Triple Trouble

Tuberculosis, HIV infection and Malnutrition

Een wetenschappelijke proeve op het gebied van de Medische Wetenschappen

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DOTTED VERSES

- in memory of many

placid,
acidic soul of pains
ravening-
for echo of geckoes
in a distant ghetto
lining-
in arms of time: the long waited…

here,
laid a disciple, sesan
as sea sands dissolves in time
trailing thy paths, so dotted
in verse

…as much
as man has dreamt
visions: missions
unfulfilled

30/7/99 Uchenna Hilary Chime

CONTENTS

Chapter 1

Introduction And Outline Of The Thesis

Introduction

Tuberculosis is the infection caused by *Mycobacterium tuberculosis,* a slowly growing acid-fast-staining bacillus. Tuberculosis is transmitted through the airborne route from contagious cases to susceptible contacts; most individuals who are infected however do not develop clinical disease. Malnutrition and immunosuppressive disorders such as human immunodeficiency virus (HIV) infection increase the risk of developing clinical disease (Figure 1). Despite effective antituberculous chemotherapy, tuberculosis is associated with wasting and high mortality. The association of poor nutrition with tuberculosis has long been evident, as older terms were used for tuberculosis such as the Greek term "phthisis" or "to waste away". In the developing world, the word "slim disease" is used for both tuberculosis and HIV infection interchangeably.

Public Health Importance

About one third of the world's population is infected with *Mycobacterium tuberculosis*, representing great numbers of individuals at risk for development of disease. Half or more of adults in sub-Saharan Africa, the Indian subcontinent, and Southeast Asia, have latent tuberculosis infection. Each year there are 8 million new cases and 3 million deaths due to tuberculosis, of which the majority occur in resource-poor countries.[1] The rates of concurrent HIV and tuberculosis are increasing worldwide, and despite chemotherapy, mortality rates remain high. In many least developed countries, where tuberculosis and HIV co-infection occur most, anti-retroviral medications is still largely unavailable. Tuberculosis is responsible for about one-quarter of all preventable deaths in developing countries, and many of these deaths are associated with underlying HIV infection.[2]

Clinical and Laboratory Diagnosis of Tuberculosis

Active tuberculosis, the form of disease that disturbs normal host physiology to produce symptoms, is generally classified as either pulmonary or extra-pulmonary disease. Pulmonary disease is the most common form of active tuberculosis, accounting for approximately 80% of cases. Cough is the most common symptom of pulmonary tuberculosis, occurring in over 95 % of cases. The cough is usually chronic, lasting more than one month, and it is most often

productive of sputum. Occasionally hemoptysis (coughing up blood), dyspnea (shortness of breath), and chest pain develop. Other symptoms include fevers, night sweats, and weight loss. The physical examination for tuberculosis is non-specific. The chest radiograph is often used to confirm the presence of pulmonary lesions and assess the extent of disease. The diagnosis of tuberculosis is based on the clinical syndrome and the identification of *M. tuberculosis* in appropriate specimens taken from the patient. Staining techniques (such as Ziehl Neelsen and fluorescent staining) are the most widely used methods, as they are inexpensive, readily available, and easily performed. Culture of *M. tuberculosis* is more laborious and expensive, but important for epidemiological reasons and determination of susceptibility to antimycobacterial drugs. Newer techniques using molecular tools are still largely beyond the reach of developing countries.
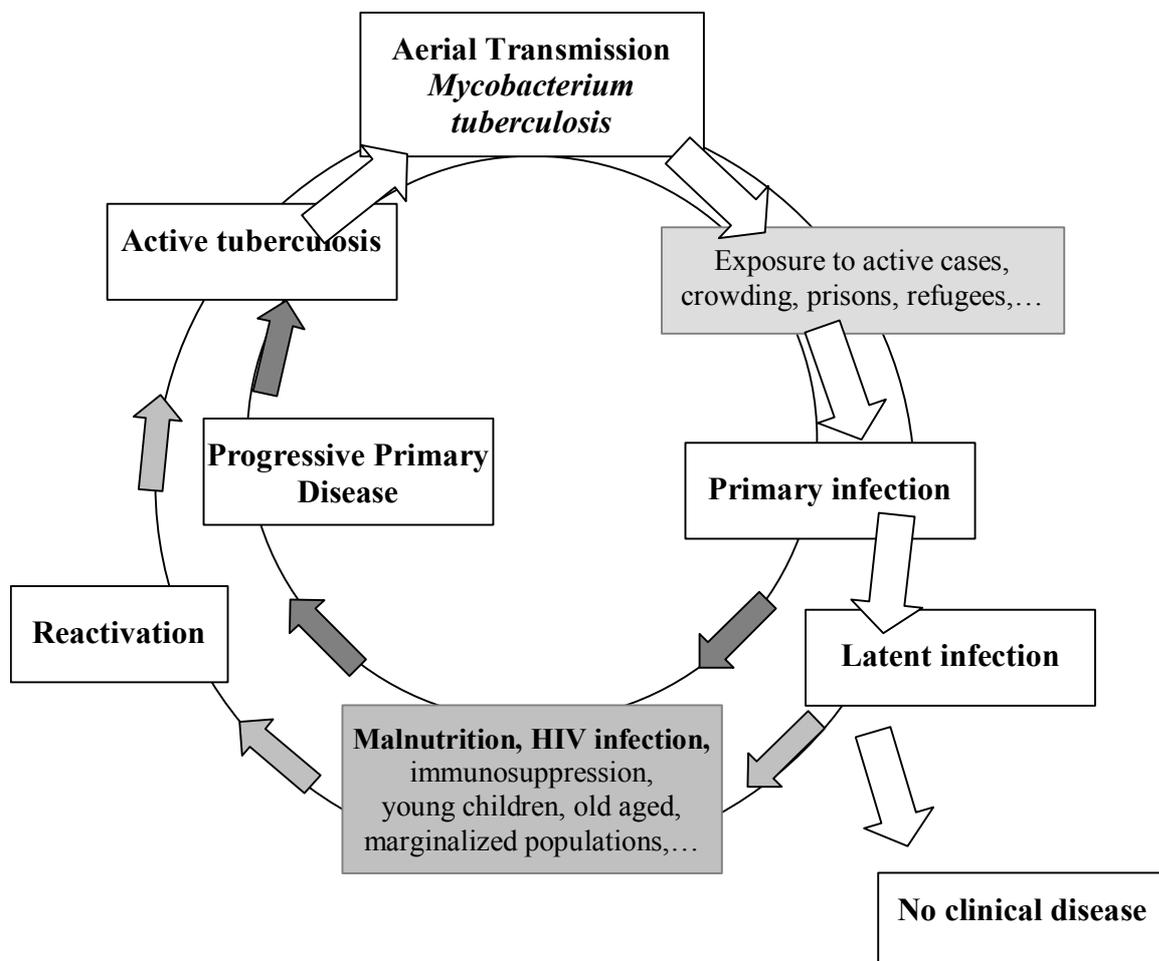

Figure 1. Model for tuberculosis infection and common risk factors. Adapted from Whalen. (Whalen 2001)

Risk Factors and Magnitude of HIV Co-infection

The risk for tuberculosis infection relates to the probability of contact with an infected case, and the risk for developing disease after infection relates to the competence of the host immune response in suppressing the infection. Figure 1 shows common risk factors for tuberculosis infection and disease. Once infected, an individual has a 10% lifetime risk of developing active tuberculosis.[2] For people co-infected with HIV, the lifetime risk of developing disease has been estimated at about 60%.[3] Common conditions associated with the risk of developing active tuberculosis are conditions that affect host defense against mycobacteria (e.g. HIV infection and malnutrition).

HIV infection presents the greatest known risk for the development of both progressive primary disease and reactivation tuberculosis. In sub-Saharan Africa, HIV infection is now strongly associated with tuberculosis. In 1989, already 52% of tuberculosis patients were reported HIV-positive in a rural hospital in Malawi.[4] In 1993-1994 in a central hospital in Malawi, 75% of tuberculosis patients were found HIV-positive.[5]

Malnutrition

Malnutrition has long been recognized among individuals with tuberculosis. In *Epidemics III* of the Hippocratic corpus from the fifth century, B.C., a clinical description was given consistent with pulmonary tuberculosis. "Phthisis," "tabes," and "marasmus" were used to describe diseases characterized by emaciation, including tuberculosis. These terms are close in suggesting wasting away, weakness, and decay. In the 18th and 19th centuries, tuberculosis became known as "consumption". Recently, in the developing world, the word "slim disease" has been applied for both tuberculosis and HIV infection interchangeably, because of the wasting effects of the disease. Uncertainty remains surrounding the mechanisms as well as the effect of this wasting syndrome.

Tuberculosis, HIV infection and Malnutrition

The association between tuberculosis and malnutrition has long been accepted; malnutrition predisposes individuals to the development of clinical disease, and tuberculosis

contributes to malnutrition. Infection leads to malnutrition through loss of appetite, loss of nutrients, changes in metabolism and malabsorption, and changes in feeding practices. (Figure 2) In addition, protein-energy malnutrition and deficiencies in micronutrients such as iron, vitamin A and zinc are known to affect immunity unfavorably [6-9], contributing to the increase in incidence, severity and duration of disease. Iron and other nutrient deficiencies, together with chronic disease such as tuberculosis and HIV infection, may cause anemia. Therefore anemia may perhaps be seen as both an outcome and marker of malnutrition.

Co-infection of HIV and tuberculosis introduces an extra dimension to the pathophysiology of malnutrition, worsening the malnutrition seen in tuberculosis or HIV infection alone.[10]

A combination of HIV and tuberculosis with malnutrition may therefore represent "triple trouble."
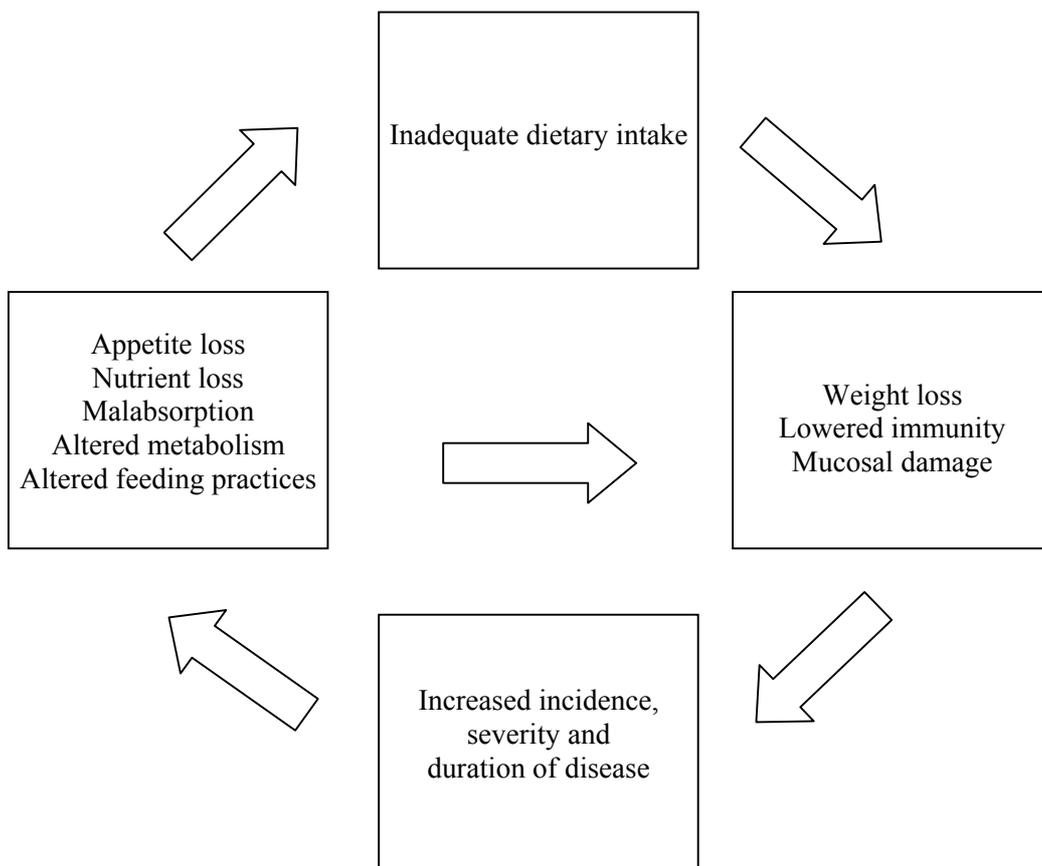

Figure 2. Infection /malnutrition cycle. Adapted from Tomkins and Watson 1998.

Although wasting or malnutrition seems a fundamental sign of tuberculosis in both HIV-positive and HIV-negative patients, and a major determinant of disease severity and outcome, the pathogenesis of malnutrition and/or wasting has not yet been clarified. It is likely that several mechanisms contribute to weight loss by affecting energy intake, raising energy expenditure, accelering muscle degradation and inducing changes in lipid, carbohydrate and protein metabolism. At a molecular level many enzymes, mediators and receptors are involved in these processes, including inflammatory cytokines and the fat derived hormone leptin. It is unclear however to what extent these different components contribute to tuberculosis-associated wasting. Before one tries to approach these pathogenetic mechanisms in a direct fashion, more information is needed on the nutritional status of patients with tuberculosis with and without HIV-co-infection. Increased understanding of the etiology of tuberculosis-associated wasting may help to provide new information about the pathophysiology of this common and serious infection and may help to design new intervention strategies.

Study Site

The studies described in this thesis are part of a large and still ongoing randomized, double-blind, placebo controlled micronutrient supplementation trial in Malawi. The specific aim of this clinical trial is to determine whether a daily multivitamin supplement can reduce morbidity and mortality in adults with tuberculosis and HIV infection. The rationale for evaluating this low cost intervention was based on data that suggest that adults with HIV/AIDS and adults with pulmonary tuberculosis are at high risk of multiple micronutrient deficiencies. Such deficiencies, associated with immune suppression, altered nutrient metabolism, anemia, impaired growth, and increased oxidative stress, may ultimately result in increased morbidity and mortality. The main outcome measure of the ongoing trial is mortality. Secondary outcome measures are body composition assessed by bioelectrical impedance analysis (BIA) and anthropometry, relapse rate of pulmonary tuberculosis as indicated by sputum relapse, progression of HIV as assessed by plasma HIV load, and biochemical indicators of nutritional status and anemia.

The participants enrolled in the trial receive either a multivitamin (vitamins and minerals) or placebo for twenty-four months, beginning with the commencement of tuberculosis chemotherapy. The study population is drawn from the Zomba District, and participants are followed at the Johns Hopkins/Malawi College of Medicine Project study clinic at Zomba

Central Hospital. HIV-negative adults with pulmonary tuberculosis were included in the protocol to mask the HIV status of the participants to the community and to reduce stigmatization.

Baseline data, regarding socio-demographic information, plasma HIV-load, body composition, chest radiography and biochemical indicators of nutritional status and anemia, of the first approximately 800 participants recruited between July 1999 and April 2003 were used in the studies presented in this thesis.

MALAWI
Statistics 2000, WHO:

Total population:	11.5 million
GDP per capita (Intl $)*:	500
Life expectancy at birth m/f (years):	36/37
Healthy life expectancy at birth m/f (years):	29/31
Child mortality m/f (per 1000)**:**	261/240
Adult mortality m/f (per 1000):	695/636
Total health expenditure as % of GDP:	7.6

* Intl $: a common currency unit that takes into account differences in the relative purchasing power of various currencies.

Figure 3.

The study site is situated in Zomba, in the Southern region of Malawi (Figure 3). Malawi is situated in South East Africa and is bordered by Tanzania in the north, Zambia in the west and Mozambique in the south and east. The climate is tropical continental and most people depend on farming for income and food.

According to the last official population census carried out in 1998, the total population was 9.9 million. 46% of the total population was under the age of 15. Zomba district counted 550

thousand inhabitants. The annual population growth has been estimated at 2%. Of the total population in Malawi, 86% lived in rural areas. Literacy rates among males and females stood at 64 and 51 percent for males and females respectively. Statistics and health indicators (see figure 3) indicate Malawi as one of the poorest countries in Sub-Saharan Africa, and in fact worldwide.

OUTLINE OF THE THESIS

Below, a brief outline of the thesis is given. For each chapter the subject of the research described in the paper is specified.

In Chapter 2 we asked the question which nutritional indicators are important in tuberculosis. Therefore we reviewed the current state of knowledge regarding the nutritional status of patients co-infected with tuberculosis and HIV, describing changes in nutrition indicators during tuberculosis chemotherapy, and examining the relationship between nutrition and clinical outcomes.

In Chapter 3 we dealt with the question whether there is a relationship between malnutrition and the severity of lung damage in tuberculosis. This was approached by relating the extent of malnutrition to the extent of lung disease in adults with pulmonary tuberculosis with and without HIV infection. Chest radiographs, anthropometric and BIA measurements were obtained. Lung disease in chest radiographs was categorized according to a conventional classification system.

In Chapter 4 we tested the hypothesis that micronutrient malnutrition is associated with wasting and higher plasma HIV load in adults with tuberculosis. We focused on associations between anthropometry, plasma HIV load and plasma micronutrient concentrations (retinol, α-tocopherol, carotenoids, zinc, and selenium) in adults with pulmonary tuberculosis with and without HIV infection. We examined the risk of micronutrient deficiencies at different severity levels of wasting.

In Chapter 5 we attempted to answer the question to what extent iron deficiency, micronutrient malnutrition and infection contribute to anemia in pulmonary tuberculosis. This was approached by relating hemoglobin concentrations to erythropoietin, ferritin, interleukin-6 (IL-6), HIV load and plasma micronutrient concentrations in adults with pulmonary tuberculosis.

In Chapter 6 we dealt with the question whether the fat derived hormone leptin plays a role in tuberculosis-associated wasting. We explored the relationships between plasma leptin concentrations and self-reported loss of appetite, body composition, IL-6 and HIV load in adults with pulmonary tuberculosis with and without HIV infection.

In Chapter 7 the main findings of the investigations presented are discussed, and conclusions are drawn with implications for intervention strategies and future research.

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Chapter 2

Triple Trouble: The Role of Malnutrition in Tuberculosis and Human Immunodeficiency Virus Co-infection

Monique van Lettow, Wafaie Fawzi and Richard Semba.

Abstract

Worldwide, the number of individuals who are co-infected with human immunodeficiency virus (HIV) and tuberculosis is increasing greatly. The "triple trouble" of HIV and tuberculosis infection and malnutrition may put those infected at greater risk than those with any of the three conditions alone. Further investigation is needed to evaluate the prophylactic and therapeutic potential of nutritional interventions for co-infection with HIV and tuberculosis.

Introduction

Tuberculosis, an infection caused by *Mycobacterium tuberculosis*, is the leading cause of infectious mortality worldwide, accounting for three million deaths per year.[1] The link between tuberculosis and malnutrition has long been recognized; malnutrition may predispose people to the development of clinical disease, and tuberculosis can contribute to malnutrition.[2] Nearly one third of the world's population—1.8 billion people—are infected with *Mycobacterium tuberculosis*, and the majority of these individuals live in developing countries where the prevalence of human immunodeficiency virus (HIV) infection is also high.[3,4] The risk of developing clinical tuberculosis is greater among individuals with immunosuppression, which explains the increase in the prevalence of tuberculosis in association with HIV infection. In some countries in sub-Saharan Africa, the HIV seroprevalence rate among tuberculosis patients is reportedly more than 75%.[5–7]

Co-infection with HIV and tuberculosis introduces an extra dimension to the pathophysiology of wasting, exacerbating the wasting seen in tuberculosis or HIV infection alone.[2,8] Nutritional alterations in tuberculosis, HIV infection, or tuberculosis and HIV combined include increased energy expenditure, nutrient malabsorption, micronutrient malnutrition, and increased production of inflammatory cytokines with lipolytic and proteolytic activity.[5,9] Although the association between malnutrition and co-infection with tuberculosis and HIV has been described, it is uncertain whether improved nutrition reduces the risk of developing active disease or improves the clinical outcome during treatment.[10] Despite adequate chemotherapy, morbidity and mortality are still high among patients with tuberculosis, especially in those co-infected with HIV. In popular public health terms, HIV and tuberculosis co-infection are "double trouble," and a combination of HIV and tuberculosis with malnutrition may well represent "triple trouble." The goal of this review is to present the current state of knowledge regarding the nutritional status of patients co-infected with tuberculosis and HIV, to describe changes in nutrition indicators during tuberculosis chemotherapy, and to examine the relationship between nutrition and clinical outcomes.

Micronutrient Malnutrition

A number of micronutrient deficiencies have been described in individuals with tuberculosis[11–21] and in those with HIV infection.[22–28] Several cross-sectional studies suggest that patients with tuberculosis suffer from deficiencies of vitamin A,[12,16,17] thiamin,[18] vitamin B$_6$,[19] folate,[11,20] and vitamin E.[21] Deficiencies of vitamin A, vitamin E, thiamin, riboflavin, vitamin B$_6$, and vitamin C are more prevalent among HIV-infected adults than in those without HIV infection.[24–28] Among these micronutrients, perhaps vitamins A and D have received the most attention in patients with tuberculosis. Much of the interest in vitamins A and D relates to historic use of cod-liver oil as treatment for tuberculosis prior to the era of antibiotics.[10]

Vitamin A

Vitamin A deficiency, manifested in night blindness, Bitot spots, low hepatic vitamin A stores, and low plasma vitamin A concentrations, has been described among children and adults with tuberculosis.[17,29–35] Vitamin A is essential to normal function of T and B lymphocytes, macrophage activity, and generation of antibody responses.[36] In experimental studies, vitamin A–deficient animals showed increased susceptibility to infection with *Mycobacterium* compared with pair-fed or ad libitum–fed control animals.[37–39] Supplemental vitamin A appears to increase survival among chicks infected with *M tuberculosis*[40] and enhances both T lymphocyte and antibody responses to *M tuberculosis*.[41]

Vitamin A status has been examined in different populations infected with HIV; these studies generally show that lower plasma or serum vitamin A concentrations are found in developing countries compared with in developed countries.[42] There have been few studies that have examined vitamin A status among individuals co-infected with tuberculosis and HIV. In a cross-sectional study in Rwanda, 29% of adults with both HIV and tuberculosis had serum vitamin A concentrations indicative of deficiency (<1.05 μmol/L).[43] These data suggested that women had lower mean serum vitamin A concentrations than men (1.22 ± 0.45 vs. 1.47 ± 0.68 μmol/L, $P = 0.07$). In a longitudinal study of 519 adults co-infected with tuberculosis and HIV in Kampala, Uganda, 36% had serum vitamin A concentrations consistent with deficiency (<1.05 μmol/L) at the time of presentation (Langi P, Semba RD, Whalen C, unpublished data, 2002). During follow-up time (median) of 17 months, 30% of subjects with serum vitamin A <1.05 μmol/L, and 17% of subjects with serum vitamin A <1.05 μmol/L died ($P <0.01$). In multivariate analyses that adjusted for CD4$^+$ lymphocyte count, age, sex, body mass index, and diarrheal

morbidity, vitamin A deficiency was associated with increased risk of death (relative risk = 1.78, 95% confidence interval [CI] 1.2–2.6).

Vitamin D

Vitamin D plays a role in the function of macrophages, key factors in host resistance to tuberculosis.[44] Abnormalities in vitamin D status have been reported in tuberculosis, but there are many dietary, genetic, and environmental factors, such as exposure to sunlight, that may influence vitamin D status. Recently, genetic variations in the vitamin D receptor were identified as a major determinant of the risk for tuberculosis in Africans.[45] By contrast, a case-control study among Indians living in London showed only a marginal influence of these receptor genotypes, and vitamin D deficiency itself was shown to be a risk factor for tuberculosis.[46] Adults with untreated tuberculosis Indonesia were shown to have significantly lower 25-hydroxyvitamin D compared with controls.[47] In a study of 15 adults with untreated pulmonary tuberculosis and 15 healthy controls, 25-hydroxyvitamin D concentrations were not significantly different, but 1,25-dihydroxyvitamin D concentrations were significantly lower among those with tuberculosis than in controls.[48] Studies have yielded inconsistent findings regarding serum or plasma calcium concentrations during tuberculosis.[49–52] In HIV-infected adults, low serum levels of 1,25-dihydroxyvitamin D_3, the biologically active form of vitamin D, have been described in the presence of normal levels of 25-hydroxyvitamin D.[53] Vitamin D status has not yet been examined among individuals co-infected with tuberculosis and HIV.

Vitamin E

Vitamin E may play a potential role in tuberculosis and HIV infection through its antioxidant properties. Increased oxidative stress may predispose individuals to failure of T lymphocyte–mediated immunity.[54] In the era prior to highly active antiretroviral therapy, low plasma or serum vitamin E concentrations were reported among 4% of heterosexual adults and in 10 to 20% of homosexual men and intravenous drug users.[23,24,55,56] In a study of HIV-infected adults in Brazil, approximately 41% of patients had vitamin E levels <18 μmol/L.[54] Supplementation with vitamin E (800 mg/day) plus vitamin C (1 gm/day) reduced indicators of oxidative stress in HIV-infected adults in Toronto.[57] Vitamin E status has not yet been characterized among individuals co-infected with tuberculosis and HIV.

B-Complex Vitamins

There is limited data regarding the status of B-complex vitamins in individuals with tuberculosis. In a study in Nigeria, serum vitamin B_{12} concentrations were not significantly different between adults with tuberculosis and asymptomatic controls.[4] Normal serum vitamin B_{12} concentrations were described in a study in Finland among adults with tuberculosis,[11] and increased concentrations of vitamin B_{12} were described in pulmonary tuberculosis patients in South Africa.[58] Low plasma folate concentrations are common in adults infected with tuberculosis.[4,11–13] Low serum folate concentrations thought to represent incipient folate depletion were found in 45% of untreated British tuberculosis patients,[20] whereas extremely low mean values (<4.5 nmol/L) were detected in Nigerian patients with tuberculosis.[4] Cameron and Horne suggested that that excessive utilization of folate by the active inflammatory process contributes to folate deficiency.[13] By contrast, among Nigerian adults suffering form pulmonary tuberculosis, the extent of the inflammatory process did not appear to influence folate status to any significant degree, nor was there any relationship found between red cell or serum folate and severity of anaemia.[4,58]

Iron

Anemia is highly prevalent among adults with pulmonary tuberculosis and also among adults with HIV infection.[15,58–61] Although anemia of chronic disease accounts for a large part of the anemia found in tuberculosis, iron deficiency may also be a contributing factor.[61] In a study conducted in Ghana, 50 adults with pulmonary tuberculosis had significantly lower hemoglobin than healthy matched controls.[62] In Uganda, mean hemoglobin concentrations were lower among HIV-infected adults with tuberculosis than in HIV-uninfected adults with tuberculosis.[63] Low plasma transferrin concentrations have been described among HIV-infected adults with tuberculosis.[64] Further studies are needed to characterize the relative contribution of iron deficiency to the anemia found in co-infection with tuberculosis and HIV.

Other Nutrients

In countries were maize is the predominant staple food, Sammon speculated that the pattern of disease is largely determined by a change in immune profile caused by metabolites of dietary linoleic acid.[65] High intake of linoleic acid in a diet deficient in polyunsaturated fatty acids and riboflavin may result in high tissue production of T-helper-1-like lymphocytes, which

are primarily involved in mediation of cellular immunity. Such dietary immune modulation may affect resistance to tuberculosis in certain geographic areas.[65] In Indonesia, patients with tuberculosis had significantly lower plasma zinc concentrations.[15] A case-control study from Miami showed that 12 people with tuberculosis were more likely to have low plasma selenium concentrations than 32 healthy controls.[66]

Little is known about vitamin C status in tuberculosis. In a large longitudinal study from a low-income area of Philadelphia, low plasma concentrations of vitamins A and C were associated with an increased risk of developing tuberculosis.[67] Indian immigrants in London have been studied to reveal the impact of a vegetarian diet upon the risk of tuberculosis. Two separate case-control studies confirmed that lacto-vegetarians are at greater risk than meat or fish eaters. Low vitamin B_{12}, iron, and vitamin D intake may explain this association.[10,14,69–71]

Protein-Energy Status

Protein-energy malnutrition is common among adults with tuberculosis and HIV infection. Several studies have shown significant differences in serum albumin concentrations between adults with untreated tuberculosis and healthy controls (Table 1). Mean ($\pm$ SD) albumin concentrations range from 3.0 ± 0.7 g/dL among people with tuberculosis in Kenya to a mean of 3.9 ± 0.6 g/dL among people with tuberculosis in England.[48–50,52,58,72,73] Table 2 shows the comparisons made between serum albumin concentrations among adults co-infected with tuberculosis and HIV and among those with tuberculosis alone. The differences were significant in all cases; mean serum albumin concentrations among adults co-infected with tuberculosis and HIV ranged from 2.0 g/dL in Burundi and Haiti to 2.9 g/dL in Uganda.[7,63,74] A descriptive study showed hypoalbuminemia (<3.0 g/dL) in 72% of 265 patients with pulmonary tuberculosis in South Africa.[58] These various studies demonstrate that hypoalbuminemia is common in people co-infected with tuberculosis and HIV. Other factors can influence serum albumin concentrations, including the acute-phase response.[75]

Other studies have used prealbumin and transferrin in addition to albumin as serum indicators of protein status. In Burundi, the mean ($\pm$ SD) serum prealbumin concentrations among 22 HIV-infected adults with tuberculosis were 0.09 ± 0.06 g/L versus 0.12 ± 0.05 g/L among adults with tuberculosis alone. Mean serum transferrin concentrations were 1.3 ± 0.5 g/L among HIV-infected adults with tuberculosis compared with 2.0 ± 0.7 among those with tuberculosis alone ($P = 0.006$).[7] Lower serum transferrin concentrations are consistent with

decreased hepatic synthesis of proteins, and these effects may be linked to the acute protein malnutrition that occurs during the acute-phase response.[54] Among Indian patients with tuberculosis, whole-body protein metabolism has been studied using ^{13}C-labelled leucine.[76] A greater proportion of ingested protein was oxidized in patients with tuberculosis during the fed state compared with the fasting state; this indicates a resistance to the anabolic effects of food. This "anabolic block" may represent one of the mechanisms for wasting. Energy metabolism was not measurably disturbed; patients with tuberculosis showed the same down-regulation of resting energy expenditure with a loss of lean body mass as undernourished healthy controls.[76] In two studies that used total protein as an indicator of protein nutrition, no significant differences were found between HIV-infected adults with tuberculosis, healthy controls, and HIV-infected adults without tuberculosis.[48,77]

Table 1. Serum Albumin Concentrations in Adults with Untreated Pulmonary Tuberculosis with Unknown HIV Status Compared with Healthy Controls

Location	Mean Serum Albumin (g/dL ± SD)		*P*	Reference
	TB cases [*n*]	Healthy Controls [*n*]		
England	3.9 ± 0.6 [47]	4.5 ± 0.3 [47]	<0.001	48
Kenya	3.0 ± 0.7 [15]	4.1 ± 0.3 [15]	<0.001	49
Belgium	3.5 ± 0.8 [22]	4.1 ± 0.3 [27]	<0.001	50
China	3.6 ± 0.7 [25]	4.3 ± 0.4 [25]	<0.001	52
Malawi	3.7 ± 0.7 [104]	4.0 ± 0.3 [104]	<0.001	72
England	3.7 ± 0.7 [30]	4.6 ± 0.2 [30]	<0.001	73

Table 2. Serum Albumin Concentrations in Adults with Pulmonary Tuberculosis with and without HIV Infection

Location	Mean Serum Albumin (g/dL ± SD)		*P*	Reference
	HIV-positive [*n*]	HIV-negative [*n*]		
Burundi	2.0 ± 0.6 [22]	2.9 ± 0.4 [11]	<0.001	7
Uganda				
men	2.9 ± 0.4 [163]	3.2 ± 0.4 [199]	<0.001	63
women	2.9 ± 0.5 [98]	3.2 ± 0.5 [79]	<0.001	
Haiti*				
men	2.1 ± 0.15 [27]	2.8 ± 0.1 [56]	<0.01	74
women	2.0 ± 0.16 [21]	2.6 ± 0.1 [46]	<0.01	

* SEM

Anthropometry And Body Composition

Undernutrition is often defined as a having a low body mass index (BMI kg/m^2) of <18.5.[78] Several studies have shown that BMI is lower in adults with pulmonary tuberculosis than in healthy controls (Table 3),[72,73,76,79] and that BMI is lower in HIV-infected adults with tuberculosis than in HIV-negative adults with tuberculosis (Table 4).[5,7,63,80] In Uganda, no difference was found in BMI between HIV-positive and HIV-negative adults with pulmonary tuberculosis.[63] Among HIV-positive subjects with tuberculosis, BMI was significantly lower among those with CD4+ lymphocyte count ≤200 cells/μL compared with those who had >200 cells/μL.[63] In Brazil, no significant differences were found in BMI between HIV-positive adults with and without pulmonary tuberculosis,[77] but the number of subjects in both groups was small.

Arm muscle circumference (AMC, calculated as arm circumference – [3.14 × triceps skinfold thickness]),[81] has been used in a few studies evaluating nutritional status in adults with pulmonary tuberculosis. Mean (± standard deviation) AMC values have ranged from 14.8 ± 2.7 cm among 32 female Nigerians to 20.4 ± 2.5 cm among male Malawians.[72,82] Two studies in Haiti and England showed significant differences between AMC in adults with tuberculosis and healthy controls.[73,74] Mid–upper arm circumference (MUAC) was significantly lower among adults with pulmonary tuberculosis than in healthy controls, and was significantly lower among HIV-positive adults with tuberculosis than in HIV-negative adults with tuberculosis.[5,74,79,82] A mean MUAC of 19.5 ± 2.6 cm was reported among 239 HIV-infected adults with tuberculosis in Ethiopia.[79] Mean triceps skinfold thickness is also lower in adults with pulmonary tuberculosis than in healthy controls, and in HIV-positive adults with tuberculosis than in HIV-negative adults with tuberculosis.[5,73,74]

Bioelectric impedance analysis or water dilution methods have been used to examine the body composition in pulmonary tuberculosis patients (Table 5).[5,7,63,64,77] Overall, there do not appear to be large differences in body composition between HIV-infected adults with tuberculosis and HIV-negative adults with tuberculosis. No significant differences have been found in absolute measures of body cell mass, total body water, or extracellular water between HIV-positive and HIV-negative adults with tuberculosis.[5,7,63] Among adults with pulmonary tuberculosis in Uganda, the ratio of intracellular water to extracellular water (ICW:ECW) and phase angle was lower among men who were HIV-positive than in men who were HIV-negative.[63] Among the HIV-positive adults, the ICW:ECW ratio, body cell mass, fat mass, and phase angle were significantly lower among those with CD4$^+$ lymphocyte count ≤200 cells/μL

than in those who had >200 cells/µL.[63] A study conducted in Brazil among homeless HIV-infected adults showed significantly lower body cell mass, ICW, and ECW among HIV-infected adults with tuberculosis than in HIV-infected adults without tuberculosis.[77]

Table 3. Body Mass Index (BMI) in Adults with Untreated Active Pulmonary Tuberculosis with Unknown HIV Status Compared with Healthy Controls

Location	Mean BMI (kg/m^2 ± SD)		P	Reference
	TB cases [*n*]	Healthy controls [*n*]		
Malawi				
men	17.3 ± 2.2 [73]	21.7 ± 2.6 [73]	<0.01	72
women	18.3 ± 2.9 [49]	23.5 ± 3.0 [49]	<0.01	
England	19.3 ± 3.7 [30]	22.2 ± 2.6 [30]	<0.01	73
India	17.7 ± 2.5 [9]	22.6 ± 1.5 [7]	<0.01	76
Ethiopia	16.7 ± 2.3 [239]	21.8 ± 2.4 [239]	<0.01	79

Table 4. Body Mass Index (BMI) in Adults with Untreated Active Pulmonary Tuberculosis with and without HIV Infection

Location	Mean BMI (kg/m^2 ± SD)		P	Reference
	HIV-positive [*n*]	HIV-negative [*n*]		
Burundi	16.7 ± 2.2 [56]	19.6 ± 2.8 [11]	<0.01	5
Burundi	15.1 ± 2.8 [22]	18.6 ± 2.4 [11]	<0.01	7
Uganda				
men	18.1 ± 1.8 [163]	18.1 ± 2.2 [199]	NS	63
women	19.8 ± 3.4 [98]	19.3 ± 2.7 [79]	NS	
Tanzania				
men	16.4 ± 1.7 [32]	17.6 ± 1.8 [67]	<0.01	80
women	18.2 ± 2.8 [21]	17.6 ± 1.9 [28]	NS	

NS = not significant.

Changes In Nutritional Status During Tuberculosis Chemotherapy

Several studies have shown that nutritional indicators, such as anthropometric measurements, improve during tuberculosis chemotherapy.[72,73,83–85] A study conducted in Malawi showed that among 1181 adults with tuberculosis, weight significantly increased after 4 weeks of treatment.[85] HIV-negative adults with tuberculosis gained significantly more weight than HIV-positive adults with tuberculosis during the same period.[84,85] By contrast, a report from Tanzania suggested that HIV-positive patients gained more weight than HIV-negative patients during tuberculosis chemotherapy.[80] Although nutritional status seems to improve during tuberculosis chemotherapy, weight loss was observed in most of the 200 patients after completing treatment, and the length of hospital stay appeared to be the most important determinant of weight gain.[80] Serum albumin concentrations and MUAC may remain subnormal after completion of tuberculosis chemotherapy, suggesting that body protein reserves may not fully recover.[10,62,72,73] Hemoglobin concentrations also appear to improve during the first months of tuberculosis chemotherapy.[62]

Nutritional Status And Clinical Outcomes

Nutritional status appears to be an important determinant of clinical outcome during tuberculosis. In an influential study conducted in India, 163 patients with tuberculosis were treated either in a sanatorium with a well-balanced diet or at home on a markedly poorer diet.[83] The overall treatment response was similar in both groups; however, those receiving better nutrition tended to show more rapid clearance of bacteria and radiographic changes in addition to greater weight gain.[83] (Serum albumin and hemoglobin concentrations are strong predictors of survival in adults with pulmonary tuberculosis.[86]) In a study in Malawi, among 1181 patients with tuberculosis, of whom 80% were HIV-positive, 8% of patients died within the first 4 weeks of treatment. Risk factors for early mortality included age >35 years, HIV seropositivity, and a high degree of malnutrition.[87] Among adults with moderate to severe malnutrition, 10.9% died in the first 4 weeks of treatment as opposed to 6.5% death rate in adults who were normal or had mild malnutrition (odds ratio 1.8, 95% CI, 1.1–2.7). Among HIV-infected patients with tuberculosis, a BMI <17.0 appears to be a strong predictor of early mortality.[87]

Table 5. Body Composition in Adults with Untreated Active Pulmonary Tuberculosis with and without HIV Infection

Location	Indicator	Mean ± SD		P	Reference
		HIV-positive [*n*]	HIV-negative [*n*]		
Burundi	BCM (kg)	36.3 ± 4.0 [56]	40.4 ± 4.3 [11]	NS	5
Burundi	BCM/H^2	8.2 ± 1.7 [22]	9.6 ± 1.4 [11]	NS	7
Uganda	BCM (kg)				
men		21.5 ± 3.4 [163]	21.9 ± 3.8 [199]	NS	63
women		16.2 ± 2.8 [98]	16.5 ± 2.2 [79]	NS	
Burundi	TBW (L)	27.0 ± 5.6 [22]	30.0 ± 6.1 [11]	NS	7
Uganda	TBW (L)				
men		33.1 ± 4.0 [163]	33.0 ± 4.2 [199]	NS	63
women		27.3 ± 3.7 [98]	27.4 ± 3.0 [79]	NS	
Burundi	ECW (L)	13.6 ± 2.2 [22]	14.6 ± 1.6 [11]	NS	7
Uganda	ECW (L)				
men		13.4 ± 1.9 [163]	12.8 ± 1.9 [199]	NS	63
women		12.5 ± 1.6 [98]	12.3 ± 1.4 [79]		
Burundi	ICW/H^2	1.3 ± 0.4 [22]	1.5 ± 0.4 [11]	NS	7
Uganda	ICW (L)				
men		19.6 ± 3.0 [163]	20.0 ± 3.4 [199]	NS	63
women		14.8 ± 2.6 [98]	15.0 ± 2.1 [79]	NS	
Burundi	ICW:ECW	1.1 ± 0.2 [22]	1.1 ± 0.3 [11]	NS	7
Uganda	ICW:ECW				
men		1.48 ± 0.26 [163]	1.59 ± 0.48 [199]	<0.01	63
women		1.19 ± 0.16 [98]	1.23 ± 0.15 [79]		
Burundi	FM (kg)	10.4 ± 4.0 [56]	13.8 ± 4.3 [11]	<0.01	5
Uganda	FM (kg)				
men		4.23 ± 2.72 [163]	4.40 ± 3.32 [199]	NS	63
women		13.0 ± 7.2 [98]	12.2 ± 5.4 [79]	NS	
Uganda	FM (% of weight)				
men		7.7 ± 4.4 [163]	7.8 ± 5.4 [199]	NS	63
women		24.8 ± 9.5 [98]	23.9 ± 7.7 [79]	NS	
Burundi	FM/H^2	2.3 ± 1.7 [22]	3.8 ± 4.4 [11]	<0.01	7
Uganda	phase angle α (°)				
men		5.42 ± 1.05 [163]	5.76 ± 1.3 [199]	<0.01	63
women		5.35 ± 1.27 [98]	5.43 ± 0.93 [79]	NS	

BCM = body cell mass, H = height, TBW = total body water, ECW = extracellular water, ICW = intracellular water, FM = fat mass.

The Role of Nutritional Supplements

A number of studies have examined the role of nutritional supplements in tuberculosis. In a recent placebo-controlled trial from Indonesia,[88] daily supplements of vitamin A and zinc given to adults with pulmonary tuberculosis led to significant improvements in the lesion area observed on chest radiographs after 2 months of tuberculosis chemotherapy; this was not the case at the six-month follow-up. The supplements resulted in earlier clearance of tubercle bacilli from sputum. However, no effects of the supplements were observed on the number of cavities, the surface area of cavities, hemoglobin concentrations, or different anthropometric indicators of nutritional status. In another trial among 110 new cases of active tuberculosis, subjects received tuberculosis chemotherapy alone or in addition to injectable thiamin, vitamin B_6, or vitamin C, or an oral multivitamin supplement. All groups receiving any vitamin supplementation had significantly better lymphocyte proliferation responses (in response to phytohemagglutinin or purified protein derivative) than the arm of the study receiving no vitamin supplementation.[89] There were no differences in responses at baseline between the treatment arms. Another trial showed that vitamins C and E were effective in improving immune responses to tuberculosis when given as adjuncts to multidrug tuberculosis therapy.[90]

Data from asymptomatic HIV-positive individuals in observational studies indicate that higher intakes of individual micronutrients and multivitamins are related to better outcomes in terms of $CD4^+$ lymphocyte counts, clinical progression to AIDS, and overall mortality.[91] Serum concentrations of vitamins A, B_{12}, and E were also associated with HIV disease progression.[56,92–94] Recently, plasma selenium concentrations were found to be associated with increased risk of mycobacterial infection among HIV-infected intravenous drug users in Miami.[95] Nearly all of the above studies adjusted for potential confounding by other variables including use of prophylactic medications and antiretroviral therapy, baseline $CD4^+$ lymphocyte counts, and albumin concentrations. It is difficult to exclude the possibility of residual confounding by other causal variables that were not measured. In addition, biochemical markers of nutritional status have some limitations. Low plasma vitamin A and zinc concentrations can be depressed in the presence of an acute-phase response despite adequate body stores. Dietary intake studies are also limited because dietary patterns may change as a result of disease; reverse causality may therefore explain the associations between dietary intake and stage of disease. Randomized controlled trials will still provide the best causal evidence to examine the issues related to nutritional status and outcomes among patients with tuberculosis and HIV infection.

Conclusion

Although malnutrition appears to play an important role in the clinical course of co-infection with tuberculosis and HIV, further studies are needed to characterize the potential role of micronutrients—vitamin E, vitamin C, zinc, and B-complex vitamins—in the pathogenesis of tuberculosis and HIV co-infections. Malnutrition may influence the outcome of HIV infection and tuberculosis, accelerating the progression of immunosuppression, but it is unclear whether nutritional intervention will slow progression of disease. For example, it is not known whether the addition of a low cost vitamin and mineral supplement to tuberculosis chemotherapy would help improve morbidity and mortality. The repletion of tissue mass in patients recovering from tuberculosis may not be attained without relatively high levels of nutritional intake.[96] Controlled clinical trials currently in progress in developing countries should help provide insight into the role of micronutrient supplementation for adults co-infected with pulmonary tuberculosis and HIV.

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Chapter 3

Malnutrition And The Severity Of Lung Disease In Adults With Pulmonary Tuberculosis In Malawi

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Abstract

There is limited insight into the relationship between malnutrition and the clinical features of tuberculosis. The relationship between malnutrition and the severity of lung disease was examined in human immunodeficiency virus (HIV)-positive and negative adults with pulmonary tuberculosis (PTB) in Zomba Central Hospital, Zomba, Malawi. Chest radiographs and anthropometric measurements were obtained and bioelectrical impedance analysis (BIA) was conducted in sputum-positive patients with PTB. Lung disease in chest radiographs was graded as normal, minimal, moderately advanced, and far advanced according to a conventional classification system. Among 319 adults with PTB with or without HIV co-infection, body mass index (BMI), fat mass, and phase angle were independently associated with increasing severity of lung disease.   Multiple logistic regression analyses showed that BMI, fat mass, and phase angle were associated with increasing severity of lung disease among 236 HIV-positive adults, when adjusted for sex, age, and plasma HIV load. The severity of lung disease in adults with PTB is associated with the extent of malnutrition, as reflected by BMI and body composition studies using BIA.

Introduction

According to the World Health Organization (WHO), about one third of the world's population is infected with *Mycobacterium tuberculosis*, and the majority live in less developed countries where human immunodeficiency virus (HIV) infection is spreading rapidly. In sub-Saharan Africa, the Indian subcontinent, and southeast Asia, half or more of adults have latent tuberculosis infection. The WHO estimated that the number of new cases of tuberculosis, and the proportion with coexisting HIV infection will continue to increase.[1] The association between tuberculosis and malnutrition has long been recognized, as malnutrition predisposes to the development of clinical disease, and tuberculosis often exacerbates malnutrition.[2,3] Individuals with immunosuppression have a greater risk of developing clinical tuberculosis, which explains the increased prevalence of tuberculosis in association with HIV infection. In some countries in sub-Saharan Africa, including Malawi, the HIV seroprevalence rate among tuberculosis patients is over 75%.[4-7] Co-infection with HIV and tuberculosis may result in an exacerbation of wasting seen in tuberculosis or HIV infection alone.[2,8] However, there is limited insight into the relationship between malnutrition and the clinical features of tuberculosis.

Although nutritional status is known to be a risk factor for pulmonary tuberculosis (PTB), the relationship between nutritional status and the severity of the disease has not been well characterized. We hypothesized that more advanced lung disease, as assessed by chest radiographs, was associated with more severe malnutrition. To test this hypothesis, we conducted a cross-sectional study to examine the relationship between malnutrition and the extent of lung disease in adults with PTB with and without HIV infection. In addition to body mass index (BMI), body cell mass, fat mass, and phase angle derived from bioelectrical impedance analysis (BIA) were used to assess the extent of malnutrition in this study. BIA, a simple and non-invasive technique, has been recommended for nutritional studies in HIV-infected individuals and has been shown to be sufficiently precise for body composition analysis.[8,9,10] BIA was shown to be a good predictor and superior to BMI as an estimator of body fat [10] Body cell mass, the total mass of all the cellular elements in the body, represents the metabolically active component of the body. It comprises those tissues that are most likely to be affected by physical activity, nutrition, or disease, and appears to be an independent predictor of mortality among HIV-infected adults in the era prior to highly active antiretroviral therapy.[11-14] Phase angle, the relationship between resistance and reactance obtained from BIA, is considered

to reflect water distribution between extra- and intracellular spaces and has been shown to be an independent predictor of mortality during HIV infection.[13,15,16]

Methods

The study population consisted of adults who presented with new sputum-positive PTB in Zomba Central Hospital between July 1999 and December 2000. Subjects were offered HIV testing and were screened for HIV antibodies after written informed consent had been obtained. All subjects were given appropriate pre- and post-test HIV counselling. At enrollment, basic demographic information and a medical history were collected and a standardized physical examination was conducted. Subjects received standard short course chemotherapy for tuberculosis as per guidelines of the Malawi National Tuberculosis Programme.[17] Adults with a previous history of treated PTB were excluded. Three sputum samples from each subject were examined with Auramine-O dark-fluorescent staining method. Sputum positive PTB was considered proven when at least one out of three sputum stains showed acid-fast bacilli. HIV infection was diagnosed on the basis of a positive rapid test (Determine 1 / 2 Rapid test by Abbott, Johannesburg, South Africa) and confirmed by a positive enzyme-linked immunosorbent assay for HIV-1 antibodies (Wellcozyme; Wellcome Diagnostics, Dartford, Kent, UK). Plasma HIV load was measured using quantitative HIV-1 RNA PCR (Roche Amplicor Monitor, version 1.5, Branchburg, NJ, USA) with a sensitivity limit of 400 HIV RNA copies/mL. The protocol was approved by the institutional review boards at the Johns Hopkins School of Medicine (Baltimore, Maryland, USA) and the College of Medicine, University of Malawi (Blantyre, Malawi), with final approval by the Office for Protection from Research Risk of the National Institutes of Health.

Nutritional assessment

Body weight was determined to the nearest 0.1 kg using an adult balance (Seca 700 balance, Seca Corporation, Hanover, MD, USA), and standing height was determined to the nearest cm. Single-frequency BIA was performed at 50 kHz and 800 µA (RJL Systems, Inc., Detroit, MI, USA) with standard tetrapolar lead placement.[18] BIA measurements were performed in triplicate for each subject. The reproducibility on repeated BIA measurements was >99%. Impedance (Z) was calculated as $(resistance^2 \times reactance^2)^{0.5}$. Body cell mass was calculated as $0.76 [Ht^{1.60} / Z(parallel)^{0.50} \times 59.06] + 18.52 \, Wt - 386.66/ 120$ for males, and as $0.96 [$

Ht$^{2.07}$ / Z(parallel)$^{0.36}$ x 1.30] + 5.79 Wt − 230.51/120 for females. Fat-free mass was calculated as 0.50 [Ht$^{1.48}$ / Z$^{0.55}$ x 1.0 / 1.22] + 0.42 Wt + 0.49 for males, and as 0.88 [Ht$^{1.97}$ / Z$^{0.49}$ x 1.0 / 22.22] + 0.081 Wt + 0.07 for females. Fat mass derived from BIA measures was calculated as body weight minus fat-free mass. Phase angle was calculated as α = arctangent (reactance / resistance).[19] These equations were previously cross-validated in a sample of white, black and Hispanic patients with and without HIV infection[8] and have been applied elsewhere in sub-Saharan Africa.[20]

Radiographical findings

A standard posterior-anterior chest radiograph was taken of each subject. All radiographs were examined by an experienced clinical officer in the Malawi National Tuberculosis Control Programme. Lung disease was graded according to an international classification of tuberculosis [21]: (1) *minimal lung disease* was defined as infiltrates of slight to moderate density; disease present in a small portion of both lungs; the total volume of infiltrate(s) being the volume of one lung present above the 2nd chondrosternal junction and the spine of the fourth junction or the body of the 5th thoracic vertebra and no cavitations present; (2) *moderate advanced disease* was defined as: disease present in one or both lungs; the total extend not more than: a) scattered lesions of slight to moderate density do not involve more than the total volume of one lung or the equivalent volume of both lungs, b) dense, confluent lesions do not involve more than one third of the volume of one lung, c) the total diameter of the cavities are not greater than 4 cm; and (3) *far advanced lung disease* was defined as: lesions more extensive than moderate advanced disease. All chest radiographs were interpreted by a reader blinded to the HIV status and clinical status of the subjects. To lessen inter- and intra-observer differences, the chest radiographs were also read by a tuberculosis specialist (C.C.W.), and in cases of discordance in readings, films were reviewed and final classifications were reached by consensus.

Data and statistical analysis

Data and statistical analysis were conducted using SPSS 9.0 (SPSS, Inc., Chicago, IL, USA). Comparisons between groups were made using *t*-tests and exact tests. Univariate analysis of variance was used to test for linear trends across categories of lung disease. Nutritional status was assessed in adults with PTB with and without HIV co-infection. Subjects were then separated into two groups according to the extent of malnutrition. BMI (wt/ht^2)<19 was considered consistent with malnutrition.[22] The proportion of adults with phase angle <5.3° was examined, as this cut-off was previously shown to be predictive of mortality among

HIV-infected adults.[23]   Body cell mass and fat mass were divided into quartiles, with the lowest quartile considered the most consistent with wasting. Logistic regression models were fitted with BMI <19.0, phase angle <5.3º and the lowest quartile of fat mass and body cell mass as the outcome variable. Multiple logistic regression models were conducted to adjust for sex, age, HIV co-infection and plasma HIV load. A test for trend across the categories of extent of lung disease was performed by assigning the subjects the mean value of their allocated category and then entering this as a continuous variable into the logistic regression model.   A significance level of P <0.05 was used in this study.

Results

Characteristics of patients with pulmonary tuberculosis

The study population consisted of 236 HIV-positive and 83 HIV-negative adults with sputum -positive PTB. The overall HIV prevalence among the male and female participants was 67%, and 80%, respectively. The mean age among all subjects was 33 years, ranging from 18 to 58 years. Education levels were significantly lower among female participants (32.5% of female versus 8.7% of male never attended school, P <0.01). Of female participants 17.2% had continued education after primary school, versus 31.5% of male participants (P <0.01). There were no significant differences in education levels by HIV status. HIV-positive men with PTB had lower mean hemoglobin than HIV-negative men (Table1).

Table 1. Characteristics of HIV-positive and HIV-negative adults presenting with pulmonary tuberculosis in Zomba, Malawi

Characteristics[*]	Men			Women		
	HIV-positive ($n = 100$)	HIV-negative ($n = 49$)	P	HIV-positive ($n = 136$)	HIV-negative ($n = 34$)	P
Age (years)	36 (8)	34 (12)	0.28	32 (9)	30 (11)	0.18
Able to read (%)	93	87.8	0.29	70.9	55.9	0.09
Primary education or higher (%)	30	34.7	0.56	17.5	20.5	0.67
Hemoglobin (g/L)	96 (26)	111 (31)	0.01	92 (23)	99 (27)	0.13
Anemic (%)**	91	67.3	0.01	86	85.3	0.91
Plasma HIV load (copies x 10^3/mL)*** median (25th, 75th percentiles)	278 (134, 703)			228 (94, 630)		

*Mean (SD) for continuous variables.

**Hemoglobin <120 g/L for females and <130 g/L for males.

***HIV load not measured in 3 male and 10 female subjects.

Nutritional status

The majority of subjects were malnourished, as overall 61% of subjects had a BMI <19. There were no significant differences found in weight, mean BMI or the proportion of individuals with BMI <19 between HIV-positive and -negative individuals. There were no significant differences in resistance, impedance, fat-free mass or fat mass between individuals with or without HIV infection. Reactance, body cell mass, and phase angle was significantly lower in HIV-positive male participants than in HIV-negative male participants. The proportion of participants with phase angle <5.3º was significantly higher in HIV-positive than in HIV-negative male participants. These differences were not observed between the HIV-positive and HIV-negative female participants (Table 2).

Table 3 shows the linear trends across categories of lung disease associated with nutritional indicators in HIV-positive and -negative subjects. These data show that among the HIV-positive individuals, more advanced lung disease was shown to be associated with lower BMI, body cell mass, fat mass, and phase angle. Among the HIV-negative individuals, more advanced lung disease was associated with lower BMI and lower phase angle.

Table 2. Body composition among HIV-positive and HIV-negative adults with pulmonary tuberculosis

Characteristics*	Men			Women		
	HIV-positive (n = 100)	HIV-negative (n = 49)	*P*	HIV-positive (n = 136)	HIV-negative (n = 34)	*P*
Weight (kg)	52.7 (7.7)	52.0 (7.8)	0.62	45.8 (8.3)	45.7 (7.3)	0.96
Height (cm)	169.4 (6.1)	167.3 (6.0)	0.04	157.4 (5.7)	156.2 (5.9)	0.28
Body mass index	18.3 (2.3)	18.6 (2.4)	0.56	18.4 (2.9)	18.7 (2.7)	0.59
Body mass index <19 (%)	62.0	65.3	0.69	61.0	61.7	0.93
Reactance (ohms)	48 (14)	54 (14)	0.02	54 (16)	55 (14)	0.72
Resistance (ohms)	573 (105)	570 (81)	0.91	675 (114)	667 (95)	0.71
Impedance	575 (105)	573 (81)	0.90	678 (114)	670 (95)	0.71
Body cell mass (% of weight)	39.2 (3.1)	40.6 (3.8)	0.02	33.0 (3.4)	32.8 (2.8)	0.70
Fat-free mass (% of weight)	93.1 (5.2)	92.7 (5.0)	0.64	80.5 (8.7)	79.6 (9.2)	0.58
Fat mass (% of weight)	6.9 (5.2)	7.3 (5.0)	0.64	19.5 (8.7)	20.4 (9.2)	0.58
Phase angle (degrees)	4.8 (1.2)	5.4 (1.4)	0.01	4.6 (1.3)	4.7 (1.2)	0.55
Phase angle <5.3° (%)	61.0	42.9	0.04	64.7	72.1	0.40

*Mean (SD) for continuous variables.

Table 3. Relationship of body composition and extent of lung disease in HIV-positive and HIV-negative adults with pulmonary tuberculosis

Extent of lung disease*	HIV-positive adults				HIV-negative adults			
	Body mass index	Body cell mass	Fat mass	Phase angle	Body mass index	Body cell mass	Fat mass	Phase angle
Normal	19.0 (3.3)	34.8 (4.5)	18.7 (10.2)	5.0 (1.2)	22.1 (2.3)	42.1 (2.0)	13.8 (3.0)	7.2 (0.7)
Minimal	18.7 (2.5)	35.5 (4.7)	13.4 (9.8)	4.8 (1.2)	19.6 (2.5)	37.1 (5.0)	16.6 (10.1)	5.6 (0.8)
Mod-adv.	18.2 (2.6)	35.9 (4.5)	13.0 (9.5)	4.6 (1.3)	18.8 (2.8)	37.3 (6.0)	13.4 (11.3)	5.2 (1.4)
Far adv.	17.6 (2.1)	34.4 (3.8)	13.6 (8.5)	4.2 (1.2)	17.3 (1.3)	36.8 (4.7)	9.6 (7.7)	4.4 (1.3)
P for trend	0.05	0.05	0.02	0.01	0.01	0.14	0.06	0.01

*Normal (n = 36, n =6), minimal (n = 95, n = 18), moderate-advanced (n = 61, n = 25), far advanced (n = 44, n = 34) for HIV-positive and HIV-negative adults, respectively.

**Mean (SD) for all body composition measures show.

Table 4 shows crude and adjusted odds ratios (O.R.) and 95% confidence intervals (C.I.) for associations between extent of lung disease and lower BMI, body cell mass, fat mass, and phase angle in adults with PTB, with and without HIV co-infection. When compared with normal lung appearance, far advanced lung disease was associated with lower BMI, fat mass, and phase angle as shown by crude and adjusted O.R. The same applied for minimal and moderate advanced lung disease, which were associated with lower fat mass and BMI, respectively. There was no significant relationship between body cell mass and extent of lung disease in adults with PTB with or without HIV co-infection in logistic regression analyses. In considering the most marked comparison, for HIV-positive individuals with far advanced lung disease, the odds ratio for an independent association with lower BMI was 6.88 (95% C.I. 2.37-19.93), with lower fat mass 9.98 (95% C.I. 2.01-49.70), and with lower phase angle 3.98 (95% C.I. 1.37-11.55) when adjusted for sex, age, and plasma HIV load (Table 5).

Table 4. Extent of lung disease and risk of low body mass index, body cell mass, fat mass, and phase angle in adults with pulmonary tuberculosis

Extent of lung disease	Body mass index <19		Body cell mass, lowest quartile		Fat mass, lowest quartile		Phase angle <5.3	
	Crude O.R. (95% C.I.)	Adjusted O.R.* (95% C.I.)	Crude O.R. (95% C.I.)	Adjusted O.R.* (95% C.I.)	Crude O.R. (95% C.I.)	Adjusted O.R.* (95% C.I.)	Crude O.R. (95% C.I.)	Adjusted O.R.* (95% C.I.)
Normal N = 42	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Minimal N = 113	2.02 (0.97-4.16)	1.97 (0.95-4.09)	0.85 (0.38-1.93)	1.21 (0.47-3.09)	3.17 (1.04-9.66)	2.54 (0.78-8.26)	1.43 (0.70-2.93)	1.54 (0.73-3.25)
Mod-advanced N = 86	2.79 (1.31-5.97)	2.86 (1.33-6.19)	0.75 (0.32-1.77)	0.84 (0.32-2.28)	3.89 (1.26-11.06)	4.15 (1.24-13.82)	1.81 (0.86-3.81)	2.11 (0.95-4.65)
Far advanced N = 78	8.12 (3.43-19.23)	8.83 (3.64-21.42)	1.25 (0.54-2.90)	1.5 (0.56-4.03)	3.73 (1.19-11.69)	4.94 (1.44-17.01)	3.11 (1.40-6.88)	4.32 (1.81-10.32)
P for trend	0.0001	0.0001	0.19	0.51	0.02	0.007	0.003	0.0004

*Adjusted for age, sex, and HIV infection.

Table 5. Extent of lung disease and risk of low body mass index, body cell mass, fat mass, and phase angle in HIV-positive adults with pulmonary tuberculosis

Extent of lung disease	Body mass index <19		Body cell mass, lowest quartile		Fat mass, lowest quartile		Phase angle <5.3	
	Crude O.R. (95% C.I.)	Adjusted O.R.* (95% C.I.)	Crude O.R. (95% C.I.)	Adjusted O.R.* (95% C.I.)	Crude O.R. (95% C.I.)	Adjusted O.R.* (95% C.I.)	Crude O.R. (95% C.I.)	Adjusted O.R.* (95% C.I.)
Normal N = 35	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Minimal N = 95	1.76 (0.81-3.83)	1.77 (0.76-4.15)	0.69 (0.30-1.63)	1.24 (0.45-3.40)	3.06 (0.98-9.50)	4.11 (0.98-17.26)	1.38 (0.63-3.04)	2.08 (0.86-5.06)
Mod-advanced N = 61	2.12 (0.92-4.89)	2.33 (0.94-5.80)	0.62 (0.24-1.57)	0.73 (0.25-2.11)	3.09 (0.95-10.06)	6.22 (1.36-28.37)	1.5 (0.64-3.51)	2.15 (0.83-5.61)
Far advanced N = 44	4.86 (1.82-13.0)	6.88 (2.37-19.93)	1.57 (0.62-3.99)	1.47 (0.50-4.27)	2.67 (0.77-9.25)	9.98 (2.01-49.70)	2.43 (0.92-6.40)	3.98 (1.37-11.55)
P for trend	0.0002	0.0003	0.09	0.3	0.05	0.02	0.08	0.02

*Adjusted for age, sex, and plasma HIV load.

Discussion

This study demonstrates that the extent of pulmonary disease, as assessed by chest radiographs, is associated with the severity of malnutrition. Few studies in sub-Saharan Africa have examined the relationship between malnutrition and the severity of lung disease. To our knowledge, the present study is the first to examine the relationship between body composition using BIA and the severity of lung disease among HIV-positive and -negative adults with PTB. One of the advantages of BIA over BMI alone is that BIA can differentiate the degree of body fat and body cell mass in individuals. However, BMI was still a sensitive indicator of malnutrition in this study and may be considered as an adequate indicator in a resource-poor setting. In a previous study from Nigeria, patients with PTB and more severe lung disease had lower weight, arm circumference, and hand grip strength than those with limited lung disease.[24] Another study from Malawi showed that nutritional status declined with the extent of lung disease and in the presence of cavitation.[25]

The main alterations associated with advanced lung disease in both HIV-positive and -negative adults with PTB were lower BMI, fat mass, and phase angle. A mean BMI of 17 in HIV-negative adults among adults with far advanced pulmonary disease are consistent with extremely poor nutritional status. Several studies have shown that BMI is lower among adults with PTB compared with healthy controls,[25-28] and that BMI is lower in HIV-infected adults with tuberculosis compared with HIV-negative adults with tuberculosis.[4,6,20,29] The data from the present study are consistent with other studies that have described body composition in adults with PTB.[3,24,25] A study among Ethiopian PTB patients showed that malnutrition influences the clinical and radiographic features, and the risk for atypical presentation of PTB was high among severely malnourished HIV-infected patients.[26]

Phase angle, the relationship between resistance and reactance, is considered a marker for cell membrane integrity and has been shown to be an independent predictor of mortality during HIV infection. [15,23] It is still unclear why this derived indicator from BIA is such a strong predictor of survival during HIV infection. In healthy adults, the phase angle at 50 kHz is usually in the range of 8-15° [30] Among HIV-infected adults on triple anti-retroviral therapy,[16] the phase angle was 6.2° compared with a mean phase angle of 4.2° in HIV-positive and 4.4° in HIV-negative adults with far advanced pulmonary disease described in the present study.

A limitation of the present study is that the assessment of lung disease through chest radiographs, although done with a descriptive grading scheme, is subjective. The cross-sectional

design of this study restricts our conclusions and does not provide information on whether poor nutritional status is a predictor of more severe PTB. In a recent study from Thyolo District in southern Malawi, moderate to severe malnutrition as assessed by BMI, was a risk factor for early death, although the reasons are unknown.[7] Wasting is a fundamental sign of tuberculosis in both HIV-positive and HIV-negative patients, and the etiology of the wasting has not been elucidated. Further studies are needed to examine the role of oxidative stress and antioxidant micronutrients, the role of inflammatory cytokines, and the relationship of severe lung disease to mortality. It is unclear whether nutritional interventions will slow progression of disease or reduce morbidity and mortality if added to tuberculosis chemotherapy. Controlled clinical trials currently in progress in developing countries should help provide insight into the role of micronutrient supplementation for adults with PTB, with and without HIV co-infection.

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Micronutrient Malnutrition And Wasting In Adults With Pulmonary Tuberculosis With And Without HIV Co-Infection In Malawi

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Abstract

Wasting and micronutrient malnutrition have not been well characterized in adults with pulmonary tuberculosis. We hypothesized that micronutrient malnutrition is associated with wasting and higher plasma human immunodeficiency virus (HIV) load in adults with pulmonary tuberculosis. In a cross-sectional study involving 579 HIV-positive and 222 HIV-negative adults with pulmonary tuberculosis in Zomba, Malawi, anthropometry, plasma HIV load and plasma micronutrient concentrations (retinol, α-tocopherol, carotenoids, zinc, and selenium) were measured. The risk of micronutrient deficiencies was examined at different severity levels of wasting. Body mass index (BMI), plasma retinol, carotenoid and selenium concentrations significantly decreased by increasing tertile of plasma HIV load. There were no significant differences in plasma micronutrient concentrations between HIV-negative individuals and HIV-positive individuals who were in the lowest tertile of plasma HIV load. Plasma vitamin A concentrations <0.70 μmol/L occurred in 61%, and zinc and selenium deficiency occurred in 85% and 87% respectively. Wasting, defined as BMI<18.5 was present in 59% of study participants and was independently associated with a higher risk of low carotenoids, and vitamin A and selenium deficiency. Severe wasting, defined as BMI<16.0 showed the strongest associations with deficiencies in vitamin A, selenium and plasma carotenoids. These data demonstrate that wasting and higher HIV load in pulmonary tuberculosis are associated with micronutrient malnutrition.

Introduction

Approximately one-third of the world's population is infected with *Mycobacterium tuberculosis*, and the majority live in less developed countries where human immunodeficiency virus (HIV) infection is spreading rapidly. The World Health Organization (WHO) estimates that the number of new cases of tuberculosis and the proportion with coexisting HIV infection will continue to increase.[1] Immunosuppression increases the risk of developing clinical tuberculosis, which contributes to the increased prevalence of tuberculosis in association with HIV infection. Malnutrition and wasting are associated with tuberculosis, and co-infection with HIV and tuberculosis may potentially exacerbate the wasting that occurs in tuberculosis or HIV infection alone.[2–5] Micronutrient deficiencies have been described in individuals with tuberculosis[6-17] and in those with HIV infection.[17-23] Several cross-sectional studies suggest that patients with tuberculosis are at high risk of deficiencies of vitamin A[7,10,11,12], thiamin[13], vitamin B_6 [14], folate[6,15], vitamin E[16], and zinc[10]. Poor selenium status has recently been shown to increase the risk of developing mycobacterial disease among HIV-infected injection drug users[24], but selenium status among HIV-infected adults with pulmonary tuberculosis has not been well characterized. Selenium plays an important role in the selenoenzyme glutathione peroxidase that protects cells against free radical damage and oxidative stress.

The relationship between severity of HIV disease and micronutrient malnutrition needs further elucidation. Such information would help identify subgroups that might benefit the most from nutritional interventions. Plasma HIV load was used as an indicator of severity of HIV disease, as HIV load tends to be higher in more active HIV disease. We hypothesized that wasting in pulmonary tuberculosis is associated with micronutrient malnutrition and that HIV-infected adults with pulmonary tuberculosis who have more active HIV disease, as reflected by higher HIV load, also have more severe micronutrient malnutrition. To test these hypotheses, we conducted a cross-sectional study to examine the relationship between wasting, HIV load and micronutrient malnutrition in HIV-positive and HIV-negative adults with pulmonary tuberculosis in Zomba, Malawi.

Methods

The study population consisted of adults who presented with new sputum-positive pulmonary tuberculosis in Zomba Central Hospital between July 1999 and April 2003. Subjects were offered HIV testing and were screened for HIV antibodies after signing a written informed consent form. All subjects were given appropriate pre- and post-test HIV counselling. Subjects commenced treatment after enrollment and received standard short course chemotherapy for tuberculosis as per guidelines of the Malawi National Tuberculosis Program.[25] Adults with a previous history of treated pulmonary tuberculosis were excluded. Three sputum samples from each subject were examined with Auramine-O dark-fluorescent staining method. Sputum positive pulmonary tuberculosis was considered proven when at least one out of three sputum stains showed acid-fast bacilli. HIV infection was diagnosed on the basis of a positive rapid test (Determine 1/2 Rapid test by Abbott, Abbott Laboratories, Johannesburg, SA) and confirmed by a positive enzyme-linked immunosorbent assay for HIV-1 antibodies (Wellcozyme; Wellcome Diagnostics, Dartford, Kent, UK). Plasma HIV load was measured using quantitative HIV-1 RNA PCR (Roche Amplicor Monitor, version 1.5, Branchburg, NJ, USA) with a sensitivity limit of 400 HIV RNA copies mL. CD4$^+$ lymphocyte counts were not conducted due to limited resources. None of the participants were taking antiretroviral treatment.

The protocol was approved by the institutional review boards at the Johns Hopkins School of Medicine (Baltimore, Maryland, USA) and the College of Medicine, University of Malawi (Blantyre, Malawi), with final approval by the Office for Protection from Research Risk of the National Institutes of Health.

Nutritional assessment

Body weight was determined to the nearest 0.1 kg using an adult balance (Seca 700 balance, Seca Corporation, Hanover, MD, USA), and standing height was determined to the nearest cm. Body mass index (BMI) was calculated as body weight/height^2.

Plasma Micronutrient Concentrations

A venous blood sample was collected by venipuncture (Sarstedt Monovette, Newton, NC). Blood samples were shielded from bright light and immediately aliquoted and stored in

cryotubes at -70° C. α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein, zeaxanthin, retinol, and α-tocopherol concentrations were measured in 100 uL of plasma by high performance liquid chromatography using a modified method from the Nutrition Laboratory, Inorganic Toxicology and Nutrition Branch Division of Laboratory Sciences, National Center of Environmental Health, Centers of Disease Control and Prevention (Rosemary Schleicher, personal communication).[26] Total plasma carotenoids were defined as the sum of α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein and zeaxanthin in μmol/L. Plasma trace element concentrations were measured using a Perkin Elmer model AAnalyst 600 atomic absorption spectrometer equipped with Zeeman background correction, a THGA graphite furnace, and an AS800 auto sampler (Perkin Elmer Corp., Norwalk, CT). Quality control was assessed by repeated analysis of pooled human plasma controls run at the beginning and the end of each analysis. Standard curves were run periodically using standard reference material 986C (National Institute of Standards and Technology, Gaithersburg, MD). Throughout all analyses, the plasma samples were run in a masked fashion.

Data and Statistical Analysis

Data and statistical analysis were conducted using SAS 8.01 (SAS Institute Cary, NC, USA) and SPSS 9.0 (SPSS, Inc., Chicago, IL, USA). Comparisons between groups were made using *t*-tests and nonparametric Mann-Whitney *U*-tests. Univariate analysis of variance was used to test for linear trends across categories of plasma HIV load and BMI.

HIV load was categorized into tertiles. HIV negative subjects were assigned a fourth category of HIV load (category 0) when groups were merged for analysis. Nutritional status was assessed in adults with pulmonary tuberculosis with and without HIV co-infection. Subjects were separated into groups according to their extent of wasting. Mild wasting was defined as BMI 17.0-18.49, moderate wasting as BMI 16.0-16.99, and severe wasting as BMI <16.0, conform the WHO strata for BMI grading of severity of malnutrition.[27]

Plasma retinol <0.70 μmol/L was considered consistent with vitamin A deficiency.[28] Vitamin E deficiency was defined as plasma α-tocopherol <11.6 μmol/L.[28] Zinc deficiency was defined as plasma zinc <11.5 μmol/L and selenium deficiency as plasma selenium <0.89 μmol/L.[28] Because there is no standard cut-off for deficiency of carotenoids, we divided total

plasma carotenoids into quartiles, with the lowest quartile considered to be the most consistent with deficiency.

To examine the risk of micronutrient deficiencies at different severity level of wasting, logistic regression models were fitted with retinol <0.70, α-tocopherol <11.6, zinc <11.5, selenium <0.89, and the lowest quartile of total carotenoids as the outcome variable. Multivariate logistic regression models were conducted to adjust for sex, age and HIV load. A significance level of P <0.01 was used in this study.

Results

The study population consisted of 579 HIV-positive and 222 HIV-negative adults with sputum-positive pulmonary tuberculosis. Among the total study population, 66% (232/352) of male and 77% (347/449) of female participants were HIV-positive. The mean age among all subjects was 33 years (range 18-59 years). The majority of subjects were wasted, as 59% of subjects had a BMI <18.5, 32% of subjects had a BMI <17.0, and 17% of all subjects were severely wasted as defined by BMI<16.0. Plasma retinol concentrations <0.70 μmol/L and <1.05 μmol/L occurred in 61% and 84% of all subjects, respectively. Vitamin E, zinc, and selenium deficiency occurred in 13%, 85% and 87% respectively.

Table 1 shows characteristics of study participants, such as sex, age, BMI, and plasma carotenoids, retinol, α-tocopherol, zinc and selenium by categories of plasma HIV load. BMI, plasma retinol, total carotenoids and selenium concentrations decreased by increasing plasma HIV load. Age, the proportion of individuals with BMI <18.5, BMI <16.0 and selenium deficiency were increased with increasing plasma HIV load. Plasma α-tocopherol, zinc or the proportion of individuals with vitamin A, vitamin E, or zinc deficiencies were not significantly different across the categories of plasma HIV load. When exploring across the categories separately, there were no significant differences between HIV-negative individuals compared with HIV-positive individuals in the lowest tertile of viral load.

Table 1. Characteristics of adults presenting with pulmonary tuberculosis in Zomba, Malawi by plasma HIV load

Characteristic[1]	HIV negative	HIV positive*			P-value**
		Plasma HIV Load (copies/mL)			
	0	≤ 133 200	133 200 - 406 000	> 406 000	
	n=222	n=185	n=186	n=186	
Sex (% Female)	45.9	63.2	58.1	55.9	0.07
Age (years)	33 ± 12	32 ± 12	33 ± 12	33 ± 12	0.01
Body mass index (BMI) (wt/ht^2)	18.6 ± 2.9	19.0 ± 2.6	18.3 ± 3.0	17.3 ± 2.7	0.0001
Wasting:[2] No, BMI ≥ 18.5	42.3	51.4	41.9	30.1	0.005
Mild, BMI 17.0-18.49 (%)	32.0	27.0	22.6	24.2	0.04
Moderate, BMI 16.0-16.99 (%)	13.1	10.3	17.2	18.8	0.04
Severe, BMI <16.0 (%)	12.6	11.4	18.3	26.9	0.001
Retinol (μmol/L)	0.636 (0.367, 1.104)	0.603 (0.336, 1.085)	0.585 (0.321, 1.066)	0.522 (0.157, 1.738)	0.001
Vitamin A deficiency, retinol <0.70 μmol/L (%)[3]	58.6	57.3	64.0	66.7	0.05
Total Carotenoids (μmol/L)[4]	0.846 (0.490, 1.459)	0.795 (0.476, 1.329)	0.700 (0.385, 1.279)	0.509 (0.279, 0.929)	0.0001
α-Tocopherol (μmol/L)	15.18 (11.71, 19.65)	14.90 (11.60, 19.16)	15.66 (11.85, 20.71)	16.07 (11.53, 22.40)	0.02
Vitamin E def., α-tocopherol <11.6 μmol/L (%)[3]	13.1	14.6	11.8	12.4	0.67
Zinc (μmol/L)	8.95 (7.01, 11.43)	8.83 (6.94, 11.25)	8.49 (6.44, 11.19)	9.15 (6.47, 12.94)	0.82
Zinc deficiency, zinc <11.5 μmol/L (%)[3]	84.2	88.1	87.6	77.4	0.09
Selenium (μmol/L)	0.687 ± 0.23	0.664 ± 0.22	0.624 ± 0.22	0.559 ± 0.21	0.0001
Selenium deficiency, selenium<0.89 μmol/L (%)[3]	79.7	84.9	90.3	92.5	0.001

[1] Mean ±SD for continues variables with normal distribution, geometric mean (lower, upper SD) when distribution was not normal

[2] Grading based on WHO Expert report, reference 27.

[3] Cut-offs based on reference 28.

[4] α-carotene + β-carotene + β-cryptoxanthin + lycopene + lutein + zeaxanthin

* HIV load could not be determined for 21 individuals.

** ANOVA, linear trend across the 4 categories of plasma HIV load.

Table 2 shows adjusted odds ratios (O.R.) and 95% confidence intervals (C.I.) for independent associations between wasting and micronutrient deficiencies. Wasting defined as BMI<18.5 was associated with vitamin A deficiency, low plasma carotenoids and selenium deficiency. The odds ratio for an independent association with vitamin A deficiency was 2.86 (95% C.I. 2.11-3.89) when adjusted for sex, age, and plasma HIV load. The adjusted odds ratio for an independent association with the lowest quartile of total carotenoids was 2.96 (95% C.I. 1.99-4.44). The adjusted odds ratio for an independent association with selenium deficiency was 1.59 (95% C.I. 1.04-2.43) (Data not shown). When separating severity levels of wasting; mild wasting did not show association with deficiencies, moderate wasting was associated with vitamin A deficiency and severe wasting was significantly associated with vitamin A deficiency, low plasma carotenoids and selenium deficiency. (Table 2) Wasting was not associated with vitamin E or zinc deficiency.

Table 2. Risk of micronutrient deficiencies at different severity levels of wasting in adults with pulmonary tuberculosis with and without HIV co-infection.

Deficiency	Mild wasting BMI 17.0-18.49		Moderate wasting BMI 16.0-16.99		Severe wasting BMI <16.0	
	O.R. (95% C.I.)*	*P*-value	O.R. (95% C.I.)*	*P*-value	O.R. (95% C.I.)*	*P*-value
Vitamin A deficiency	0.81 (0.58-1.13)	0.20	1.59 (1.03-2.47)	0.03	3.51 (2.19-5.72)	0.0001
Lowest quartile of Total Carotenoids	0.92 (0.62-1.36)	0.67	2.46 (1.57-3.85)	0.0001	1.82 (1.18-2.83)	0.007
Vitamin E deficiency	0.86 (0.54-1.37)	0.54	1.24 (0.70-2.18)	0.46	1.13 (0.64-1.97)	0.68
Zinc deficiency	0.71 (0.45-1.15)	0.17	0.61 (0.37-1.00)	0.05	0.76 (0.46-1.24)	0.27
Selenium deficiency	1.59 (1.04-2.43)	0.03	1.16 (0.62-2.17)	0.65	3.25 (1.38-7.62)	0.007

* Adjusted for sex (male), age (per year) and HIV load (quartiles, where category 0 is HIV negative and category 3 is the highest)

Figures 1, 2 and 3 show plasma retinol, total plasma carotenoids, and plasma selenium concentrations with 95% C.I by severity of wasting and categories of plasma HIV load. Plasma retinol concentrations significantly decreased with the increase of plasma HIV load among non-wasted adults with pulmonary tuberculosis ($P=0.004$). Total carotenoid concentrations significantly decreased with the increase of plasma HIV load among non-wasted, mildly wasted, moderately wasted and severely wasted adults ($P=0.0001$, $P=0.002$, $P=0.001$ and $P=0.001$, respectively). Selenium concentrations decreased significantly with the increase of plasma HIV load among non-wasted and severely wasted adults with pulmonary tuberculosis ($P=0.0001$ and $P=0.03$, respectively). Among the HIV negative adults and those in the 1st and 2nd tertile of HIV load, plasma retinol, total carotenoids and selenium concentrations significantly decrease with the increasing severity of wasting. Among those in the 3rd tertile of HIV load, only plasma retinol concentrations significantly decreased with the increasing severity of wasting. This trend did not reach significance for plasma carotenoid and selenium concentrations.
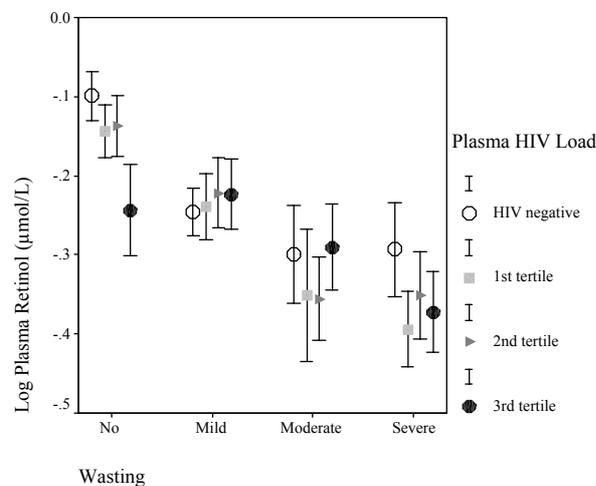

Figure 1. Log-transformed mean plasma retinol concentrations with 95% C.I. are depicted by severity of wasting and plasma HIV load. Among the not-wasted adults with pulmonary tuberculosis log mean plasma retinol concentration significantly decrease with the increase of plasma HIV load ($P=0.004$). Among those with mild, moderate and severe wasting this linear trend did not reach significance. Among the HIV negative adults with pulmonary tuberculosis, log mean plasma retinol concentration significantly decrease with the increasing severity of wasting ($P=0.0001$). The same trend appears among those in the 1st, 2nd and 3rd tertile of HIV load; $P=0.0001$, $P=0.0001$ and $P=0.01$ respectively.
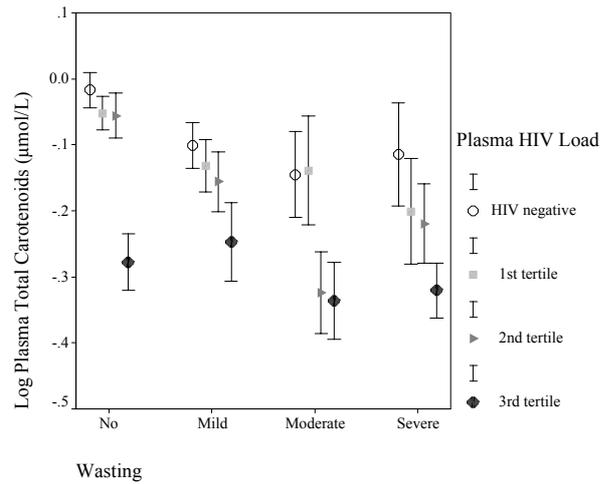
Figure 2. Log-transformed mean plasma total carotenoid concentrations with 95% C.I. are depicted by severity of wasting and plasma HIV load. Among not-wasted, mildly wasted, moderately wasted and severely wasted log mean plasma total carotenoid concentrations significantly decrease with the increase of plasma HIV load (P=0.0001, P=0.002, P=0.001 and P=0.001, respectively). Among the HIV negative adults, and those in the 1st and 2nd tertile of plasma HIV load, log mean plasma total carotenoid concentrations significantly decreased with the increasing severity of wasting (P=0.007, P=0.002 and P=0.0001, respectively). This trend did not reach significance among those in the 3rd tertile of plasma HIV load.
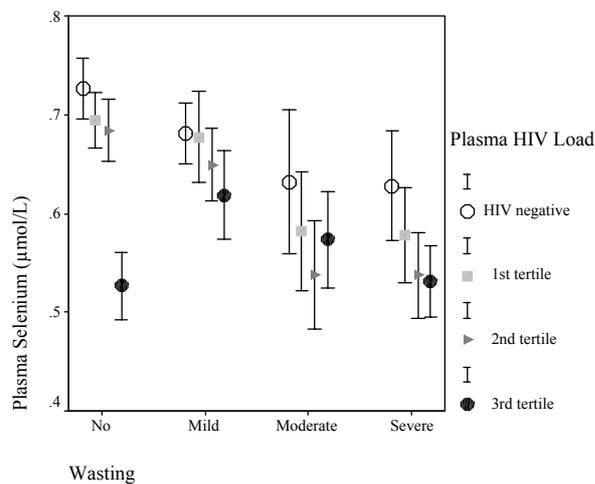

Figure 3. Mean plasma selenium concentrations with 95% C.I. are depicted by severity of wasting and plasma HIV load. Among not-wasted and severely wasted adults, mean plasma selenium concentrations significantly decrease with the increase of plasma HIV load (P=0.0001 and P=0.03, respectively). This trend did not reach significance among those with mild and moderate wasting. Among the HIV negative adults, and those in the 1st and 2nd tertile of plasma HIV load, mean plasma selenium concentrations significantly decreased with the increasing severity of wasting (P=0.02, P=0.008 and P=0.0001, respectively). This trend did not reach significance among those in the 3rd tertile of plasma HIV load.

Discussion

The present study shows that micronutrient malnutrition and wasting are more severe in adults with pulmonary tuberculosis who have higher plasma HIV load. The association between high plasma HIV load and micronutrient deficiencies was strongest for the major plasma carotenoids and selenium. Overall in this study population, both HIV-positive and HIV-negative adults with pulmonary tuberculosis were extremely malnourished as indicated by BMI and plasma micronutrient concentrations. About one-third of the adults in this study had a BMI <17.0, a cut-off that is predictive of mortality in adults co-infected with tuberculosis and HIV.[29]

To our knowledge, this is the first study to demonstrate that selenium status is extremely poor among HIV-infected adults with pulmonary tuberculosis, and that the extent of selenium deficiency is associated with higher plasma HIV load. This observation may be of potential importance because selenium deficiency has been associated with increased mortality during HIV infection [30], and selenium supplementation for HIV-infected adults has been shown to reduce morbidity.[31] In the present study, selenium deficiency occurred in 87% of the participants, which, to our knowledge, may be the highest prevalence of selenium deficiency reported in an HIV-infected group of patients. It is unknown whether selenium supplementation will reduce morbidity and mortality among HIV-infected adults with pulmonary tuberculosis.

Carotenoids are among the most important dietary antioxidants found in human plasma, and this study shows that poor carotenoid status was associated with higher HIV load and with wasting. Plasma carotenoid concentrations are widely considered to be the most accurate indicator of dietary carotenoid intake.[32] It is not known whether adults with pulmonary tuberculosis and higher HIV load have lower plasma carotenoid concentrations because of increased oxidative stress, or whether these individuals are sicker and unable to consume enough carotenoid-rich foods. Further studies are needed in the future to address dietary intake of carotenoids in HIV-infected adults with pulmonary tuberculosis.

Low BMI is a known risk factor for mortality[5,29], and the present study showed that the risk of micronutrient deficiencies is highest in those with low BMI.

HIV-infected adults with wasting and high viral load were at the highest risk of more severe micronutrient malnutrition, suggesting that this subgroup might potentially benefit the greatest from nutritional interventions.

The cross sectional design of this study restricts our conclusions and does not provide information on whether poor nutritional status is a predictor of more severe pulmonary tuberculosis. It is unknown whether nutritional interventions will slow progression of disease or reduce wasting associated with morbidity and mortality if added to tuberculosis chemotherapy. Controlled clinical trials currently in progress in developing countries should help provide insight into the role of micronutrient supplementation for HIV-positive and HIV-negative adults with pulmonary tuberculosis.

The present study shows that micronutrient malnutrition and wasting are more severe in adults with pulmonary tuberculosis who have higher HIV load. The association between high plasma HIV load and nutrient deficiencies was strongest for the major plasma carotenoids and selenium. Further longitudinal investigations are needed to determine whether deficiencies in micronutrients are independent risk factors for increased morbidity and mortality.

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Chapter 5

Low Plasma Selenium Concentrations, High Plasma HIV Load And High Interleukin-6 Concentrations Are Risk Factors Associated With Anemia In Adults Presenting With Pulmonary Tuberculosis In Zomba District, Malawi

Monique van Lettow, Clive West, Jos van der Meer, Frank Wieringa and Richard Semba

Abstract

Although anemia is common among adults with pulmonary tuberculosis and human immunodeficiency virus (HIV) infection in sub-Saharan Africa, the factors contributing to its pathogenesis have not been well characterized. The purpose of this study was to characterize the antioxidant micronutrient status, IL-6 concentrations, and HIV load in relationship with anemia in adults with pulmonary tuberculosis. Erythropoietin, interleukin-6 (IL-6), plasma HIV load and markers of micronutrient status (hemoglobin (Hb), plasma concentrations of retinol, α-tocopherol, carotenoids, ferritin, zinc, and selenium) were measured in 500 adults who presented with pulmonary tuberculosis in Zomba Central Hospital, Malawi. Among 370 HIV-positive and 130 HIV-negative adults, the prevalence of anemia was 88% and 77%, respectively (P=0.002), and moderate to severe anemia (Hb <80 g/L) occurred in 30% and 15%, respectively (P=0.001). Geometric mean IL-6 concentration was 21.1 pg/mL, with no difference between HIV-positive and HIV-negative adults. The erythropoietin response to anemia was not different between adults with elevated IL-6 and those with lower IL-6 concentrations. In a multivariate logistic regression model, HIV load and lower plasma selenium concentrations were associated with moderate to severe anemia. In a final multivariate linear regression model, IL-6, plasma HIV load and plasma selenium concentrations were associated with hemoglobin concentrations. This study suggests that low selenium concentrations, high HIV load and high IL-6 concentrations are associated with anemia in adults with pulmonary tuberculosis in sub-Saharan Africa.

Introduction

Anemia is commonly associated with fatigue, poor quality of life and increased mortality, and also with human immunodeficiency virus (HIV) infection.[1,2] Anemia is highly prevalent among HIV-infected adults with pulmonary tuberculosis and can be relatively severe.[1,3-7] The pathogenesis of anemia associated with pulmonary tuberculosis has not been well characterized, especially in sub-Saharan Africa where there are the greatest number of cases of HIV infection and pulmonary tuberculosis.[8]

The etiology of anemia during tuberculosis and HIV infection is thought to be multifactorial. Although the anemia of infection, a subset of the anemia of chronic disease,[9] may account for a large part of the anemia found in pulmonary tuberculosis, iron deficiency,[1,10,11] poor antioxidant status,[1] and vitamin A deficiency [2,12] may potentially contribute to the anemia. A blunted response of erythropoietin to anemia may occur in the anemia of infection [9] and has been described in HIV-infected adults[13,14] and children[15]. Interleukin-6 (IL-6), a pro-inflammatory cytokine, is a marker for the severity of infection[16] and has been shown to modulate the anemia of chronic disease[17].

The purpose of this study was to characterize the antioxidant micronutrient status, IL-6 concentrations, and HIV load in relationship with anemia in adults with pulmonary tuberculosis. We hypothesized that poor antioxidant micronutrient status is associated with anemia in HIV-infected and uninfected adults with pulmonary tuberculosis and that the response of erythropoietin to anemia is blunted among those with high levels of inflammation, characterized by elevated plasma IL-6 concentrations. To test these hypotheses, we measured erythropoietin, IL-6, plasma HIV load and markers of micronutrient status, such as hemoglobin and plasma concentrations of retinol, α-tocopherol, carotenoids, ferritin, zinc, and selenium.

Methods

Study setting and population

The study population consisted of 500 adults who presented with new sputum-positive pulmonary tuberculosis in Zomba Central Hospital between July 1999 and September 2001. Subjects were offered HIV testing and were screened for HIV antibodies after signing a written informed consent form. All subjects were given appropriate pre- and post-test HIV counseling.

Subjects received standard short course chemotherapy for tuberculosis as per guidelines of the Malawi National Tuberculosis Program.[18] Adults with a previous history of treated pulmonary tuberculosis were excluded. Three sputum samples from each subject were examined with Auramine-O dark-fluorescent staining method. Sputum-positive pulmonary tuberculosis was considered proven when at least one out of three sputum stains showed acid-fast bacilli. HIV infection was diagnosed on the basis of a positive rapid test (Determine 1/2 Rapid test by Abbott, Abbott Laboratories, Johannesburg, SA) and confirmed by a positive enzyme-linked immunosorbent assay for HIV-1 antibodies (Wellcozyme; Wellcome Diagnostics, Dartford, Kent, UK).

Anthropometry and laboratory measurements

Body weight was determined to the nearest 0.1 kg using an adult balance (Seca 700 balance, Seca Corporation, Hanover, MD, USA), which was attuned systematically and standing height was determined to the nearest cm. Body mass index (BMI, wt/ht^2) <18.5 was considered consistent with wasting.[19]

Blood samples were obtained by venipuncture (Sarstedt Monovette, Newton, NC). Hemoglobin concentrations were measured using a hemoglobinometer (HemoCue Inc, Mission Viejo, CA, USA). Aliquots of plasma were made in trace element-free cryovials, and samples were stored in liquid nitrogen. Plasma samples were kept in liquid nitrogen or at −70º C until the time of laboratory analyses. Plasma HIV load was measured using quantitative HIV-1 RNA PCR (Roche Amplicor Monitor, version 1.5, Branchburg, NJ, USA) with a sensitivity limit of 400 HIV RNA copies mL. Plasma erythropoietin (ALPCO, Windham, NH), ferritin (Human Ferritin Enzyme Immunoassay Test Kit, ALPCO, Windham, NH), and IL-6 (Human IL-6, R & D Systems, Minneapolis, MN) concentrations were measured by ELISA.

Concentrations of α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein, zeaxanthin, retinol, and α-tocopherol were measured in 100 uL of plasma by high performance liquid chromatography using a modified method from the Nutrition Laboratory, Inorganic Toxicology and Nutrition Branch Division of Laboratory Sciences, National Center of Environmental Health, Centers of Disease Control and Prevention.[20,21] Quality control was assessed by repeated analysis of pooled human plasma controls run at the beginning and the end of each analysis. Standard curves were run periodically using standard reference material 986C (National Institute of Standards and Technology, Gaithersburg, MD).

Plasma trace element concentrations were measured using a Perkin Elmer model AAnalyst 600 atomic absorption spectrometer equipped with Zeeman background correction, a THGA graphite furnace, and an AS800 auto sampler (Perkin Elmer Corp., Norwalk, CT). For both selenium and zinc, two pooled human plasma controls, which were run at the beginning and end of each batch of samples, as well as "Seronorm" Trace Elements Serum (Accurate Chemical and Scientific Corp., Westbury, NY, USA), which was run periodically throughout the analysis, were used to determine within and between run CV. Throughout all analyses, the plasma samples were run in a masked fashion. Due to the unavailability of some sample aliquots, plasma erythropoietin, ferritin, IL-6, zinc, and HIV load could not be measured in 2, 1, 1, 1, and 16 samples respectively.

Statistical analysis

Anemia was defined as hemoglobin concentrations<120 g/L for females and <130 g/L for males, as per convention.[10] Moderate to severe anemia was defined as hemoglobin concentrations <80 g/L for both sexes according to the AIDS Clinical Trials Group classification.[22] Iron deficiency was defined as plasma ferritin concentrations <30 μg/L. We have chosen this cut-off based on earlier work in Malawi.[23] This higher cut-off was shown to be appropriate in an HIV infected population with elevated concentrations of acute phase proteins. Because there is no standard cut-off for elevated plasma IL-6 concentrations, we divided plasma IL-6 into tertiles and made comparisons of the highest two tertiles of IL-6 with the lowest tertile, where tertiles were defined as lowest (< 15.0 pg/mL), middle ($\geq$ 15.0 to < 40.0 pg/mL), and highest ($\geq$ 40.0 pg/mL). Vitamin A deficiency was defined as plasma retinol concentrations <0.70 μmol/L, vitamin E deficiency as plasma α-tocopherol concentrations <11.6 μmol/L, zinc deficiency as plasma zinc concentrations <10.71 μmol/L and selenium deficiency as plasma selenium concentrations <0.89 μmol/L.[24] Total plasma carotenoids were defined as the sum of α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein and zeaxanthin in μmol/L. Provitamin A carotenoids were defined as the sum of α-carotene, β-carotene, and β-cryptoxanthin. Non-provitamin A carotenoids were defined as the sum of lycopene, lutein and zeaxanthin.

Comparisons of categorical data were made using χ-square tests. Comparisons between continuous variables were made using univariate analysis of variance. One-way analysis of variance was applied to test for linearity. Appropriate variable transformations were made to reduce skewness of data, such as $\log_{10}$ transformation for concentrations of erythropoietin, IL-6,

retinol, total carotenoids, α-tocopherol, zinc, and HIV load. Geometric means were shown for skewed data were applicable.

A linear regression model was used to compare the relationship between plasma erythropoietin and hemoglobin concentrations among adults who were in the lowest and two highest tertiles of plasma IL-6, and among adults with and without HIV infection. Univariate and multivariate logistic regression models were fitted with moderate to severe anemia as the outcome variable. Linear regression models were used to validate the associations. A significance level of $P <0.05$ was used in this study. Statistical analyses were conducted using software packages SAS 8.01 (SAS Institute Cary, NC, USA) and SPSS 9.0 (SPSS, Inc., Chicago, IL, USA). The protocol was approved by the institutional review boards at the Johns Hopkins School of Medicine in Baltimore, MD and the College of Medicine, University of Malawi in Blantyre, Malawi, with final approval by the Office for Protection from Research Risk of the National Institutes of Health, Bethesda, MD.

Results

The study population consisted of 370 HIV-positive and 130 HIV-negative adults with sputum-positive pulmonary tuberculosis. Among the total study population, 69% (156/227) of male and 78% (214/273) of female participants were HIV-positive. The mean age among HIV-positive and HIV-negative individuals was 34 and 32, respectively (P=0.05). Mean hemoglobin concentrations among HIV-positive and HIV-negative individuals was 93.8 g/L and 106.6 g/L, respectively (P=0.0001). Among HIV-positive and HIV-negative adults, the prevalence of anemia was 88.4% and 76.9%, respectively (P=0.002). Moderate to severe anemia occurred in 30.0% of HIV-positive versus 14.6% of HIV-negative adults (P=0.001). The majority of subjects were wasted, as 56% of subjects had a BMI <18.5. Geometric mean plasma ferritin concentrations among HIV-positive and HIV-negative adults was 248.6 and 119.4 µg/L, respectively (P=0.001) Among HIV-positive and HIV-negative adults, iron deficiency, defined as plasma ferritin concentrations <30µg/L, occurred in 5% and 12%, respectively (P=0.009). Geometric mean IL-6 concentration was 21.1 pg/mL, with no difference between HIV-positive and HIV-negative subjects. Vitamin A, E, zinc, and selenium concentrations considered consistent with deficiency, occurred in 59%, 12%, 80% and 88% of all subjects, respectively, with no significant differences between HIV-positive and HIV-negative subjects. Characteristics, such as sex, mean age, BMI, plasma selenium concentrations, and geometric

mean plasma ferritin, erythropoietin, IL-6, micronutrient concentrations and HIV load of HIV-negative and HIV positive individuals are shown by degree of anemia in **Table 1** and **Table 2,** respectively. Among both HIV-negative and HIV positive individuals BMI, plasma retinol, provitamin A carotenoids, non provitamin A carotenoids, total carotenoids and selenium concentrations decreased by increasing degree of anemia. Plasma ferritin, erythropoietin, IL-6, and the proportion of individuals with vitamin A and selenium deficiency were increased with increasing degree of anemia. In addition, among HIV-positive individuals, educational level was increasingly lower, and the proportion of individuals with BMI<18.5 and plasma HIV load were increasingly higher with increasing degree of anemia.

The relationship between $\log_{10}$ plasma erythropoietin and hemoglobin concentrations among those in the highest two tertiles versus the lowest tertile of IL-6 was examined. The regression lines were $\log_{10}$ plasma erythropoietin = 2.18 – 0.07 * hemoglobin for those in the highest two tertiles of IL-6 and $\log_{10}$ plasma erythropoietin = 1.88 – 0.06 * hemoglobin for those in the lowest tertile of IL-6. The slopes of the regression lines between $\log_{10}$ plasma erythropoietin and hemoglobin among adults in the lowest tertile versus the higher two tertiles of IL-6 were not different (P= 0.49). The relationship between $\log_{10}$ erythropoietin and hemoglobin concentrations was also compared between HIV-positive and HIV-negative adults with pulmonary tuberculosis. The regression lines were $\log_{10}$ plasma erythropoietin = 2.22 – 0.08 * hemoglobin for HIV-positive adults and $\log_{10}$ plasma erythropoietin = 2.15 – 0.07 * hemoglobin for HIV-negative adults. The slopes of the regression line between $\log_{10}$ plasma erythropoietin and hemoglobin were not significantly different between HIV-positive and HIV-negative adults with pulmonary tuberculosis (P= 0.36).

Table 1. Characteristics of HIV negative adults with pulmonary tuberculosis in Zomba, Malawi, with and without anemia

Characteristic[1]	No anemia[2] (n = 30)	Mild to moderate anemia[3] (n = 81)	Moderate to severe anemia[4] (n = 19)	P-value
Sex (% Female)	33.3	49.4	47.4	0.25
Age (years)	32 ± 10	32 ± 12	30 ± 11	0.55
Primary education or higher (%)	46.7	34.6	47.4	0.84
Body mass index (wt/ht^2)	19.8 ± 2.7	18.5 ± 2.9	17.7 ± 2.6	0.009
Wasting, body mass index <18.5 (%)	36.7	60.5	57.9	0.08
Ferritin (µg/L)	69.9 (27.5,173.8)	141.12 (44.7,446.7)	136.58 (43.7,436.5)	0.05
Iron deficiency, ferritin <30 µg/L (%)	23.3	8.6	10.5	0.11
Erythropoietin (mU/mL)	18.5 (5.8,58.9)	24.5 (7.8,77.6)	71.2 (22.4,223.9)	0.003
IL-6 (pg/mL)	5.6 (0.9,35.5)	30.9 (12.3,77.6)	28.1 (11.2,70.8)	0.007
Retinol (µmol/L)	1.01 (0.58,1.76)	0.54 (0.32,0.92)	0.54 (0.29,1.00)	0.001
Vitamin A deficiency, retinol <0.70 µmol/L (%)	23.3	67.9	68.4	0.001
Provitamin A carotenoids (µmol/L)	0.36 (0.19,0.71)	0.26 (0.11,0.64)	0.22 (0.09,0.54)	0.02
Non-provitamin A carotenoids (µmol/L)	0.62 (0.42,0.92)	0.43 (0.26,0.69)	0.41 (0.22,0.79)	0.001
Total carotenoids (µmol/L)	1.02 (0.67,1.55)	0.72 (0.43,1.19)	0.65 (0.32,1.32)	0.002
α-Tocopherol (µmol/L)	16.0 (11.9,21.6)	15.0 (11.7,19.4)	17.9 (14.3,22.6)	0.35
Vitamin E deficiency, α-tocopherol <11.6 µmol/L (%)	13.3	14.8	5.2	0.51
Zinc (µmol/L)	9.8 (8.2,11.8)	8.4 (6.4,11.1)	8.9 (6.9,11.5)	0.12
Zinc deficiency, zinc <10.71 µmol/L (%)	77.5	73.7	76.9	0.72
Selenium (µmol/L)	0.729 ± 0.23	0.639 ± 0.25	0.554 ± 0.22	0.01
Selenium deficiency, selenium <0.89 µmol/L (%)	73.3	85.2	94.7	0.04

[1] Mean ±SD for continues variables with normal distribution, geometric mean (lower, upper SD) when distribution was not normal.

[2] Hemoglobin ≥120 g/L for females and ≥130 g/L for males.

[3] Hemoglobin <120 g/L for females and <130 g/L for males, and ≥ 80 g/L for both sexes.

[4] Hemoglobin < 80 g/L for both sexes.

Table 2. Characteristics of HIV positive adults with pulmonary tuberculosis in Zomba, Malawi, with and without anemia

Characteristic1	No anemia2 (n = 43)	Mild to moderate anemia3 (n = 216)	Moderate to severe anemia4 (n = 111)	P-value
Sex (% Female)	65.1	52.8	64.9	0.43
Age (years)	32 + 8	34 + 9	34 + 8	0.33
Primary education or higher (%)	55.8	48.6	34.2	0.005
Body mass index (wt/ht2)	19.9 + 3.1	18.5 + 2.8	17.6 + 2.2	0.001
Wasting, body mass index <18.5 (%)	39.5	54.2	69.4	0.001
Ferritin (μg/L)*	132.3 (41.7,416.9)	226.1 (70.8,708.0)	381.6 (151.4,955.0)	0.001
Iron deficiency, ferritin <30 μg/L (%)	9.3	6.0	2.7	0.08
Erythropoietin (mU/mL)*	18.9 (6.0,60.3)	20.1 (6.3,63.1)	55.6 (17.8,177.8)	0.001
IL-6 (mU/mL)*	11.4 (2.9,45.7)	19.1 (6.0,60.3)	33.3 (13.2,83.2)	0.001
Retinol (μmol/L)	0.71 (0.37,1.36)	0.64 (0.35,1.20)	0.47 (0.12,1.91)	0.001
Vitamin A deficiency, retinol <0.70 μmol/L (%)	46.5	53.2	75.7	0.001
Provitamin A carotenoids (μmol/L)	0.28(0.12,0.64)	0.24(0.11,0.54)	0.20(0.09,0.47)	0.02
Non-provitamin A carotenoids (μmol/L)	0.47 (0.27,0.82)	0.40 (0.24,0.69)	0.32 (0.19,0.55)	0.001
Total carotenoids (μmol/L)	0.78 (0.42,1.45)	0.68 (0.38,1.20)	0.55 (0.30,1.00)	0.002
α-Tocopherol (μmol/L)	15.9 (12.1,20.9)	16.0 (11.8,21.5)	16.8 (12.2,23.2)	0.20
Vitamin E deficiency, α-tocopherol <11.6 μmol/L (%)	4.6	11.6	11.1	0.23
Zinc (μmol/L)	9.8 (7.5,12.9)	8.5 (6.3,11.5)	8.5 (6.3,11.5)	0.09
Zinc deficiency, zinc <10.71 μmol/L (%)	83.0	77.5	81.3	0.22
Selenium (μmol/L)	0.724 + 0.22	0.633 + 0.20	0.513 + 0.20	0.001
Selenium deficiency, selenium <0.89 μmol/L (%)	79.1	90.3	94.6	0.006
Log10 HIV load (copies *103/mL)*	77.3 (9.8,616.6)	169.0 (44.7,7.7.9)	298.0 993.3,933.3)	0.001

1 Mean ±SD for continuous variables with normal distribution, geometric mean (lower, upper SD) when distribution was not normal

2 Hemoglobin >120 g/L for females and >130 g/L for males

3 Hemoglobin > 80 g/L to <120 g/L for females and > 80 g/L to <130 g/L for males

4 Hemoglobin < 80 g/L for both sexes

* Ferritin, Erythropoietin, IL-6, zinc and HIV load was not measured in 1,2,1,1 and 16 HIV-positive subjects, respectively.

Univariate and multivariate logistic regression models were used to examine the relationship between, age, sex, BMI, plasma ferritin, IL-6, micronutrient concentrations, and HIV load, with moderate to severe anemia (**Table 3**). In univariate models, sex, BMI, IL-6, retinol, total carotenoids, selenium, and HIV load were significantly associated with moderate to severe anemia. In multivariate logistic analysis, low plasma selenium concentrations and high HIV load were independently associated with moderate to severe anemia. In a final multivariate linear regression model that adjusted for BMI, micronutrient concentrations, sex and age; $\log_{10}$ IL-6 (P=0.03) and $\log_{10}$HIV load (P=0.001) were negatively associated with hemoglobin concentrations, whereas plasma selenium (P=0.0001) was positively associated with hemoglobin concentrations.

Table 3. Risk factors for moderate to severe anemia1 in adults with pulmonary tuberculosis with and without HIV co-infection.

Risk Factor	Univariate O.R. (95% C.I.)	*P*-value	Multivariate O.R. (95% C.I.)*	*P*-value
Male sex	0.65 (0.43-0.98)	0.04	0.63 (0.34-1.12)	0.12
Age (per year)	0.99 (0.98-1.02)	0.90	1.01 (0.98-1.04)	0.58
Body mass index (wt/ht^2)	0.85 (0.78-0.92)	0.001	1.02 (0.91-1.13)	0.76
$\log_{10}$ IL-6 (mU/mL)	3.02 (1.90-4.80)	0.001	1.97 (0.84-4.63)	0.12
$\log_{10}$ Retinol (μmol/L)	0.16 (0.07-0.34)	0.001	0.46 (0.12-1.84)	0.27
$\log_{10}$ Total carotenoids (μmol/L)	0.20 (0.09-0.43)	0.001	0.61 (0.18-2.07)	0.43
$\log_{10}$ α-Tocopherol (μmol/L)	6.83 (1.40-33.21)	0.02	7.02 (0.83-59.45)	0.07
$\log_{10}$ Zinc (μmol/L)	0.65 (0.13-3.24)	0.60	1.45 (0.19-10.81)	0.72
Selenium (μmol/L)	0.04 (0.01-0.13)	0.001	0.10 (0.03-0.41)	0.001
$\log_{10}$ HIV load (copies *10^3/mL)	2.83 (1.73-4.63)	0.001	2.05 (1.21-3.50)	0.008

[1] Hemoglobin < 80 g/L for both sexes.

* adjusted for all other variables in the model.

Discussion

This study suggests that there is an association between low selenium concentrations and anemia. The prevalence of anemia is extremely high among adults with pulmonary tuberculosis in Malawi, as 88% of HIV-positive adults and 77% of HIV-negative adults were anemic. In comparison, 60% of malnourished tuberculosis patients with unknown HIV status were anemic in Indonesia[5], and 71% of adults co-infected with tuberculosis and HIV were anemic in Uganda.[6] The relatively higher prevalence of anemia in Malawi is consistent with the observation that malnutrition and wasting are also higher in this study population compared with other populations of adults with pulmonary tuberculosis (van Lettow et al. 2004, submitted).

In this study we demonstrate an association between selenium deficiency and anemia in adults with pulmonary tuberculosis. Selenium is contained in glutathione peroxidase, an enzyme that plays a major role in protection against free radicals and oxidative stress.[25,26] Dietary selenium markedly increased activity of plasma glutathione peroxidase in rat erythrocytes. Accordingly, higher concentrations of selenium in rat erythrocytes were considered to be responsible for increased resistance against oxidative stress.[27]

Selenium deficiency may potentially contribute to anemia through increased oxidative stress, but further studies are needed to confirm the association between selenium deficiency and anemia in adults with pulmonary tuberculosis.

It was difficult in this study to assess iron deficiency using plasma ferritin in these subjects, as ferritin is a positive acute phase reactant.[28] Many subjects with pulmonary tuberculosis had evidence of an acute phase response, as reflected by elevated IL-6 concentrations. Thus, the prevalence of iron deficiency, as measured by plasma ferritin in this study, is probably greatly underestimated. Iron deficiency and iron deficiency anemia are known to be common among women in Malawi [23], and this is consistent with our observation in univariate analysis that women were at higher risk of anemia than men. A limitation of our study is that malaria parasitemia was not assessed in all subjects at enrollment. Malaria is endemic in this study population, but there was no justification from a clinical standpoint at the time of enrollment to perform malaria smears on all patients who were commencing therapy for pulmonary tuberculosis.

Although wasting, as shown by low BMI, was associated with moderate to severe anemia in univariate analyses, this relationship was no longer significant after adjusting for sex, age,

plasma HIV load, IL-6 and micronutrient concentrations. Similarly, antioxidant nutrients such as plasma carotenoids, retinol, and α-tocopherol concentrations were associated with moderate to severe anemia in univariate analyses, but these relationships were no longer significant after adjusting for BMI, sex, age, plasma HIV load, IL-6 and micronutrient concentrations. Inflammation, as reflected by IL-6 concentrations, was not associated with moderate to severe anemia in multivariate logistic analysis. However, in multivariate linear regression analysis that adjusted for sex, age, BMI, plasma HIV load, and micronutrient concentrations; IL-6 was associated with hemoglobin concentrations. We may therefore conclude that the anemia of infection does partly account for the anemia found in tuberculosis. There is evidence from Malawi that patients with tuberculosis may have asymptomatic bacteraemia.[29]

To our knowledge, this is the first study to characterize the erythropoietin response to anemia among HIV-positive adults with pulmonary tuberculosis. This study shows that the erythropoietin response to anemia was not different between adults with elevated IL-6 and those with lower IL-6 concentrations. One study from South Africa described a blunted response to erythropoietin among adults with pulmonary tuberculosis, when comparing with matched individual with uncomplicated iron deficiency.[30] The blunted response was explained by the inhibitory effects of inflammatory cytokines. In the present study all individuals had relatively high levels of inflammation, which could explain that we did not find any difference between adults with elevated IL-6 and those with lower but still relatively high IL-6 concentrations.

Similarly, there were no differences in the erythropoietin response to anemia between HIV-positive and HIV-negative adults with pulmonary tuberculosis. This is in contrast with previous studies that have shown a blunted erythropoietin response to anemia in HIV-positive compared with HIV-negative adults.[13,14] One possible explanation is that both HIV-positive and HIV-negative adults had high levels of inflammation at the time of commencement of tuberculosis chemotherapy in the present study, and that the inflammation associated with tuberculosis and HIV co-infection, rather than HIV seropositivity alone, is the main factor in suppressing the erythropoietin response to anemia.

Anemia might be a secondary effect of a chronic disease such as tuberculosis and its socio-economic implications that lead to inadequate dietary intake. It likely that iron and folate deficiencies contribute to the anemia in HIV and tuberculosis, and further studies are needed to gain insight into this issue.

Despite important advances in HIV therapies and treatment of associated infections, the prevalence of anemia does not appear to have changed greatly over the last several years.[31] Anemia is one of the most common but neglected problems associated with HIV infection[31], and the present study shows that the anemia can be especially severe with tuberculosis and HIV co-infection. High mortality is the most important challenge facing tuberculosis programs in sub-Saharan Africa and it is likely that anemia plays an important role in this event. This study shows that there is an association between high HIV load and anemia. Early recognition of HIV through voluntarily counselling and testing and ultimately highly active antiretroviral treatment are important ways towards addressing this problem. A recent study from Uganda, showed evidence that cotrimoxazole had stabilizing effects on HIV load and CD4-cell counts.[32] Daily cotrimoxazole prophylaxis was associated with reduced mortality and has been recommended as a basic component of Tuberculosis and HIV care throughout Africa.[32,33] Recombinant erythropoietin therapy has been shown to be effective in treating the anemia associated with HIV infection, however, the cost of the medication alone for a usual 12-week treatment can be U.S. $4,000-5,000, which is beyond the resources of many in developing countries. Other strategies, such as micronutrient supplementation, are inexpensive strategies that need further evaluation as possible approaches for the anemia associated with pulmonary tuberculosis and HIV infection.

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Chapter 6

Plasma Leptin Concentrations, Interleukin-6 And HIV Load
In Relation To Loss Of Appetite And Wasting
In Adults With Pulmonary Tuberculosis In Malawi

Monique van Lettow, Jos van der Meer, Clive West, Reinout van Crevel and Richard Semba

Abstract

Wasting is a prominent feature of tuberculosis and may be more severe among individuals with human immunodeficiency virus (HIV) co-infection. It is likely that several biological mechanisms, including the anorexia of infection are contributing to wasting. The purpose of this study was to determine whether leptin concentrations, in relation to the inflammatory cytokine response and level of HIV infection, are contributing to loss of appetite and wasting in adults with pulmonary tuberculosis and HIV infection. We characterized plasma leptin concentrations in relationship with self-reported loss of appetite, body mass index (BMI), fat mass (FM), interleukin-6 (IL-6) and HIV load in a cross-sectional study of 500 adults who presented with pulmonary tuberculosis in Zomba, Malawi. Plasma leptin concentrations, associated with FM, significantly decreased by increasing tertile of plasma HIV load (P=0.0001). Leptin concentrations were inversely associated with plasma IL-6 concentrations after adjusting for sex, age, FM and HIV load. Plasma leptin concentrations were neither associated with loss of appetite nor with wasting. Inflammation, reflected by increased IL-6 concentrations, was associated with loss of appetite (OR = 3.41; 95% confidence interval [CI], 1.91-6.09), when adjusted for sex, age, FM, leptin concentrations and HIV load. A high plasma HIV-load was associated with severe wasting, defined as BMI<16.0 (OR = 2.14; 95% CI, 1.09-4.19), when adjusted for sex, age, IL-6, FM and leptin concentrations.

Introduction

Tuberculosis has re-emerged with the acquired immune deficiency (AIDS) pandemic to become the world's leading cause of death from a single infectious agent, accounting for a quarter of the avoidable adult deaths in the developing world.[1,2] Wasting is a prominent feature of tuberculosis and is probably one of the determinants of disease severity and outcome. Co-infection with human immunodeficiency virus (HIV) and tuberculosis may worsen the wasting seen in tuberculosis or HIV infection alone.[3] It is likely that several biological mechanisms, including the anorexia of infection are contributing to wasting. Inflammatory cytokines are principal candidates as mediator of the metabolic changes resulting in tuberculosis-associated wasting. Inflammatory cytokines trigger the host's acute phase response (APR) and possibly also the anorexia during infections.[4] Several microbial products, as well as leptin and cytokines reduce food intake after parental administration, suggesting a role of these substances in the anorexia of infection.[4,5]

The fat-derived hormone leptin is best known as a key mediator of energy metabolism.[6] High leptin concentrations signal the presence of sufficient energy stores to the hypothalamus, which respond by reducing appetite and increasing energy expenditure. Leptin concentrations are proportional to fat mass and are reduced in starvation.[7] In addition, leptin is now also recognized as both a recipient and an effector of immune stimuli, belonging to the same class of cytokines as interleukin-6 (IL-6).[8] Animal studies have suggested that leptin mediates anorexia in chronic inflammatory states.[9] On these bases, leptin may be involved in the cross-regulation between nutritional status and the immune response in tuberculosis.

Leptin concentrations may be low, as a result of low body fat in tuberculosis, or high as a result of the host inflammatory response. High leptin concentrations in tuberculosis patients, could suppress appetite and food intake, which could be one of the contributing factors of weight loss and wasting. Low leptin concentrations may suppress immunity and worsen disease outcome. Two studies from Turkey suggested high leptin concentration in adults with tuberculosis[10,11], while one study from Indonesia found low leptin concentrations in adults with tuberculosis.[12]

The role of leptin and the inflammatory cytokine response in the anorexia and wasting of patients with tuberculosis and HIV infection has not been characterized. The purpose of this study was to characterize plasma leptin concentrations in HIV-infected and uninfected adults

with pulmonary tuberculosis, in relation to nutritional status, level of inflammation and level of HIV-infection. Moreover, we aimed to determine whether leptin concentrations, in relation to inflammation and level of HIV infection are contributing to loss of appetite and wasting.

To address these objectives, we characterized plasma leptin concentrations in relationship with self-reported loss of appetite, body mass index (BMI), fat mass (FM) derived from bioelectrical impedance analysis (BIA), plasma interleukin-6 (IL-6), and plasma HIV load in a cross-sectional study of 500 adults who presented with pulmonary tuberculosis in Zomba, Malawi.

Methods

The study population consisted of 500 adults who presented with new sputum-positive pulmonary tuberculosis in Zomba Central Hospital between July 1999 and September 2001. This cross-sectional sample was drawn from a micronutrient supplementation study. Subjects were offered HIV testing and were screened for HIV antibodies after written informed consent. All subjects were given appropriate pre- and post-test HIV counselling. Subjects received standard short course chemotherapy for tuberculosis as per guidelines of the Malawi National Tuberculosis Program.[13] Adults with a previous history of treated pulmonary tuberculosis were excluded. Three sputum samples from each subject were examined with Auramine-O dark-fluorescent staining method. Sputum-positive pulmonary tuberculosis was considered proven when at least one out of three sputum stains showed acid-fast bacilli. HIV infection was diagnosed on the basis of a positive rapid test (Determine 1/2 Rapid test by Abbott, Abbott Laboratories, Johannesburg, SA) and confirmed by a positive enzyme-linked immunosorbent assay for HIV-1 antibodies (Wellcozyme; Wellcome Diagnostics, Dartford, Kent, UK).

Body weight was determined to the nearest 0.1 kg using an adult balance (Seca 700 balance, Seca Corporation, Hanover, MD, USA), and standing height was determined to the nearest cm. Wasting was defined as BMI (wt/ht^2) <18.5, and severe wasting as BMI <16.0, conform the WHO strata for BMI grading for severity of malnutrition.[14]
Single-frequency BIA was performed at 50 kHz and 800 μA (RJL Systems, Inc., Detroit, MI, USA) with standard tetrapolar lead placement. BIA measurements were performed in triplicate for each subject. The reproducibility on repeated BIA measurements was >99%. To calculate FM and body cell mass (BCM), equations were used that were validated in a sample of adults

with and without HIV-infection.[15,16]. A standard questionnaire, with closed questions, was used to determine loss of weight and loss of appetite. Loss of appetite was considered positive when the subject gave loss of appetite as a reason for the observed loss of weight in the lost month.

Blood samples were obtained by venipuncture (Sarstedt Monovette, Newton, NC) at initial diagnosis of tuberculosis. Subjects were not asked about prior food intake. Aliquots of plasma were made in trace element-free cryovials, and samples were stored in liquid nitrogen. Plasma samples were kept in liquid nitrogen or at $-70º$ C until the time of laboratory analyses. Plasma HIV load was measured using quantitative HIV-1 RNA PCR (Roche Amplicor Monitor, version 1.5, Branchburg, NJ, USA) with a sensitivity limit of 400 HIV RNA copies mL.

Plasma leptin concentrations were measured by ELISA using R&D Systems,Inc., Human Leptin Quantikine Colorimetric Sandwich ELISA kit (Minneapolis, MN, USA). Plasma IL-6 concentrations were measured by ELISA. (Human IL-6, R & D Systems, Minneapolis, MN. Quality control was assessed by repeated analysis of pooled human plasma controls run at the beginning and the end of each analysis. Standard curves were run periodically using standard reference material 986C (National Institute of Standards and Technology, Gaithersburg, MD). Throughout all analyses, the plasma samples were run in a masked fashion. Due to the unavailability of some sample aliquots, plasma IL-6 and HIV load could not be measured in 1 and 16 samples respectively.

Comparisons of categorical data were made using χ-square tests. Comparisons between continuous variables were made using t-tests. Appropriate variable transformations were made to reduce the skewness of the data, such as log_{10} transformation for leptin, IL-6 and HIV load. Univariate analysis of variance was used to test for linear trends of plasma leptin concentrations across categories of plasma HIV load. Linear regression models were used to explore the relationships between plasma leptin concentrations and FM, IL-6 and HIV load. Univariate and multivariate logistic regression models were used to evaluate determinants of self-reported loss of appetite, and to evaluate associations with severe wasting.

A significance level of $P <0.05$ was used in this study. Statistical analyses were conducted using software packages SAS 8.01 (SAS Institute Cary, NC, USA) en SPSS 9.0 (SPSS, Inc., Chicago, IL, USA). The protocol was approved by the institutional review boards at the Johns Hopkins School of Medicine in Baltimore, MD and the College of Medicine, University of Malawi in Blantyre, Malawi, with final approval by the Office for Protection from Research Risk of the National Institutes of Health, Bethesda, MD.

Results

The study population consisted of 370 HIV-positive and 130 HIV-negative adults with sputum-positive pulmonary tuberculosis. Of all participants, 69% (156/227) of men and 78% (214/273) of women were HIV-positive. **Table 1** shows characteristics of study participants by sex and HIV status; such as age, body composition, IL-6, HIV load and plasma leptin concentrations.

Table 1. Characteristics of adults with and without human immunodeficiency virus (HIV) infection presenting with pulmonary tuberculosis in Zomba, Malawi

Characteristic[1]	Male		*P*-value	Female		*P*-value [2]
	HIV-negative n = 71	HIV-positive n = 156		HIV-negative n = 59	HIV-positive n = 214	
Age (years)	33.1 ± 11.6	36.0 ± 7.9	0.03	30.5 ± 11.2	32.2 ± 8.6	0.18
Loss of appetite (% reported)	22.5	39.7	0.01	28.8	41.1	0.24
Loss of weight (% reported)	74.6	81.4	0.30	81.4	86.9	0.24
Body mass index (wt/ht^2)	18.7 ± 3.1	18.5 ± 2.6	0.58	18.6 ± 2.7	18.3 ± 2.8	0.40
Wasting, body mass index <18.5 (%)	54.9	54.5	0.95	54.2	58.4	0.57
Wasting, body mass index <16.0 (%)	14.7	12.7	0.84	21.0	10.2	0.06
Body cell mass (% of weight)	41.0 ± 3.8	39.4 ± 3.1	0.001	33.6 ± 3.2	33.2 ± 3.2	0.39
Fat mass (% of weight)	7.2 ± 4.8	7.4 ± 5.2	0.81	19.9 ± 8.8	19.0 ± 8.7	0.49
Log_{10} IL-6 (pg/mL)	1.28 ± 0.6	1.32 ± 0.5	0.54	1.36 ± 0.5	1.33 ± 0.5	0.67
Log_{10} HIV load	__	5.32 ± 0.5		__	5.22 ± 0.6	
Log_{10} Leptin (pg/mL)	2.45 ± 0.5	2.33 ± 0.6	0.17	3.12 ± 0.6	3.02 ± 0.5	0.20

[1] Mean ±SD for continues variables; [2] *P*-values assessed by t-tests for continuous variables, χ-square tests for categorical data.

[3] Hemoglobin <120 g/L for females and <130 g/L for males

When comparing men and women (data not shown), men were older and had lower FM (P=0.0001), $\log_{10}$ leptin concentrations (P=0.0001) and higher BCM (P=0.0001) than women. There was no difference in BMI or the proportion of individuals with wasting between men and women. There was no difference in mean $\log_{10}$ IL-6 concentrations between men and women, and no difference in mean $\log_{10}$ HIV load between male and female HIV-positive adults.

Figures 1 shows plasma leptin concentrations with 95% C.I by sex and categories of plasma HIV load. This figure illustrates that plasma leptin concentrations were not different between HIV negative subjects and HIV positive subjects in the lowest tertile of HIV load. However, plasma leptin concentrations significantly decreased by increasing tertile of plasma HIV load.
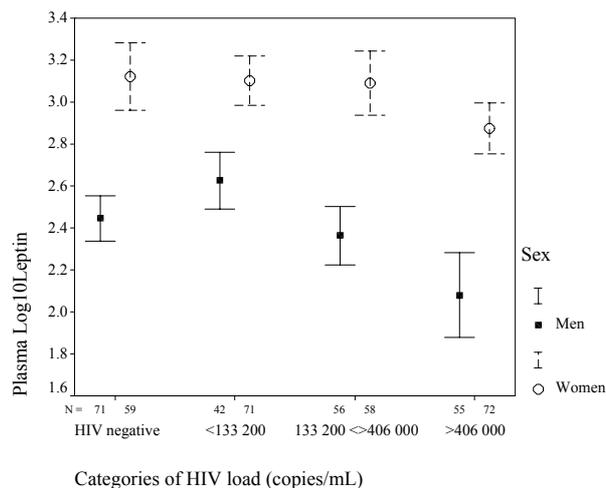
Fig 1. Log-transformed mean plasma leptin concentrations with 95% C.I. are depicted by sex and plasma HIV load.

Among all participants with pulmonary tuberculosis, men had significant lower $\log_{10}$ leptin concentrations than women. There was no significant difference in mean $\log_{10}$ leptin concentrations between HIV-negative individuals compared with HIV-positive individuals in the lowest tertile of HIV load. However, mean $\log_{10}$ leptin concentrations significantly decreased with the increase of plasma HIV load, in both men and women (P=0.0001 and P=0.01, respectively).

<p style="text-align:center">Table 2. Factors associated with plasma leptin concentrations</p>
<p style="text-align:center">in adults with pulmonary tuberculosis</p>

Variable	Regression coefficient, β	95% CI	*P*	Regression coefficient, β	95% CI	*P*
	Univariate[*]			Multivariate[*]		
Fat mass (% of weight)	5.00	4.57 – 5.43	0.0001	4.18	3.57 – 4.80	0.0001
Log_{10} IL-6 (pg/mL)	-0.34	-0.45 – -0.23	0.0001	-0.10	-0.18 – -0.01	0.02
Log_{10} HIV Load	-0.28	-0.39 – -0.17	0.0001	-0.08	-0.07 – 0.01	0.06

[*]Univariate and multivariate linear regression; adjusted for sex, age, and all other variables in the model.

The linear relationships between plasma leptin concentrations and FM, IL-6 and HIV load are shown in **Table 2**. In order to determine an independent association of FM, inflammation and HIV infection with leptin concentrations, we examined the relationships in multivariate linear models that adjusted sex, age and all variables in the model. In univariate linear regression analysis, FM (*P*=0.0001) was positively correlated with log_{10} leptin concentrations, while log_{10} IL-6 (*P*=0.0001) and log_{10} HIV load (*P*=0.0001) were inversely correlated with log_{10} leptin concentrations. In multivariate linear regression; that adjusted for sex, age and all other variables in the model, log_{10} leptin concentrations remained independently associated with FM (*P*=0.0001) and inversely with log_{10} IL-6 concentrations (*P*=0.02). The adjusted regression coefficient β for a linear association with log_{10} leptin concentrations was 4.18 (95% C.I.3.57 – 4.80) for FM and -0.10 (95% C.I.-0.18 – -0.01). for log_{10} IL-6 concentrations. The association between log_{10} HIV load with log_{10} leptin concentrations did not reach significance in multivariate analysis (*P*=0.06).

Figure 2 shows that plasma leptin concentration increases proportionally with percentage of body fat. In univariate analysis, the fitted regression lines for male and female were log_{10} leptin =1.86 +0.07 * fat mass and log_{10} leptin =2.26 +0.04 * fat mass, respectively, with a difference in the slope of the regression lines between log_{10} plasma leptin and fat mass among males and females (*P*= 0.001).
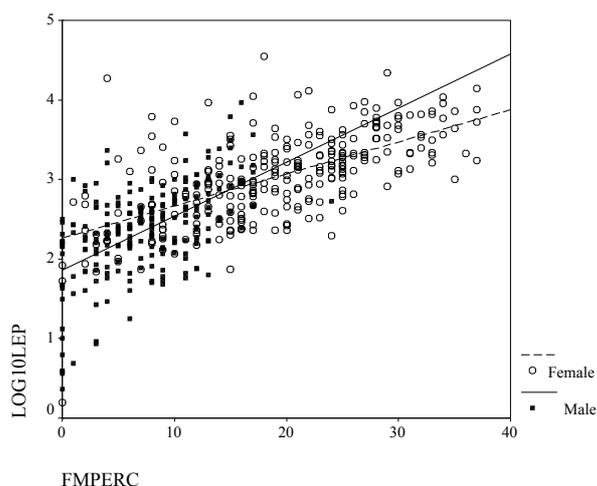
Fig 2. Relationship between $\log_{10}$ plasma leptin and percentage of body fat among males (squares, solid line) and females (circles, broken line) with pulmonary tuberculosis. The fitted regression lines were $\log_{10}$ leptin =1.86 +0.07 * fat mass and $\log_{10}$ leptin =2.26 +0.04 * fat mass, respectively, with a difference in the slope of the regression lines between $\log_{10}$ plasma leptin and fat mass among males and females ($P=$ 0.001)

Determinants of appetite and wasting

The relationships between self-reported loss of appetite and plasma leptin concentrations, IL-6 and HIV load are shown in **Table 3.** In order to determine which factors contribute to loss of appetite, we examined the relationships in multivariate logistic regression models that adjusted for sex, age, FM and all other variables in the model. This table shows that plasma leptin concentrations were not associated with loss of appetite. In univariate logistic regression analysis $\log_{10}$-IL-6 ($P=0.0001$) and $\log_{10}$ HIV load ($P=0.03$) were associated with loss of appetite. In multivariate logistic regression analysis only higher $\log_{10}$ IL-6 ($P=0.0001$) remained associated with loss of appetite. The adjusted O.R. for an independent association of $\log_{10}$ IL-6 with loss of appetite was 3.41 (95% C.I. 1.91-6.09).

Table 3. Factors associated with self-reported loss of appetite

in adults with pulmonary tuberculosis

Risk Factor	Univariate O.R. (95% C.I.)	P-value	Multivariate O.R. (95% C.I.)*	P-value
Log_{10} leptin (pg/mL)	0.82 (0.62-1.09)	0.17	1.06 (0.65-1.72)	0.81
Log_{10} IL-6	2.60 (1.73-3.91)	0.0001	3.41 (1.91-6.09)	0.0001
Log_{10} HIV load**	1.52 (1.04-2.23)	0.03	1.38 (0.90-2.12)	0.14

Univariate and multivariate logistic analysis; adjusted for sex, age, FM and all other variables in the model.

The relationships between severe wasting and plasma leptin concentrations, IL-6 and HIV load are shown in **Table 4.** In order to determine which factors contribute to severe wasting, we examined the relationships in multivariate logistic regression models with severe wasting, defined as BMI<16, as the outcome variable. In univariate analyses, log_{10} leptin (P=0.0001), log_{10} IL-6 (P=0.0001) and log_{10} HIV load (P=0.0006) were associated with severe wasting. In multivariate analysis that adjusted for sex, age, the interaction between leptin and FM, and all other variables in the model only higher HIV load (P=0.03) remained associated with severe wasting. The adjusted O.R. for an independent association of log_{10} HIV load with severe wasting was 2.14 (95% C.I. 1.09-4.19).

Table 4. Factors associated with severe wasting (BMI<16.0)

in adults with pulmonary tuberculosis

Risk Factor	Univariate O.R. (95% C.I.)	P-value	Multivariate O.R. (95% C.I.)*	P-value
Log_{10} leptin (pg/mL)	0.40 (0.28-0.59)	0.0001	1.00 (0.47-2.15)	0.99
Log_{10} IL-6	3.71 (2.09-6.59)	0.0001	2.03 (0.93-4.42)	0.07
Log_{10} HIV load**	3.03 (1.61-5.72)	0.0006	2.14 (1.09-4.19)	0.03

* Univariate and multivariate logistic analysis; adjusted for sex, age, the interaction between FM and leptin and all other variables in the model.

Discussion

This study demonstrates that plasma leptin concentrations are associated with FM and with the inflammatory cytokine (IL-6) response. Leptin reflected the percentage of FM and decreased with the increase of HIV load. Inflammation, characterized by IL-6 concentrations, was associated with loss of appetite, and level of HIV-replication, characterized by plasma HIV load, was associated with severe wasting. This study suggests that leptin does not account for the anorexia and weight loss in tuberculosis. The anorexia and wasting in patients with tuberculosis and HIV co-infection seem primarily determined by the level of inflammation and the level of HIV infection.

The role of leptin in tuberculosis has been limited to adults without tuberculosis and HIV co-infection.[10-12,17] In 30 patients with tuberculosis in Turkey, higher leptin concentrations were described among those with active tuberculosis compared with controls.[10] Similar results were reported in another study from Turkey involving 25 patients with tuberculosis.[11] One study from Indonesia, found lower leptin concentrations in 60 HIV-negative patients with active tuberculosis compared to 30 healthy controls.[12] And in effect, leptin concentrations in the untreated tuberculosis patients from Indonesia were similarly low as those in our study. In the present study, plasma leptin concentrations were not different between HIV negative subjects and HIV positive subjects in the lowest tertile of HIV load. However, plasma leptin concentrations significantly decreased by increasing tertile of plasma HIV load. The lower leptin concentrations in tuberculosis patients with high plasma HIV load may simply be attributed to further deprived nutritional status, as body fat is the most important determinant of plasma leptin concentrations. This is consistent with the observation that the nutritional status in patients with pulmonary tuberculosis in Malawi significantly decreases by increasing HIV load. (van Lettow et al. 2004, in print)

The study from Indonesia, reported a negative association between C-reactive protein (CRP) and leptin, while our study demonstrates a negative association between the inflammatory cytokine (IL-6) response and leptin concentrations in adults with pulmonary tuberculosis (with and without HIV infection). Experimental and animal studies have shown that inflammatory mediators are able to increase leptin production leptin.[4,10,18,19] However, the acute inflammatory response is different from the more chronic inflammatory response in tuberculosis. The results from this study support the hypothesis of Van Crevel and colleagues[12] that the prolonged inflammatory response in tuberculosis may deplete or "exhaust" leptin production.

Theoretically, decreased leptin concentrations could impair protective cellular immunity to *Mycobacterium tuberculosis*[12], as leptin is necessary for an effective cell-mediated immune response.[20] For instance, CD4 T lymphocyte activities are suboptimal in the absence of leptin.[7,20] The present study suggests that the inflammatory response may deplete leptin production, which theoretically suppresses immunity. Low leptin levels, as a result of poor nutritional status and chronic inflammatory response may be a contributing factor in suppressing the immune function and worsening the outcome of tuberculosis. Further studies are needed to clarify whether this hypothesis is true.

Conversely, in theory, low plasma leptin concentrations should increase appetite and decrease energy expenditure. However, the present study shows that leptin was not associated with loss of appetite, after adjusting for other factors, and this finding reinforces the conclusion of Schwenk and colleagues[17] that leptin does not account for the weight loss and anorexia in tuberculosis.

We demonstrated that leptin concentrations were not, but IL-6 was associated with loss of appetite. Experimental studies showed that parenteral administration of cytokines (including IL-6) reduce food intake, suggesting a role in the anorexia during infection.[4,5] Further research is needed into the role of cytokines in the physiological control of eating and energy balance during acute and chronic infection.

In conclusion, this study suggests that leptin does not play a role in the anorexia and wasting in tuberculosis, but that it may play a role as a mediator between nutritional status and host defense, which could explain thin people's susceptibility to tuberculosis or the link between malnutrition and disease outcome. In addition, IL-6 production was associated with loss of appetite. Current knowledge[4] on the different mechanisms involved in the anorexia of infection suggests some therapeutic options for treatment, including substances that antagonize cytokine action in combination with nutritional support.

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Chapter 7

Summary And General Discussion

Tuberculosis has emerged as the second commonest cause of death from infectious disease worldwide, after HIV/AIDS, killing nearly 2 million people each year. Most cases occur in less-developed countries. The human immunodeficiency virus (HIV) is the greatest single risk factor for the development of active tuberculosis in adults. Hence, over the past decade, tuberculosis incidence has increased in Africa, mainly as a result of the burden of HIV infection.[1]

The association between tuberculosis and wasting has long been recognized, and is probably one of the determinants of disease severity and outcome. Yet, little is known about the etiology of tuberculosis-associated wasting.

The nutritional status in relation to disease of patients with pulmonary tuberculosis with and without HIV co-infection, form the basis of this thesis. The studies described in this thesis were carried out in newly diagnosed adult pulmonary tuberculosis patients in Zomba Central Hospital, in the Southern region of Malawi.

In Chapter 1, a general introduction is given and an outline of the thesis. Tuberculosis is discussed as a communicable disease. Risk factors and magnitude of HIV co-infection are depicted, globally, in Africa and in particular in Malawi. The "triple trouble" of tuberculosis, HIV and malnutrition is introduced. This concept suggests that HIV and tuberculosis infection and malnutrition may put those affected at greater risk than those with any of the three conditions alone. Some information about Malawi is given and the study site is presented.

In Chapter 2, a review is presented on the current state of knowledge regarding the nutritional status of patients co-infected with tuberculosis and HIV. Changes in nutrition indicators during chemotherapy for tuberculosis are described, and the relationship between nutrition and clinical outcomes are examined. In brief, several cross-sectional studies suggest that patients with tuberculosis suffer from deficiencies of vitamin A, thiamin, vitamin B6, folate, and vitamin E. Some deficiencies are more prevalent among HIV-infected adults than in those without HIV infection. Other overall findings were that protein-energy malnutrition and anemia are common among adults with tuberculosis and HIV infection. Several anthropometrical studies showed that body mass index (BMI) is lower in adults with pulmonary tuberculosis than in healthy controls. Bioelectrical impedance analysis (BIA) has been used to examine the body composition in pulmonary tuberculosis patients, and overall, large differences in body

composition between HIV-positive and HIV-negative adults with pulmonary tuberculosis do not seem to be present. Several studies showed that nutritional indicators, such as anthropometric measurements, improve during chemotherapy for tuberculosis. In addition, hemoglobin concentrations appear to improve, while nutritional status appears to be an important determinant of clinical outcome during chemotherapy for tuberculosis. A number of studies have examined the role of nutritional supplements in tuberculosis and in HIV. The outcome of these studies are discussed.

In Chapter 3, a study is presented that dealt with the question whether there is a relationship between malnutrition and the severity of lung damage in tuberculosis. In this study we related the extent of malnutrition to the extent of lung disease. Lung disease in chest radiographs was graded according to a conventional classification system. We found that among 319 adults with pulmonary tuberculosis with or without HIV co-infection, BMI, fat mass, and phase angle (the angle between resistance and reactance, derived from BIA) were independently associated with increasing severity of lung disease, when adjusted for sex, age, and plasma HIV load. These results support the hypothesis that the severity of lung disease is associated with the extent of malnutrition in adults with pulmonary tuberculosis.

In Chapter 4, a study is presented that tested the hypothesis that micronutrient malnutrition is associated with wasting and higher plasma HIV load in adults with tuberculosis. In a study involving 801 adults with pulmonary tuberculosis, we found that BMI, plasma retinol, carotenoid and selenium concentrations significantly decreased by increasing tertile of plasma HIV load. There were no significant differences in plasma micronutrient concentrations between HIV-negative individuals and HIV-positive individuals with plasma HIV load $\leq$133 200 copies/mL. Plasma vitamin A concentrations <0.70 μmol/L occurred in 61%, and zinc and selenium deficiency occurred in 85% and 87% respectively. Wasting, defined as BMI <18.5, was present in 59% of study participants and was independently associated with a higher risk of low carotenoids, and vitamin A and selenium deficiency. Severe wasting, defined as BMI<16.0 showed the strongest associations with deficiencies in vitamin A, selenium and plasma carotenoids. These data demonstrate that wasting and higher HIV load in pulmonary tuberculosis are associated with micronutrient malnutrition.

In Chapter 5, a study is presented in which we assessed the antioxidant micronutrient status, IL-6 concentrations, and HIV load in relationship with anemia in adults with pulmonary tuberculosis. We found that among 370 HIV-positive and 130 HIV-negative adults with pulmonary tuberculosis, the prevalence of anemia was 88% and 77%, respectively. Moderate to severe anemia (hemoglobin <80 g/L) occurred in 30% and 15%, respectively. It was difficult to assess iron deficiency using plasma ferritin, as many subjects had evidence of an acute phase response, as reflected by elevated Interleukin-6 (IL-6) concentrations. Thus, the prevalence of iron deficiency in this study is probably greatly underestimated. In a multivariate logistic regression model, HIV load and lower plasma selenium concentrations were associated with moderate to severe anemia. In a final multivariate linear regression model, HIV load, selenium and IL-6 were associated with hemoglobin concentrations. The results of this study suggest that low selenium concentrations, high HIV load and high IL-6 concentrations are associated with anemia in adults with pulmonary tuberculosis.

In Chapter 6, a study is presented in which we investigated whether leptin concentrations, in relation to the inflammatory cytokine response and level of HIV infection, are contributing to loss of appetite and wasting in adults with pulmonary tuberculosis and HIV infection. We found that among 500 adults with pulmonary tuberculosis, plasma leptin concentrations were associated with fat mass and inversely with the inflammatory cytokine (IL-6) response. Leptin reflected the percentage of fat mass and decreased with the increase of plasma HIV load. Inflammation, reflected by increased IL-6 concentrations, was associated with loss of appetite, and the level of HIV infection, reflected by plasma HIV load, was associated with severe wasting. Leptin concentrations were neither associated with loss of appetite nor with wasting. This study suggests that leptin does not play a role in the anorexia and wasting in tuberculosis, but that it may play a role as a mediator between nutritional status and host defense, which could explain thin people's susceptibility to tuberculosis or the link between malnutrition and disease outcome.

In conclusion, both HIV-positive and HIV-negative adults with pulmonary tuberculosis were extremely malnourished as indicated by body composition and plasma micronutrient concentrations. A limitation of our findings is that we did not have a control group of healthy Malawian adults. However, one earlier study from Malawi showed that BMI was significantly

lower in adults with pulmonary tuberculosis than in healthy controls.[2]   And in effect, mean BMI in male and female tuberculosis patients were similarly low as those in our studies.

Nutritional status between HIV-negative and HIV-positive adults appeared similar. However, in a study involving 801 adults, we showed that nutritional status was similar between HIV-negative adults and HIV-positive adults who were in the lowest tertile of plasma HIV load; but that nutritional status significantly decrease by increasing HIV disease. Anemia, which may perhaps be seen as both an outcome and marker of malnutrition, was also especially severe in adults with tuberculosis and HIV co-infection. We can therefore conclude that co-infection with tuberculosis and HIV does exacerbate the malnutrition seen in tuberculosis infection alone.

We used BMI and indices derived from BIA measurements to illustrate the body composition of adults with pulmonary tuberculosis. One of the advantages of BIA over BMI alone is that BIA can differentiate the degree of body fat and body cell mass in individuals. However, BMI was still a sensitive indicator of malnutrition in our investigations and may be considered as an adequate indicator in resource-poor settings.

The association between high plasma HIV load and micronutrient deficiencies was strongest for selenium and the major plasma carotenoids. This observation may be of potential importance because selenium deficiency has been associated with increased mortality during HIV infection.[3] In our study population, selenium deficiency occurred in 90% of HIV-positive individuals, which may be the highest prevalence of selenium deficiency reported in HIV-infected adults. Our data also suggested that there is an association between selenium deficiency and anemia in adults with tuberculosis. To our knowledge, this is the first study to demonstrate an association between selenium and hemoglobin concentrations. Selenium deficiency may potentially contribute to anemia through increased oxidative stress, but further studies are needed to confirm the association. It is unknown whether selenium supplementation could reduce morbidity and mortality in adults with pulmonary tuberculosis.
We showed that poor carotenoid status is associated with both advanced HIV disease and with wasting. Carotenoids are among the most important dietary antioxidants found in human plasma. It is not known whether adults with pulmonary tuberculosis have low plasma carotenoid concentrations because of increased oxidative stress, or whether these individuals are merely unable to consume enough carotenoid-rich foods. Further studies are needed to address dietary intake of carotenoids in adults with pulmonary tuberculosis.
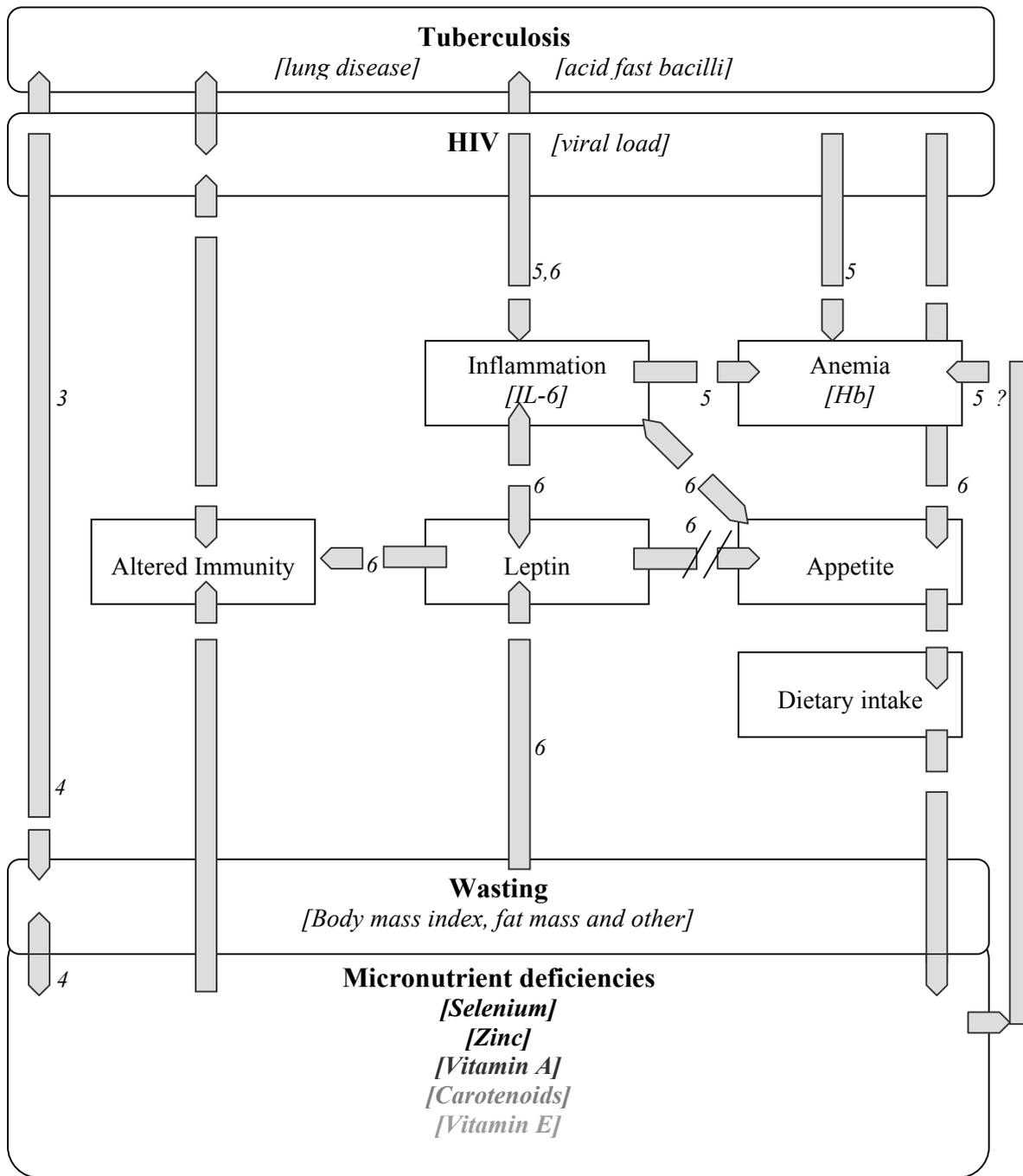
Fig 1. Associations that may contribute to malnutrition and disease (severity) in tuberculosis and HIV co-infection. (*n*) = Chapter in this thesis that describes the examined association.

Figure 1 shows the associations discussed in this thesis that may contribute to malnutrition and (severity of) disease in tuberculosis and HIV co-infection. We demonstrated that malnutrition in pulmonary tuberculosis is associated with the severity of lung disease; that micronutrient concentrations are related to wasting; that micronutrient malnutrition, anemia and wasting are more severe in adults with pulmonary tuberculosis who have higher HIV load and that low selenium concentrations, high HIV load and high IL-6 concentrations are associated with anemia in adults with pulmonary tuberculosis. We showed that plasma leptin concentrations are associated with the percentage of fat mass and with the inflammatory cytokine response. We demonstrated that leptin was not, but IL-6 was, associated with loss of appetite and concluded that the anorexia and wasting in patients with tuberculosis seem primarily determined by the level of inflammation and the level of HIV infection. Yet, further research is needed into the role of cytokines in the physiological control of eating and energy balance in pulmonary tuberculosis. We hypothesized that low leptin levels, as a result of poor nutritional status and chronic inflammatory response may be a contributing factor in suppressing the immune function and worsening the outcome of tuberculosis. Further research is needed to clarify the mechanisms as well as the effects of malnutrition, in relation to immunity and disease outcome of tuberculosis.

The outcome of our studies contribute to an increased understanding of the etiology of tuberculosis-associated wasting. However, the cross-sectional design of the studies presented in this thesis restrict our conclusions and do not provide information on associations between nutritional status and (re) activation of (latent) tuberculosis infection, or whether poor nutritional status is a predictor of more severe pulmonary tuberculosis. Succeeding longitudinal studies are needed to clarify such associations.

Considering that Malawi is one of the poorest countries in Sub-Saharan Africa, the nutritional status in this ill and impoverished patient group may be low merely because of low dietary intake. Therefore, the link between tuberculosis, HIV, malnutrition and poverty should not be underestimated. Malnutrition, attributable to poverty, may increase the risk or rate of progression of HIV and therefore indirectly predispose those with latent tuberculosis to active tuberculosis.[4,5]

In our study population, both HIV-positive and HIV-negative adults with pulmonary tuberculosis were extremely micronutrient deficient. Micronutrient deficiencies associated with wasting, depleted leptin production, immune suppression, anemia, and increased oxidative stress

may results in increased morbidity and mortality. It is unknown whether nutritional interventions will slow progression of disease or reduce malnutrition associated with morbidity and mortality. Adults with pulmonary tuberculosis and advanced HIV disease are at the highest risk of more severe micronutrient malnutrition, suggesting that this subgroup might potentially benefit the greatest from nutritional interventions. The controlled clinical trial currently in progress in our study population should help to provide insight into the role of micronutrient supplementation for adults with pulmonary tuberculosis.

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Nederlandse Samenvatting

Tuberculose, de infectieziekte die veroorzaakt wordt door Mycobacterium tuberculosis is, na AIDS, momenteel de belangrijkste doodsoorzaak door infectieziekten. Elk jaar sterven er wereldwijd bijna 2 miljoen mensen aan tuberculose, waarvan het overgrote deel in minder ontwikkelde landen. Het humane immunodeficiëntie virus (HIV), de verwekker van AIDS, vormt de grootste onafhankelijke risicofactor voor de ontwikkeling van actieve tuberculose bij volwassenen. Tuberculose komt de laatste decennia aanzienlijk meer voor in Afrika, hoofdzakelijk tengevolge van de HIV pandemie.[1]

De relatie tussen tuberculose en ondervoeding is sinds lange tijd bekend. Ondervoeding is vermoedelijk één van de bepalende factoren voor de ernst en het klinisch verloop van ziekte. In tegenstelling tot wat algemeen wordt aangenomen, is er nog weinig bekend over de oorzaak van de met tuberculose gepaard gaande ondervoeding.

De voedingstoestand van patiënten met longtuberculose, met en zonder HIV-infectie, vormt de basis van dit proefschrift. De onderzoeken die in dit proefschrift zijn beschreven werden uitgevoerd onder volwassen patiënten bij wie longtuberculose net was vastgesteld. Zij werden onderzocht in *Zomba Central Hospital*, in het zuidelijke deel van Malawi.

In Hoofdstuk 1 wordt een algemene inleiding en een overzicht van de achtergronden van dit proefschrift beschreven. Tuberculose wordt beschreven als besmettelijke ziekte. Risicofactoren en de omvang van tevens aanwezige HIV-infectie ("co-infectie") worden besproken, mondiaal, in Afrika, en in het bijzonder in Malawi. Het begrip "triple trouble" (ofwel het "driedubbel drama") van tuberculose, HIV en ondervoeding wordt uitgelegd. Dit begrip veronderstelt dat HIV én tuberculose én ondervoeding tezamen, grotere gezondheidsrisico's veroorzaken dan wanneer slechts één van de drie aanwezig is. Dit hoofdstuk geeft ook informatie over Malawi en de plaats waar de onderzoeken zijn uitgevoerd.

In Hoofdstuk 2 wordt een literatuuronderzoek gepresenteerd, waarin de actuele kennis over de voedingstoestand van patiënten met tuberculose en HIV-co-infectie beschreven wordt. Veranderingen in voedingstoestand tijdens de behandeling voor tuberculose en de samenhang tussen voedingstoestand en het ziekteverloop worden beschreven. Verschillende onderzoeken beschrijven dat patiënten met tuberculose, tekorten hebben in vitamine A, thiamine, vitamine B6, foliumzuur en vitamine E. Verscheidene van deze tekorten komen gemiddeld meer voor bij HIV-

geïnfecteerde tuberculosepatiënten dan bij tuberculosepatiënten zonder HIV-co-infectie. Andere bevindingen zijn dat eiwit- en energietekort, en bloedarmoede vaak voorkomen bij volwassenen met tuberculose en HIV-infectie. Metingen tonen dat de *body mass index* (*BMI*) (het gewicht in kilogram gedeeld door de lengte in het kwadraat) bij volwassenen met longtuberculose lager is dan bij gezonde volwassenen. Uit sommige onderzoeken die gebruik maakten van *bioelectrische impedantie analyse* (*BIA*) (een methode die de elektrische weerstand van het lichaam meet, waardoor vervolgens onder andere het lichaamsvetpercentage als onderdeel van de lichaams-samenstelling kan worden berekend), blijkt dat er geen grote verschillen in lichaams-samenstelling zijn tussen HIV-positieve en HIV-negatieve volwassenen met longtuberculose. Enkele onderzoeken tonen aan dat de voedingstoestand, afgemeten aan de *BMI*, verbeterden tijdens de behandeling van tuberculose. Ook de bloedarmoede lijkt te verbeteren. De voedingstoestand lijkt een belangrijke bijdrage te leveren aan het ziekteverloop tijdens de behandeling voor tuberculose. Een aantal onderzoekingen hebben de rol van aanvullende voeding in de vorm van micronutriënten (vitaminen en mineralen) supplementen bij tuberculose en HIV onderzocht. De bevindingen van deze onderzoeken worden besproken.

In Hoofdstuk 3 wordt een onderzoek naar de relatie tussen ondervoeding en longtuberculose gepresenteerd. We hebben onderzocht of er een verband bestaat tussen de ernst van ondervoeding en de ernst van longziekte. De ernst van longziekte werd bepaald met behulp van een klassieke methode voor het beoordelen van longfoto's. Wij hebben 319 volwassenen met longtuberculose met en zonder HIV-co-infectie onderzocht en aangetoond dat er een onafhankelijk verband bestaat tussen *BMI*, vetmassa en de z.g. *phase angle* (een maat afgeleid van de *BIA* methode) enerzijds, en de ernst van longziekte anderzijds. De analyses waren gecorrigeerd voor geslacht, leeftijd en de hoeveelheid HIV in het bloed. Deze resultaten steunen de veronderstelling dat de ernst van longtuberculose samenhangt met de ernst van ondervoeding bij volwassenen met longtuberculose.

In Hoofdstuk 4 wordt een onderzoek beschreven dat de veronderstelling toetst dat tekorten aan micronutriënten samenhangen met *wasting* (vermagering) en met meer actieve HIV-infectie bij volwassenen met tuberculose. We onderzochten 801 volwassenen met longtuberculose en vonden bij 59% van alle volwassenen een BMI van minder dan 18.5, ofwel *wasting*. We toonden aan dat *BMI,* en retinol-, caroteen- en seleniumconcentraties in het bloed beduidend afnamen met het toenemen van de hoeveelheid HIV in het bloed. Er waren geen

significante verschillen tussen HIV-negatieve en HIV-positieve volwassenen bij wie de hoeveelheid HIV in het bloed minder was dan 133200 virus kopieën per milliliter. Vitamine A tekort, gedefinieerd als retinol in het bloedplasma van minder dan 0.70 micromol per liter, werd bij 61% van alle volwassenen gevonden. Bij 85% werd een tekort aan zink en bij 87% van alle volwassenen werd een tekort aan selenium geconstateerd. We toonden aan dat er een verband is tussen *wasting* en een verhoogd risico voor tekorten aan caroteen, vitamine A and selenium. Deze verbanden bleken het sterkst voor patiënten met ernstige *wasting,* gedefinieerd als *BMI* van minder dan 16.0. De resultaten van dit onderzoek tonen aan dat er een verband is tussen *wasting,* meer actieve HIV-infectie en tekorten aan micronutriënten bij longtuberculose.

In Hoofdstuk 5 wordt een onderzoek gepresenteerd waarin is onderzocht of tekorten aan micronutriënten, ontstekingsreactie en de hoeveelheid HIV in het bloed bijdragen aan bloedarmoede bij volwassenen met longtuberculose. Bij 88% van de 370 HIV-positieve, en 77% van de 130 HIV-negatieve volwassenen met longtuberculose werd bloedarmoede geconstateerd. Matig-tot-ernstige bloedarmoede (hemoglobine minder dan 80 gram per liter) werd bij 30% van de HIV-positieven en bij 15% van de HIV-negatieven gevonden. De meerderheid van de volwassenen vertoonde verhoogde *Interleukin-6* (*IL-6*) concentraties, wat veronderstelt dat er sprake is van een ontstekingsreactie. Statische analyses toonden dat de hoeveelheid HIV in het bloed en verlaagde seleniumconcentraties in het bloed samen hangen met matig-tot-ernstige bloedarmoede. De hoeveelheid HIV-, selenium- en *IL-6* concentraties bleken samen te hangen met hemoglobineconcentraties. De resultaten van dit onderzoek tonen dat verlaagde seleniumconcentraties, verhoogde hoeveelheid HIV in het bloed en verhoogde *IL-6* concentraties gerelateerd zijn aan bloedarmoede bij volwassenen met longtuberculose.

In Hoofdstuk 6 wordt een onderzoek gepresenteerd naar de rol van leptine in verband met ondervoeding in tuberculose. Leptine, een hormoon dat door vetcellen wordt aangemaakt, reguleert de eetlust en ondersteunt in de afweer. Bij 500 volwassenen met longtuberculose, hingen lage leptineconcentraties samen met lage vetmassa, en met hoge *IL-6* concentraties. Verlies van eetlust bleek samen te hangen met verhoogde plasma *IL-6* concentraties. We zagen een verband tussen ernstige *wasting* en een verhoogde hoeveelheid HIV in het bloed. Hoewel leptine voornamelijk bekend is als eetlust regulerend hormoon, konden wij geen verband aantonen tussen leptineconcentraties en het verlies van eetlust of de mogelijk daarmee samengaande *wasting*. De resultaten van dit onderzoek veronderstellen dat de eetlust

vermindering veroorzaakt door infectie hoofdzakelijk verantwoordelijk is voor de ondervoeding bij tuberculose. Leptine zou mogelijk wel een rol kunnen spelen in de het verband tussen ondervoeding en afweer. Mogelijk draagt lage leptine concentraties bij tot een slechte afloop van het ziekteproces in tuberculose, maar verder onderzoek is nodig om deze veronderstelling te toetsen.

Concluderend, gebaseerd op lichaamssamenstelling en micronutriënten in het bloed, waren zowel HIV-positieve als HIV-negatieve volwassenen met longtuberculose ernstig ondervoed. Het is jammer dat wij geen groep van gezonde Malawiaanse volwassenen hadden om de voedingsstatus te vergelijken met die van onze patiëntengroep. Een eerder onderzoek uit Malawi toonde echter aan dat volwassenen met longtuberculose een beduidend lagere *BMI* hadden dan gezonde volwassenen.[2] Opmerkelijk is dat de gemiddelde *BMI* van de tuberculosepatiënten in dit eerdere onderzoek vergelijkbaar is met de gemiddelde *BMI* van de tuberculosepatiënten in onze onderzoeken.

De voedingstoestand van HIV-positieve volwassenen leek vergelijkbaar met de voedingstoestand van HIV-negatieve volwassenen. Echter, in een onderzoek onder 801 tuberculosepatiënten, toonden we aan dat de voedingstoestand van HIV-negatieve volwassenen vergelijkbaar was met de voedingstoestand van HIV-positieve volwassenen met een geringe hoeveelheid HIV in het bloed, maar dat de voedingstoestand beduidend afneemt met de toename van HIV infectie. Bloedarmoede (wat mogelijk zowel als gevolg, als als bewijs van ondervoeding gezien kan worden) kwam ook aanzienlijk meer voor bij volwassenen met tuberculose en HIV-co-infectie. We kunnen daaruit concluderen dat co-infectie met tuberculose en HIV de ondervoeding verergert, vergeleken met de ondervoeding bij tuberculose-infectie alleen.

In onze onderzoeken werd gebruik gemaakt van *BMI* en maten afgeleid van *BIA*-metingen, om de lichaamssamenstelling van volwassenen met longtuberculose te illustreren. Eén van de voordelen van de BIA-methode boven het bepalen van *BMI* alleen, is dat men met *BIA* een onderscheid kan maken tussen lichaamsvet en lichaamscelmassa bij individuen. In onze onderzoeken was *BMI* echter een goede maat voor ondervoeding, en kan wellicht beschouwd worden als een geschikte maat voor ondervoeding in situaties met minder beschikbare middelen.

Het verband tussen toegenomen hoeveelheid HIV in het bloed en tekort aan micronutriënten was het sterkst voor selenium en caroteen. Deze waarneming kan belangrijk zijn, gezien eerder onderzoek een verband aantoonde tussen seleniumtekort en verhoogde sterfte bij HIV-geinfecteerden.[3] In onze onderzoekspopulatie had 90% van de HIV-positieve tuberculosepatiënten een seleniumtekort. Dit is wellicht de hoogst gerapporteerde prevalentie van seleniumtekort bij HIV-geïnfecteerde volwassenen. Onze gegevens veronderstellen tevens dat er een relatie bestaat tussen seleniumtekort en bloedarmoede bij volwassenen met tuberculose. Naar ons weten, is dit het eerste onderzoek dat een verband heeft aangetoond tussen selenium- en hemoglobineconcentraties. Selenium heeft een anti-oxidatieve werking en seleniumtekort zou mogelijk kunnen bijdragen aan bloedarmoede door toegenomen oxidatieve processen, maar verder onderzoek is noodzakelijk om het verband tussen seleniumtekort en bloedarmoede te bevestigen. Het is nog onbekend of het verstrekken van voedingssupplementen met selenium het ziekteproces en sterfte bij volwassenen met longtuberculose zou kunnen beïnvloeden.

Wij hebben aangetoond dat er een verband is tussen verlaagde caroteenconcentraties in het bloed enerzijds en meer actieve HIV-infectie en *wasting* anderzijds. Caroteen is één van de belangrijkste voedings-gerelateerde antioxidanten die in het menselijke bloed gevonden worden. Het is niet bekend of patiënten met longtuberculose lage caroteenconcentraties hebben tengevolge van toegenomen oxidatieve processen of dat deze personen eenvoudigweg de mogelijkheid niet hebben om genoeg caroteenrijk voedsel te consumeren. Verder onderzoek is nodig met betrekking tot de hoeveelheid caroteen in de voeding van volwassenen met longtuberculose.

In het voorgaande hoofdstuk (de Engelstalige samenvatting) wordt een figuur gepresenteerd, dat de in dit proefschrift beschreven relaties die bijdragen aan ondervoeding en (omvang van) ziekte in tuberculose en HIV-co-infectie visualiseert. Samenvattend hebben onze onderzoeken aangetoond dat er een verband is tussen ondervoeding bij volwassenen met longtuberculose en de ernst van de longziekte; dat tekort aan micronutriënten in het bloed samenhangt met *wasting*; dat de mate van tekorten aan micronutriënten, bloedarmoede en *wasting* ernstiger zijn bij volwassenen met longtuberculose en tevens meer actieve HIV-infectie; en dat verlaagde seleniumconcentraties, verhoogde IL-6 concentraties en verhoogde hoeveelheid HIV in het bloed gerelateerd zijn aan bloedarmoede. We toonden aan dat er een verband bestaat tussen leptineconcentraties en de voedingstoestand en tussen leptineconcentraties en ontstekingsreactie in volwassenen met longtuberculose. Ons onderzoek wees dat er geen verband

was tussen verlies van eetlust en leptineconcentraties. Daarentegen, toonden we aan dat verhoogde plasma *IL-6* concentraties samen hingen met verlies van eetlust, en zagen we een verband tussen hoge hoeveelheid HIV in het bloed en ernstige *wasting*. We concludeerden hieruit dat de eetlust vermindering veroorzaakt door infectie hoofdzakelijk verantwoordelijk lijkt te zijn voor de ondervoeding bij tuberculose. Verder onderzoek is nodig om een beter inzicht te krijgen in de mechanismen die een rol spelen bij ondervoeding, evenals het effect van ondervoeding in relatie tot immuniteit en ziekteverloop bij tuberculose.

De resultaten uit onze onderzoeken dragen bij tot een beter begrip over de met tuberculose gepaard gaande ondervoeding. Echter, de *cross-sectionele* opzet van de onderzoeken die in dit proefschrift zijn beschreven, beperkt onze conclusies en geeft geen informatie over verbanden tussen voedingstoestand en (re) activering van (latente) tuberculose-infectie, noch of een slechte voedingstoestand voorspellend is voor een ernstiger ziekteproces. Verdere *longitudinale* onderzoeken zijn nodig om deze verbanden te onderzoeken.

Rekening houdend dat Malawi één van de armste landen in Afrika is, is de slechte voedingstoestand van deze zieke en arme patiëntenpopulatie mogelijk eveneens het gevolg van ontoereikend voedsel. De schakel tussen tuberculose, HIV, ondervoeding én armoede moet niet worden onderschat. Ondervoeding, als gevolg van armoede, kan het risico op (verergeren van) HIV-infectie vergroten, wat indirect de kans vergroot dat latente tuberculose actieve tuberculose wordt.[4,5]

In onze onderzoekspopulatie waren zowel HIV-positieve als HIV-negatieve volwassenen met longtuberculose ernstig ondervoed. Tekorten aan micronutriënten en *wasting,* lage leptineconcentraties, een verlaagde immuniteit, bloedarmoede en toegenomen *oxidatieve* processen kunnen een toename van ziekte en sterfte veroorzaken. Het is niet bekend of het geven van voedingssupplementen de voedingstoestand en het ziekteverloop van patiënten met tuberculose kunnen beïnvloeden. Volwassenen met longtuberculose en meer actieve HIV-infectie vormen de groep met het hoogste risico voor ernstige tekorten aan micronutriënten. Dit veronderstelt dat deze subgroep het meest baat zou kunnen hebben bij voedingsinterventies. Het placebo-gecontroleerd klinisch onderzoek, dat momenteel nog gaande is in onze patiëntenpopulatie, zal verder inzicht verschaffen over het effect van micronutriëntensupplementen op de voedingstoestand en het klinisch verloop van ziekte bij volwassenen met longtuberculose.

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Curriculum Vitae

Monique van Lettow werd op 6 december 1964 geboren in Rotterdam. In 1983 behaalde zij haar Havo-diploma aan de Albert Einstein Scholengemeenschap in Hoogvliet. In 1984 begon zij met haar opleiding fysiotherapie aan de Hogeschool Rotterdam. In 1988 behaalde zij haar Bachelor's degree. Zij werkte enkele maanden als assistent onderzoeker in het anatomisch laboratorium van het Erasmus Universitair Medisch Centrum in Rotterdam. Na een jaar als praktizerend fysiotherapeut in Rotterdam vertrok zij in januari 1990 als vrijwilliger naar Transkei om daar als fysiotherapeut in een orthopedisch centrum in Umtata te werken. Eind 1991 volgde zij de tropencursus voor paramedici bij het Koninklijk Instituut voor de Tropen in Amsterdam. Begin 1992 vertrok zij naar Malawi, uitgezonden door MEMISA, als fysiotherapeut en trainer voor Malawi Against Polio (een lokaal niet-gouvernementele organisatie). Zij assisteerde in het operationaliseren van een nationaal (*outreach based*) revalidatieprogramma, hulpverlenend aan Malawianen en Mozambikaanse vluchtelingen. Eind 1995 keerde zij terug naar Europa om in Engeland een 1-jarig master's programma in Public Health te volgen aan de Liverpool School of Tropical Medicine. Zij behaalde haar Master's degree in december 1996 en werd begin 1997 aangesteld als Public Health consultant voor Christoffel Blinden Mission (CBM). Zij evalueerde verscheidene door CBM gefinancierde projecten in Ghana en Ethiopië en assisteerde bij de rapportage aan de WHO alliance GET 2020 (Global Elimination of Trachoma by the year 2020). Vervolgens vertrok zij met haar partner naar Kameroen, waar zij meerdere korte contracten had als freelance Public Health consultant. In januari 1999 keerde zij terug naar Malawi om daar, aangesteld door de Johns Hopkins Universiteit, als researchmanager en senior research assistent van Richard Semba, een placebo-gecontroleerd klinisch onderzoek op te zetten onder tuberculosepatiënten in Zomba district. Zij was verantwoordelijk voor het algemeen management, het opzetten van een onderzoekscentrum, de aanstelling en training van het onderzoeksteam, het ontwikkelen van studieprotocollen, het operationaliseren van het onderzoek, datamanagement, evaluatie en rapportage. Na vier jaar van intensieve betrokkenheid in het onderzoeksproject koos zij voor een nieuwe uitdaging; het analyseren en publiceren van de tot dan toe verzamelde data. Daaruit is dit proefschrift voortgekomen.

Sinds februari 2004 woont zij in Overijse (België) tezamen met haar echtgenoot Jean Bourgeois en hun twee kinderen; Biko en Julie.