuals get diarrhoea compared with less than half in developed countries (*17*), and reactivation of latent tuberculosis is common (*18*). Although prevention or treatment of tuberculosis and other infections may delay HIV progression (*19*), the patient will eventually become wasted and die if antiretroviral (ARV) drugs are unavailable.

3.2. Methodological issues

Defining the intake of specific micronutrients necessary to prevent deficiencies among people living with HIV is important. However, the interpretation of currently available data is impeded by a number of methodological difficulties.

The presence of micronutrient deficiencies in HIV-positive individuals cannot be attributed to direct biological effects of HIV infection. Selection bias is a major problem in most studies, but even if micronutrient status was accurately assessed in random samples of HIV-positive and -negative individuals, it would not be possible to attribute any difference in status to HIV infection because HIV infection does not occur randomly. This is particularly so in underprivileged populations where lack of shelter, food and money is associated with unprotected sex. Confounding is therefore likely to be a major problem because poor socioeconomic status is associated with HIV infection and is also a strong determinant of food and nutrition insecurity. Finally, reverse causation cannot be excluded if micronutrient deficiencies increase susceptibility to HIV infection.

3.2.1. Assessment of micronutrient status

A major limitation of assessment is the lack of adequate markers of micronutrient status. For some micronutrients no satisfactory marker exists even under ideal conditions. For other micronutrients reasonable markers exist but fail to reflect micronutrient status in the presence of infectious and other diseases eliciting an acute phase response.

The acute phase response, accompanying generalized and severe infections, mediates the effect of infections on micronutrient status but also disproportionately affects the level of the markers of micronutrient status (*20–22*). The validity of these markers is therefore impaired during the acute phase response. For example, serum retinol is reduced during an acute phase response to infection. This partly reflects a true effect of the infection on vitamin A status and partly an apparent effect due

to a transient decline in serum retinol binding protein, the carrier protein of retinol. Serum ferritin increases during an acute phase response, which may partly reflect an increased iron sequestration when haemoglobin declines and partly that serum ferritin behaves as an acute phase reactant. Adjusting for one or more acute phase reactants in multivariable analysis has been recommended as a way to control for the potential confounding effect of the acute phase response (*23–25*). However, which acute phase reactant or combination of reactants to use is not known. In fact, the effect of the acute phase response on a specific micronutrient may well differ not only with respect to magnitude but also duration and different acute phase reactants may have to be used.

3.2.2. Assessment of HIV stage

Another limitation is the lack of accurate information on the stage of HIV infection in most published studies. That information is important because the effects of HIV infection on micronutrient status depend on the stage of infection as well as on the occurrence of common and opportunistic infections and whether the patient is receiving ARV treatment (ART). With information about viral load, CD4+ counts and coinfections, it may be possible to better understand the change in micronutrient status with advancing HIV infection and the underlying mechanism. It is likely that the low status seen in early asymptomatic HIV is mainly due to reduced absorption whereas reduced intake become increasingly important with advancing infection and increased utilization and loss of micronutrients when diarrhoea and other coinfections become frequent.

3.3. Possible effects and current knowledge

3.3.1. Primary HIV infection

The effects of primary HIV infection on micronutrient status have not been studied. Nonetheless, it is conceivable that the acute HIV syndrome, with fever, anorexia, nausea, and diarrhoea followed by weight loss, may impair micronutrient status. However, because the acute stage is transient, it is mainly of concern in individuals with prior poor micronutrient status or lack of access to an adequate convalescent diet. Such deficiencies, precipitated or exacerbated by a symptomatic primary HIV infection, could be pivotal by affecting the viral load set point and host defence and thereby affecting HIV transmission and progression.

3.3.2. Asymptomatic HIV infection

Little acute phase response occurs during the long asymptomatic stage of HIV infection (*25*), but viral replication occurs continuously, leading to the slow but relentless increase in viral load over a number of years. Changes in the structure and function of the intestinal tract seem to occur relatively early in HIV infection. An HIV enteropathy characterized by villous atrophy and crypt hyperplasia and accompanied by malabsorption has been described in HIV-positive individuals (*26*). Reduced absorption likely leads to impaired micronutrient status at this stage, which may be important because of the stage's long duration.

Few studies on micronutrient status have been conducted with asymptomatic HIV-positive individuals and appropriate comparison groups. However, some studies have been done in developing countries in pregnant women attending antenatal care. HIV-positive pregnant women are usually at an early stage of infection, partly because even early HIV infection reduces fertility and increases foetal loss (*27,28*). Accordingly, among 1669 Zimbabwean pregnant women, those with HIV infection had mean viral load of 3.85 log (*29*) and morbidity, body composition and serum α_1-antichymotrypsin similar to values for HIV-negative women (*24,25,30*). Nonetheless, serum retinol and β-carotene were considerably lower and α-tocopherol, ferritin and folate were slightly but significantly lower after adjustment was made for elevated acute phase proteins. These differences most likely reflect increased requirements in HIV-positive individuals but this could not be substantiated because intake was not controlled for.

In this developing country setting, most women seek antenatal care when pregnant and are rarely aware of their HIV status. Although selection bias is therefore not likely to be a problem, HIV-positive and -negative women may not have comparable socioeconomic backgrounds. Confounding cannot be excluded because poor socioeconomic status may be associated with unprotected sex and HIV infection and be a cause of poor micronutrient status. Controlling for dietary intake and possibly other socioeconomic factors is therefore critical.

3.3.3. Symptomatic HIV infection

During symptomatic HIV infection, the effects of HIV in the gastrointestinal tract are more severe. The increasingly frequent enteric and other infections result in both acute phase responses and localized lesions which further exacerbate an impaired micronutrient status.

A number of early studies from developed countries, before the use of ARV drugs, reported low serum levels of several micronutrient indicators, such as vitamin A; carotenoids; vitamins B_6, B_{12}, C and E; folate; as well as selenium and zinc in adults (*31–36*) and children (*37*). However, these studies were mostly hospital based and contained little information about the stage of HIV infection and how HIV-positive and -negative controls were selected. Furthermore, the acute phase response was not controlled for, which leads to overestimation of the association between HIV and deficiencies for some of the micronutrients. Many patients may have taken supplements in response to their HIV diagnosis and coinfections. This may have led to gross underestimation of the effect of HIV infection on micronutrient status, making it difficult to base conclusions on these data.

One study attempted to control for the intake of micronutrients and will therefore be mentioned in more detail. This cross-sectional study was conducted in 108 HIV-positive homosexual men in United States (*31*). Serum vitamins A, B_6, B_{12} and E and serum zinc were assessed and compared with values for 38 HIV-negative homosexual men. All subjects were selected from hospitals, clinics or community programs. All were free from other diseases, but 19% of the HIV-positive men had symptoms and 90% had normal weight. HIV-positive men had higher triceps skinfold thickness than did the HIV-negative men. More HIV-positive men took supplements and had a higher total intake of all micronutrients than did HIV-negative men. In fact, most HIV-positive men had intakes above the recommended dietary allowance. Intakes at or above the recommended dietary allowance were associated with normal plasma levels in the HIV-negative men. In contrast, in HIV-positive men even intakes several times the recommended dietary allowance were not associated with adequate serum levels. No attempt was made to control for the acute phase response, and data were not given separately for subjects with and without symptoms. The authors concluded that intake of nutrients at levels recommended for the general population did not appear adequate for HIV-1–

positive men (*36*). Despite its limitations, this study has contributed considerably to the widespread notion that HIV- positive individuals need multiples of recommended dietary allowances.

Prevention and prompt treatment of opportunistic infections and effective ART will most likely reduce the effect of HIV infection on micronutrient status. For example, antioxidant status is considerably improved in patients on protease inhibitors (*38*). Nevertheless, drugs often have adverse effects, such as nausea, vomiting and diarrhoea, or affect micronutrient metabolism, resulting in a negative effect on micronutrient status.

3.4. Conclusion and research priorities

In analogy with other infections and on the basis of knowledge about the natural history of HIV infection, it is expected that HIV infection, through various mechanisms operating at different levels, impairs micronutrient intake and status. Accordingly, available data suggest that HIV infection impairs the status of a range of micronutrients and that HIV-positive individuals may have increased requirements. However, because the methodological shortcomings mentioned above, the available data do not allow development of specific, evidence-based dietary guidelines. In particular, it is not clear whether micronutrient requirements can be met through the diet.

Studies are needed to determine the levels of intake of specific micronutrients that are required to prevent clinical and biochemical signs of deficiencies in people with HIV infection. For micronutrients for which status cannot be assessed (e.g., zinc), a factorial approach should be used. Such studies should be conducted in HIV-positive patients at different stages of disease, as assessed using viral load, and also taking the occurrence of coinfections and ART into consideration. The appropriate design of such studies should be discussed and standard protocols should be developed. Key concerns are the control of dietary intakes of individual micronutrients and the acute phase response.

Recommendations on micronutrient intakes for people with HIV infection should address levels that prevent clinical and biochemical signs of deficiency. They should also be based on the effects of micronutrient status and intake on HIV progression and morbidity, and possibly transmission, as discussed below.

4. Effects of micronutrients on HIV infection

Micronutrient deficiencies as well as interventions to increase micronutrient intake may be determinants of susceptibility to HIV infection, transmission and progression, including risk of opportunistic and other infections (**Table 1**) as well as of a range of non-HIV outcomes that will not be considered further here (**Table 2**). Although the micronutrient requirements are likely to be reduced when the HIV patient is put on ARV, micronutrient deficiencies may persist and may affect absorption, pharmacokinetics and hence toxicity and efficacy of the drugs, as discussed elsewhere (*39*).

Given the routes of transmission of HIV infection, it is likely that micronutrient deficiencies may impair the epithelial integrity, the differentiation of target cells and other host defence mechanisms of an exposed sexual partner or offspring, thereby facilitating viral entry and replication.

Micronutrient deficiencies may affect viral load of the HIV-positive individual, systemically or locally in genital secretions and breast milk, and thus affect both HIV progression and infectivity. These effects are mediated by oxidative stress and impaired immune functions (**Figure 3)**. The oxidative stress may lead to activation of the nuclear transcription factor NF-κB, resulting in increased viral replication (*40*). The impaired immune functions resulting from lack of essential micronutrients have been called nutritionally acquired immune deficiency syndrome, or NAIDS (*41*). NAIDS may contribute to the depletion and dysfunction of CD4+ cells but also makes the host susceptible to other infections which may increase viral replication and hence quicken HIV progression (*19*). Plasma viral load is a strong determinant of HIV progression but also of viral load in cervicovaginal secretions, semen and breast milk although local infections may further boost local viral replication or shedding (*42,43*). Thus, viral load is also a determinant of infectivity with respect to sexual and mother-to-child HIV transmission.

These relationships need to be carefully studied in order to develop effective interventions. However, methodological issues complicate the design and interpretation of not only observational studies but even of RCTs.

4.1. Observational studies

Several observational studies have attempted to relate micronutrient status or intake to mother-to-child and sexual HIV transmission and to HIV progression.

4.1.1. Methodological issues

By including individuals with a range of exposure levels (i.e., micronutrient status or intake), observational studies may provide information about dose-response relationships that cannot be obtained from a RCT. However, because of methodological problems, observational studies alone cannot provide the evidence on which recommendations must be based.

Assessing the relationship between micronutrient status and HIV outcomes is difficult because HIV infection and other infections affect the markers for micronutrient status (*22*). Thus, the unavoidable misclassification of micronutrient status is differential, as it is likely to be worse in those with more advanced disease. Serum retinol declines during infections (*25*), which may create a false or overestimate a true association between low serum retinol and HIV outcome (i.e., progression or transmission). In contrast, serum ferritin increases during infections (*24*), and a differential misclassification may create a false or overestimate a true association between high serum ferritin and HIV outcome. Similarly, in studies relating dietary and supplemental intake of micronutrients to HIV outcome, the actual or reported dietary intake may be affected by knowledge about the stage of HIV infection. This may lead to false associations due to reverse causality and misclassification bias.

4.1.2. Review of observational studies

4.1.2.1. Mother-to-child HIV transmission

Serum retinol (vitamin A) was assessed in 338 HIV-positive, pregnant, Malawian women attending antenatal care (*44*). Mothers and infants were followed for mortality, and infants alive at 12 months of age were tested for HIV infection. The transmission rate was 7.2% for women with normal serum retinol (>1.40 μmol/L) and increased linearly with decreasing serum retinol category to 32.4% for those with low serum retinol (<0.70 μmol/L). The strong inverse relationship between serum retinol and mother-to-child HIV transmission persisted after controlling for maternal age, body mass index and CD4+ count at inclusion and corresponded to a 0.56-times reduction in risk of mother-to-

child HIV transmission for each 0.45 μmol/L increase in maternal serum retinol. An inverse relationship was also found between maternal serum retinol and infant mortality (*45*).

Similar relationships between maternal serum retinol and mother-to-child HIV transmission were found in a study in Rwanda (*46*), and in two studies in the United States (*47,48*). A third study from United States found no effects of maternal serum retinol, β-carotene and vitamin E (*49*). Cross-sectional studies in Kenya found that low maternal serum retinol was a strong predictor for the presence of HIV in breast milk (*50*) and genital shedding of HIV (*51*), suggesting a plausible mechanism for an effect of vitamin A on mother-to-child transmission through breastfeeding and intrapartum routes, respectively.

Although attempts were made to control for stage of HIV disease and other potential confounding factors, neither the acute phase response nor socioeconomic factors including access to health care were controlled for. For example, low serum retinol could merely be a marker of another micronutrient deficiency which increased mother-to-child HIV transmission and mortality.

4.1.2.2. Sexual transmission

Sexual transmission depends on infectivity of the HIV-positive individual as well as susceptibility of the exposed HIV-negative partner. No studies have addressed the effect of micronutrient intake and status in HIV-positive individuals and sexual transmission on clinical outcomes. However, a few studies addressed the relationship between micronutrient status and genital shedding of HIV. In a cross-sectional study of pregnant HIV-positive women in Nairobi, Kenya, low serum retinol was a predictor of HIV DNA in vaginal but not cervical secretions (*51*). In contrast, no relationship was found between serum retinol and HIV RNA in cervicovaginal lavage in women in New York, USA (*52*). Similarly, low serum retinol was also a predictor of vaginal HIV shedding among nonpregnant HIV-positive women who attended a sexually transmitted diseases clinic in Mombasa, Kenya (*42*). In a subgroup of women in the same study, low serum selenium was also a predictor of vaginal HIV shedding after adjustment for the previously found effect of low serum retinol (*53*).

Three nested case-control studies addressed the role of vitamin A and other micronutrients regarding the risk of acquiring HIV infection for sexually active adults, with quite conflicting results.

In Kigali, Rwanda, a cohort of sexually active women was followed every 6 months for 24 months (*54*). No differences were noted for serum concentrations of retinol, carotenoids, vitamin E, ferritin and selenium between 45 women who seroconverted and 74 randomly selected women who did not. HIV-negative adults attending sexually transmitted disease clinics in Pune, India, were enrolled in a cohort and followed every 3 months for HIV infection (*55*). Serum retinol, various carotenoids and vitamin E concentrations were determined for 44 participants who later seroconverted and for 44 matched HIV-negative controls. The time between the visit when vitamin status was determined and the visit when the participant first was found to have HIV seroconverted was 6 months. Serum β-carotene below 0.075 μmol/L (i.e., the upper tertile) was associated with an increased risk of seroconversion (odds ratio [OR] 4.67; 95% confidence interval [CI] 1.34, 16.24), which increased further after adjustment for risk behaviour, age and other confounders. Serum retinol and vitamin E were respectively associated with non-statistically significant increased (adjusted OR=2.96, p=0.34) and decreased (adjusted OR 0.35, p=0.12) risks of seroconversion. Finally, a study was conducted among sexually active men in Nairobi, Kenya (*56*). A cohort of HIV-negative men seeking treatment for an acute genital ulcer was established. After treatment, the men were tested for HIV infection at 3-month intervals; mean follow-up time was 6 months. For each participant who seroconverted, two or three consecutive participants who remained HIV negative were included as controls. Surprisingly, although there were no differences in socioeconomic status and history of unprotected sex, the 38 seroconverters had higher baseline serum retinol values than did the 94 controls. Serum retinol greater than 0.70 μmol/L was associated with a greater than two-fold increased risk of seroconversion. The authors suggest that the results may be due to an effect of vitamin A on differentiation of target cells of the monocyte/macrophage lineage in the mucosa, as previously reported (*57*).

The outcome of these studies was risk of HIV infection rather than susceptibility per se because data on exposure to HIV are difficult to obtain and the acute phase response was not controlled for. The status of other micronutrients likely to be associated with vitamin A and of importance to risk of HIV infection was not assessed.

4.1.2.3. Progression and morbidity

The role of micronutrient status on progression and morbidity was addressed in several cohort or nested case-control studies. Several studies examined the role of vitamin A status in later progression of HIV based on serum retinol determinations. Two reports from the same cohort of intravenous drug users in the United States found that low serum retinol was associated with increased mortality after controlling for low CD4+ counts (*58,59*).

Several studies were based on the U.S. Multicenter AIDS Cohort (MAC) Study. In a nested case-control design, serum copper was found to be higher and serum zinc lower in 54 HIV-positive homosexual men who progressed to AIDS compared with 54 who did not progress within the mean 2.5-year follow-up (*60*). There were no differences in the zinc and copper concentrations of toenail samples or in zinc and copper intake. However, the associations most likely reflect the effects of advancing HIV infection on serum zinc and copper. Data from 311 HIV-positive homosexual men from the same cohort showed that high serum vitamin E was associated with a reduced risk of progression to AIDS or to death during a mean of 9 years follow-up whereas serum retinol was not associated with progression (*61*). In the same study participants, low serum concentrations of vitamin B_{12} but not vitamin B_6 and folate were associated with increased progression (*62*).

A relationship between low serum selenium and HIV progression was reported by several prospective cohort studies in different study populations. Among 95 adult homosexual men in France, low serum selenium was associated with the risk of death after CD4+ counts were controlled for (*63*). Baum et al. (*64*) followed 125 HIV-positive U.S. drug users for 3.5 years. Low serum retinol, vitamin B_{12}, zinc and selenium were all associated with progression to death after CD4+ counts were controlled for, but in the final multivariable model only low serum selenium was a predictor (relative risk [RR] 10.8; 95% CI 2.4, 49.2). Similar results were obtained by the same group from studies of HIV-positive homosexual men (*65*) and children with symptomatic HIV infection (*66*). Recently, observational data from the Tanzania Vitamin and HIV Trial (*67*) was reported (*68*), showing that low serum selenium assessed during pregnancy was a predictor of mortality over 5.7 years of follow-up among HIV-positive women in Tanzania after adjustment for the trial interventions. However, serum selenium seems to be a negative acute phase reactant (*69*), and there were no attempts to control for

the acute phase response in these studies. Low serum selenium has also been suggested as a risk factor of mycobacterial disease in HIV-positive individuals (*70*).

Several studies addressed the role of iron in HIV infection. A study was conducted in Italy, Greece and France of HIV-positive patients with thalassaemia major, a haemoglobinopathy with increased iron absorption and risk of iron overload. HIV progressed significantly faster in those prescribed a low dose of an iron chelator than in those prescribed an adequate dose (*71*), and the effect seemed to be explained by higher serum ferritin levels (*72*). In a study of 348 HIV-1–positive U.S. adults who had diagnostic bone marrow aspirates done, iron status was assessed through iron staining of the bone marrow (*73*). Those with high iron stores had shorter survival from the time of diagnosis than did those with normal or low iron stores. Although direct assessment of iron stores is a better measure of iron status than serum ferritin, the association may be due to reverse causality in that iron is sequestered and accumulates in the stores with progression of HIV. Other studies assessed the effect of iron indirectly. Haptoglobin is an acute phase protein which removes free haemoglobin (*74*). Because heme and iron are prooxidants and catalyse production of free radicals, haptoglobin is an important endogenous antioxidant. Although haptoglobin phenotype (Hp) 2-2 may have beneficial immunological properties, it has less affinity for haemoglobin. Any harmful effects of Hp 2-2 are therefore likely to reflect the local effects of iron due to iron-driven formation of free radicals. Interestingly, a cohort study among HIV-1–positive adults in Europe found that those with Hp 2-2 had a higher viral load, higher iron stores and significantly shorter survival times (7 years) than did those with Hp 1-1 and 2-1 (11 years) (*75*). In a study among pregnant women in Zimbabwe, Hp 2-2 and nondepleted iron stores were found to be independent predictors of viral load after the acute phase response was controlled for (*29*). Although the data could reflect that storage iron and iron not removed by haptoglobin increase viral replication, no inference about cause and effect can be made from this study. A similar study of pregnant women in Malawi did not reveal any associations between iron status and HIV-1 viral load (*76–78*).

Given the lack of valid markers of micronutrient status, especially in people with infections, data from these observational studies should be interpreted cautiously. Furthermore, because micronutrient deficiencies usually coexist, and most studies only assess the status of a single or few

micronutrients, confounding by other micronutrients is likely. Studies assessing micronutrient intake rather than status may be better able to account for the many micronutrients but have other limitations.

The role of micronutrient intake in progression of HIV to AIDS was studied in 281 homosexual or bisexual HIV-positive men taking part in the MAC Study (*79*). Daily dietary and supplemental intakes of micronutrients were assessed using a self-administered semiquantitative food frequency questionnaire at inclusion and every 6 months, and the participants were followed for a median of 6.8 years. After symptoms, CD4+ counts, *Pneumocystis carinii* pneumonia prophylaxis and energy intake were controlled for, vitamin A, niacin, and zinc were predictors of progression whereas vitamin C intake was only marginally significant. Compared with the lowest three quartiles combined, the highest quartile of intake was associated with lower risk for both niacin (0.51; 95% CI 0.29, 0.92) and vitamin C (0.59; 95% CI 0.34, 1.03). In contrast, for vitamin A, the second and third quartiles combined, compared with the lowest, were associated with reduced risk (0.57; 95% CI 0.35, 0.91) but the highest quartile was not (0.95; 95% CI 0.54, 1.69). This led the authors to suggest a U-shaped relationship between vitamin A and HIV progression. Surprisingly, the risk of progression to AIDS increased with increasing zinc intake. The third and fourth quartiles were associated with relative risks of 1.85 (95% CI 1.03, 3.31) and 2.97 (95% CI 1.59, 5.56), respectively (*79*). With time-to-death as the outcome (*80*), intakes of thiamin, riboflavin, vitamin B_6 and niacin were all associated with reduced risk of death. Because these four B vitamins were strongly correlated, vitamin B_6 was chosen to represent the B vitamins in the final multimicronutrient model: vitamin B_6 in the fourth (0.45; 95% CI 0.28, 0.73) and β-carotene in the third (0.60; 95% CI 0.37, 0.98) quartiles were associated with reduced risk whereas intakes of zinc in the third (1.84; 95% CI 1.16, 2.93) and fourth (2.44; 95% CI 1.51, 3.95) quartiles were associated with increased risks. In fact, use of zinc supplements was associated with increased risk of death (1.49; 95% CI 1.02, 2.18).

However, the study does not address the effects of low intakes. For example, over 75% of the study participants had intakes of vitamin A above 180% of the recommended dietary allowance. Although half consumed less than the recommended dietary allowance for zinc, some had very high intakes of supplemental zinc. Because the bioavailability of zinc is high in a balanced Western diet, some individuals may have had high intakes of zinc causing immunosuppression. These findings may

not be applicable to developing countries, where the intake and bioavailability of zinc and other micronutrients are low.

No effect of zinc intake was observed in the San Francisco Men's Health Study (*81*). In this prospective cohort study, dietary and supplemental intake of various micronutrients was assessed and the participants were followed for 6 years for progression to AIDS. After the disease stage at enrollment was controlled for via a composite measure of symptoms and CD4+ count, high intakes of iron, vitamin E and riboflavin were associated with reduced progression to AIDS. High intakes of vitamin A, thiamin and vitamin C were associated with reduced progression but were only marginally significant. The daily intake of a micronutrient supplement was also a negative predictor of progression (*81*).

4.2. Intervention studies

RCTs to assess the effect of micronutrient interventions have become increasingly common the past two decades. With a randomized, placebo-controlled, double-blind trial, it is possible to efficiently control confounding and reduce bias, and this type of trial is therefore the strongest tool for establishing a cause-effect relationship. Nonetheless, in nutrition research difficulties exist with respect to both design of the intervention and interpretation of the results.

4.2.1. Methodological issues

Most micronutrient trials are based on the implicit but often wrong assumptions that all study participants are initially deficient with respect to one or more micronutrients and are successfully repleted by the micronutrient intervention, although it is possible that supplementation may improve immune functions and clinical outcomes in those who are not deficient. A simple linear or threshold dose-response relationship cannot be assumed because different doses may have different and even opposite effects, and the effect of the same dose may depend on baseline micronutrient intake or status. For example, a zinc supplement may increase immune function in individuals with low baseline intake, have no effect in those with adequate intake and impair immune function in those with a high intake.

Micronutrients often interact. This means that the effect of a micronutrient deficiency—and of a micronutrient supplement—depends on the status and intake of other micronutrients. An example of

a micronutrient-micronutrient interaction is copper deficiency, which, although rarely a problem unless the intake of zinc is very high, may lead to iron deficiency anaemia because copper is essential for the enzyme responsible for transport of iron into haemoglobin (*82*). Iron supplementation may affect the distribution of vitamin A, and zinc deficiency impairs the conversion of β-carotene to vitamin A and mobilization of vitamin A from the stores (*82,83*). The antioxidant vitamin C in the diet increases absorption of nonheme iron (*84*) and also restores the radical-scavenging activity of vitamin E (*85*). Because both vitamin E and selenium scavenge reactive oxygen species, a higher intake of one reduces the requirements for the other (*86*) whereas the requirements for both are increased if the intake of the prooxidant iron is high (*87*). Accordingly, data from a randomized, controlled micronutrient trial may have high validity but the generalizability to different populations should be carefully considered.

4.2.2. Review of intervention studies

4.2.2.1. Mother-to-child transmission and other pregnancy outcomes

An observational study in Malawi showed that low serum vitamin A was a strong, negative predictor of mother-to-child HIV transmission (*44*). On the basis of this study, three large micronutrient supplementation trials were conducted among pregnant HIV-positive women in South Africa (*88*), Malawi (*89*) and Tanzania (i.e., the Tanzania Vitamin and HIV Trial referred to above) (*90–92*). These trials all assessed the effect of maternal vitamin A supplementation but were different with respect to baseline vitamin A status, study intervention and co-interventions (i.e., micronutrient interventions provided to all study participants).

Vitamin A status at inclusion seemed to be considerably lower among women in Malawi (mean serum retinol 0.72 μmol/L) than in women in South Africa (0.95 μmol/L) and Tanzania (0.90 μmol/L). The study interventions were different with respect to dose, form, duration and regimen (**Table 3**). For example, vitamin A was given as preformed vitamin A supplements in the Malawian trial whereas a combination of preformed vitamin A and the provitamin A carotenoid β-carotene was given with a postpartum megadose of vitamin A in the South African and Tanzanian trials. In Malawi and South Africa the daily supplement was only given prenatally (i.e., from recruitment until delivery) whereas it was given perinatally (i.e., from recruitment through pregnancy and throughout lactation)

and for several years after in the study in Tanzania. The trials also differed with respect to when vitamin A was provided to all study participants. In Malawi, all mothers were given 100 000 IU at 6 weeks postpartum, and in Tanzania all infants received 100 000 IU at 6 months and thereafter double that amount every 6 months. Iron and folic acid supplements were provided to all study participants but the doses differed: in Malawi, South Africa and Tanzania, the daily doses of iron and folic acid were 30/0.4, 60/5 and 120/5 mg/mg, respectively. The Tanzanian trial used a two-by-two factorial trial whereby the effects of two interventions could be assessed simultaneously. The other intervention was a multivitamin supplement containing six B vitamins and vitamins C and E in doses between 3 and 10 times the recommended dietary allowance. Details about the design of these trials are presented in Table 3.

Considering the results of observational studies, the effects of the similar, albeit not identical, vitamin A interventions were surprising. In South Africa, vitamin A supplementation reduced the risk of preterm delivery but had no effect on mother-to-child HIV transmission when assessed at 3 months of age (*88*). Because a considerable proportion of mothers did not breastfeed their infants, as reflected by the relatively low transmission rates at 3 months, effects of vitamin A supplementation were not assessed at later times in South Africa. In Malawi vitamin A supplementation reduced risk of low birth weight and infant anaemia but had no effects on preterm delivery or mother-to-child HIV transmission at 6 weeks and 12 and 24 months of age (*89*). In Tanzania no effects on non-HIV pregnancy outcomes were found (*90*). Transmission over the first 24 months was increased by almost 40% (RR 1.38 [95% CI 1.09, 1.76]) in mothers receiving vitamin A (*93*). There were also higher relative risks of infant HIV infection at birth and 6 weeks of age (*91*) of about the same magnitude as the total relative risk over the first 2 years of life, although these effects were only marginally significant. Significantly more women receiving vitamin A supplements had detectable HIV in cervicovaginal lavage (*92*). The effects of the vitamin A interventions are presented in **Table 4**. The relative risks were consistently below 1 in the South African (0.91) and the Malawian trials (0.84–0.96), but above 1 in the Tanzanian trial (1.22–1.49) at all follow-up points, although only the latest at 24 months (1.38) was significant. The differences in the vitamin A regimens in the three studies do

not seem to explain the differences in results. Rather, the effect of vitamin A in the Tanzanian study seems to be basically different from the effects in the two other studies, even at birth and 6 weeks.

The inverse association between serum retinol and mother-to-child HIV transmission in observational studies and the lack of beneficial effects of vitamin A supplementation in these trials was surprising. However, it underscores the potential for confounding in observational studies, especially when micronutrient status is used as the exposure. Because serum levels of several micronutrients are correlated and affected in the same direction by the acute phase response and advancing HIV infection, confounding is obviously a major potential problem.

The apparent lack of effect of vitamin A supplementation in the trials in Malawi and South Africa and the adverse effect in the trial in Tanzania are difficult to explain. Confounding and bias may occur in RCTs. Confounding may occur if the randomization fails to balance other risk factors of the outcome, either by chance or because of flaws in the randomization process, or if inappropriate comparisons are made, and bias may occur because of a lack of randomization concealment, blinding and follow-up. Nonetheless, although chance cannot be excluded, there seems to be no reason to question the validity of the findings. Thus, the inconsistency between trial results may more likely be due to effect modification, such as the presence—in Tanzania, but not in Malawi and South Africa, or vice versa—of a factor that modifies the effect of the vitamin A intervention on mother-to-child HIV transmission. One factor that differed among sites was iron supplementation, which according to national policy was 120 mg/day in Tanzania whereas only 30 and 60 mg/day in Malawi and South Africa. Whether a high iron intake may modify the effect of vitamin A or β-carotene is not known. However, there is some evidence that the antioxidant vitamin C may acquire prooxidant properties in the presence of iron (*94*), and one may speculate whether in this trial β-carotene had adverse effects at the time of delivery and during lactation as a result of a build up of iron stores by the high-dose prenatal iron supplements. Vitamin A has been suggested to interact with childhood vaccinations with respect to mortality (*95*), but it is not clear whether the immunization schedule differed among trials. Other potential effect modifiers could be malaria treatment and prophylaxis, dietary intake of other nutrients, other infections, etc.

The multivitamin intervention of the Tanzanian trial considerably reduced adverse pregnancy outcome, such as foetal loss (RR 0.61; 95% CI 0.39, 0.94), small for gestational age (RR 0.57; 95% CI 0.39, 0.82) and low birth weight (RR 0.56; 95% CI 0.38, 0.82), and increased haemoglobin concentration and CD4+ count (*90*). In contrast, the relative risks of multivitamins on mother-to-child HIV transmission at birth, 6 weeks and 24 months were 1.54 (95% CI 0.94, 2.51, p=0.08), 1.17 (95% CI 0.81, 1.70) and 1.04 (95% CI 0.82, 0.1.32), respectively. The high RR at birth is noteworthy but may, as suggested by the authors, be attributable to survival bias (i.e., that the reduction in fetal loss is mirrored by more children being born with HIV).

However, among children found HIV negative at 6 weeks, multivitamins were associated with reduced transmission in mothers with low lymphocyte count (0.37; 95% CI 0.16, 0.87, interaction p=0.03) and low haemoglobin (0.48; 95% CI 0.24, 0.93, interaction p=0.06) although not low CD4+ count (0.93; 95% CI 0.55, 1.58, interaction p=0.69). Interestingly, maternal multivitamin supplementation also improved health of the offspring as it significantly increased CD4+ count and reduced diarrhoeal morbidity (*96*).

The effect of a prenatal multimicronutrient supplement on birth size was assessed in a randomized, controlled effectiveness trial among Zimbabwean women (*97*). Multimicronutrient supplementation was associated with 49 g (95% CI -6, 104) higher birth weight. The effect was 101 g (95% CI -3, 205) for the 33% of the population that was HIV positive and only 26 g (95% CI -38, 91) for the HIV-negative women, although the interaction was not significant. The effect on mother-to-child HIV transmission could not be assessed.

A large trial on the effect of postpartum maternal and infant megadose vitamin A supplementation, using a factorial design, was conducted among 14110 mother-infant pairs in Zimbabwe (*98*). The mother-infant pairs were randomised to maternal postpartum vitamin A (400.000 IU) or placebo, and infant vitamin A (50.000 IU) or placebo (*99*). Among infants found HIV negative at 6 weeks, there were no effects of maternal and infant supplementation on HIV infection or HIV infection and death at 6, 12 and 18 months after adjustment for maternal CD4, early breast feeding pattern and other factors (*98*).

4.2.2.2. Sexual transmission

No RCTs have been conducted to assess the effect of micronutrients in sexual transmission. Although effects on both infectiousness and susceptibility would be of interest, conducting such studies using clinical outcomes would be difficult for ethical and scientific reasons. However, studies using viral load in cervicovaginal secretion or semen as proxy endpoints of female-to-male and male-to-female transmission, respectively, are feasible.

4.2.2.3. Progression and morbidity

The effect of a daily multimicronutrient supplement on HIV progression and survival was evaluated in a randomized, placebo-controlled, double-blind trial in Thailand (*100*). The 481 HIV-positive participants had CD4+ counts between 50 and 550 x 10^6 cells/μL and had not taken antiretroviral drugs for the 30 days before recruitment. The commercial supplement contained 18 micronutrients as well as magnesium and cystine. The study participants were advised to take two tablets daily for 48 weeks. The supplemental intake of most micronutrients exceeded the recommended dietary allowances. Although only 23 died, the mortality ratio was 0.53 (95% CI 0.22, 1.25) but was 0.37 (95% CI 0.13, 1.06) in those with CD4+ counts below 200 and 0.26 (95% CI 0.07, 0.97) in those with counts below 100. However, there were no effects on CD4+ count or viral load. This trial was the first to be reported on the effect of micronutrients on progression in adults. Although the results are encouraging, effects with reasonable statistical certainty were only seen in subgroups with low CD4+ counts. Finally, effects on CD4+ count and viral load did not accompany the effect on mortality. Although effects on CD4+ count and viral load would have been biologically plausible and reassuring, they are not necessarily part of the causal pathway. The effect on mortality could reflect effects on risk of other infections or maintenance of lean body mass.

In the Tanzania Vitamin and HIV Trial, the HIV-positive women continued to receive daily supplements of vitamin A and multivitamins for several years in order to assess the effect on the mothers' HIV progression (*101*). In the primary analysis the effect of multivitamins alone, vitamin A alone and both were compared with placebo for both interventions. Multivitamin supplementation alone was shown to reduce progression of HIV to AIDS or death from AIDS-related causes by 59% (RR 0.41 [95% CI 0.20, 0.85]) over the first 2 years and by 29% (RR 0.71 [95% CI 0.51, 0.98]) over

the whole 4–8 year supplementation and follow-up period. The reduction in progression of HIV was accompanied by reductions in episodes of HIV-related morbidity. The effects of multivitamin supplementation on the clinical outcomes were probably mediated by viral load and CD4+, because there was a 0.18 $\log_{10}$ reduction in viral load and a 48 (95% CI 10, 85) x 10^6 cell/L increase in CD4+ count throughout the study. Vitamin A alone had no effect on the primary outcome, progression to AIDS or AIDS-related death, neither over the first 2 years (RR 0.76 [95% CI 0.42, 1.37]) or over the whole period (RR 0.88 [95% CI 0.64, 1.19]), but was associated with a reduced risk of more than two stages of progression and progression to stage 3 or more. However, vitamin A had not effect on HIV-related morbidity, CD4+ count or viral load.

A randomized, controlled vitamin A supplementation trial was conducted with 687 Tanzanian children between 6 and 60 months of age who were hospitalized with pneumonia in Dar es Salaam (*102,103*). Vitamin A supplementation (200 000 IU on the day of admission and the following day, half to those below 12 months of age) had no significant effect on case fatality (RR 1.63; 95% CI 0.67, 3.97) (*102*), but additional doses after 4 and 8 months reduced all-cause mortality over 2 years after discharge (RR 0.51; 95% CI 0.29, 0.90). Among the 58 (9%) of the children found HIV positive, all-cause mortality was reduced by 63% (RR 0.37; 95% CI 0.14, 0.95) (*103*).

The effect of vitamin A supplementation on morbidity was assessed in an RCT in 118 children of HIV-positive mothers in Durban, South Africa (*104*). The children received vitamin A (50 000 IU at 1 and 3 months, 100 000 IU at 6 and 9 months and 200 000 IU at 12 and 15 months of age) or placebo. Vitamin A reduced overall morbidity by 31% (OR 0.69; 95% CI 0.48, 0.99) and diarrhoeal morbidity by 49% (OR 0.51; 95% CI 0.27, 0.99) in 28 children known to be HIV positive.

In contrast, no effect on diarrhoeal morbidity or mortality was found from supplementing HIV-positive adults with high doses of vitamins A, C and E; zinc; and selenium for 2 weeks (*105*). However, the patients had advanced HIV disease with persistent diarrhoea and may not have absorbed the micronutrients. A reduction in hospitalization rate due to daily selenium supplementation was reported from an RCT among 187 HIV-positive U.S. adults (*106*).

A study was conducted in Mwanza, Tanzania, among patients with pulmonary TB, of which almost half were HIV co-infected, with an aim to assess the effect on sputum culture conversion and

weight gain (*107*). The patients were randomised to receive two daily tablets containing zinc (45 mg of elementary zinc) or placebo and multimicronutrients (vitamins A, B, C, D, E, and selenium and copper, in 3-10 times the RDA) or placebo, throughout TB treatment. While there was no effect on culture conversion (*108*), zinc and multimicronutrients together, but neither alone, increased the weight gain by 2.37 kg (95% CI: 0.91; 3.83). Among HIV co-infected patients, zinc and multimicronutrients combined reduced mortality (RR 0.29; 95% CI: 0.10, 0.80), although there were no effects on viral load. However, mortality was not a primary outcome and effects were only seen in subgroups, so the findings need to be confirmed in different settings (*107*).

4.2.2.4. HIV viral load

RCTs using clinical end-points, such as transmission or progression, require long follow-up and large sample sizes and may be ethically difficult. HIV load in various body fluids and secretions have therefore been used as proxy end-points for transmission and progression (Table 1). This seems reasonable in view of the convincing data showing that plasma HIV load is a strong determinant of progression of HIV infection to death (*109*), as well as sexual (*110*) and mother-to-child transmission (*111,112*). However, a micronutrient intervention may have beneficial effects on clinical outcomes despite a lack of effect on viral load, as was seen in the trial from Thailand (*100*) and the trial among HIV-co-infected TB patients in Tanzania (*107*). It is not clear whether this is because the effect is mediated through mechanisms other than viral replication, such as maintenance or gain in lean body mass. Alternatively, it is possible that viral load is too insensitive a measure of viral replication, and that change in viral load after ex vivo stimulation of whole blood may be a better measure. However, viral load in cervicovaginal secretion and semen is likely a stronger determinant of intrapartum mother-to-child and sexual transmission, respectively, and milk viral load is likely a stronger determinant of postnatal mother-to-child transmission than is plasma viral load.

Several RCTs have assessed the effect of a micronutrient supplement on viral load (**Table 5**). The effect of vitamin A was studied in four trials. Neither a single megadose of 200 000 IU given postpartum to 24 pregnant South African women (*113*) nor a single megadose of 300 000 IU given postpartum to 40 Zimbabwean women (*114*) had any effect on plasma HIV load. Similarly, 10 000 IU vitamin A daily given over 6 weeks to 400 Kenyan women who were not pregnant had no effect on

plasma viral load or genital HIV DNA or RNA (*115*). In 120 intravenous drug users, a single dose of 200 000 IU had no effect on viral load assessed after 2 and 4 weeks (*116*). However, a study in Canadian HIV-positive individuals found that daily supplementation with large doses of vitamins C and E (1000 mg and 800 IU, respectively) for 12 weeks was associated with a considerable but nonsignificant reduction in plasma viral load (*117*): the mean plasma viral load increased 0.5 log in the placebo and declined 0.45 log in the intervention group. Based on a randomised iron supplementation trial in Kenya, no effect of 60 mg iron given twice weekly on viral load was seen among 25 HIV-positive individuals (*118*). The effect of selenium and multivitamins was assessed as part of the Kenyan vitamin A trial mentioned above (*115*), using the same placebo group (*119*). There was no effect on vaginal HIV shedding but the intervention was reported to increase cervical shedding. However, although the proportions of women shedding HIV from the cervical tract at follow-up were 31% and 17% in the intervention and placebo groups, respectively, the proportions at baseline were 25% and 18%. In the Thai trial mentioned above (*100*) the viral load was also assessed in semen and cervico-vaginal secretions, but no effects were found (*120*). As mentioned, in the trial among patients with pulmonary TB and HIV, no effect of daily supplements with 45 mg of zinc, and of a multimicronutrient supplement, on plasma viral load were found (*107*).

5. Current evidence of role of individual micronutrients

5.1. Vitamin A

In view of the importance of vitamin A to immune functions and childhood infections (*121,122*) and mortality (*8,9*), it is plausible that vitamin A is beneficial in HIV infection. However, in vitro studies in different cell lines suggest that vitamin A may both reduce (*123*) and increase viral replication (*57*).

Despite the biological plausibility of a role for vitamin A in epithelial integrity, no data support the hypothesis that increased vitamin A intake or status reduces susceptibility to infection through sexual transmission. Vitamin A supplementation does not seem to affect viral load in plasma. Maternal vitamin A supplementation during pregnancy and postpartum was not found to affect

mother-to-child HIV transmission in two trials (*88,89*). However, in the Tanzanian trial, vitamin A supplementation increased risk of HIV shedding in cervicovaginal lavage and mother-to-child HIV transmission (*92,93*). It is not clear which component of the vitamin A intervention had adverse effects (i.e., preformed vitamin A or β-carotene) and if the adverse effect was due to interaction with other factors.

Despite the lack of effect on plasma viral load in adults, regular megadoses of vitamin A to HIV-positive children under 5 years of age have been shown to reduce diarrhoeal morbidity (*104*), diarrhoeal- and AIDS-specific mortality and all-cause mortality (*103*). However, these effects were only demonstrated in subgroups with relatively few children, where confounder control is less efficient, although consistent with effects seen in settings in Asia and Africa where HIV was not prevalent (*8*).

5.2. Vitamins B, C and E

Several of the B vitamins as well as vitamins C and E have been associated with reduced risk of HIV progression in observational studies (*61,62,79–81*). If the associations reflect causal relationships, this is most likely due to their strong antioxidant properties, although effects on the immune system, in particular of vitamin E, may also play a role. Because antioxidants interact, studying the effect of some or all of these micronutrients given together is rational. A small RCT found a large, albeit not significant, reduction in viral load after large doses of vitamin C and E (*117*). A large RCT in Tanzania showed that a daily prenatal multivitamin supplement containing 3–10 times the recommended dietary allowance doses of six B vitamins and vitamins C and E reduced the risk of adverse pregnancy outcome (*90*), postnatal mother-to-child transmission and child mortality (i.e., in subgroups) (*93*), child morbidity and progression to AIDS or death among adults (*101*).

5.3. Iron

Iron deserves special mention because both the effect of HIV infection on status and the effect of status and intake on HIV infection seem to be different from those of other micronutrients. Iron stores decline in early asymptomatic HIV infection probably because of impaired absorption (*24*) but increase with advancing HIV infection as iron accumulates in macrophages and other cells (*124*). Iron may have adverse effects in HIV and other viral infections (*125,126*). For example, iron increases and

iron chelation reduces HIV replication in in vitro studies (*127,128*). Nonetheless, although data from observational studies are in accordance with the hypothesis that iron may have adverse effects, iron supplementation twice weekly did not increase viral load (*118*). Because iron is widely administered in much higher doses to patients with anaemia and routinely to pregnant women, its effect on plasma viral load and clinical end-points needs to be assessed. Additionally, data suggest that iron may increase the susceptibility to and severity from common and opportunistic infections, such as tuberculosis (*129–131*). Studies are needed to clarify the effect of iron status and intake, including supplementation, on HIV transmission and progression and risk of tuberculosis and other secondary infections.

5.4. Zinc

Zinc is a component of both structural and catalytic proteins of HIV. Zinc is required for the activity of reverse transcriptase and the production of infectious virus (*132*) and may inhibit HIV replication through binding to the catalytic site of HIV protease (*133*). The association between zinc intake and increased progression to AIDS and death, found in one cohort study in U.S. patients with a high intake of highly bioavailable zinc (*79,80*) but not in another (*81*), may have delayed assessment of the effect of zinc supplementation in populations at risk of zinc deficiency.

Given the considerable importance of zinc for immune functions (*4*) and in the prevention of diarrhoea and respiratory tract infections (*134*), it is likely to play a role in HIV infection (*135,136*). A RCTs in adults with pulmonary TB co-infected with HIV found no significant effect of 45 mg of zinc daily during TB treatment on viral load. In fact, zinc in combination with other micronutrients considerably increased weight gain and seemed to increase survival during treatment (*107*).

5.5. Selenium

Studies in laboratory animals showed that the passage of a benign virus through a selenium-deficient host changed the viral genome and converted it to virulence (*137*). The effect was considered to be due to oxidative stress because vitamin E deficiency and iron overload had the same effects (*138)*. Because HIV and other viruses encode for selenoproteins, host selenium status has been postulated to be involved in regulation of viral replication (*139,140*). However, despite the finding from several observational studies that low serum selenium is strongly associated with HIV

progression (*63–66*), there are no reports of the effect of selenium supplementation on viral load or clinical HIV endpoints although reduced hospitalization rate was reported (*109*).

6. Research gaps

The main research priorities are the following:

- Establish micronutrient requirements among people with HIV infection.
- Develop micronutrient interventions to reduce transmission and progression of HIV
- Establish how micronutrients with potential adverse effects on HIV infection can be safely given.

A more comprehensive list of research priorities is given in Table 6.

7. Summary

Data confirm that micronutrients play an important role in HIV infection. Like other infections, HIV infection seems to impair micronutrient status, and micronutrient status and intake may affect HIV transmission, progression and morbidity. However, data also confirm that although some micronutrient supplements may be beneficial, others may have adverse effects, and the same micronutrient supplement may even be beneficial in some settings and have adverse effects in others.

Although conclusive evidence is lacking, some data suggest that increased iron intake may be detrimental in HIV infection, selenium may be beneficial, and zinc could prove beneficial, although adverse effects cannot be precluded.

As for effects on transmission of HIV, evidence from one RCT showed that supplementation with preformed vitamin A and β-carotene increased mother-to-child HIV transmission, but two other vitamin A trials found no evidence of adverse effects. Less strong evidence, based on subgroup effects from RCTs, showed that supplementation of pregnant women with high doses of vitamins B, C and E may reduce postnatal mother-to-child HIV transmission.

With respect to effects on progression of HIV, evidence from RCTs shows that regular megadose vitamin A supplementation may reduce diarrhoeal morbidity and mortality and all-cause mortality in children younger than 5 years of age. Furthermore, daily supplementation with a broad

multimicronutrient or a high-dose multivitamin supplement have been shown to reduce progression and mortality among adults.

The effect of a given micronutrient intervention will depend on the background dietary status and intake in the study population. It is important to determine appropriate markers of micronutrient status in an RCT so that the effect of the intervention can be assessed at different levels of baseline status. In addition, the status and intake of other nutrients may modify the effect of the micronutrient intervention. Therefore, different effects of the same intervention, even effects in different directions, are conceivable. Factorial designs can be used to address the issue of effect modification or interactions between micronutrients. Such effect modification cannot always be identified within a study but may occur between studies. Therefore, recommendations should preferably be based on more than one trial.

A major concern is the apparently adverse effect of vitamin A supplementation on mother-to-child HIV transmission. Because vitamin A is an essential nutrient and vitamin A deficiency is widespread in populations afflicted by HIV, there is an urgent need to find out whether the adverse effect is due to the form or dose of vitamin A given or the presence of a factor that adversely modifies its effect. If this can be clarified, then the global commitment to combat vitamin A deficiency may be continued without the risk of increasing mother-to-child HIV transmission.

The findings from the same trial, that a daily high-dose multivitamin supplement may not only reduce adverse pregnancy outcome and mother-to-child transmission but also considerably reduce progression of HIV, are promising.

Nevertheless, the generalizability of the findings remains to be determined. Although it may be reasonable to expect a similar effect among pregnant HIV-positive women elsewhere in Tanzania, it is not clear whether the findings can be generalized to pregnant HIV-positive women in other countries, women who are not pregnant, men and children, tuberculosis patients coinfected with HIV, those on ARV therapy, etc. The main concern is that it is an unbalanced, high-dose supplement. As a pre- or perinatal supplement, lower doses of the vitamins and inclusion of zinc and other essential minerals would have been preferable if the same or greater effects could have been achieved. That

would make it possible to safely give it to all women attending antenatal care, even in settings without access to HIV testing.

Although not seen in the present study, adverse effects of the high-dose multivitamin supplement may occur when it is given to individuals with lower requirement, higher dietary intake or other background characteristics. Conversely, the high-dose multivitamin supplement is most likely inadequate when used in populations with low intake of zinc and other minerals.

It would be useful if studies using clinical end-points could assess the effect on viral load in plasma, cervicovaginal secretions and breast milk and the relationship between viral load and the clinical outcomes. If it can be shown that these variables are valid as proxies for clinical outcomes, then they could be used as such in studies on the effect of different micronutrient supplements. This will allow identification of the optimal composition and doses. Testing the effect of each single micronutrient may not be realistic. However, micronutrients with potential adverse effects may need to be addressed separately, preferably using factorial designs. For example, a factorial trial on the effects of preformed vitamin A, β-carotene and iron on viral load may allow identification of the micronutrient or the potential micronutrient–micronutrient interaction responsible for the adverse effect on mother-to-child transmission.

8. References

1. Scrimshaw NS, Taylor CE, Gordon JE. *Interactions of nutrition and infection.* Geneva, World Health Organization, 1968.
2. Tomkins A, Watson F. Malnutrition and infection. A review. Geneva, ACC/SCN, 1989.
3. Friis H. Micronutrients and infections: an introduction. In: Friis H, ed. *Micronutrients and HIV infection*. Boca Raton, CRC Press, 2001:1-21.
4. Shankar AH, Prasad AS. Zinc and immune function: the biological basis of altered resistance to infection. *American Journal of Clinical Nutrition,* 1998, 68:447S-463S.
5. Chandra RK. Excessive intake of zinc impairs immune responses *JAMA*, 1984, 252:1443-1446.

6. Black RE, Morris SS, Bryce J. Where and why are 10 million children dying every year? *Lancet*, 2003, 361:2226-2234.
7. Jones G, et al. How many child deaths can we prevent this year? *Lancet*, 2003, 362:65-71.
8. Fawzi WW et al. Vitamin A supplementation and child mortality. A meta-analysis. *JAMA*, 1993, 269:898-903.
9. Glasziou PP, Mackerras DE. Vitamin A supplementation in infectious diseases: a meta-analysis. *BMJ*, 1993, 306:366-370.
10. Humphrey JH, West Jr KP, Sommer A. Vitamin A deficiency and attributable mortality among under-5-year-olds. *Bulletin of the World Health Organization*, 1992, 70:225-232.
11. Alnwick DJ. Combatting micronutrient deficiencies: problems and perspectives. *Proceedings of the Nutrition Society*, 1998, 57:137-147.
12. Gibson RS, Ferguson EL. Assessment of dietary zinc in a population. *American Journal of Clinical Nutrition*, 1998, 68:430S-434S.
13. Bhutta ZA et al. Prevention of diarrhea and pneumonia by zinc supplementation in children in developing countries: pooled analysis of randomized controlled trials Zinc Investigators' Collaborative Group. *Journal of Pediatrics*, 1999, 135:689-697.
14. Bhutta ZA et al. Therapeutic effects of oral zinc in acute and persistent diarrhea in children in developing countries: pooled analysis of randomized controlled trials *American Journal of Clinical Nutrition,* 2000, 72:1516-1522.
15. Fauci AS, Lane HC. Human immunodeficiency virus (HIV) disease: AIDS and related disorders Harrison's Online, 2003 (http://harrisons.accessmedicine.com/harrisons/public/index.html, accessed 6 October 2004).
16. Pantaleo G et al. HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease *Nature*, 1993, 362:355-358.
17. Smith PD et al. NIH conference. Gastrointestinal infections in AIDS [see comments]. *Annals of Internal Medicine*, 1992, 116:63-77.
18. Frieden TR et al. Tuberculosis. *Lancet*, 2003, 362:887-899.

19. Whalen CC et al. Impact of pulmonary tuberculosis on survival of HIV-infected adults: a prospective epidemiologic study in Uganda. *AIDS*, 2000, 14:1219-1228.
20. Brown KH et al. Potential magnitude of the misclassification of a population's trace element status due to infection: example from a survey of young Peruvian children. *American Journal of Clinical Nutrition*, 1993, 58:549-554.
21. Filteau SM, Tomkins AM. Micronutrients and tropical infections. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1994, 88:1-3, 26.
22. Tomkins A. Assessing micronutrient status in the presence of inflammation. *Journal of Nutrition*, 2003, 133:1649S-1655S.
23. Filteau SM et al. Influence of morbidity on serum retinol of children in a community-based study in northern Ghana. *American Journal of Clinical Nutrition*, 1993, 58:192-197.
24. Friis H et al. HIV and other predictors of serum folate, serum ferritin, and hemoglobin in pregnancy: a cross-sectional study in Zimbabwe. *American Journal of Clinical Nutrition*, 2001, 73:1066-1073.
25. Friis H et al. HIV and other predictors of serum beta-carotene and retinol in pregnancy: a cross-sectional study in Zimbabwe. *American Journal of Clinical Nutrition*, 2001, 73:1058-1065.
26. Ullrich R et al. Small intestinal structure and function in patients infected with human immunodeficiency virus (HIV): evidence for HIV-induced enteropathy. *Annals of Internal Medicine*, 1989, 111:15-21.
27. Glynn JR et al. Decreased fertility among HIV-1-infected women attending antenatal clinics in three African cities. *Journal of Acquired Immune Deficiency Syndromes*, 2000, 25:345-352.
28. Ryder RW et al. Effect of HIV-1 infection on tuberculosis and fertility in a large workforce in Kinshasa, Democratic Republic of the Congo. *AIDS Patient Care and STDs*, 2000, 14:297-304.
29. Friis H et al. Iron, haptoglobin phenotype, and HIV-1 viral load: a cross-sectional study among pregnant Zimbabwean women. *Journal of Acquired Immune Deficiency Syndromes*, 2003, 33:74-81.

30. Friis H et al. HIV-1 viral load and elevated serum alpha(1)-antichymotrypsin are independent predictors of body composition in pregnant Zimbabwean women. *Journal of Nutrition*, 2002, 132:3747-3753.

31. Baum M et al. Inadequate dietary intake and altered nutrition status in early HIV-1 infection. *Nutrition*, 1994, 10:16-20.

32. Beach RS et al. Specific nutrient abnormalities in asymptomatic HIV-1 infection. *AIDS*, 1992, 6:701-708.

33. Bogden JD et al. Micronutrient status and human immunodeficiency virus (HIV) infection. *Annals of the New York Academy of Sciences*, 1990, 587:189-195.

34. Coodley GO, Coodley MK, Nelson HD, Loveless MO. Micronutrient concentrations in the HIV wasting syndrome. *AIDS*, 1993, 7:1595-1600.

35. Sappey C et al. Vitamin, trace element and peroxide status in HIV seropositive patients: asymptomatic patients present a severe beta-carotene deficiency. *Clinica Chimica Acta*, 1994, 230:35-42.

36. Baum MK et al. Micronutrients and HIV-1 disease progression *AIDS*, 1995, 9:1051-1056.

37. Periquet BA et al. Micronutrient levels in HIV-1-infected children. *AIDS*, 1995 9:887-893.

38. Tang AM et al. Improved antioxidant status among HIV-infected injecting drug users on potent antiretroviral therapy. *Journal of Acquired Immune Deficiency Syndromes*, 2000, 23:321-326.

39. Raiten DJ. Nutritional considerations in the use of ARV/HAART in resource-limited settings. 2005, World Health Organization.

40. Schreck R, Rieber P, Baeuerle PA. Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-kappa B transcription factor and HIV-1. *EMBO Journa*l, 1991, 10:2247-2258

41. Beisel WR. Nutritionally acquired immune deficiency syndromes. In: Friis H, ed. *Micronutrients and HIV infection*. Boca Raton, CRC Press, 2001:23-42.

42. Mostad SB et al. Hormonal contraception, vitamin A deficiency, and other risk factors for shedding of HIV-1 infected cells from the cervix and vagina. *Lancet*, 1997, 350:922-927.

43. Semba RD et al. Human immunodeficiency virus load in breast milk, mastitis, and mother- to-child transmission of human immunodeficiency virus type 1. *Journal of Infectious Diseases*, 1999, 180:93-98.

44. Semba RD et al. 1994b Maternal vitamin A deficiency and mother-to-child transmission of HIV-1. *Lancet*, 343:1593-1597.

45. Semba RD et al. Infant mortality and maternal vitamin A deficiency during human immunodeficiency virus infection. *Clinical Infectious Diseases*, 1995, 21:966-972.

46. Graham N, Bulterys M, Chao A. Effect of maternal vitamin A deficiency on infant mortality and perinatal HIV transmission National Conference on Human Retroviruses and Related Infection Baltimore, Johns Hopkins University,1993.

47. Burns DN et al. Vitamin A deficiency and other nutritional indices during pregnancy in human immunodeficiency virus infection: prevalence, clinical correlates, and outcome. Women and Infants Transmission Study Group. *Clinical Infectious Diseases*, 1999, 29:328-334.

48. Greenberg BL et al. Vitamin A deficiency and maternal-infant transmissions of HIV in two metropolitan areas in the United States. *AIDS*, 1997, 11:325-332.

49. Burger H et al. Maternal serum vitamin A levels are not associated with mother-to-child transmission of HIV-1 in the United States. *Journal of Acquired Immune Deficiency Syndromes*, 1997, 14:321-326.

50. Nduati RW et al. Human immunodeficiency virus type 1-infected cells in breast milk: association with immunosuppression and vitamin A deficiency. *Journal of Infectious Diseases*, 1995, 172:1461-1468.

51. John GC et al. Genital shedding of human immunodeficiency virus type 1 DNA during pregnancy: association with immunosuppression, abnormal cervical or vaginal discharge, and severe vitamin A deficiency. *Journal of Infectious Diseases*, 1997, 175:57-62.

52. French AL et al. Vitamin A deficiency and genital viral burden in women infected with HIV-1. *Lancet*, 2002, 359:1210-1212.

53. Baeten JM et al. Selenium deficiency is associated with shedding of HIV-1--infected cells in the female genital tract. *Journal of Acquired Immune Deficiency Syndromes*, 2001 26:360-364.

54. Moore PS et al. Role of nutritional status and weight loss in HIV seroconversion among Rwandan women. *Journal of Acquired Immune Deficiency Syndromes*, 1993, 6:611-616.

55. Mehendale SM et al. Low carotenoid concentration and the risk of HIV seroconversion in Pune, India. *Journal of Acquired Immune Deficiency Syndromes*, 2001, 26:352-359.

56. MacDonald KS et al. Vitamin A and risk of HIV-1 seroconversion among Kenyan men with genital ulcers. *AIDS*, 2001, 15:635-639.

57. Turpin JA, Vargo M, Meltzer MS. Enhanced HIV-1 replication in retinoid-treated monocytes Retinoid effects mediated through mechanisms related to cell differentiation and to a direct transcriptional action on viral gene expression. *Journal of Immunology*, 1992, 148:2539-2546.

58. Semba RD et al. Increased mortality associated with vitamin A deficiency during human immunodeficiency virus type 1 infection. *Archives of Internal Medicine*, 1993, 153:2149-2154.

59. Semba RD et al. Vitamin A deficiency and wasting as predictors of mortality in human immunodeficiency virus-infected injection drug users. *Journal of Infectious Diseases*, 1995, 171:1196-1202.

60. Graham NM et al. Relationship of serum copper and zinc levels to HIV-1 seropositivity and progression to AIDS. *Journal of Acquired Immune Deficiency Syndromes*, 1991, 4:976-980.

61. Tang AM et al. Association between serum vitamin A and E levels and HIV-1 disease progression. *AIDS*, 1997, 11:613-620.

62. Tang AM et al. Low serum vitamin B-12 concentrations are associated with faster human immunodeficiency virus type 1 (HIV-1) disease progression. *Journal of Nutrition*, 1997 127:345-351.

63. Constans J et al. Serum selenium predicts outcome in HIV infection. *Journal of Acquired Immune Deficiency Syndromes*, 1995, 10:392.

64. Baum MK et al. High risk of HIV-related mortality is associated with selenium deficiency. *Journal of Acquired Immune Deficiency Syndromes*, 1997, 15:370-374.

65. Baum MK et al. Selenium deficiency and HIV-1 related mortality in homosexual men. *FASEB Journal*, 1998, 12(4):A525.

66. Campa A et al. Mortality risk in selenium-deficient HIV-positive children. *Journal of Acquired Immune Deficiency Syndromes*, 1999, 20:508-513.

67. Fawzi WW et al. Rationale and design of the Tanzania Vitamin and HIV Infection Trial. *Controlled Clinical Trials,* 1999, 20:75-90.

68. Kupka R et al. Selenium status is associated with accelerated HIV disease progression among HIV-1-infected pregnant women in Tanzania. *Journal of Nutrition,* 2004, 134:2556-2560.

69. Nichol C et al. Changes in the concentrations of plasma selenium and selenoproteins after minor elective surgery: further evidence for a negative acute phase response? *Clinical Chemistry,* 1998, 44:1764-1766.

70. Shor-Posner G et al. Impact of selenium status on the pathogenesis of mycobacterial disease in HIV-1-infected drug users during the era of highly active antiretroviral therapy. *Journal of Acquired Immune Deficiency Syndromes*, 2002, 29:169-173.

71. Costagliola DG et al. Dose of desferrioxamine and evolution of HIV-1 infection in thalassaemic patients. *British Journal of Haematology*, 1994, 87:849-852.

72. Salhi Y et al. Serum ferritin, desferrioxamine, and evolution of HIV-1 infection in thalassemic patients. *Journal of Acquired Immune Deficiency Syndromes*, 1998, 18:473-478.

73. de Monye C et al. Bone marrow macrophage iron grade and survival of HIV-seropositive patients. *AIDS*, 1999, 13:375-380.

74. Langlois MR, Delanghe JR. 1996 Biological and clinical significance of haptoglobin polymorphism in humans. *Clinical Chemistry*, 42:1589-1600.

75. Delanghe JR et al. Haptoglobin polymorphism, iron metabolism and mortality in HIV infection. *AIDS*, 1998, 12:1027-1032.

76. Semba RD et al. Iron Status and Indicators of Human Immunodeficiency Virus Disease Severity among Pregnant Women in Malawi. *Clinical Infectious Diseases*, 2001 32:1496-1499.

77. Weinberg GA et al. Iron status and the severity of HIV infection in pregnant women. *Clinical Infectious Diseases*, 2001, 33:2098-2100.

78. Semba RD. Iron status and the severity of HIV infection in pregnant women. Reply. *Clinical Infectious Diseases*, 2001, 33:2099-2100.

79. Tang AM et al. Dietary micronutrient intake and risk of progression to acquired immunodeficiency syndrome (AIDS) in human immunodeficiency virus type 1 (HIV-1)-infected homosexual men *American Journal of Epidemiology*, 1993, 138:937-951.

80. Tang AM, Graham NM, Saah AJ. Effects of micronutrient intake on survival in human immunodeficiency virus type 1 infection *American Journal of Epidemiology*, 1996, 143:1244-1256.

81. Abrams B, Duncan D, Hertz-Picciotto I. A prospective study of dietary intake and acquired immune deficiency syndrome in HIV-seropositive homosexual men. *Journal of Acquired Immune Deficiency Syndromes*, 1993, 6:949-958.

82. Lonnerdal B. Zinc metabolism during pregnancy--interactions with vitamin A. *Bibliotheca Nutritio et Dieta*, 1998, 54:93-102.

83. Wieringa FT et al. Redistribution of vitamin A after iron supplementation in Indonesian infants. *American Journal of Clinical Nutrition*, 2003, 77:651-657.

84. Allen LH. Iron-ascorbic acid and iron-calcium interactions and their relevance in complementary feeding. In: Micronutrient interactions: impact on child health and nutrition. Washington, DC, International Life Sciences Institute, 1998:11-20.

85. Niki E. Antioxidants in relation to lipid peroxidation. *Chemistry and Physics of Lipids*, 1987, 44:227-253

86. Halliwell B, Gutteridge JM. The antioxidants of human extracellular fluids. Archives of Biochemistry and Biophysics, 1990, 280:1-8

87. Srigiridhar K, Nair KM. Supplementation with alpha-tocopherol or a combination of alpha-tocopherol and ascorbic acid protects the gastrointestinal tract of iron-deficient rats against iron-induced oxidative damage during iron repletion. *British Journal of Nutrition*, 2000, 84:165-173

88. Coutsoudis A et al. 1999 Randomized trial testing the effect of vitamin A supplementation on pregnancy outcomes and early mother-to-child HIV-1 transmission in Durban, South Africa South African Vitamin A Study Group, *AIDS*, 13:1517-1524

89. Kumwenda N et al. 2002 Antenatal vitamin A supplementation increases birth weight and decreases anemia among infants born to human immunodeficiency virus-infected women in Malawi. *Clinical Infectious Diseases*, 35:618-624

90. Fawzi WW et al. 1998b Randomised trial of effects of vitamin supplements on pregnancy outcomes and T cell counts in HIV-1-infected women in Tanzania. *Lancet*, 351:1477-1482

91. Fawzi WW et al. 2000 Randomized trial of vitamin supplements in relation to vertical transmission of HIV-1 in Tanzania. *Journal of Acquired Immune Deficiency Syndromes*, 23:246-254

92. Fawzi W et al. Effect of prenatal vitamin supplementation on lower-genital levels of HIV type 1 and interleukin type 1 beta at 36 weeks of gestation. *Clinical Infectious Diseases,* 2004, 38:716-722.

93. Fawzi WW et al. 2002 Randomized trial of vitamin supplements in relation to transmission of HIV-1 through breastfeeding and early child mortality, *AIDS*, 16:1935-1944

94. Food and Nutrition Board, Institute of Medicine. Vitamin C. In: Dietary reference intakes for vitamin C, vitamin E, selenium and carotenoids. Washington, DC, National Academy Press, 2000:95-185.

95. Benn CS, et al. 2003 Hypothesis: vitamin A supplementation and childhood mortality: amplification of the non-specific effects of vaccines? Int J Epidemiol 32:822-828

96. Fawzi WW et al. Effect of providing vitamin supplements to human immunodeficiency virus-infected, lactating mothers on the child's morbidity and CD4+ cell counts. *Clinical Infectious Diseases*, 2003, 36:1053-1062

97. Friis H et al. Effect of multimicronutrient supplementation on gestational length and birth size: a randomized, placebo-controlled, double-blind effectiveness trial in Zimbabwe. *American Journal Clinical Nutrition,* 2004, 80:178-184.

98. Iliff P et al. Early exclusive breastfeeding reduces the risk of postnatal HIV-1 transmission and increases HIV-free survival. 2005, 19:699-798.

99. Malaba L et al. Impact of post-partum maternal or neonatal vitamin A supplementation on infant mortality among infants born to HIV-negative mothers in Zimbabwe. American Journal of Clinical Nutrition, 2005, 81:454-460.

100. Jiamton S et al. A randomized trial of the impact of multiple micronutrient supplementation on mortality among HIV-infected individuals living in Bangkok. *AIDS*, 2003, 17:2461-2469.

101. Fawzi WW et al. A randomized trial of multivitamin supplements and HIV disease progression and mortality. *New England Journal of Medicine,* 2004, 351:23-32.

102. Fawzi WW et al. Vitamin A supplementation and severity of pneumonia in children admitted to the hospital in Dar es Salaam, Tanzania. *American Journal of Clinical Nutrition*, 1998, 68:187-192.

103. Fawzi WW et al. A randomized trial of vitamin A supplements in relation to mortality among human immunodeficiency virus-infected and uninfected children in Tanzania. *Pediatric Infectious Disease Journal*, 1999, 18:127-133.

104. Coutsoudis A et al. The effects of vitamin A supplementation on the morbidity of children born to HIV-infected women. American Journal of Public Health, 1995, 85:1076-1081.

105. Kelly P et al. Micronutrient supplementation in the AIDS diarrhoea-wasting syndrome in Zambia: a randomized controlled trial. AIDS, 1999, 13:495-500.

106. Burbano X et al. Impact of a selenium chemoprevention clinical trial on hospital admissions of HIV-infected participants. HIV Clinical Trials, 2002, 3:483-491.

107. Friis H et al. The effect of adjunctive zinc and multi-micronutrient supplementation during treatment of pulmonary TB: a randomised, controlled trial in Mwanza, Tanzania. VIIth Conference of the International Society for Trace Elements Research in Humans, Bangkok, November 2004.

108. Range N et al. The effect of micronutrient supplementation on treatment outcome in patients with pulmonary tuberculosis: a randomised controlled trial in Mwanza, Tanzania. Tropical Medicine and International Health, in press.

109. Mellors JW et al. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. Science, 1996, 272:1167-1170.

110. Gray RH et al. Probability of HIV-1 transmission per coital act in monogamous, heterosexual, HIV-1-discordant couples in Rakai, Uganda. Lancet, 2001, 357:1149-1153.

111. O'Shea S et al. Maternal viral load, CD4 cell count and vertical transmission of HIV-1. Journal of Medical Virology, 1998, 54:113-117.

112. Fawzi W et al. Predictors of intrauterine and intrapartum transmission of HIV-1 among Tanzanian women. AIDS, 2001, 15:1157-1165.

113. Coutsoudis A et al. Effects of vitamin A supplementation on viral load in HIV-1-infected pregnant women. Journal of Acquired Immune Deficiency Syndromes, 1997, 15:86-87.

114. Humphrey JH et al. Short-term effects of large-dose vitamin A supplementation on viral load and immune response in HIV-infected women. Journal of Acquired Immune Deficiency Syndromes, 1999, 20:44-51.

115. Baeten JM et al. Vitamin A supplementation and human immunodeficiency virus type 1 shedding in women: results of a randomized clinical trial. Journal of Infectious Diseases, 2002, 185:1187-1191.

116. Semba RD et al. Vitamin A supplementation and human immunodeficiency virus load in injection drug users. Journal of Infectious Diseases, 1998, 177:611-616.

117. Allard JP et al. Effects of vitamin E and C supplementation on oxidative stress and viral load in HIV-infected subjects. AIDS, 1998,12:1653-1659.

118. Olsen A et al. Low dose iron supplementation does not increase HIV-1 viral load. Journal of Acquired Immune Deficiency Syndromes, 2004, 36:637-638.

119. McClelland RS et al. Micronutrient supplementation increases genital tract shedding of HIV-1 in women. Journal of Acquired Immune Deficiency Syndromes, 2004, 37:1657-1663.

120. Jiamton S et al. A randomized placebo-controlled trial on the impact of multiple micronutrient supplementation on HIV-1 in genital sheddings among Thai subjects. Journal of Acquired Immune Deficiency Syndrome, 2004,37:1216-1218

121. Semba RD. Vitamin A, immunity, and infection. Clinical Infectious Diseases, 1994, 19:489-499.

122. Stephensen CB. Vitamin A, infection, and immune function. Annual Review of Nutrition, 2001, 21:167-192.

123. Poli G et al. 1992 Retinoic acid mimics transforming growth factor beta in the regulation of human immunodeficiency virus expression in monocytic cells. Proceedings of the National Academy of Sciences U S A, 89:2689-2693.

124. Boelaert JR, Weinberg GA, Weinberg ED. Altered iron metabolism in HIV infection: mechanisms, possible consequences, and proposals for management. Infectious Agents and Diseases, 1996, 5:36-46.

125. Weinberg ED. Iron withholding: a defense against viral infections. Biometals, 1996, 9:393-399.

126. Weinberg GA, Boelaert JR, Weinberg ED. Iron and HIV infection. In: H Friis, ed. Micronutrients and HIV infection. Boca Raton, CRC Press, 2001:135-157.

127. Georgiou NA et al. Inhibition of human immunodeficiency virus type 1 replication in human mononuclear blood cells by the iron chelators deferoxamine, deferiprone, and bleomycin. Journal of Infectious Diseases, 2000181:484-490.

128. Sappey C et al. NF-kappa B transcription factor activation by hydrogen peroxide can be decreased by 2,3-dihydroxybenzoic acid and its ethyl ester derivative. Archives of Biochemistry and Biophysics, 1995, 321:263-270.

129. Dhople AM et al. Role of iron in the pathogenesis of Mycobacterium avium infection in mice. Microbios, 1996, 87:77-87.

130. Gangaidzo IT et al. Association of pulmonary tuberculosis with increased dietary iron. Journal of Infectious Diseases, 2001, 184:936-939.

131. Schaible UE et al. Correction of the iron overload defect in beta-2-microglobulin knockout mice by lactoferrin abolishes their increased susceptibility to tuberculosis. Journal of Experimental Medicine, 2002, 196:1507-1513.

132. Tanchou V et al. Role of the N-terminal zinc finger of human immunodeficiency virus type 1 nucleocapsid protein in virus structure and replication. Journal of Virology, 1998, 72:4442-4447.

133. Zhang ZY et al. Zinc inhibition of renin and the protease from human immunodeficiency virus type 1. Biochemistry, 1991, 30:8717-8721.

134. Black RE. Zinc deficiency, infectious disease and mortality in the developing world. Journal of Nutrition, 2003, 133:1485S-1489S.

135. Friis H, Sandström B. Zinc and HIV infection. In: Friis H, ed. Micronutrients and HIV infection. Boca Raton, CRC Press, 2001:159-181.

136. Kupka R, Fawzi W. Zinc nutrition and HIV infection. Nutrition Reviews, 2002, 60:69-79.

137. Beck MA et al. Rapid genomic evolution of a non-virulent coxsackievirus B3 in selenium-deficient mice results in selection of identical virulent isolates. Nature Medicine, 1995, 1:433-436.

138. Beck MA, Handy J, Levander OA. Host nutritional status: the neglected virulence factor. Trends in Microbiology, 2004, 12:417-423.

139. Zhang W et al. Selenium-dependent glutathione peroxidase modules encoded by RNA viruses. Biological Trace Element Research, 1999, 70:97-116.

140. Zhao L et al. Molecular modeling and in vitro activity of an HIV-1-encoded glutathione peroxidase. Proceedings of the National Academy of Sciences U S A, 2000, 97:6356-6361.

Table 1. HIV outcomes with clinical and proxy end-points.

	Clinical end-points	Proxy end-points
Susceptibility	Seroconversion	-
Transmission	Seroconversion	Viral load in
Sexual	Partner	Plasma
Female-male	Male	Cervicovaginal
Male-female/male	Female/male	Semen
Mother-child	Offspring	Plasma
In utero/intrapartum	< ? days	Cervicovaginal
Postnatal	> ? days	Milk
Progression		
Opportunistic infections	Disease incidence	
AIDS	Clinical definition	Viral load / CD4+ count
Survival	Death	

Table 2. Non-HIV outcomes in children and adults, and during pregnancy and lactation

Children	**Adults**	**Pregnant or lactating women**
Growth	Weight maintenance	Pregnancy outcome
Development	Working capacity	Foetal loss
Physical activity	Morbidity and mortality	Preterm delivery
Morbidity and mortality	Quality of life	Intrauterine growth retardation
Quality of life		Growth and development of offspring
		Morbidity and mortality of offspring

Table 3. Mother-to-child HIV transmission: design, interventions and co-interventions of randomized maternal micronutrient supplementation trials.

Country	Recruitment	N	Study intervention (placebo controlled)			Co-interventions (to all)	
			Micronutrient	Daily	Postpartum [1]	Iron/folate (mother)	Vitamin A
South Africa [2]	28-32 wk	728	Vitamin A	5.000 IU + 30 mg β-carotene	200.000 IU	60/5 mg/day	
Malawi [3]	18-28 wk	697	Vitamin A	10.000 IU	-	30/0.4 mg/day	100.000 IU at 6 weeks (mother)
Tanzania [4]	14-27 wk	1075	Vitamin A Multivitamins	5.000 IU + 30 mg β-carotene Vitamins B, C and E	200.000 IU continued	120/5 mg/day	100.000 IU at 6, 12, 18 months (infant)

[1] Vitamin A was given once postpartum but multivitamins were given for several years.

[2] Coutsoudis et al. (*88)*. Study intervention and iron/folate given from recruitment until delivery.

[3] Kumwenda et al. (*89*). Study intervention and iron/folate given from recruitment until delivery.

[4] Fawzi et al. (*91*); Fawzi et al. (*93*). Two-by-two factorial design, i.e. two placebo-controlled interventions. Study intervention and iron/folate given from recruitment and throughout and several years after lactation. Multivitamins included 20 mg thiamin, 20 mg riboflavin, 25 mg vitamin B_6, 50 μg vitamin B_{12}, 100 mg niacin, 0.8 mg folate, 500 mg vitamin C, and 30 mg vitamin E.

Table 4. Mother-to-child HIV transmission: estimates of the effect of maternal vitamin A supplementation from randomized, controlled trials. [1]

Country	**Effect estimates** [2]			
	Birth	**6 weeks–3 months**	**12 months**	**24 months**
South Africa [3]	-	0.91 (20.3/22.3)		-
Malawi [4]	-	0.96 (26.6/27.8)	0.85 (27.3/32.0)	0.84 (27.7/32.8)
Tanzania [5]	1.49 (10.0 /6.7)	1.22 (22.2 /18.2)		1.38 (34.2/25.4) *

[1] Testing the null-hypothesis, that the transmission rates in vitamin A and placebo groups are similar. * $P < 0.05$.

[2] Effect estimates given as relative risks (transmission rate [%] in vitamin A / placebo group).

[3] Coutsoudis et al. (*88*).

[4] Kumwenda et al. (*89*).

[5] Fawzi et al. (*91*); Fawzi et al. (*93*).

Table 5. HIV viral load: randomized, controlled micronutrient supplementation trials.

Country	Participants	N	Micronutrient	Regimen	Effects			Reference
					Plasma	Cervico-vaginal	Milk	
South Africa	Pregnant women	24	Vitamin A	200 000 IU once postpartum; 1 week	None	-	-	Coutsoudis et al. (*113*)
Zimbabwe	Lactating women	40	Vitamin A	300 000 IU once postpartum; 8 weeks	None	-	-	Humphrey et al. (*114*)
Kenya	Nonpregnant women	400	Vitamin A	10 000 IU daily for 6 weeks	None	None	-	Baeten et al. (*115*)
USA	Intravenous drug users	120	Vitamin A	200 000 IU once; 4 weeks	None	-	-	Semba et al. (*116*)
Canada	Adults	49	Vitamins C + E	500 mg C + 800 mg E daily, 3 months	Decline	-	-	Allard et al. (*117*)
Kenya	Adults	32	Iron	60 mg twice weekly, 4 months	None	-	-	Olsen et al. (*118*)
Tanzania	Pregnant women	393	Vitamin A	5000 IU preformed + 30 mg β-carotene	-	Increase	-	Fawzi et al. (*92*)
			Vitamins B, C, E	8 vitamins in high doses, 15 weeks	-	None	-	
Thailand	Adults	112	Multi-micronutrients	18 micronutrients in high doses, 48 weeks	None	-	-	Jiamton et al. (*100*) Jiamton et al. (*120*)
Kenya	Nonpregnant women	400	Selenium and	0.2 mg selenium +	-	Increase	-	McClelland et al. (*119*)
			Vitamins B, C, E	8 vitamins in high doses, 6 weeks				
Tanzania	TB/HIV patients	213	Zinc	45 mg during first 2 months treatment	None	-	-	Friis et al. (*107*)
			Multi-micronutrients	vitamins and minerals in high doses, during first 2 months treatment	None	-	-	

Table 6. Research priorities

Micronutrient requirements

- Establish the required intake of individual micronutrients to maintain normal status in people living with HIV at different stages, with and without antiretroviral treatment.

Sexual HIV transmission

- Establish the optimal dietary and supplemental intake of vitamins and minerals to reduce infectiousness among HIV-positive people.
- Establish the optimal dietary and supplemental intake of vitamins and minerals to reduce susceptibility to HIV infection among HIV-negative people.

Mother-to-child HIV transmission

- Establish the optimal composition and doses of vitamins and minerals in pre/perinatal supplements and duration of supplementation to reduce risk of mother-to-child transmission.
- Clarify whether lower doses of vitamins B, C and E are effective and safe.
- Clarify what form and doses of vitamin A can be safely given to pregnant HIV-positive women
- Identify potential effect modifiers responsible for apparently different effects of vitamin A.
- Establish the generalizability of the findings regarding effects of perinatal supplements.
-

HIV progression

- Establish the optimal dietary and supplemental intake of vitamins and minerals to reduce progression of HIV to AIDS and mortality.
- Clarify whether lower doses of vitamins B, C and E are effective and safe.
- Establish the optimal dietary and supplemental intake of vitamins and minerals to reduce risk of opportunistic and other infections.
- Establish the optimal dietary and supplemental intake of vitamins and minerals to improve pharmacokinetics and drug efficiency and reduce risk of metabolic and other adverse effects.

Non-HIV outcomes

- In HIV-positive children, establish the optimal dietary and supplemental intake of vitamins and minerals to optimize growth, development and physical activity.
- In HIV-positive pregnant and lactating women, establish the optimal dietary and supplemental intake of vitamins and minerals to reduce risk of adverse pregnancy outcome improve growth and development of the offspring.
- In nonpregnant HIV-positive adults, establish the optimal dietary and supplemental intake of vitamins and minerals to maintain body weight and lean body mass, physical activity and working capacity.

Potentially adverse effects and interactions

- Establish the effects and safety of iron supplementation on HIV load and progression.
- Establish whether the beneficial or adverse effects of specific micronutrients depend on the intake and status of other micronutrients.

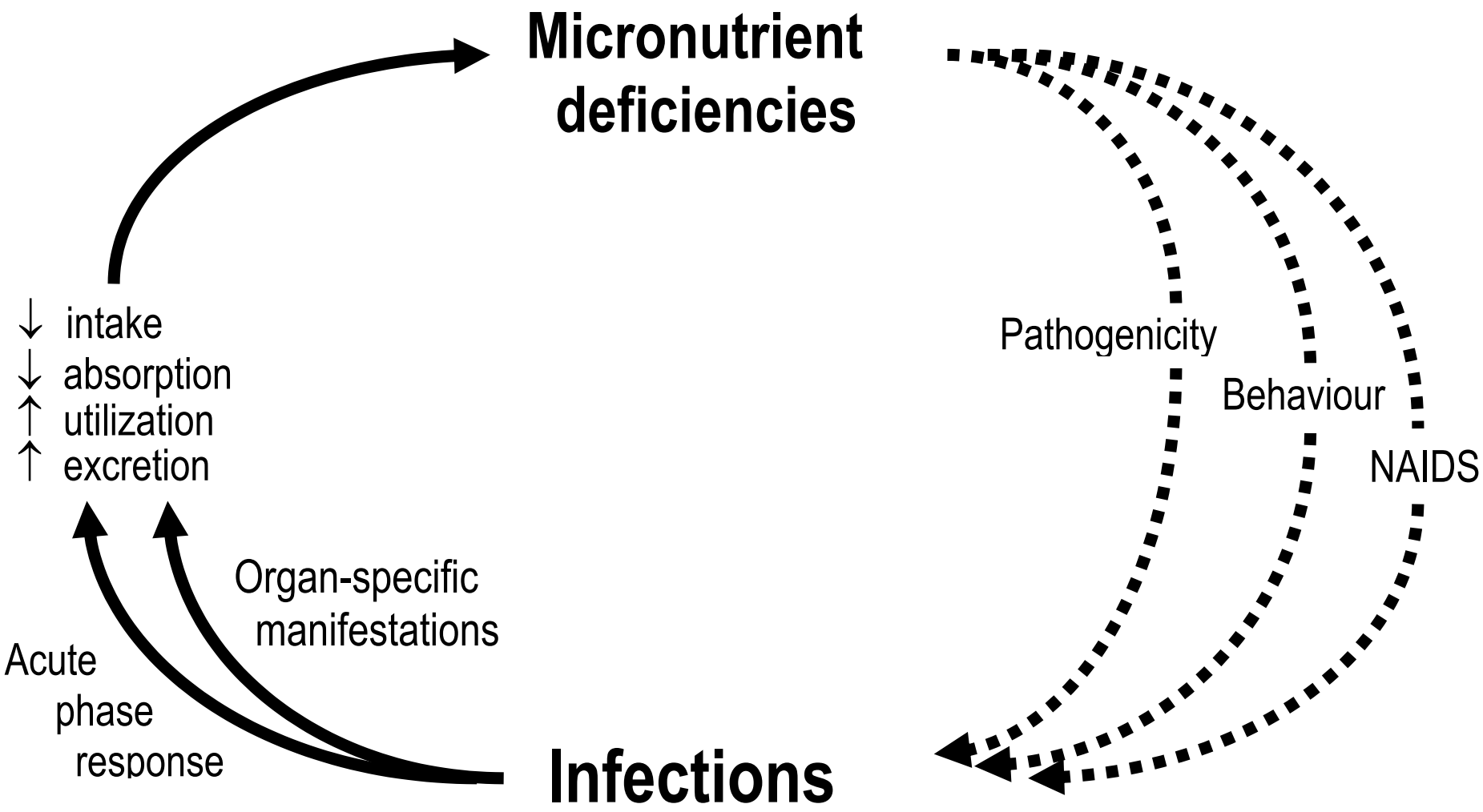

Figure 1. The two-way relationship between micronutrient deficiencies and infections. NAIDS, nutritionally acquired immune deficiency syndrome.

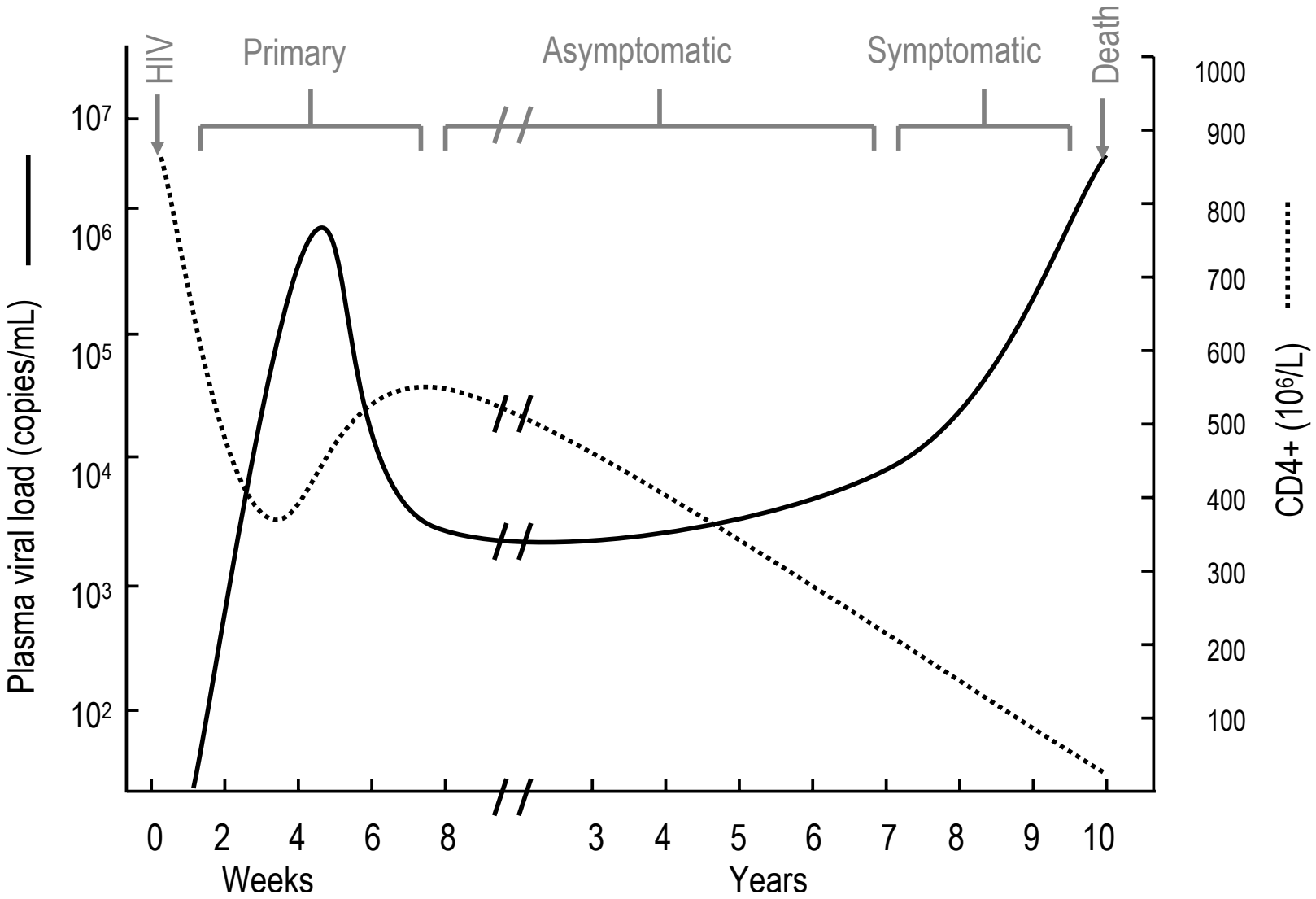

Figure 2. The natural history of HIV infection and time course of viral load and CD4+ counts.

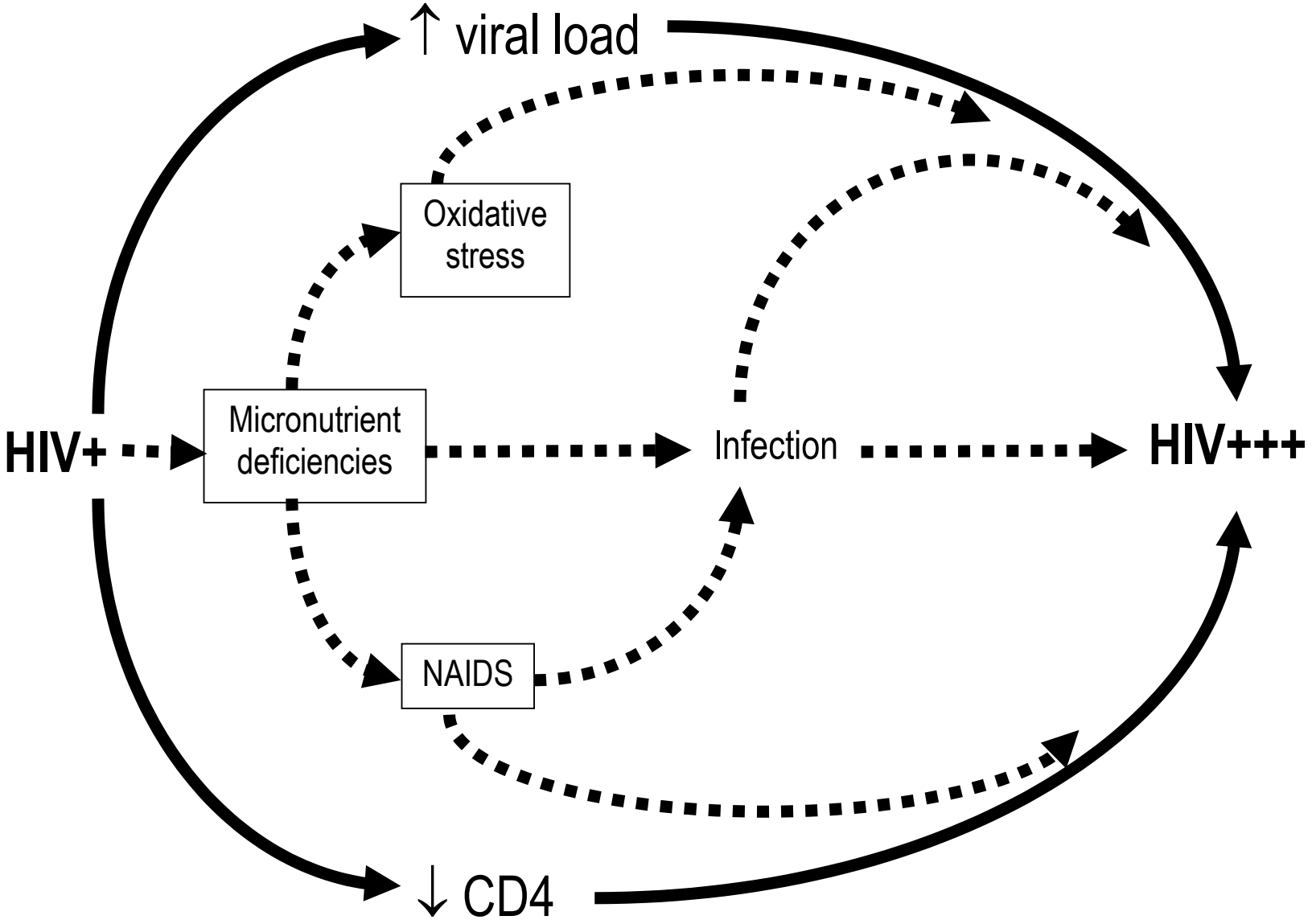

Figure 3. Possible role of micronutrient deficiencies in HIV progression. NAIDS, nutritionally acquired immune deficiency syndro