INVESTIGATION INTO PLASMA LEVELS OF SELENIUM AND GLUTATHIONE IN SMEAR-POSITIVE ADULT TUBERCULOSIS PATIENTS AND HEALTHY CONTROLS IN THE MANGOCHI DISTRICT, MALAWI

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She came to Gjøsegaarden (Sanatorium) in 1901. Dr. Holst Jonassen examined her, saying: “You have TB, so we have to feed you!” She stayed at Gjøsegaarden for some months and returned home as plump and round as a doughnut. She never experienced any relapses. At that time it was -- of course -- logical medical thinking to place the patients on a high calorie diet, based on the idea that they needed extra energy if and when the disease once again became active. Being plump and round like a doughnut was considered a sign that the patient was (and could very well remain) well and healthy.

Dag Skogheim
“Life at the Sanatorium”
Abstract

**Title**
Investigation into plasma levels of selenium and glutathione in smear-positive adult tuberculosis patients and healthy controls in the Mangochi district, Malawi.

**Student**
Heidi Arntsen

**Supervisors**
Gunnar Bjune, Asim Duttaroy, Kenneth Maleta

**Background**
Knowledge on the concentrations of selenium and glutathione in patients and controls are scarce. We hypothesized that tuberculosis patients had significantly lower levels of selenium and glutathione than those in healthy controls. Also, we wanted to find out whether low levels were due to low intake or high turn-over.

**Objectives**
The objective was to study selenium and glutathione levels in tuberculosis patients on treatment and in apparently healthy controls and their association to nutritional status and clinical presentation. We also wanted to compare the plasma levels of selenium and cysteine to dietary intake of selenium and cysteine respectively in these groups.

**Methodology**
A case control study was conducted where 19 cases and 15 controls participated. The patients were recruited from 2 hospitals in the Mangochi district. The controls were selected from the patients’ village, matched for age and sex. Due to low sample size, matching was not done in the statistical analysis. In addition to dietary interviews and blood tests, the participants’ height and body weight were measured.

**Results**
The cases had significantly lower plasma concentrations of selenium than the controls, but there were no significant differences in plasma concentrations of glutathione between the two groups. Body mass index was significantly lower in the case group compared with the control group. No relationship was found between Body mass index and selenium levels in both groups whereas an association was found between Body mass index and glutathione in the two groups. Regarding clinical presentation, most patients experienced cough and fever and all lost weight. Mean duration of cough was 7 weeks and more than 60% had a high number of bacilla in their sputum tests. Very weak association was found between selenium and glutathione and clinical presentation. No difference was found in socio-economic status between cases and controls. There was no difference in food intake of selenium and cysteine in the two groups. Lower levels of plasma selenium in TB patients can be connected to altered digestion absorption and/or increased oxidative stress rather than lower food intake.

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University of Oslo

**Presentation**
Part of this paper was presented at the annual meeting for public health nurses who are working with TB in Oslo and Akershus.
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### Abbreviations

<table>
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<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>AIDS</td>
<td>Acquired immune deficiency syndrome</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>CD4</td>
<td>Cluster of differentiation 4</td>
</tr>
<tr>
<td>CD8</td>
<td>Cluster of differentiation 8</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DOTS</td>
<td>Direct observed therapy short course</td>
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<tr>
<td>GSH</td>
<td>Glutathione</td>
</tr>
<tr>
<td>GSSG</td>
<td>Glutathione disulfide</td>
</tr>
<tr>
<td>γ-GCS</td>
<td>Gamma glutamylcysteine synthetase</td>
</tr>
<tr>
<td>HIV</td>
<td>Human immunodeficiency virus</td>
</tr>
<tr>
<td>ICP-MS</td>
<td>Inductively coupled plasma mass spectrometer</td>
</tr>
<tr>
<td>IFγ</td>
<td>Interferon gamma</td>
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<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>IUATLD</td>
<td>International Union Against Tuberculosis and Lung Disease</td>
</tr>
<tr>
<td>M</td>
<td>Molar</td>
</tr>
<tr>
<td>M.tb</td>
<td>Mycobacterium tuberculosis</td>
</tr>
<tr>
<td>MDR-TB</td>
<td>Multi-drug resistant tuberculosis</td>
</tr>
<tr>
<td>MHC</td>
<td>Major histocompatibility complex</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>NK</td>
<td>Natural killer</td>
</tr>
<tr>
<td>PEM</td>
<td>Protein-energy malnutrition</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>RNS</td>
<td>Reactive nitrogen species</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical package for social sciences</td>
</tr>
<tr>
<td>TB</td>
<td>Tuberculosis</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumor necrosis factor</td>
</tr>
<tr>
<td>Th</td>
<td>T helper</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>XDR-TB</td>
<td>Extensively drug-resistant tuberculosis</td>
</tr>
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Chapter 1: Background theory and problem statement

1.1 Purpose of the study

The purpose of the study was to identify whether tuberculosis (TB) patients are low in selenium and glutathione (GSH). Low levels would impair the immune system and consequently have an impact on the outcome of disease and treatment. To this end, we wanted to compare their plasma levels with the intakes of these two nutrients in patients and apparently healthy controls. This could tell us whether low levels were due to low intake or high turn-over.

Our findings may provide a basis for future prospective studies where supplementary interventions can be carried out. Whether supplementation will improve TB outcome or has a preventive effect, can only be definitively concluded through such studies.

1.2 Hypotheses

H0: There is no significant difference in the mean values for blood selenium and GSH levels between the two groups.

H1: There is a significant difference in the mean values for selenium and GSH between the two groups.

1.3 Problem statement

Low plasma levels of selenium and GSH will compromise the immune system and prolong the recuperation process in TB patients. Both protein-energy malnutrition (PEM) and deficiencies in micronutrients are associated with a significant impairment of various interactions and functions of the cell-mediated immune system(1). *Mycobacterium tuberculosis* (*M. tb*) is a classic example of a pathogen where the protective response relies on the cellular immune response(2). A study in Malawi showed that selenium deficiency occurred in 87% in TB patients (3). A study in Ethiopia on antioxidants status in untreated TB patients, showed that these patients were deficient in GSH compared to healthy controls(4).

1.4 Rationale

In the mid-twentieth century, it was widely believed that advances in antituberculous chemotherapy and radiographic diagnosis might result in eradication of TB; this hope has not been realized(5). The burden of TB continues to rise globally, mainly as a result of the HIV epidemic(6). Malawi has one of the highest levels of HIV-infections in the world and TB
notification rates have risen accordingly(7). There is a mutual interaction between TB and HIV; the immunosuppression induced by HIV modifies the clinical presentation of TB and TB influences the progression of HIV infection(8).

In the past it was believed that good nutrition was one way to treat TB patients(5). After the introduction of chemotherapy the nutritional aspect seems to be forgotten. With the emergence of multi drug-resistance tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB), new patterns of disease may arise and chronic infection may become more common. Hence research into nutritional aspects in TB is highly needed(9). Malnutrition is a serious problem among children in Malawi (10) and a food crisis threatened half the population in the autumn of 2005(11). More than half of the TB patients participating in a cross-sectional study in 2004 were malnourished(12).

Selenium is an essential trace element involved in several metabolic activities. Adequate levels are necessary to protect against oxidative damage and to regulate immune functions. (13), both in the innate and acquired immune system(14). The tripeptide thiol GSH is one of the most important antioxidants in cells(15). GSH regulates a number of immune cell functions as well as being important for the regulation of the cellular immune system(16).

In Malawi, there exists information on the levels of selenium in TB patients, and HIV-positive patients had lower levels of selenium than did HIV-negative patients(3,17). In Ethiopia, TB patients were found to have lower levels of GSH than did healthy Ethiopians(4) Information on selenium status in healthy controls in Malawi seems to be lacking as well as information on the antioxidant GSH both in TB patients and in controls. Also, studies on whether low plasma levels of selenium and GSH are due to low intake or altered digestion absorption and/or increased oxidative stress, seem to be lacking. Information regarding the plasma levels of GSH and selenium in inhabitants of Malawi may create the basis for further intervention studies and nutritional advice.

TB is associated with poverty. Of the 2 million annual deaths from TB, 98% occur in developing countries. Poverty leads to vulnerability to TB infection (18) and it is also a predisposing factor to poor nutritional status and impaired immune function.(9) In Malawi, nearly half of the population is living below the poverty line(19).

1.5 Objectives of the study

1.5.1 Main objective
To study selenium and GSH status in TB patients on treatment and in apparently healthy controls.

1.5.2 Specific objectives
A) To determine and compare plasma levels of selenium and GSH in TB patients and controls.
B) To describe nutritional status in both groups and relate the BMI status to selenium and GSH
C) To describe clinical signs of TB and their association to selenium and GSH status
D) To describe socio-economic status in both groups.
*E) To compare the plasma levels of selenium and cysteine with the dietary intake of selenium and cysteine respectively in the two groups.

*The study has been cooperation between Frode Eick (termed co-investigator) and the investigator and is comprised of three parts; collection of food items, dietary interviews and blood sampling and analysis. Frode Eicks’ thesis deals with food intakes of selenium and sulphur amino acids and the figures used in this context are from his work. Collection of food items has been solely the task of Frode Eick. The dietary interviews have been a dual task whereas blood sampling has been solely the task of the investigator.
Chapter 2

2.1 Country profile

2.1.1 Geography
Malawi is a landlocked country south of the equator in sub-Saharan Africa. It is bordered to the north and northeast by the United Republic of Tanzania; to the east, south, and southwest by the People’s Republic of Mozambique; and to the west and northwest by the Republic of Zambia.

The country has a total area of 118,484 square kilometres of which 94,276 square kilometres is land area. Lake Malawi, which is about 475 kilometres long, composes most of the remaining area and runs down Malawi’s eastern boundary with Mozambique. To the west and south of the lake lie fertile plains. The Shire River drains the water from the lake into the Zambezi River in Mozambique.

Malawi has a tropical climate with moderate rainfall, and the temperature varies depending on altitude and proximity to the lake. From May to August, the weather is cool and dry. The hot season lasts from September to November, while the rainy season begins in October or November and continues until April(20).

Fig. 1 Map of Malawi
2.1.2 History
Malawi became independent in 1964 after being under British rule since 1891. During colonial times the country was known as Nyasaland Protectorate. From 1953 until 1964 Malawi was part of the Federation of Rhodesia and Nyasaland which consisted of today’s Zimbabwe, Zambia and Malawi(20). The country became independent in 1964 with Dr. Banda as appointed Prime Minister. Dr. Banda instituted an authoritarian one-party state and became president for life in 1971. Growing protests against the government repression regime resulted in a referendum in 1993 where the majority chose multi-party democracy.(21) A multiparty system and a strategy to eradicate poverty were adopted in 1994. The country has introduced free primary school education, a free market economy, a bill of rights, and a parliament with three main parties. Migration from rural to urban areas has increased over the past ten years (20). Today, Dr. Mutharika, an economist, is the country’s president, having been elected in 2004(21).

2.1.3 Population and demographic characteristics
Malawi has experienced rapid population growth over the last years. The population grew from 8.0 million people in 1987 to 11.2 million people in 2002. This represents a population growth rate of 3.3 % per year(20). The Second Malawi Integrated Household Survey conducted between 2004 and 2005, revealed that 88 % live in rural areas whereas 12 % live in urban areas(22).

The population is unevenly distributed. In 1998 47 % lived in the Southern Region, 41 % lived in the Central Region and 12 % lived in the Northern Region(23).

Relatively speaking, Malawi has a larger population in the younger age groups than in the older age groups; in 2004 46.2% were <15, 45.4 % were between 15 and 59 years of age and 5.5 % were 60 years of age and above(22).

2.1.4 Administration
The country is divided into three regions; the Northern, Central, and the Southern Regions. There are 28 districts in the country. Six districts are in the Northern Region, nine are in the Central Region and 13 are in the Southern Region. For administrative purposes, the districts are subdivided into traditional authorities which are presided over by chiefs. The villages are the smallest administrative unit within the TA and are presided over by village headmen(20).

2.1.5 Economy
Malawi is classified as a low-income country and ranks amongst the poorest in Africa(24). Agriculture forms the backbone of the economy and agricultural produce accounted for 70 percent of the country’s exports in 2004. The major export commodities are tobacco, tea, and sugar(20). The country has no mineral wealth, and there is limited fishing and industry(25). Malawi has a gross national per capita income of around 170 US$. The World Health Organization (WHO) states that the population living below the poverty line is 41.7%. To live below the poverty line is defined as living on less than 1 dollar a day(19).

2.1.6 Poverty and literacy levels
The Southern Region has the largest poverty rate (60%) which means that three out of five people live in poverty. The Central Region has the lowest proportion (44%). Female-headed households tend to have higher poverty rates than male-headed ones, and education is also found to be highly correlated with poverty; poverty is more severe in households whose head have no formal education(26).
The health indicators reflect the depth and the severity of poverty in the country. A study published in 2000 showed that socio-economic support for good health in the Lungwena area (which is part of our study district, Mangochi) was insufficient to meet local needs(27).

The overall literacy rate in the country is 64.1% (2000-2004)(19). In the Lungwena area, 14% of the women and 43% of their husbands were found to be literate. Half of the household had no literate members. Of the 795 women who participated in the study, 83% of the women and 63% of their husbands had no schooling. Only 1% of the women and 4% of the men had secondary school education(27)

2.2 Overview of health issues in Malawi

2.2.1 General health indicators
Health indicators for Malawi have generally reflected its level of poverty. The life expectancy has (in the past few years) declined to 39 years, mainly as a result of the HIV/AIDS epidemic(28).

The under-five mortality rate was 133 per 1000 live births in 2004. This figure represents a decline by 30 percent over the last 15 years as the rate was 190 per 1000 in 1989(29). The maternal mortality rate was 984 per 100 000 in 2004 compared to 1120 per 100 000 in 2000 which represents a decline of 12.1%(30). The total fertility rate is 6 children per woman(28).

2.2.2 Tuberculosis and HIV indicators
The incidence of TB was estimated by WHO to be 413 per 100 000 in 2004, and prevalence 501 per 100 000. TB mortality rate was 97 per 100 000. The estimate of new TB cases that were multi drug-resistant was 1.7%. The trend in incidence is falling by 1.1% yearly. Prevalence of HIV in adult TB patients was 52%(31) and could be as high as 66% for males and 77% for females(3). National TB coordinator in Malawi, Felix Salaniponi, estimates that close to 70% of TB patients in the country are HIV-positive (no official source).

Malawi has one of the highest levels of HIV infections in the general population in the world and the prevalence is currently 15%(32). AIDS is now the leading cause of death among adults. HIV has had a great impact upon the emergence of TB cases; between 1985 and 2003 the TB case notification rose by 500%(7).

2.2.3 Health care services
Health care service providers in the country can be divided into the traditional and modern sectors. These sectors are used simultaneously or consecutively by many people, and they complement each other. Traditional healers and traditional birth attendants represent the two main categories of traditional providers. Within the modern health sector, there are three main providers: the public sector, non-profit private sector and the private for-profit sector. The Ministry of Health is the largest provider of public health services(33).

Of the 617 health facilities registered in 2003, 60% of them were operated by the Ministry of Health(28). The non-profit private sector is grouped under the Christian Health Association of Malawi (33) and operates 25% of the reported facilities. The user fees charged by the Ministry of Health of Malawi were a serious threat to equity amongst the rural population and the signing of the Memorandum of Understanding between Christian Health Association of
Malawi and the Ministry of Health was an important step towards improved access to health services. The agreement ensures access to health care regardless of payment ability(28). The private-for-profit sector represents the remaining 15% of the health facilities and is a rapidly growing business. The health delivery system consists of maternity units, dispensaries, health centres, district and central hospitals which are linked through a referral system. At the grassroots level community services are delivered by a network of community-based units consisting of Health Surveillance Assistants and other community volunteers(33).

2.2.4 Health care services and policies for treatment of tuberculosis
There are 44 non-private hospitals that register and treat TB patients; including four central hospitals and 22 district hospitals. These hospitals are supported by the government. In addition there are 18 mission hospitals. (34) Very few patients are treated in the private for-profit sector. (35) For the last six weeks of the initial phase of treatment patients get their medication from health centres or guardians. (36) Sputum smear examination is the cornerstone of diagnosis of pulmonary TB and also for the follow-up of the treatment. In new smear-positive patients, sputum smears are examined at the end of the initial phase of treatment (2 months), and on one or two occasions during the continuation phase. (34) The results of the microscopy are reported according to the International Union Against Tuberculosis and Lung Disease (IUATLD) grading scheme(37;38).

Since 1984, Malawi has implemented a directly observed treatment, short course, (DOTS) in its TB programme(39). From the mid-2001 the country has practiced a treatment regimen consisting of 2 weeks of in-hospital treatment with daily DOTS. Patients are then given the option of staying in the hospital or going home. If fit enough, they go home receiving their treatment from the TB-ward as an out-patient, from a health centre or from a guardian. For the last 6 weeks of the initial phase, they receive supervised treatment three times weekly. In the continuation phase, which consists of 6 months, the treatment is given daily, and is unsupervised(36;37). Drugs, consultations and diagnostic tests are provided free of charge by the public health sector(18).

2.2.5 Human resources in health services
There is a human resource crisis in the health sector in Malawi. Since the late 1990s declining human resource levels have fuelled a collapse of the public health services. The country struggles to keep pace with the demand for services; especially because of high population growth and high incidence of HIV/AIDS. In 2004, Malawi had 1.1 physician per 100,000 population and 25.5 nurses per 100,000 population. The government has, together with donors, responded to the crisis by developing an Emergency Human Resources Programme. The Programme will (by various measures) help the country to deliver required health services to the population. The government has also launched the Essential Health Package in an effort to improve health outcomes(24). Prevention and treatment of TB and related complications are parts of this health initiative(40).

Regarding resources for TB control activities in the Southern Region (where our study district is) the number of registered TB cases per year per one full-time hospital TB officer was 522 in 2003. The report concludes that this region has TB-specific human resource gaps which will result in a poorer quality of TB control compared to the other regions which are better staffed(35).
2.3 Overview of nutrition issues in Malawi

2.3.1 Food situation
Food insecurity is a serious threat to the country’s population. Agriculture, the backbone of the economy, is susceptible to droughts; and lack of rain has, therefore, a huge impact upon agricultural production. Hence inadequate rainfall as well as insufficient access to agricultural inputs during the 2004-2005 season led to the worst critical food crisis since 1994. The high prevalence of HIV/AIDS and chronic poverty adds to the problem. In 2005 the production of maize, the county’s most important staple food, decreased with 29% compared to the already-experienced poor harvest of 2004. The country was only able to produce 55% of the maize needed for national consumption(41;42). In a study from the Lungwena area in the Mangochi district, more than 25% of the 795 households that participated had no means of securing enough food for all family members.(27)

2.3.2 Nutritional status
The nutritional status of women in Malawi has remained constant since 2000. The mean height is 156 cm and the mean Body Mass Index (BMI) is 22 kg/m\(^2\). Seventy-seven percent of the women were classified as normal whereas 9% had a BMI below 18.5 kg/m\(^2\). Regarding children under the age of five, 48% are stunted; too short for their age, 5% are wasted or too thin and 22% are underweight. All figures are from 2004. (43)

A study in 2004 comprising 236 HIV-positive and 83 HIV-negative pulmonary TB patients showed that the majority were malnourished with a BMI under 19 kg/m\(^2\). There were no significant differences found in weight and mean BMI between HIV-positive and HIV-negative persons(12).

2.4 Mangochi District - The Study Area
Mangochi is a district in the Southern Region, on the tip of Lake Malawi. The total area is 6,273 square km and the estimated population in 2007 is 693,496. Mangochi Township is the biggest town in the district with a population of 44,720 followed by Monkey Bay having a population of 12,513(44). Mangochi district consists of nine Traditional Authorities.
Mangochi District Hospital is situated in Mangochi Township and we recruited most of our patients from this hospital. The other hospital, St. Martin’s Hospital was 20 minutes drive away, along the east coast of Lake Malawi. In addition to the hospital, the infrastructure in Mangochi Township consists of a town hall, district administration offices, banks, museum, shops, a few restaurants and internet-cafes. The rural areas have no electricity or infrastructure as found in Mangochi Township. The central roads are tarmac roads and the rest are dirt roads which can be inaccessible during heavy rain falls. The Shire River runs on the outskirts of the town and much used for fishing and bathing. The district has holiday resorts and lodges along the lakeside. The ship Ilala travels from Monkey Bay, and in addition to being an essential lifeline service to the communities it is also a cruise liner for tourists.
Chapter 3: Literature review

3.1 The natural history of tuberculosis

TB is spread by airborne droplet nuclei, which are small particles containing M. tb. Because of their small size, the particles can remain airborne for minutes to hours after expectoration by people with pulmonary or laryngeal tuberculosis during coughing, sneezing, singing or talking(45). These droplets lodge in the alveoli or terminal air passages of the lung and establish a local focus of disease called the Ghon focus. Bacilli are transported to the lymph nodes at the hilum of the lung, where additional foci of disease develop.

The Ghon focus, together with the hilar lymphadenopathy, is termed the primary complex. The bacilli disseminate by the lymphatic and the bloodstream and lodge in different organs in the body. TB is hence a systemic infection.

Primary infection leads to the development of tuberculin positivity 3-8 weeks after infection. In about 95% of infected immunocompetent persons, the primary complex resolves spontaneously and the infection will remain dormant unless factors that compromise the immune system occur. TB will only develop in 5% of those who overcome their primary infection and this could be due to endogenous reactivation or exogenous re-infection. For the remaining 5% of the cases, various forms of primary TB develop. The Ghon focus may enlarge and rupture causing pleurisy. The enlargement of the lymph node may compress a bronchus, causing collapse of a lobe of the lung. Also, foci of infection in more distant organs may progress and cause meningeal, bone or renal TB(46).

3.1.1 Clinical presentation

According to WHO, any person who is afflicted with cough for more than 2 weeks and who may be coughing blood, has chest pain, is breathless and tired, has fever and night sweats and who has significant weight loss as a consequence of loss of appetite, should be regarded as a suspected TB case(47). The time perspective varies for when a person is considered to be a TB “suspect”. While WHO (as stated above) stipulates cough for more than 2 weeks as stated, IUATLD stipulates cough for 3 weeks or more(38). Prolonged cough is therefore an important symptom, and people who are afflicted with cough, should have access to quality-assured sputum microscopy. From a public health perspective, proper diagnosis and treatment of TB is necessary to decrease disease transmission within communities(48).

TB cough is constant and irritating and may be non-sputum-producing in the beginning. As the disease progresses, it may become very sputum-producing. Rupture of a blood vessel may lead to haemoptysis as the disease progresses. It may seem dramatic but is rarely life-threatening.

Fever is normally low grade, rarely rising above 40ºC.

Weight loss is gradual, but if treatment is not started, may become dramatic over a space of a few months. The patient can lose 50% of body mass or more.

The onset of fatigue may come gradually but can rapidly lead to demotivation in the patient, so after a few weeks the patient wants to do nothing but rest.

Night sweats may be profuse and may go on for some months after treatment has started. (49)

In a study from Ethiopia comprising 81 TB patients, 80% reported cough, 73% fever and 72% weight loss(50). In relation to selenium status, a study from Ecuador concluded unexpectedly
that the concentration of selenium was relatively high in patients who had haemoptysis and fever (51).

In a study from Brazil, it was found that lowering the current threshold for screening for TB would significantly increase the number of TB cases without increasing the workload for the clinic and laboratory personnel. The current guidelines in the country state that only patients with cough for more than 3 weeks should be screened for TB(52).

3.2 Tuberculosis situation globally

There were an estimated 8.8 million new cases of TB in 2003 including 3.9 million smear positive cases. The number of TB cases that occur in the world each year is still growing, although the rate of increase is slowing; the incidence rate of TB was growing at a maximum of 1.5% per year in 1995, but less than 1% per year by 2003.

The African Region, the Southeast Asia Region, and the Western Pacific Region accounted for 82% of all notification cases and a similar proportion amongst new smear-positive cases. In the African Region of WHO, the TB case rate continues to increase rapidly, mainly because of the HIV epidemic and the poor or absent primary-care services throughout the region(6;53).

Fatal outcomes of TB treatment were most common in the African Region where a high fraction of cases are HIV-positive and in the European Region where a higher proportion of cases are drug resistant(53).

The WHO Stop TB Department estimates the number of incident cases of multi drug-resistant tuberculosis (MDR-TB) to be 458,000 in 2003. Prevalent cases could be two to three times higher than the number of incident cases(54). MDR-TB are organisms that are resistant to the first line drugs isoniazid and rifampicin. In March 2006, a new term was introduced; extensively drug-resistant tuberculosis (XDR-TB). This form of tuberculosis is resistant to first-line drugs as well as second-line drugs(55). So far, 35 countries have confirmed XDR-TB(56).

3.3 Immunity and tuberculosis

3.3.1 Innate immunity and the reduction-oxidation defence system

Innate immunity is non-specific and is effective immediately after exposure to microbes. It consists of host defences such as barriers to infection, cells like macrophages, neutrophils and dendritic cells and proteins like e.g. interferons. It also includes processes like phagocytosis and inflammation. Killing invading microbes and activating the acquired immune system are the major functions of the innate system(57).

Droplets infected with \(M.\text{tb}\) are inhaled and lodge in the alveoli in the distal airways(45). Phagocytosis of the bacilli by alveolar macrophages is the first event in the host defence against the disease. Likewise are neutrophils and Natural Killer (NK) cells early actors at the site of infection. During early infection, NK cells are capable of activating phagocytic cells
and a significant reduction in NK cells’ activity is associated with MDR-TB. NK cells are also important in the sense that they cause apoptosis by their cytotoxicity and they are also able to produce the cytokine gamma interferon (IF\(\gamma\)) and lyse mycobacterium pulsed target cells(2).

Macrophages have three main functions: phagocytosis, antigen presentation and cytokine production. Neutrophils and dendritic cells have also phagocytic abilities. Macrophages and dendritic cells are the most important antigen-presenting cells. This activation of helper T-cells requires that the T-cell recognizes a complex on the surface of the macrophage. This complex consists of both the antigen and a class II major histocompatibility complex (MHC) protein (58). This process activates the acquired immune system and hence creates a bridge between both arms of the immune system(57). After ingestion, the cellular vacuole containing the microbe fuses with an organelle called lysosome. The microbe is killed within this phagolysosome by reactive oxygen species and reactive nitrogen species(58).

In general, lung diseases are related to inflammatory processes that generate increased reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS like superoxide and hydrogen peroxide have important physiological functions, but can be destructive if produced in excessive amounts. The same applies for RNS such as nitric oxide which is both physiologically necessary and potentially destructive. In order to minimize the damage caused by oxidation of organic compounds, the lung is endowed with an integrated antioxidant system. The lung contains enough intracellular antioxidant enzymes to maintain a normal redox state, but the alveolar space can recruit additional antioxidant activity from the epithelial lining fluid which contains large amounts of GSH. An imbalance between the production of ROS and RNS and antioxidant capacity leads to a situation called “oxidative stress”. This situation contributes to the damaging of lipids, proteins and DNA(59).

Macrophages produce several cytokines where interleukine-1 (IL-1) and Tumor Necrosing Factor (TNF) are the most important. IL-1 has a function in the activation of helper T-cells and TNF is an inflammatory mediator. Macrophages also produce interleukine-8 (IL-8) which attracts neutrophiles and T cells to the site of infection. When \(M.\ tb\) bacilli enter the body and are broken down, fragments or antigens from the bacilli are presented on the surface of the cell, for instance on a macrophage. These antigens, in association with class II (MHC) proteins, create an antigen-class II MHC protein complex which interacts with an antigen-specific receptor on the surface of a helper T lymphocyte. Interleukins like IL-1, produced by macrophages and IL-2, produced by lymphocytes, are the main producers of this antigen-specific helper T-cell activation. These activated helper T-cells are an important faction of the cellular immune system response called the delayed hypersensitivity reaction(57).

### 3.3.2 The acquired immune system

\(M.\ tb\) is a classic example of a pathogen where the response relies upon the cellular immune system(2). Stem cells create the basis for the immune response, and they differentiate into different series of cells; lymphoid cells being one of them. Lymphoid cells evolve into T lymphocyte and B lymphocytes. After several events, T cells differentiate into helper T cell (CD4) and cytotoxic T cell (CD8). CD4-positive T cells and macrophages are the main components of the cell-mediated immune response. T cells perform several functions which can be divided into two categories: regulatory functions which are mediated by CD4 T cells and effector functions which are mediated by CD8 T cells. In the activation of T cells, CD4 cells interact with class II MHC protein, CD8 cells interact with class I MHC proteins.
There are two subpopulations of CD4 T cells; T helper-1 (Th1) cells and T helper-2 cells (Th2). In order to mount a protective immune response against *M. tb*, it is important that the appropriate subpopulation is activated; the Th1 cells. It is a lipoprotein from the bacterium that stimulates a specific “toll-like receptor” on the antigen-presenting cell. This receptor signals the cell to synthesize interleukin-12 (IL-12). In turn, IL-12 induces helper T cell to differentiate into the Th-1 type of helper T cells that are essential in the cell-mediated response. An important feature of the Th-1 cells is that they produce the protein IFγ. This protein activates macrophages and this “IL-12-IFγ axis” enhances the body’s ability to control the infection(58).

#### 3.4 Selenium

**3.4.1 An introduction**

Selenium is an essential dietary trace element that enters the food chain through plants, which take it up from the soil. Human dietary intake will therefore range from high to low according to geography(60). Selenium is taken up with the diet mainly as selenoamion acids in which selenium replaces sulphur; mainly as selenomethionine and selenocysteine(61).

Selenomethionine can represent more than 50% of the total selenium of the plant.(62) The term “selenoproteins” refers to selenocysteine-containing proteins like the selenoenzyme glutathione peroxidase. Selenium metabolism is complex and comprises several steps. The selenium containing compounds that are derived from the diet, undergo different processes depending on their bioavailability.(61) Bioavailability is defined as the proportion of an ingested nutrient that is absorbed and used for normal physiological functions(62). With adequate intake and regular functioning of the organs, most selenium is taken up by the liver and used for synthesis of hepatic selenoenzymes(61).

It is believed that it is through its incorporation into selenoproteins that selenium exerts its effect. Glutathione peroxidase was the first selenoprotein to be discovered 30 years ago; and selenium is now found to be an essential component of at least four glutathione peroxidase enzymes as well as three selenoprotein enzymes that regulate the thyroid hormone metabolism. Today 25 selenoproteins are identified, some very recently(63). It is estimated that half of the selenoproteins have antioxidant functions. For glutathione peroxidase, the major physiological role is to maintain low levels of the ROS hydrogen peroxide within the cell, and then decrease potential free radical damage. Further, it provides a second line of defence against hydroperoxides which can damage membranes and other cell structures.(62)

Selenium offers protection from cancer and other diseases like cardiovascular disease. An intervention study from China provides evidence that nutritional supplement with β-carotene, vitamin E and selenium may lower the risk of cancer. Mortality from cancer was significantly lower in the population that received supplements than in the group that did not(64).

Recommended intakes of selenium for adults vary; WHO/FAO recommend 30–40 μg/day(65) and USA/Canada 55 μg/day(66). Both values are based on the intake needed to maximize the selenoprotein glutathione peroxidase. In addition to the daily intake, supplementation with 200 μg selenium for 8 weeks has considerable immunoenhancing effects(67). However, selenium can be toxic if intake exceeds 350-700 μg per day(62). Liver and seafood have the
highest content of selenium. Meat and cereals/grain are also good sources whereas dairy products and fruits/vegetables are low in selenium(13).

Regarding the “normal” reference ranges, there are no accepted values because selenium status will vary from country to country. A blood selenium concentration of 1.0 to 1.2 μmol/L is sufficient for maximization of glutathione peroxidase. Whether deficiency is likely to occur at concentrations below 1.0 μmol/L is unresolved; partly because there are no good clinical markers for selenium deficiency(66).

3.4.2 Selenium and the immune system
Selenium deficiency is suggested to be accompanied by loss of immunocompetence, as both cell-mediated and B-cell functions can be impaired as well as the body’s redox’ status. Hence selenium behaves both as an antioxidant and anti-inflammatory agent. Being a part of glutathione peroxidase, it can reduce hydrogen peroxide and lipid hydroperoxides; thereby quenching the enhancement of free radicals and reactive oxygen species. Furthermore, in its antioxidant role, selenium can reduce hydroperoxide species and thereby diminish the production of inflammatory prostaglandins and leukotrienes. As a result, glutathione peroxidase modulates this respiratory burst by removing the hydrogen peroxide and reducing the superoxide production.(60) Selenium is not working alone to reduce the damage made by the formation of ROS; selenium and vitamin E (α-tocopherol) are working synergistically by related but independent mechanisms to reduce damage to lipid membranes(68).

In a study where the relationship between selenium status, nitric oxide synthase expression and nitrogen oxide production in selenium deficient and selenium supplemented macrophages was investigated, the model system showed a 17-fold decrease in glutathione peroxidase activity with selenium-deficient macrophages when compared with those values observed in cells that were supplemented with selenium. The model also showed that levels of nitric oxide produced in selenium deficient cells upon lipopolysaccharide stimulation were significantly higher than in the selenium-supplemented cells. (69)

Selenium-deficient lymphocytes were less able to proliferate in response to mitogen(14). This was shown in an intervention study from China where healthy individuals were grouped and given micronutrient supplements. The men in the group who were given supplements of β-carotene, selenium and α-tocopherol showed significantly higher T lymphocyte response to the mitogen phytohemagglutinin compared with those who did not(70).

Selenium is also important for achieving optimal functionality of neutrophils. A study showed that selenium deficiency can impair the ability of mouse neutrophils to kill Candida albicans in in vitro tests. (71)

Low plasma selenium concentrations, high plasma HIV load and high IL-6 concentrations are risk factors associated with anaemia in adults with TB in Malawi(17). Significant risk for development of TB was associated with selenium levels <=135 microgram/L (1.70 μmol/L), CD4 counts <200/mm³ and malnutrition. This was shown in a follow-up study over 2 years in the USA among HIV-positive drug users(72).
3.5 Glutathione

3.5.1 An introduction
GSH is a tripeptide comprised of the amino acids glutamate, cysteine and glycine. GSH is present in most cells where it functions as an antioxidant protecting cells from toxic effects of ROS and RNS(16). In these reactions GSH is oxidized to form glutathione disulfide (GSSG), which is then reduced to GSH by using glutathione reductase. GSH/GSSG is regarded as the most important redox couple and serves crucial roles in (for instance) antioxidant defence and nutrient metabolism(73). It is mainly in its reduced form that GSH is transported around the body, both in plasma and in cells(74).

The synthesis of GSH from the 3 amino acids is catalyzed sequentially by two enzymes: $\gamma$-glutamylcysteine synthetase (GCS) and GSH synthetase. This process occurs in almost all cell types, with the liver being the major producer and exporter of GSH(73). Under normal physiological conditions there is feedback on the activity of the enzyme GCS by GSH. Therefore, the conversion of cysteine to GSH is strongly influenced by the rate of utilization and transport of GSH within and between the cells of the body, provided that cysteine is available(74). In a situation of oxidative stress, GSH is first consumed in order to protect the cell. To avoid depletion, most cells increase their synthesis of GSH. During continuous stress, cellular activity is not sufficient to restore the consumption of GSH, reducing the total amount of cell and plasma GSH.(75) GSH synthesis is, however, complex partly due to the many substrates involved and to their metabolism at both the organ and sub cellular levels(73).

Cysteine, the sulphur containing amino acid is incorporated into proteins and hence taken up from the diet. Cysteine is also converted from the essential amino acid methionine(73). The conversion of methionine to cysteine is an irreversible process, which accounts for the principle that cysteine is not a dietary essential amino acid provided that adequate methionine is available. Methionine is, however, an essential amino acid, regardless of cysteine availability(76). The liver is considered to be the primary site of dietary methionine and cysteine metabolism in the body(77). Cysteine plays a key role in cellular redox status which is linked to the sulphydryl group as well as being the precursor of GSH. An adequate provision of sulfur-amino acids is therefore essential for the maximization of GSH synthesis(73).

In a study among malnourished children with severe oedema one group was given N-acetylcysteine (NAC) and the other alanine. Erythrocyte cysteine and GSH concentrations were measured three times after hospital admission showing a significant increase in the erythrocyte concentration of GSH by day 9 in the NAC group compared to the increase in the control group. This finding was accompanied by marked increases in erythrocyte concentration of cysteine and in the rate of synthesis of GSH. The researchers suggest that a shortage of intracellular cysteine is the underlying cause of the slower rate of GSH synthesis and hence the low intracellular GSH concentration(78).

Glutamate plays a regulatory role in GSH synthesis through the uptake of cysteine and the prevention of GSH inhabitation of GCS. Glycine availability may be reduced in response to protein malnutrition, sepsis and inflammatory stimuli(73).

Animal protein is generally considered to be a better source of sulphur-amino acids than vegetable protein. This is primarily because the biological value of animal protein is higher than that of vegetable protein(79).
According to WHO’s table for energy and protein requirements the average intake of dietary cysteine and methionine for an adult is 13mg/kg/day(80). For a person weighing 60kg the intake should be 780 mg.

Plasma GSH levels varies from 2.79 to 11.36 μmol/L(81).

3.5.2 Glutathione and the immune system
GSH deficiency contributes to oxidative stress, which is demonstrated in an in vitro study of rat hepatocytes. It was hypothesized that the level of redox stress would be a determinant of nitric oxide (NO)-mediated toxicity in the cells. NO has been shown to affect GSH synthesis, and here GSH-depleting agents were used to induce stress in the cultured cells. The results were that NO increased cytotoxicity and oxidative stress and reduced adenosine triphosphate content as well as the mitochondrial membrane potential. This disruption of cellular redox homeostasis by GSH depletion leads hepatocytes to be more susceptible to NO and finally necrotic cell death(15).

GSH may improve immunologic and virologic indexes in HIV-patients and this was tested in a pilot study involving 8 patients. All patients were given N-acetylcysteine and vitamin C for 6 days. The results showed a number of immunologic and virologic effects in patients with low CD4+ counts. A significant increase in CD4+ was observed after 6 days and the increase persisted after 13 days. A reduction in HIV RNA plasma level, an enhanced lymphocyte proliferation and an increased level of intracellular GSH in CD4+ lymphocytes were also found(82).

GSH has an important role in determining whether Th1 or Th2 response patterns predominate. In an experimental study different methods to deplete GSH from T cell in mice were used and the responses to different antigens were studied in vivo and/or in vitro. The researchers show that GSH levels in antigen-presenting cells determine whether Th1 or Th2 response patterns predominate; in all cases, GSH depletion inhibits Th1- associated cytokine production and/or favours Th2-associated responses(83).

GSH and S-nitrosoglutathione; glutathione carrying NO, are directly toxic to mycobacteria. This was shown in an experiment where survival of BCG and a permease mutant of BCG were studied. The study was performed in macrophages from mice and humans. It was shown that stimulating macrophages from mice with IFN-γ and lipopolysaccaride resulted in significant eradication of BCG. The researchers concluded that GSH levels play an important role in regulating directly or indirectly, antimicrobial activity in immune cells(16). The same laboratory also demonstrated that GSH at a 5mM concentration is bacteriostatic to a virulent laboratory strain of M. tb(84).

3.6 Selenium and Glutathione status in TB patients
Previous studies have documented that TB patients have low blood levels of selenium (3;17;50;51;85) and GSH (4;86). A study from USA showed that selenium <1,70μmol/L (135μg/L), malnutrition and disease status predisposes to TB in HIV-positive drug users(72). In Malawi low selenium concentrations, high human immunodeficiency virus load were among the risk factors associated with anaemia in TB patients(17). Neither of these studies
has dealt with selenium and GSH status in TB patients and healthy controls nor with selenium and sulphur amino acids in the diet of these two groups.

The studies that have examined selenium and GSH levels can be categorized into the following main areas: selenium and GSH status in TB patients and controls (4;50;51;85-87), selenium status in TB patients (3) and selenium levels, GSH levels and nutritional status in TB patients (3;4;17;50;72).

3.6.1 Selenium and Glutathione status in TB patients and controls
Several studies have shown that blood levels of selenium were significantly lower in the patient groups compared with selenium levels in the control groups (50;51;85).

In a study done in Turkey, it was found that the mean selenium level was 46.0 μgram/dl (5.82 μmol/L) in the control group whereas it was 37.86 μgram/dl (4.79 μmol/L) in the patient group. After 60 days on treatment the TB patients had a mean level of 35.7 μgram/dl (4.52 μmol/L) which indicates that there was no progression in the level of selenium and that 2 months treatment seems to be insufficient to evaluate the changes in this trace element(85). A similar study was done in Ethiopia with the same findings as in Turkey when comparing newly diagnosed patients with healthy controls. However, an increase in selenium levels was seen after 2 months of anti-tuberculosis treatment(50). In Ecuador, the selenium levels were lower in patients than in control subjects, but the values were higher than those generally reported from Europe(51).

Studies of GSH in TB patients and controls, the findings show that TB patients had significantly lower levels of GSH than the control group(4;86). Also the total antioxidant level was lower in TB patients than in community controls(87). A study done in Ethiopia in TB patients and controls, show that the mean GSH level was 1.07 μmol/L whereas in the healthy control group was 1.37 μmol/L. In the healthy Norwegian control group, the mean was 5.01 μmol/L(4). Also in the study from India, lower values of GSH were found in this patient group compared with the healthy control subjects(86).

3.6.2 Selenium levels in TB patients
In a study from Malawi involving 801 TB patients of whom 579 were HIV-positive, selenium deficiency was found in 87 % of the participants when cut-off for deficiency was set at 0.89 μmol/L(3).

3.6.3 Selenium and Glutathione levels and nutritional status in TB patients
Studies on selenium, GSH and nutritional status have been done in Malawi (3;17) Ethiopia(4;50) and in the USA(72). Two of these studies suggest an association between wasting and selenium and GSH deficiencies; in a study from Malawi, severe wasting defined as BMI <16.0 kg/m² was associated with selenium deficiency whereas mild and moderate wasting was not(3). In a study in Ethiopia, BMI was positively correlated with concentrations of GSH, suggesting an association between GSH and malnutrition(4).

3.7 Malnutrition and TB
TB has been connected to malnutrition for very many years. Hippocrates (460-377 BC) introduced the ancient Greek term for TB, *phthisis*, which has several possible translations including “to consume”, “to spit” and “to waste away”. Much later TB became known as “consumption”; most languages that have given a name to the disease, the word used indicates “wasting away”(5).

Profound wasting is often seen in TB patients and is regarded as one of the most typical features of the disease(88). A study in Malawi found that the extent of pulmonary TB, as assessed by chest radiographs, is associated with the severity of malnutrition. Extent of malnutrition was reflected by BMI and body composition studies like fat mass calculations(12). Also from Malawi moderate to severe malnutrition was found to be a risk factor associated with early death among TB patients. The cut-off point for moderate to severe malnutrition was set at a BMI of 17.0 kg/m²(89). Co-infection with HIV and TB introduces an extra dimension to the pathophysiology of wasting. The consequence is an enhancement of the wasting seen in TB or HIV infection alone(9). Co-infection with other pathogens than HIV may affect the body’s response to TB, particularly the presence of intestinal pathogens that may enhance malnutrition(9).

In a study from Singapore, TB patients were found to have significantly lower BMI status, lean body mass and total fat mass compared with controls(88). Lower BMI status in TB patients compared to controls was also found in two separate studies from Ethiopia(4;50). Increasing evidence suggests that malnutrition – both PEM and lack of essential micronutrient like vitamins, trace minerals, essential amino acids and polyunsaturated fatty acids, constitute the underlying reason for increased susceptibility to infections.(90) Certain infectious diseases can cause malnutrition, and a vicious cycle is created(90). Hence the interaction between TB and malnutrition is two-fold: TB can affect the nutritional state of the patient, and nutrition can influence the occurrence and clinical manifestations of TB(9).

Micronutrient malnutrition is often seen in TB patients. Levels of vitamin C, E and A were lower in TB patients than in healthy controls(4;86) as well as selenium, zinc and iron(50;51). PEM malnutrition is common among adults with TB and HIV infection(91). TB patients with and without HIV infection had significantly lower values of albumin than did the healthy controls. This was shown in studies from Ethiopia and Singapore(4;88).

Regarding weight gain during TB treatment, it is understood that full recovery takes more than 12 months(9). Researchers from London studied the proportion of weight change attributable to changes in fat mass versus protein mass during the treatment period. The study showed that TB patients gained body fat mass but they did not gain protein mass during the 6-month treatment(92).

### 3.8 Malnutrition and the immune system

PEM is today cited as the major cause of immunodeficiency worldwide. This can be explained by the immune cells’ high requirement for energy and amino acids which are used in their cell division and protein synthesis(68). PEM is therefore associated with a significant impairment of cell-mediated immunity, phagocyte function, complement system and cytokine production. Delayed-hypersensitivity cutaneous responses are depressed and there is a reduction in mature, fully differentiated T lymphocytes; partly because of reduced thymic
activity. The proportion of CD4+ T cells is markedly decreased. Lymphocyte proliferation and DNA synthesis are reduced, which may be due to inhibitory factors as well as deficiency of essential nutrients. Metabolic activation and intracellular destruction of bacteria are reduced as well as the production of several cytokines, including IL-1 and IL-2 in addition to IFγ. Malnutrition changes the ability of T lymphocytes to respond appropriately to cytokines(1).

The pro-inflammatory cytokine IL-6 is a result of the activation of the immune system by phagocytic cells(93). In a study from Malawi the relationship between self-reported loss of appetite and plasma levels of IL-6 was investigated in adult pulmonary TB patients. The regression analysis showed that IL-6 was associated with loss of appetite in these patients. Inflammatory cytokines are important mediators of the metabolic changes that result in TB-associated wasting(94). Pro-inflammatory cytokines may also impair the utilization of amino acids for protein synthesis. This phenomenon has been called “anabolic block”; a study was performed in a group of Indian TB patients where whole body energy and protein metabolism was investigated. It was found that in TB patients a greater proportion of ingested amino acids was oxidized rather than being utilized for protein anabolism. The researchers concluded that feeding has a less anabolic effect in TB patients than in controls and that this mechanism may contribute to the wasting seen in TB(95). However, poverty, contaminated food and water, inadequate nutritional knowledge and poor sanitation also predispose for poor nutritional status and impaired immune function(9).

3.9 Socio-economic status

The Millennium Development Goals were adopted during the United Nations Millennium Summit in 2000,. The first goal aims at reducing extreme poverty and hunger by half in 2015. The goal addresses extreme poverty in its many dimensions: income, poverty, hunger, disease, lack of adequate shelter and exclusion(96). TB often occurs in populations suffering from poverty(9).

In a study done in Italy, a significant association between indices of socio-economic deprivation and the incidence of TB was found; neighbourhoods with higher levels of socio-economic deprivation had a greater TB incidence rate than neighbourhoods with a higher socio-economic level(97). Supporting evidence was found in a study from New York City, USA. Neighbourhood poverty, defined as the proportion of persons living below the federal poverty level, was strongly and independently associated with TB incidence which rose from 20.3% in 1980 to 46.5 % in 1990(98). A study from Lungwena area in the Mangochi district in 2000, the aim of which was to analyse the frequency of appropriate socio-economic support for good health like housing, educational level, access to modern health care and food security, found that these prerequisites for good health were generally missing in the area(27).
Chapter 4: Materials and Methods

4.1 Study area

The study was conducted in the Mangochi district. This district was chosen for the following reasons: first, cooperation was already established between the University of Oslo and the College of Medicine in Mangochi; secondly, the district forms part of the Rift Valley, an area which contains a large amount of old rocks. This type of soil is known to be low in selenium. Finally, the incidence of TB in the district is high which would allow the sample size to be met and the study to be relevant for public health in the district. The whole of the Mangochi district was included in the study.

4.2 Study design and population

4.2.1 Study design

The study used quantitative methodology and the design was case-control. A quantitative approach was chosen in order to compare TB patients and controls in relation to two exposures; selenium and sulphur amino acids. A case-control design was chosen because we wanted to compare past exposures, selenium and sulphur amino acids, to TB and apparently healthy controls; and secondly a case-control design was suitable as the prevalence of TB in Malawi is 0.5%. The controls were matched according to age and sex, and were residents of the same village as the case population.

The study was part of a larger study comprising three parts; first the measurement of selenium and sulphur amino acids in food samples from the Mangochi district. This part was the responsibility of the co-investigator. Secondly, the interactive 24-hour dietary recall field interview was shared between the co-investigator and the investigator. The blood sampling and analysis procedure was the sole task of the investigator.

4.2.2 Study population

Ideally all TB patients could be included in the study, but sputum smear positive cases were chosen due to diagnostic concerns; they were all positive on *M. tb* on at least two out of three sputum specimens. The study was conducted in the intensive phase of the treatment period, i.e. the first 8 weeks. The reasons for this choice were that the patients were likely to be more marked by the illness at this stage than later as well as practical concerns; e.g. that they during the first two weeks would be hospitalized and hence easier to find for recruitment. The final reason was connected to the recall bias; i.e. that it would be easier for them to recall the answers for the questionnaire. The cases were recruited from Mangochi District Hospital and St. Martin’s Hospital. The controls were randomly selected, but were residents of the same village as the patient in question and were matched according to age and sex.

*Inclusion criteria*

TB patients:

- Smear-positive TB patients aged 15-60 yrs
- In intensive phase of treatment, 2-8 weeks after onset of treatment
- Resident in Mangochi district during period of study
- Signed informed consent

Controls:
- Aged 15-60 yrs
- Resident in the same village as a case
- Same age as a case (or within 10 years of the age of a case)
- Signed informed consent form

To be a resident in the district was set as a criterion to ensure the comparability between plasma levels of selenium and dietary intake of the nutrient.

**Exclusion criteria**

- Previously diagnosed TB
- Extra pulmonary TB patients (treatment failures)
- Chronic tuberculosis patients
- Not a resident in Mangochi district
- Unable to comprehend the consenting procedure
- Refused consent

### 4.3 Sample size

The sample size was determined by using a model where the association between low selenium and TB was calculated in terms of an odds ratio.

It was assumed that 70% of the cases were low in selenium. A study by van Lettow et al detected 87% selenium deficiency in tuberculosis patients in Malawi(3). Hence 70% could be expected to have low selenium levels. The requirement was one control per case.

It was assumed that low selenium could triple the occurrence of TB. The odds ratio was therefore set at 3 and the confidence interval at 95%.

The confidence interval should not include 1. The power was set to 80%.

In a calculation where 70% of the cases were exposed to low selenium and 43.8% of the controls, 70 cases and 70 controls were required to find an odds ratio of 3.

Determination of the sample size should be done by using data for the most critical nutrient(99). In this study selenium is considered to be the most critical nutrient as it has an independent function as well as being contained in glutathione peroxidase.
Programme for calculation of sample size from odds ratio

<table>
<thead>
<tr>
<th>Values:</th>
<th>Names:</th>
<th>low Selen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases:</td>
<td></td>
<td>TB</td>
</tr>
<tr>
<td>Controls per case:</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Proportion of cases exposed:</td>
<td>70.0 %</td>
<td></td>
</tr>
<tr>
<td>Proportion of controls exposed:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expected OR:</td>
<td>3.00</td>
<td></td>
</tr>
<tr>
<td>Confidence level:</td>
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<td></td>
</tr>
<tr>
<td>Power:</td>
<td>80 %</td>
<td></td>
</tr>
</tbody>
</table>

<table>
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<tr>
<th>low selenium</th>
<th>Cases</th>
<th>Controls</th>
<th>sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>49</td>
<td>31</td>
<td>80</td>
</tr>
<tr>
<td>-</td>
<td>21</td>
<td>39</td>
<td>60</td>
</tr>
<tr>
<td>sum</td>
<td>70</td>
<td>70</td>
<td>140</td>
</tr>
</tbody>
</table>

Proportion exposed= 70.0 % 43.8 % 56.9 %

OR=3. 95% CI=(1.11 , 8.11), power=80%

The programme was developed by Hein Stigum at the Institute of Public Health, Oslo, Norway. It is copied with permission (personal communication).

The figures for TB patients in the Mangochi district for the last quarter are as follows:

New smear positive: 97, relapses: 12, smear negative: 157, extra pulmonary 49, and others 29. 
(Personal communication with our supervisor, Kenneth Maleta in Malawi, May 2006)

New smear positive: 97/3=32 patients a month.

70 cases and 70 controls were therefore considered to be a realistic number considering the time frame and budget.

However, the planned sample size was not achieved. Only 22 cases and 15 controls gave their permission and went through the procedure of the blood test. One of the cases had to be excluded because the blood test was mistakenly taken after 8 days of treatment and not 14 days, as set as an inclusion criteria. Another two tests had to be excluded because they were taken at the hospital on the day these two patients were discharged, and not as part of the dietary interview as the others. All blood tests were taken at home or within a distance of 40 minutes drive from the hospital. The reasons for why the planned sample size was not achieved can be categorized into matters related to time frame and budget as well as resistance to consent (please see chapter 5).
4.4 Ethical Clearance

Ethical clearance for this study was obtained from the Regional Committee for Medical Research Ethics, Norway and the Malawi College of Medicine Research Ethics Committee. The District Health Commissionaire in the Mangochi district and the Police Officer in charge at the Mangochi police station were informed. Permission to recruit patients from Mangochi District Hospital was obtained from the District Health Officer and permission was likewise sought from the director at St. Martin’s Hospital. Permission was also obtained from concerned village chiefs. Informed consent was obtained from each of the participants prior to their involvement. For the only person under the age of 18 who participated, the next of kin’s consent was obtained.

4.4.1 Ethical considerations

Blood sampling is not considered ethically correct during the holy month of Ramadan. Neither blood testing nor interviews were conducted during this period.

The Malawi College of Medicine Research Ethics Committee raised the issue of how to protect the TB patients in the villages from suspicion of also being HIV-positive when the selection of the controls was done. In the district TB was connected to HIV, and if it was known that this study included TB patients, the controls could get the impression that the TB patient also was HIV-positive. Our local supervisor suggested that two separate information letters and consent forms were elaborated. The information letter and consent form for the TB patients were kept without making any changes. The information letter and consent form for the controls were reviewed and re-written. For the controls the study was presented as a nutritional study and no information about TB was included. The study was presented to the community leaders in this way as well.

4.5 Data collection

4.5.1 Preparation for data collection

The local supervisor introduced the investigator to the District Health Officer at Mangochi District Hospital. The District Health Officer was informed about the study as well as was the TB Officer and staff on the TB wards. After obtaining ethical clearance, an information meeting was held for all staff from the male and female TB wards. At St. Martin’s Hospital the director introduced the investigator to the TB officer and an information meeting was also held for staff at the TB wards.

Research Assistants

The local supervisor introduced the investigator to the research assistant who was hired and stayed throughout the study period. There were, however, periods where the research assistant was absent, and a new one had to be hired and introduced into the field. To ensure sufficient introduction an over-lapping period of five days was arranged.

On two occasions when the aforementioned research assistant was absent, the co-investigator’s research assistant, who was very well acquainted to the procedure, was hired for one day and a secretary from the hospital, who acted as a translator, was hired for two hours. Two laboratory technicians were hired on three occasions to do the blood sampling when the research assistant was absent. Oral and written information was given on how to prepare the
samples. However, on one occasion the vials were not put in the freezer after centrifuging, but left in the cooling box for app. 90 minutes before they were put in the freezer. On another occasion the tubes were left too close to the ice pack in the cooling bag, which resulted in haemolysis of the antioxidant sample. Both incidences were noted.

4.5.2 Pre-testing
Upon request directed to the local supervisor, the investigator and the co-investigator were given permission to start pre-testing the week before ethical clearance was given. The reason for the request was lack of time.

The purpose of the pre-testing of the blood sampling procedure was to check the quality of the serine-borat buffer solution as it had gotten stiff in the tubes, and also to go through the preparation procedure. The serine-borat buffer worked well when the tube was inverted (until the buffer had dissolved), but later the tests haemolysed and a new supply had to be brought in from Norway. The investigator considered it not ethically appropriate to test the buffer solution and the procedure on any person who was not connected to the study. The bloodtesting procedure was therefore tested on the co-investigator and vice versa.

The questionnaire to the TB participants was expanded after advice from the supervisor to include questions on the experience of symptoms. A question on period of cough prior to diagnosis was also added. Finally a question on time span from diagnosis was given until start of treatment was added. The questionnaire was tested on 6 TB patients who were not included in the study. The questions were well understood. A minor change was made on the options for answering the final question to include “same day” and “day after” as some of the respondents gave this answer.

Following advice from the external examiner at the institute and the local supervisor, the data collecting tool for socio-economic status was expanded and adjusted to local conditions. In addition questions regarding educational level and housing conditions were added. The tool was tested on 9 people who were not included in the study. The list of luxury items was reduced to only include one item: a television; and possessions like shoes and domestic animals were added.

4.5.3 Selection of cases
The cases were recruited from Mangochi District Hospital and St. Martin’s Hospital TB wards. A list of sputum-positive patients was obtained every week from Mangochi District Hospital’s TB officer, and more seldomly from St. Martin’s Hospital’s officer because they only had few cases of sputum-smear positive patients. Altogether 36 patients were recruited from the former and 6 patients from the latter hospital.

Patients were recruited from the wards where the topic was presented to them. If they were interested in receiving more information and perhaps to join the study, they were invited into the laboratory allocated solely for the study and, hence, a private room.

4.5.4 Selection of controls
When doing a case-control study, a big challenge is to select the controls. We want the controls to be as similar as possible to the cases except that they do not have TB. To match is one way to make cases and controls more comparable for some variables that might confuse the comparison. Matching means that each case is individually paired with a control subject.
In this case we matched the controls on age and sex in addition to village; hence these variables cannot be investigated as a possible risk factor for the outcome.(100). However, our small sample size was small and not suitable for individual matching, but group, age and sex were adjusted for in a linear regression analyzing model.

The controls were selected from the same village as the patient. The age-span allowed for was 2-3 years. However, due to difficulties in finding an appropriate control participant, the matter of expanding the time-frame to 10 years was discussed with the supervisors and accepted on the grounds that in the Mangochi district, the diet of a 20-year old is not different than the diet of a 30-year old.

Unlike the cases, the controls were randomly selected in order to maintain confidentiality. The controls were selected according to the “random-walk method” as described by Gibson and Ferguson, 1999: When in the actual village, we will walk to the centre and randomly select a direction by spinning a pen on the ground and walk in the direction that the pen points. Then all the houses from the central area to the edge of the village will be counted. Next step is to randomly select a number from all the houses counted. This number will be the first house to visit. The second house selected will be the one whose front door is closest to the first household and the third will be the closest front door of the next household(101). This was a way to make the controls representative for the population.

4.5.5 Logistical matters in the field
The investigator was given access to a room in the TB ward where a laboratory was set up. However, towards the rainy season where the power-cuts became frequent, the investigator was given permission to move the centrifuge and essential equipment into the main laboratory at the hospital which was covered by the generator in times of power-cut. The samples were frozen at minus 20°C in the hospital’s freezer. Every third week the samples were transported to the Malawi Office of the University of North Carolina laboratory in Lilongwe to be frozen at minus 80°C.

The cases were recruited at the hospital and their addresses were obtained. An appointment for the first visit was also made at this stage. As very many of the participants were living in remote villages and the roads often were in bad condition, a car with four-wheel drive was rented. Later, another car was also rented to be used on tarmac roads and dirt roads of better condition.

Permission was sought from village chiefs before the recruitment of the controls commenced. The chiefs were informed about the study and were given evidence that the investigator indeed had obtained the required permissions from the authorities. In these meetings the chiefs were asked about the village’s demarcation lines and what was the centre of the village.

The blood test was taken at home. However, this was not always possible as the period of time from the blood test was taken until it was centrifuged should not be more than 40 minutes. This was solved in different ways: on two occasions the test was taken at the hospital upon departure and on other occasions the participants were taken to hospital and driven home afterwards. Also arrangements were made with the co-investigator to meet on the road (about a 40-minute drive from the hospital). Here the test was taken and the participant was driven back home while the sample was taken to the hospital for preparation.
A buffer-solution had to be added to the antioxidant vial to prevent the GSH from oxidizing. All vials were prepared in Norway, but at the time of data-collection the solution had gotten stiff and was no longer functioning. A new sample had to be brought in from Norway and all together 14 participants, 7 cases and 7 controls were lost in the meantime.

4.5.6 Data collecting tools
Blood sampling procedure
The site on the arm where the blood sample was taken was disinfected with an alcohol solution and left to dry before the blood was drawn. Then the site was covered with a cotton pad and secured with a bandage. The person was asked to press two fingers on the site for 5 minutes and leave the pad on for at least one hour. However, this was not always done as some participants removed the pad to avoid questions and rumours when leaving the house. The site was always checked before leaving the participant to ensure that there was no bleeding.

Seven ml blood was taken in heparin tubes and the tube was inverted 8-10 times. 2 ml of blood was transferred to an eppendorf vial containing 50 µl 2.0 M serine borat buffer for total glutathione and cysteine. The vial was inverted 8-10 times to mix the buffer with blood. The subjects had not been fasting.

The samples were stored for 40 minutes before being centrifuged and transferred into vials for freezing. An attempt was made to always protect the samples from direct sunlight. The samples were stored in a cooling box which would maintain 20 degrees under outside temperature. However, the socket in the car with four-wheel drive did not work and ice packs had to be used instead. Hence, the temperature varied from 12°C (the lowest) to approximately 22°C (the highest).

Both tubes were centrifuged in Labofuge 200 at 2500 X g for 10 minutes.

One ml of plasma for selenium analysis was transferred to an eppendorf vial.

Five hundred µl of plasma and 500 µl of erythrocytes/whole blood for glutathione peroxidase were transferred in two separate eppendorf vials. This sample did not contain serine borat.

Five hundred µl of plasma for total glutathione and cysteine was transferred to an eppendorf vial.

Fifty µl of erythrocytes/whole blood for glutathione was transferred to another eppendorf vial.

The samples obtained were frozen at -20 °C for 3 weeks and then transferred to -80 °C

Serine borat buffer:
A 100 ml solution of 2 mol/L serine borat (pH 8.5) was prepared by dissolving 9.9 g boric acid, 8.1 g anhydrous sodium tetraborat in 60 mL Milli-Q water using a magnetic mixer. After the salts were dissolved 21.0 g of L-serine and 100 mg BPDS were added and the final volume was made to 100 mL.

In order to adjust for 50 µl serine borat, every value was adjusted accordingly:
2 ml = 2000 µl + 50µl serine borat = 2050 µl
Value in µmol/L X 2050 µl /2000 µl = adjusted value.
Method for analysing GSH and cysteine
The GSH- and cysteine samples were analysed using a high-performance liquid chromatographic system which consisted of a Hewlett-Packard pump and a Hewlett-Packard fluorescence detector. The procedure is described in Bohn et al (102). The method is also validated for the measurement of cysteine.

Method for analysing selenium
Selenium was measured with Perkin Elmer, Elan® DRC™ II ICP-MS (Inductively Coupled Plasma – Mass Spectroscopy) instrument. The ICP generates singly charged ions from the elemental species within the sample. These ions were then directed into the mass spectrometer. The mass spectrometer separates the ions introduced from the ICP according to their mass-to-charge ratio. Ions of the selected mass-to-charge ratio were directed to a detector which determines the number of ions present.

The $^{82}$Se isotope was measured in standard mode (without reaction gas). External calibration was used and the standard was matched with sample-matrix by adding Selenium Pure Atomic Spectroscopy Standard (Perkin Elmer) to Autonorm™ (Sero). Samples, standard and quality controls were diluted 1:20 with H$_2$O with 0.1% Nitric acid 65% Suprapur® and 0.5% 1-Butanol. 10 ppb Rhodium was added directly to the diluent and is used as an internal standard.

Structured questionnaire
Two structured questionnaires were used for data collection: one for the TB patients only and one for both cases and controls. The questionnaires were developed in English and translated into two languages: Chichewa and Chiyao, which are the main languages spoken in the Mangochi district. Re-translation of the questionnaires from the vernacular languages into English was done and corrections were made.

The questionnaire solely for the TB patients collected information on the following:
- Experienced symptoms of TB: cough, fever, weight loss, blood in the sputum
- Weeks of coughing until diagnosis
- Time span from diagnosis was given until start of treatment

In addition the investigator collected information from the patient card on classification of TB and the results of the sputum tests. Information was also collected on date for diagnosis and on start of medication.

The questionnaire for both groups collected information on the following:
- Socio-demographic characteristics: age, sex, literacy, profession
- Economic status: assets owned, housing conditions; on source of drinking water, flooring in the house and kind of toilet used.

Anthropometry measurement tools
Body weight was determined to the nearest 0.1 kg using a bathroom scale. The participants wore light clothes and no shoes. Standing height was determined to the nearest cm.

The scales and the height measurement devices were checked four times totally. The scales were checked by weighing 5 litres of water. They were also checked before every weighing and adjusted if necessary. The height measurement devices were tested on the investigator and co-investigator and checked against a stadiometer. It turned out that device A showed on
average 2 cm too short. This was adjusted for by adding 2 cm to the measurements done with device A when entering the data into the Statistical Package for Social Services (SPSS).

Body mass index (BMI) was calculated as body weight/height². WHO’s International Classification of adult underweight and normal range was used to describe nutritional status.(103)

An interactive 24-hour recall dietary interview procedure

The dietary interview was a joint task shared between the investigator and the co-investigator and is not the main topic for this study. Hence the conversion factors for different dishes and food items that were elaborated upon in the field research will not be presented here. They are considered part of the co-investigator’s topic which is food intake of selenium and sulphur amino acids.

The 24-hour recall dietary interview procedure will therefore be presented in brief.

The interview was 3-day process where the respondent was prepared on day one; explained the purpose of the interview; asked to tell the interviewer what he/she had to eat and drink the day before the interview, was given a picture chart, bowl, plate and cup, was asked to use the separate bowl and plate and was explained the importance of following a usual eating pattern on the recording day.

On day two the respondent was recording his/her eating regime.

Day three was the day of the interview. On this day records were taken of each food and drink consumed. At the end, the respondent’s picture chart was checked to see if it corresponded with the recording form.

The size of the individual portions was estimated by direct weighing. The following five dishes were made by a cook at the campus at the College of Medicine and brought to the field for each interview: rice, beans, vegetable relish, fish relish and nsima (thick maize porridge). A 50cm long sugar cane, sugar, salt, nuts and a bottle of water were also brought in addition to cassava and seasonal fruit.

The 24-hour recall dietary interview procedure was adopted from R.S. Gibson and E.L. Ferguson(104).

4.5.7 Data handling

Recording forms for the diet interviews and questionnaires were checked on the spot at the end of each interview in case of indistinctness or if some questions had been omitted. At the end of the day, the recording forms and the questionnaires were rechecked. In the eventual case of missing data the investigator went back to the respondent to seek clarity.

4.6 Data analysis

Data was entered in Excel and later transferred to SPSS for analysis. The blood test results were entered directly to SPSS. Variables were classified as below:
4.6.1 Dependent variables
- Plasma concentrations of selenium
- Plasma concentrations of GSH
- Participant’s nutritional status (BMI)

4.6.2 Independent variables
- Age
- Sex
- Completed school years
- Reading ability
- Assets owned
- Participant’s occupation
- Acid Fast Bacilla load
- Experienced symptoms; cough, fever, weight loss, haemoptysis
- Coughing period
- Water source
- Flooring in the house
- Toilet facilities

4.6.3 Operational definitions of variables

Dependent variables

Nutritional status

WHO’s Expert Committee describes BMI with the following grades(103):
- normal range: BMI>18.5
- mild thinness: BMI 17.0-18.49
- moderate thinness: BMI 16.0-16.99
- severe thinness: BMI <16.00

Important independent variables

Acid Fast Bacilla load (AFB)

The number of AFB per immersion fields. Based on the IUATLD’s definitions(38):
- 1-AFB per 100 immersion fields = Scanty
- 10-99 AFB per 100 immersion fields = 1+
- 1-10 AFB per field = 2+
- More than 10 AFB per field = 3+

Symptoms

Number of symptoms experienced by the patient before treatment was started; cough, fever, weight loss and haemoptysis.

Coughing period

The period of time the participant was coughing before treatment was started. Based on the WHO case definitions(105):
- 2 weeks or less
- 2 weeks or more
Number of assets owned:  Assets owned by a household were classified as follows:

<table>
<thead>
<tr>
<th>Number of assets</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 1</td>
<td>Very Poor</td>
</tr>
<tr>
<td>2 – 4</td>
<td>Poor</td>
</tr>
<tr>
<td>5 - 6</td>
<td>Fair</td>
</tr>
</tbody>
</table>

Categorization was based upon number of assets owned and not the purchasing value of the item because the assets owned by the family would vary according to needs, household size and preferences.

4.6.4 Statistical methods

Descriptive statistics of the sample’s characteristics; nutritional status, selenium levels in cases and controls, GSH levels in cases and controls and clinical presentations were obtained through descriptives and frequencies. Continuous variables have been presented as means (+/-SD). Due to the low sample size, the results have been presented as numbers rather than as percentages; e.g. see the categorical variables in table 1.

The differences between cases and controls in relation to plasma selenium levels and plasma GSH levels were investigated using linear regression analysis. Here, age and sex were adjusted for. Hence the dependent variable was either selenium, GSH, BMI or socio-economic status and the independent variables were group, age and sex. The analysis was done in a stepwise process; first the number of participants was analysed, a Q-Q plot was done in order to check for outliers and a final analysis was performed. The data was checked for normality, and log transformation considered if the data was not normally distributed. The analysis on plasma GSH data obtained more normal distribution when log transformed, but log transformation had no consequences for the evaluation of significance and therefore was not done.

Correlation analysis was used to describe the strength and direction of the relationship between two variables. Here, we chose Pearson Correlation coefficient because the dependent variables were continuous, and it is also suitable if one variable is continuous and the other is dichotomous. In order to determine the strength of the relationship, the following guidelines were used:

\[
\begin{align*}
    r &= 0.10 \text{ to } 0.29 \quad \text{or} \quad r &= -0.10 \text{ to } -0.29 \quad \text{small} \\
    r &= 0.30 \text{ to } 0.49 \quad \text{or} \quad r &= -0.30 \text{ to } -0.49 \quad \text{medium} \\
    r &= 0.50 \text{ to } 1.0 \quad \text{or} \quad r &= -0.50 \text{ to } -1.0 \quad \text{large}(106)
\end{align*}
\]

Correlation analysis was used to investigate the relationship between the variables selenium and GSH and their association to variables like BMI, symptoms, coughing period as a continuous variable and AFB bacilla load in TB patients as well as the association between selenium, GSH and BMI in the control group.

Significance was measured as \( P < 0.05 \) or less.
Chapter 5: Results

5.1 Characteristics of the Study Population

The case-control study consisted of 19 cases; 6 women and 13 men and 15 controls; 5 women and 10 men.

Characteristics of the study population are shown in table 1. The mean age for women was lower, (23, +/- 6 SD) in the case group than in the control group (32 +/- 13 SD), whereas the mean age for men among the cases and controls was 30 and 32, respectively. Completed school years were the same in case and controls, 6 years. Subsistent farmer was the main occupation for both groups. The percentage of those having two jobs were higher among the controls (40%) compared to the cases (26%). Reading ability was higher in the case group (74%) compared to the control group (67%) (data not shown).

Regarding water and sanitation, all in the control group had access to safe water compared to 79% of the cases. Of the total group all had traditional pit latrines except one of the cases who had a flush toilet. The general housing standard in terms of flooring in the house was approximately the same; 68% of the cases had sand or earth flooring compared to 73% of the controls (data not shown).

In terms of socio-economic status, 74% of the cases were regarded as poor (owning 2-4 items out of totally 6 items) compared to 53% of the controls. Among the controls two persons were regarded as very poor, owning 0-1 item compared to none in the case group. Linear Regression analysis showed that there was no difference in economic status between the two groups (P=0.834) when adjusted for age and sex (data not shown).

Table 1. Characteristics of the study population in Mangochi district, Malawi

<table>
<thead>
<tr>
<th></th>
<th>Cases Women N=6</th>
<th>Cases Men N=13</th>
<th>Controls Women N=5</th>
<th>Controls Men N=10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
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<td>30 (6)</td>
<td>32 (13)</td>
<td>32 (8)</td>
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<td>Completed school years</td>
<td>6 (3)</td>
<td>5 (3)</td>
<td>5 (6)</td>
<td>6 (5)</td>
</tr>
<tr>
<td>Able to read,² Yes</td>
<td>4</td>
<td>10</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>No</td>
<td>3</td>
<td>4</td>
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<td>3</td>
</tr>
<tr>
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<td>5</td>
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<tr>
<td>Sub. farmer+formal</td>
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<td>1</td>
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<td>0</td>
</tr>
<tr>
<td>Sub.farmer+other</td>
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<td>3</td>
<td>1</td>
<td>5</td>
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<td>1</td>
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<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Very poor²</td>
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<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Poor</td>
<td>4</td>
<td>10</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Fair</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

¹ Mean +/- SD for continuous variables. (SD=Standard Deviation). ² Frequency analysis categorical variables.
5.2 Selenium

5.2.1 Mean values of selenium levels in cases and controls
Descriptive analysis demonstrated that in the group of 19 cases, the mean plasma selenium level is 1.10 \( \mu \text{mol/L} \) ranging from 0.80 – 1.55 \( \mu \text{mol/L} \) (+/- 0.22 SD).

In the group of 15 controls the mean plasma level is 1.28 \( \mu \text{mol/L} \) ranging from 0.85 – 2.00 \( \mu \text{mol/L} \) (+/- 0.27 SD). Adjusting for the outlier the mean plasma level of the remaining 14 controls is 1.23 \( \mu \text{mol/L} \) ranging from 0.85 -1.60 \( \mu \text{mol/L} \) (+/- 0.19 SD).

5.2.2 Differences between selenium levels in cases and controls
The results from the Linear Regression analysis is shown in table 2. Here the association between selenium levels in cases and controls is investigated. The model explains 19.2 % of the variance in selenium levels in cases and controls. Adjusted for age and sex, the mean difference between cases and controls is estimated to be 0.22 \( \mu \text{mol/L} \). On average the cases has a selenium concentration that is 0.22 \( \mu \text{mol/L} \) lower than the controls when adjusted for age and sex. The difference is significant (\( P=0.011 \)). Sex has no effect on the selenium status (\( P=0.762/0.384, N=34/N=33 \)) compared to the variable age where the effect is significant (\( P=0.032/0.016, N=34/33 \)).

In the group of 33 subjects (one outlier is excluded; selenium level 2.00 \( \mu \text{mol/L} \)) the model explains 22.3 % of the variance in selenium levels in cases and controls. Adjusted for age and sex, the mean difference between cases and controls is estimated to be 0.17 \( \mu \text{mol/L} \); on average the cases have a selenium concentration that is 0.17 \( \mu \text{mol/L} \) lower than the controls when adjusted for age and sex. The difference is still significant (\( P=0.024 \)).

In order to find out if there is an association between selenium levels and education in cases and controls, bivariate correlation analysis was done. Pearson correlation showed that in the case group of 18 participants (information on one subject was missing), it was a positive, but very small correlation between selenium and completed school years (\( r=0.12 \ P=0.632 \). For the group of 15 controls, there is no correlation between selenium and completed school years (\( r=0.01 \ P=0.957 \) (data not shown).

| Table 2. Relationship between selenium levels in the two groups. Mangochi district, Malawi |
|---------------------------------|-----------------|-----------------|-----------------|
| Group                          | Estimate (B)    | 95% CI for B    | P - value       |
| N=34                           | -0.22           | -0.39, -0.05    | 0.011           |
| N=33^1                         | -0.17           | -0.31, -0.02    | 0.024           |
| N=34                           | -0.02           | -0.21, 0.15     | 0.762           |
| N=33^1                         | -0.06           | -0.22, 0.08     | 0.384           |
| N=34                           | -0.011          | -0.021, -0.001  | 0.032           |
| N=33^1                         | -0.010          | -0.019, -0.002  | 0.016           |

^1Outlier excluded. Adjusted for age and sex. Dependent variable: Selenium levels. CI= Confidence Interval. P-value is significant at the 0.05 level.
In order to find out how many of the cases and controls had enough selenium to maximize the selenoprotein glutathione peroxidase, we grouped the variable using a cut-off point of 1.1 μmol/L. This cut-off point was made based on the concentration which is sufficient to maximize the selenoprotein; 1.0 to 1.2 μmol/L (66). The mean value was chosen (2.2 μmol/L/2 = 1.1 μmol/L). Of the 19 cases 9 had values that were below 1.1 μmol/L and 10 had values that were 1.1 μmol/L or higher.

Of the 15 controls, 2 had values that were below 1.1 μmol/L and 13 had values that were 1.1 μmol/L or higher (12 when the outlier is excluded).

5.2.2.1 Association between selenium levels, BMI and clinical presentation

Table 3 shows the association between selenium levels, BMI and clinical signs. The correlation was investigated using Pearson Correlation. The investigation showed no correlation between selenium and BMI in the case group (r=.05, N=19, P=.831).

The association between selenium levels and number of symptoms showed a negative correlation among the 19 subjects (r=-.26, N=19, p=.281) indicating a small correlation between the two variables and the correlation is very weak (r=-.26).

The association between selenium levels and coughing period was investigated and showed a negative correlation between the two variables (r=-.13, N=19, P=.593) The correlation between the two variables is very small (r=-.13), suggesting almost no association between selenium and coughing duration.

The association between selenium levels and the load of AFB showed a positive correlation (r=.13, N=19, P=.592). The strength of the correlation is very small (r=.13), suggesting a weak association between selenium and AFB load.

Table 3. Association between selenium, nutritional status and number of symptoms, cough duration and AFB load in pulmonary tuberculosis patients in Mangochi, Malawi.

<table>
<thead>
<tr>
<th>Selenium</th>
<th>BMI</th>
<th>Number of symptoms</th>
<th>Coughing period in weeks</th>
<th>AFB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=19</td>
<td>r=.05 P=.831</td>
<td>r=-.26 P=.281</td>
<td>r=-.13 P=.593</td>
</tr>
<tr>
<td></td>
<td>N=19</td>
<td></td>
<td>r=.13 P=.592</td>
<td></td>
</tr>
</tbody>
</table>

SE: Selenium  BMI: Body Mass Index kg/m2  AFB: Acid Fast Bacilla load  r: correlation coefficient.
Correlation is significant at the 0.05 level (2 tailed).

Correlation analysis between selenium levels and the different symptoms was investigated using Pearson Correlation. No correlation was found between selenium, cough and fever, and a small correlation between selenium and haemoptysis. Regarding selenium and weight loss, the variable could not be computed as it was constant (data not shown).
5.2.2.2 Association between selenium levels and BMI in the control group

Regarding the control group, the association between selenium and BMI was investigated using Pearson Correlation. There was a negative, small correlation between the two variables ($r=-.10$, $N=15$, $P=.72$). The correlation did not change much when omitting the outlier ($r=-.12$, $N=14$, $P=0.67$). The strength of the association is small ($r=-.10$, $r=-.12$), suggesting a very weak association between selenium and BMI in the control group (data not shown).

5.3 Glutathione (GSH)

5.3.1 Mean values of GSH levels in cases and controls

In the analysis of GSH concentrations three tests had haemolysed and had to be excluded (2 tests in the case group and 1 test in the control group). Descriptive analysis demonstrated that in the group of 17 cases the mean plasma GSH level is 3.85 μmol/L ranging from 2.40-8.61 μmol/L (+/- 1.68).

In the group of 14 controls the mean plasma level is 4.48 μmol/L ranging from 2.93 – 8.46 μmol/L (+/- 1.52 SD).

5.3.2 Differences between GSH concentrations in cases and controls

Table 4 shows the differences between GSH levels in cases and controls. The association was investigated using Linear Regression analysis.

The model explained 9.0 % of the variance in GSH levels in cases and controls. Adjusted for age and sex, the mean difference between the two groups is estimated to be 0.53 μmol/L. On average the cases have a GSH concentration that is 0.53 μmol/L lower than the controls when adjusted for age and sex. The difference is not significant ($P=0.395$). Neither sex ($P=0.820$) nor age ($P=0.541$) have any effect on GSH status in cases and controls.

As for selenium, we wanted to find out if there is an association between completed school years and GSH levels in cases and controls. Pearson correlation showed that in the case group of 18 participants (information on one subject was missing), it was a positive, small correlation between selenium and completed school years ($r=0.20$ $P=0.406$). For the group of 15 controls, there is no correlation between selenium and completed school years ($r=0.06$ $P=0.812$) (data not shown).

| Table 4 Differences between GSH levels in cases and controls, Mangochi district, Malawi |
|---------------------------------|----------|-----------|----------|
|                                | Estimate B | 95% CI for B | P-value  |
| Group                          |           |            |          |
| N=31                           | -0.53     | -1.81, 0.73 | 0.395    |
| Sex                            | -0.15     | -1.49, 1.19 | 0.820    |
| Age                            | 0.02      | -0.05, 0.09 | 0.541    |

Adjusted for age and sex. Dependent variable: GSH levels. CI= Confidence Interval. P-value is significant at the 0.05 level.
5.3.2.1 Association between GSH levels, BMI and clinical signs
Table 5 shows the association between GSH levels, BMI and symptoms of TB. The association was investigated using Pearson Correlation. The investigation showed a positive correlation between GSH and BMI (r=.41, N=17, P=.097). The correlation is of medium strength (r=.41).

The association between GSH levels and number of symptoms showed a negative correlation (r=-.19, N=17, P=.455) The strength of the correlation is small, suggesting a weak association between GSH and symptoms of TB (r=-.19)

The association between GSH levels and coughing period was investigated showing a negative correlation between the two variables (r=.12, N=17, P=.635). The strength of the correlation is small suggesting a weak association between GSH levels and weeks of coughing.

The association between GSH levels and the load of AFB showing a positive correlation (r=.08, N=17, P=.740). The strength of the correlation is very small suggesting no correlation between GSH and AFB load in TB patients.

<table>
<thead>
<tr>
<th>GSH</th>
<th>BMI</th>
<th>N=17</th>
<th>r=.41 P=.097</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH</td>
<td>Number of symptoms</td>
<td>N=17</td>
<td>r=-.19 P=.455</td>
</tr>
<tr>
<td>GSH</td>
<td>Coughing period in weeks</td>
<td>N=17</td>
<td>r=-.12 P=.635</td>
</tr>
<tr>
<td>GSH</td>
<td>AFB</td>
<td>N=17</td>
<td>r=.08 P=.740</td>
</tr>
</tbody>
</table>

Table 5. Association between GSH, nutritional status, symptoms of TB, coughing duration and AFB load in TB patients in Mangochi district, Malawi.

GSH: Glutathione  BMI: Body Mass Index kg/m²  AFB: Acid Fast Bacilla load. r: correlation coefficient. Correlation is significant at the 0.05 level (2 tailed).

Correlation analysis between GSH levels and the different symptoms was investigated by using Pearson Correlation and showed a very weak correlation between GSH, cough and fever. The correlation between GSH and heamopthysis was of medium strength (data not shown).

5.3.2.2 Association between GSH levels and BMI in the control group
The association between GSH and BMI was investigated using Pearson Correlation. In the group of 14 subjects there was a positive correlation between the two variables, (r=plus .55, N=14, P=.040). The correlation is large (r=plus .55) suggesting quite a strong association between GSH and BMI in the control group. The correlation is significant (P=0.040).

5.3.3 Differences between cysteine concentrations in cases and controls
Descriptive analysis shows that mean value for cases is 226.8 μmol/L (+/- 50.7 μmol/L) and 219.0 μmol/L for the controls (+/- 46.4 μmol/L).
The association between cysteine levels in cases and controls was investigated using Linear Regression analysis. The result is shown in table 6. Our model included the variables group, age and sex, and explained 10.4% of the variance in cysteine levels in cases and controls. Adjusted for age and sex, the mean difference between cases and controls is estimated to be 8.05 μmol/L. On average the cases have a cysteine concentration that is 8.05 μmol/L higher than the controls when adjusted for age and sex. The difference is not significant (P=0.673). Neither age (P=0.952) nor sex (P=0.985) had any effect on the cysteine status in cases and controls.

Table 6. Differences between cysteine concentrations in cases and controls in Mangochi district, Malawi.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimate (B)</th>
<th>95% CI for B</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>N=31</td>
<td>8.059</td>
<td>-46.8. 30.7</td>
</tr>
<tr>
<td>Age</td>
<td>N=19</td>
<td>0.067</td>
<td>-2.18. 2.32</td>
</tr>
<tr>
<td>Sex</td>
<td>N=17</td>
<td>-0.382</td>
<td>-41.3. 40.5</td>
</tr>
</tbody>
</table>

Adjusted for age and sex. Dependent variable: Cysteine levels. CI= Confidence Interval. P-value is significant at the 0.05 level.

Table 7 gives an overview of selenium, GSH and cysteine in cases and controls.

Table 7. Plasma concentrations of selenium, GSH and cysteine in Mangochi district, Malawi

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selenium (μmol/L)</td>
<td>N=19</td>
<td>N=14</td>
</tr>
<tr>
<td></td>
<td>1.10 (0.80-1.55)</td>
<td>1.23 (0.85-1.60)</td>
</tr>
<tr>
<td>GSH (μmol/L)</td>
<td>N=17</td>
<td>N=14</td>
</tr>
<tr>
<td></td>
<td>3.85 (2.40-8.61)</td>
<td>4.48 (2.93-8.46)</td>
</tr>
<tr>
<td>Cysteine (μmol/L)</td>
<td>N=17</td>
<td>N=14</td>
</tr>
<tr>
<td></td>
<td>50.72 (159.44-311.98)</td>
<td>46.47 (119.33-324.56)</td>
</tr>
</tbody>
</table>

Mean values and ranges.

5.4 Nutritional Status

Anthropometric indexes are shown in tables 8 and 9. TB patients, both women and men have lower values for weight and BMI than apparently healthy controls.
Table 8. Nutritional status among adults with pulmonary tuberculosis and apparently healthy controls in Mangochi, Malawi.

<table>
<thead>
<tr>
<th></th>
<th>Cases Women</th>
<th>Cases Men</th>
<th>Controls Women</th>
<th>Controls Men</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>6</td>
<td>13</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>47.0 (4.7)</td>
<td>50.0 (5.6)</td>
<td>52.4 (3.0)</td>
<td>58.8 (7.7)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>157 (0.03)</td>
<td>165 (0.05)</td>
<td>157 (0.03)</td>
<td>166 (0.07)</td>
</tr>
<tr>
<td>BMI kg/m²</td>
<td>19.07 (1.79)</td>
<td>18.19 (2.03)</td>
<td>21.11 (2.04)</td>
<td>21.04 (1.75)</td>
</tr>
</tbody>
</table>

Mean (SD) for continuous variables. SD= Standard Deviation. BMI (Body Mass Index) kg/m². Based on World Health Organization International. Classification of adult weight, reference(103).

The mean weight for cases is 49.0 kg compared to 56.7 kg for controls (data not shown). Frequency analysis of BMI status according to classification shows that 10 patients had a BMI status of 18.5 kg/m² or over, 6 were between 17.0-18.49 kg/m² and 3 were below 16.99 kg/m². Of the controls 14 had a BMI status of 18.5 kg/m² or over. One person had a BMI status that was between 16.0-16.99 kg/m².

Fifty-two percent of the patients had a BMI > 18.5 compared to 93 % of the controls (data not shown).

Table 9 shows the difference between BMI status in cases and controls. The difference was investigated using Linear Regression analysis. Our model of 34 subjects, which includes the variables group, age and sex, explains 29.6% of the variance in BMI status in cases and controls. Adjusted for age and sex, the mean difference between cases and controls is estimated to be 2.45 kg/m². That is, on average the cases have a BMI status that is 2.45 kg/m² lower than the controls (when adjusted for age and sex). The difference is highly significant (P=0.001).

Table 9. Association between BMI in tuberculosis patients and apparently healthy controls in Mangochi district, Malawi.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimate (B)</th>
<th>95% CI for B</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group N=34</td>
<td>-2.45</td>
<td>-3.83, -1.06</td>
<td>0.001</td>
</tr>
<tr>
<td>Age</td>
<td>0.34</td>
<td>-0.04, 0.11</td>
<td>0.403</td>
</tr>
<tr>
<td>Sex</td>
<td>-0.67</td>
<td>-2.14, 0.80</td>
<td>0.359</td>
</tr>
</tbody>
</table>

Dependent variable: Body Mass Index (BMI) kg/m². Adjusted for age and sex. CI= Confidence Interval. P-value is significant at the 0.05 level.

5.5 Clinical presentation

Symptoms of TB were experienced as follows: 17 experienced cough (90%), 18 fever (95%) and all experienced weight loss, 13 (68%) had haemoptysis. In order to see the distribution of symptoms we found that 1 person experienced 2 symptoms while the rest (18 persons) experienced 3-4 symptoms. 5 (26%) of the patients experienced all symptoms (cough, fever, weight loss and haemoptysis).
Regarding cough, 13 (68%) experienced coughing for more than 2 weeks before treatment was started. Mean duration of coughing was 7 weeks (+/-8 SD) (data not shown). Regarding Acid Fast Bacilla load in the sputum tests, 12 (63%) had 2+ and 3+. Table 10 gives an overview of symptoms, duration of cough and AFB load. Information is given in numbers rather than percentages due to the small sample size.

Table 10. Clinical data of adults with pulmonary tuberculosis in Mangochi district, Malawi.

<table>
<thead>
<tr>
<th></th>
<th>N=19</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cough</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Fever</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Weight loss</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Haemopt.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Cough duration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>&lt;2 weeks</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>&gt;2 weeks</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>AFB load(^2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sc</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>1+</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>2+</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>3+</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

\(^{1}\)Cough duration: Based on World Health Organization: Treatment of TB: Guidelines for National Programmes. Reference(105). \(^{2}\) Sc= scanty: 1 AFB per 100 immersion fields, 1+ =10-99 AFB per 100 immersion fields, 2+ = 1-10 AFB per field, 3+ =10 AFB per field. Reference(38).

5.6 Food intake of selenium and cysteine

The data for dietary intake of selenium and cysteine that is used in this analysis is obtained from the co-investigator Frode Eick.

5.6.1 Mean selenium intake in cases and controls

Descriptive analysis shows that mean value for 18 cases is 50.3 μg ranging from 0.73 to130 μg (+/- 31.2 μg SD) and the mean intake for the 15 controls is 56.7 μg ranging from 28.0 to 88.0 μg (+/- 18.7 μg SD).

5.6.2 Differences in food intake of selenium in cases and controls

The difference between food intake of selenium in cases and controls was investigated using Linear Regression analysis. Our model included the variables group, age and sex. The model explained 7.4 % of the variance in food intake of selenium in cases and controls. Adjusted for age and sex the estimated difference between cases and controls is -3.192 μg which indicates that the mean intake is 3.2 μg lower in cases than in controls when adjusted for age and sex. The difference is not significant (P=0.728) (data not shown).
5.6.3 Mean cysteine intake in cases and controls
Descriptive analysis shows that mean value for the 18 cases is 1147.5 mg ranging from 434.0 to 2535.0 mg (+/-599.3 mg SD) and for the 15 controls the mean intake is 1112.2 mg ranging from 388.0 to 1828.0 mg (+/-426.6 mg SD).

5.6.4 Differences in food intake of cysteine in cases and controls
The difference between food intake of cysteine in cases and controls was investigated using Linear Regression analysis. The model explained 14.2 % of the variance in cysteine levels in cases and controls. Adjusted for age and sex the estimated difference in cysteine intake between cases and controls is 97.05 mg which means that the mean intake of cysteine is 97 mg higher in cases than in controls when age and sex are adjusted for. The difference is not significant (P=0.583) (data not shown).
Chapter 6: Discussion

This study shows lower levels of selenium in the case group than in the control group, and no difference in GSH levels in the two groups. TB patients were found to have lower BMI than the healthy control group. There was a very weak association between selenium levels and BMI status in cases and controls. The strength of the association between GSH levels and BMI status were of medium to large strength in the two groups. Very weak associations were found between selenium levels and GSH levels and clinical presentation. Mean duration of cough was 7 weeks and more than 60% had a high number of bacilla in their sputum tests. No difference was found in socio-economic status. Regarding food intake of selenium and cysteine, no difference was found in the two groups.

6.1 Methodological discussion

6.1.1 Strengths of the study

**Co-investigator and investigator as enumerators**

All the interviews including the diet interviews were conducted by the co-investigator and the investigator in cooperation with the research assistants. This had a positive bearing on the reliability of the results because the investigator was well acquainted with the study objectives and could, in spite of not speaking the language, observe the respondent and notice if a question was not well understood before the answer was recorded.

Regarding the preparation of the blood tests, the investigator prepared all the blood samples herself with the exception of five preparations. Hence the matter of reliability of the results of the tests applies to this part of the study as well.

**There were two investigators cooperating**

The cooperation between the co-investigator and the investigator strengthened the study in the sense that various aspects of the methods and materials used in the field could be discussed and feedback given accordingly. It was also positive for the study that we had a common understanding of the objectives and tasks and hence could assist each other if necessary.

6.1.2 Limitations of the study

**The sample size determination was not met**

We did not manage to meet the planned number of participants; neither for the cases nor for the controls. Referring to the model for calculating the sample size; by increasing or reducing the number of cases the lower confidence interval (CI) will change. The guideline is that the CI should not include 1. With cases set at 70 the CI is 1,11. By reducing the number of cases to 60 the CI will still be >1. This implies that the sample size requirements could be 60 cases and 60 controls, not 70 in each group as stated earlier. Still, 120 participants are far from what was achieved.

Fieldwork started on September 4 because the procedure getting the Ethical Clearance took longer than expected. We did not lose any participants during the holy month of Ramadan. In this period and in the following 2-3 weeks we recruited people and made appointments which we then followed up on. Hence we were able to follow up all who had consented. However, there were occasions where people were difficult to find or had moved to another house. On
these occasions and others where patients were treated as out-patients or had been discharged earlier than expected and we had not got the chance to talk to them, more time and resources could have been spent searching for them. Due to financial constraints this was not possible. Many participants lived in remote villages and the study’s time-consuming nature added to the low number.

The time frame for data collection was set at approximately three- and-a-half months. Based on our calculations, approximately 100 TB patients should normally be available in this period which would meet the requirements for obtaining the desired sample size. When checking the hospital list of smear-positive TB patients dating from 24th of July until 22nd of November, 11 patients did not consent, 33 were not asked, 13 patients consented but were not interviewed, and of these 13 people, 2 died. Regarding the controls, 12 people consented, but were not interviewed. One main reason for why those who consented were not interviewed was that we did not manage to find them in their villages. The reason could be incomplete addresses, moved to another house or known under another name. We also experienced that one person lived very remote and the road to his village was inaccessible. Yet another person was hospitalized with a seriously ill child.

The resistance to consent for the blood test was greater than expected; of the 42 cases and 40 controls that were enrolled in the study, 20 subjects did not consent and 11 subjects withdrew their consent. A common explanation among the cases for not wanting to consent was that they were weak, wasted and had no blood to give. Another reason was that they already had taken a blood test and was not prepared to give another one (all patients were encouraged to do a HIV-test at the hospital because if proven positive, they would be offered antibiotics to prevent opportunistic infections. Later, anti-retroviral treatment would be considered). TB patients also thought that it was not wise to give blood test when ill. Most of the control participants were reluctant without giving a specific reason. Some associated the test with an HIV-test and were not ready to go through with the procedure. Others said that they would not give a blood test unless they were sick and also related the denial to religious considerations. Another explanation was that 7ml of blood was too much and that the study was not important enough to donate blood to. A general impression from the field was that rumours easily occurred in the village saying that people were coming to take blood and this was not for the good of the residents. This attitude could easily be adopted by others resulting in several not wanting to give their consent.

In addition, in the period where the serine borat buffer was lacking, we lost 14 subjects who might have been willing to consent to the test. Finally, we did not manage to take the sample from one person; and one person consented, but was absent on the day of the interview and test.

**Sample size and its implications for statistical analysis**

The first choice in choosing a statistical model for analysing our sample would be contingent logistic regression. This model would allow us to compare the pairs individually and the matching variables would be a part of the analysis. However, the small sample was not suitable for ordinary contingent logistic regression and in order to utilize all the information, linear regression analysis was chosen. The study design was case-control matching on age and sex in addition to selecting the controls from the same village as the cases. The linear regression method allowed us to estimate the effect of the two groups and to adjust for age and sex, but no for village.
For each independent variable adjusted for in a linear regression analysis, a rule of thumb is that 10 participants are needed. There are 34 subjects in our sample and hence not enough information to run analyses with four independent variables (group, age, sex and village). The alternative was to use the 7 matched pairs for selenium analysis and 5 matched pairs for GSH analysis (the number of pairs is different because 3 tests haemolyzed and therefore were not suitable for antioxidant analysis). We checked the effect of village in the analysis and found no effect (P=0.342). To conclude, we excluded “village” as an independent variable and adjusted for group, age and sex because we lacked data to run the desired model of analysis: contingent logistic regression. The decision is, however, at the expense of the validity of our study.

The small sample has also implication for the interpretation of the results. The idea behind the calculation of the sample size is that it should be representative for the population. In statistical analysis we use the sample mean as an estimate of the population mean, and the standard deviation is a measure of the variability of observations around their mean. The expected value of the standard deviation of the means of several samples is a quantity that is called standard error. The standard error is a measure of the uncertainty of a single sample mean as an estimate of the population mean. As the number of means increases so does the likelihood of achieving normal distribution. Hence the level of uncertainty will decrease as the sample size increases. The standard error is used to construct the CI and is a range of values which we can be confident includes the true value. Because the standard error is related to sample size, a small study may fail to detect a difference that is real(107). This means that a small sample size will have a large CI, and hence greater uncertainty. In our case the sample size is very small, especially for the GSH analysis and hence it is not possible to detect a difference (if there is any).

**Use of matched controls**

The aim of matching is to control for variables that can confuse the outcome of the study.(100) In this study we matched the controls for age and sex. The selection of the controls had implications for the actual selecting in the field and for the analysing procedure. First, it turned out to be very difficult as well as time consuming to find a control person that fit within the age frame which was +/- 2-3 years. This age frame was, after discussing with our supervisor, expanded to 10 years. It was argued that this was acceptable, as the diet of a 20-year old person in this society would not differ from the diet of a 30-year. Secondly, it has had implications for the analysing procedure which is presented above.

**Use of disposable pipettors**

When transferring 2 ml of blood to the tube for antioxidant analysis, a disposable pipettor was used. This type of pipette is not as precise and accurate as a non-disposable pipettor with continuous adjustable volume. Therefore the use of disposable pipettors might have been a source of bias when transferring blood from one tube to the other; and further in the analysis of the levels of GSH and cysteine. The reason for not having a non-disposable pipettor was financial constraints.

**Variation in storing temperature**

Regardless of where the blood tests were taken, a period of 40 minutes should lapse before centrifugation and preparation. This implied that the storing conditions varied, also in temperature. A cooling bag was purchased for this purpose, but as the electric socket in the car was broken and only worked on a few occasions, other solutions like ice packs had to be used. The temperature in the box was difficult to keep stable, also because the outside
temperature changed, especially in the car in the summer season. The temperature in the box could therefore vary between 10°C as the lowest to 25°C as the highest and it was impossible to keep the temperature stable. It is especially the antioxidant values that are sensitive to low temperatures. According to Amrit Kaur Saki at the Institute of Nutrition the temperature should not be below 4-5°C (personal conversation).

The samples were stored in a freezer holding -20°C. However, as the freezer was placed in a part of the hospital not covered by the generator, the temperature dropped during power-cuts. Power-cuts became frequent towards the rainy season, and on one occasion the temperature was registered at -11°C.

Every three weeks the samples were transported to the University of North Carolina Project in Lilongwe for storing at -80°C. Lilongwe is approximately a 4 hour drive from Mangochi Township. The samples were properly packed and stored, and were still frozen when reaching the laboratory in Lilongwe.

The samples were transported to Norway on dry ice with an airfreight company. The samples got lost during transportation and reached Norway after 4 days instead of 2 days (as planned). However, the samples were still frozen upon arrival as it is the company’s task to refill the dry ice when necessary.

Still, variation in storing temperature may have been a source of bias in the analysis of the samples, and may also have affected the calculation of the mean values for the groups.

**Use of bathroom scale for measuring adult weight**

Adult weight was measured using ordinary bathroom scales. More accurate values could have been obtained using digital scales. Such scales were, however, not available. The two scales used (there were two teams) were calibrated using 5 litres of water and the scales were also adjusted before every use. However, the use of these scales may have been a source of bias in the estimation of the participant’s nutritional status.

**Use of height-measuring device for measuring adult height**

Adult height was measured using ordinary measuring devices. These devices were calibrated measuring the co-investigator and the investigator’s height. In order to ensure accuracy in height measurement, we had our own height measured at Lungwena Health Centre on an advanced height measuring machine. One of our devices was proven to measure wrongly by 2 cm. This was therefore adjusted accordingly. However, the use of these devices may have been a source of bias in the estimation of the participant’s nutritional status. Also, the height measuring was done in people’s homes and often out-of-doors. As the surface was often uneven, this could have biased the results as well.

**Participant’s recall**

Information regarding the number of symptoms experienced by the patient was obtained by asking the participants if they had any of the following symptoms: cough, fever, weight loss and haemoptysis. In order to answer the question on cough duration they had to first answer a question on how long they were coughing before they got the diagnosis and then to assess how long time it took from the diagnosis was given until treatment was started. These questions relied on the participants’ ability to recall. Retrospective information is subject to recall bias especially in studies focusing on a particular disease and its potential risk factors; patients tend to report incidences in the past more often than healthy people(100). These
questions on symptoms were naturally related to the patient group only. Still, recall bias is a limitation in the study; especially related to cough duration as the patients had to give the answer in time rather than answering “yes” or “no” as they could when answering the other questions.

Use of more than one research assistant
Due to various reasons more than one research assistant had to be employed by the investigator. The primary research assistant gave notice beforehand that she needed to take a leave of absence. Another assistant was hired, and 5 days of overlapping was arranged. Hence there were three of us working together during this period and the new assistant was well prepared when the primary assistant left. She came back after three weeks and stayed for the duration. When she later needed yet another day off, the other assistant was able to replace her without great difficulty as she was now familiar with the routines. On two occasions the co-investigator’s research assistant, who was very well acquainted with the procedure, was hired for one day and a secretary from the hospital, was hired for two hours.

Also, two laboratory assistants had to be hired on three occasions to help with blood tests and preparations. The procedure was explained beforehand and written information was placed on the wall in the laboratory. However, on one occasion the tubes were accidentally stored too close to the ice pack and the test haemolysed, and on another occasion a misunderstanding occurred and the vials were stored for one- and-a-half hours in the cooling bag after preparation before being put in the freezer. The use of more than one research assistant might therefore be a limitation for the reliability of the results, especially for the results obtained in the interviews.

6.1.3 Confounding factors
Confounding effects are systematic biases that occur in a study. These confounding factors can threaten the reliability of the results(100).

HIV status
HIV status of the participants was not recorded in our study. According to WHO, 52% of TB patients in Malawi are HIV-positive(31). The National TB coordinator Felix Salaniponi said in a newspaper interview in the autumn of 2006 that there was reason to believe that close to 70% of TB patient were HIV-positive in the country (no official source). Hence at least 50% of our cases are HIV-positive. Being HIV-positive can affect the selenium level in TB patients which was shown in a study from Malawi; HIV-positive TB patients had lower levels of selenium than did HIV-negative TB patients(3). HIV infection is hence a strong confounding factor. However, we decided not to check for HIV infection and instead take a more pragmatic viewpoint; being low in selenium and GSH is a stated deficiency regardless of HIV status. Also, the logistical and financial part should not be underestimated as well as the sensitivity of the issue; some, especially among the control persons, were reluctant to participate because they thought their HIV status would be tested in spite of being informed otherwise.

Other diseases
The control participants did not go through any medical check-up. Neither the controls nor the cases were asked about their general health status. The only question related to their health status was if they were sick the day on the recording day and if “yes”, if this sickness had affected their appetite. Malaria is a major public health problem in the country(108) and could influence blood values as well as nutritional status.
**Supplements given at the hospital**

While in the hospital, plumpy-nut paste was given to the most malnourished patients and could, therefore, affect the blood values. The patients at the TB wards were also given extra food twice a day in addition to the food made by their guardians. Food supplied from the hospital was similar to the home diet (and the meals made by the guardian), e.g. nsima (the thick maize porridge), vegetable relish or fish relish. However, in our study we did not assess whether patients had been given any extras at the hospital. Hence, controlling for this factor could have given us more reliable values. We also did not ask if they had taken any mineral or vitamin supplements lately. However, it is not common for most people in this district to take daily vitamin supplements.

**6.2 Discussion of the findings of the study**

**6.2.1 Selenium concentrations in cases and controls**

In our regression analysis of 34 participants, the mean difference between cases and controls is estimated to be 0.22 μmol/L when adjusting for age and sex. That means that, on average the cases have 0.22 μmol/L lower values than the controls when age and sex are adjusted for. This difference is significant (P<0.011). One value was high, 2.0 μmol/L, and when this outlier was removed the mean difference between cases and controls was estimated to be 0.17 μmol/L when age and sex were adjusted for. The difference is still significant (P<0.024). These results correspond to findings from Ethiopia(50), Turkey(85) and Ecuador(51).

Descriptive analysis showed that the mean selenium level is 1.10 μmol/L in the case group and 1.23 μmol/L in the control group (outlier excluded).

In the study from Ethiopia the mean serum level of selenium in TB patients was 1.12 μmol/L (8.86 μg/dl) whereas it was 0.95 μmol/L (7.55 μg/dl) in HIV-positive TB patients. In the control group the mean serum level of selenium was 1.35 μmol/L (10.70 μg/dl) (50). In the study from Ecuador the mean serum level of selenium was 1.81 μmol/L in the patient group and 1.99 μmol/L in the control group(51). Compared to our study the values found in Ecuador and Ethiopia are higher for both cases and controls, with the exception of HIV-positive patients in Ethiopia.

Taking the mean values into consideration, except for the values attributed to HIV-positive TB patients in Ethiopia, all have sufficient selenium to maximize glutathione peroxidase. However, there is a significant difference between cases and controls which can be explained by the increased levels of micronutrients needed to compensate for oxidative stress during TB and/or HIV infection or to maintain immune balance(72). The impact of HIV infection should not be underestimated on the selenium status in particular and on the nutritional status in general, both in cases and controls. Information on HIV status may contribute to the understanding of metabolic processes in both groups. We have, however, not assessed HIV status in our group of participants. The reasons are explained in the methodology section in this chapter.

Because of soil variation selenium status in humans varies from country to country; hence there are no accepted “normal” reference ranges. One criterion for the assessment of physiological requirement of a nutrient is the intake needed for maximization of for instance an enzyme, in this case the selenoprotein glutathione peroxidase. The value considered to be
Sufficient for maximization of glutathione peroxidase is 1.0-1.2 μmol/L (66). Based on the mean value; 1.1 μmol/L we found that 10 patients had selenium levels that would be sufficient to maximize the selenoprotein, and 9 did not. In the control group the respective numbers were 13 and 2. It is important to note that this cut-off point does not indicate that those below 1.1 μmol/L are deficient or that those over are not. The question as to whether deficiency is likely to occur at concentrations below 1.0 μmol/L (or 1.1 μmol/L ) is unresolved, because there are no good clinical markers for selenium deficiency as yet (66).

TB is a disease where the protective response relies upon the cellular immune system (2). Almost all nutrients in the diet play a crucial role in maintaining an “optimal” immune response; and some, like selenium, may specifically modulate host defence to infectious pathogens (68).

Selenium is an essential trace element and known to play an important role in human health. Selenium is involved in several key metabolic activities via selenoproteins like glutathione peroxidase (13). TB is a lung disease that has been associated with oxidative stress (109) and extracellular glutathione peroxidase plays a significant role in preventing pulmonary oxidative damage caused by free radicals and ROS (59). Selenium is also important for the innate and acquired immune system where deficiency can reduce T-cell count, impair lymphocyte proliferation and responsiveness (67) in addition to altered redox status in macrophages (69).

Low levels of selenium, high HIV load and high IL-6 concentrations are risk factors associated with anaemia in adult TB patients (17). Subjects with selenium levels <1.70 μmol/L (135 μg/L), malnutrition and CD4 cell count of <200/mm³ were 13 times more likely to develop TB than were those with plasma selenium levels >1.70 μmol/L (135 μg/L) (72).

Supplementation with recommended doses of selenium as selenium yeast daily in addition to β-Carotene and α-Tocopherol for 5.25 years appears to result in augmentation and/or restoration of immunologic functions in the general population in a province in China (70). Improved immunologic function was also found after supplementation with 200 μg/d selenium in the form of sodium selenite for 8 weeks. The results in this study indicates that the enhancing effects of selenium on the immune system require supplementation above normal dietary intake (67). This view is supported by findings from the USA (72).

Clinically, it is interesting to see whether these improvements in immune functions have any impact on the patient’s general health status; do they experience more/less cold, flu, chest infections than before? Do they experience any change in mood? Intervention studies with selenium in various patient populations shows that selenium improves mood and decreases anxiety (110). In the study where supplementation with selenium yeast/β-Carotene/α-Tocopherol was given, a modest but significant reduction in cancer mortality was seen in the general population, but clinical observations are not characterized.

6.2.2 GSH concentrations in cases and controls

As mentioned for the antioxidant samples, three of them had haemolysed and, therefore, were not suitable for analysis.

In our regression analysis of 31 participants, the mean difference between cases and controls is estimated to be 0.53 μmol/L when adjusting for age and sex; on average the cases have 0.53 μmol/L lower values than the controls when age and sex are adjusted for. The difference is
not significant (P=0.395). This result is not in line with results found in Ethiopia(4) and India(86) where the GSH concentrations were found to be significantly lower in TB patients than in the control group.

Descriptive analysis showed that the mean plasma GSH value in the group of 17 cases was 3.85 μmol/L (+/- 1.68 SD). In the group of 14 controls, the mean value was 4.48 μmol/L (+/- 1.52 SD).

Pulmonary TB is a lung disease that is associated with oxidative stress(109) which is explained as an imbalance between the production of ROS and RNS and antioxidant capacity; that is excess amount of ROS and RNS(59). The degree of oxidative stress was significantly higher in patients than in controls in the study from Ethiopia (4) and from India(86). GSH is an effective scavenger of ROS and RNS, and hence an important antioxidant(73). GSH is also important for the cellular immune system; the level of GSH in antigen-presenting cells determine whether Th1 or Th2 response patterns predominate(83). It is important for the proliferation of lymphocytes(82) and essential for the activation of T-lymphocytes(111). It has also been demonstrated that GSH is bacteriostatic to a laboratory strain of M.tb (84).

GSH is synthesized from glutamate, glycine and cysteine. Cysteine is considered to be the limiting amino acid for GSH synthesis in humans. There are many factors that can affect the uptake of cysteine, for instance protein malnutrition and HIV infection(73). In our study, regression analysis shows that there is no significant difference in cysteine values in cases and controls (P=0.673). Descriptive analysis shows that the mean value for cases is 226.8 μmol/L (+/-50.7 μmol/L) and 219.0 μmol/L (+/-46.4 μmol/L) for the controls; the value is slightly higher in cases than in controls, which is contrary to what we expected. Madebo et al in the study from Ethiopia also measured cysteine concentrations in TB patients and controls. However, their data is log transformed and therefore not suitable for comparison(4).

The following comments may contribute to understanding why a significant difference was not found; why did we not detect a difference that, based on the theory and other study results, should exist? Accurate and precise measurement of plasma GSH is not straight forward because GSH can be easily oxidized during sample preparation giving wrong results. Plasma values are easily increased by incidental and non-reproducible leakage from red blood cells causing haemolysis(81). In our case we excluded samples that were visible haemolysed; still leakage may have occurred that we did not notice. Also, there could be factors related to the equipment used for transferring blood from one tube to the other; a disposable pipettor was used instead of a non-disposable pipettor with continuous adjustable volume. The amount of blood drawn into a disposable pipettor would not be as accurate, creating a mismatch between the amount of serine borat and blood. The enzyme γ-glutamyl transpeptidase could then degrade GSH and give erroneous results(81). The allocation of blood from one tube to the other was performed on the floor inside or outside peoples home, hence the working conditions could have added to the discrepancy (if there was any). Also, we did not manage to keep the temperature stable during the 40 minutes time laps from the sample was taken until it was prepared in the laboratory. Due to frequent power-cuts the temperature in the 20°C freezer was not stable and the samples were transported twice while frozen; the samples were first transported from Mangochi to Lilongwe, a drive that takes at least 4 hours, and then air freighted to Norway which took longer time than expected. However, to our knowledge, the samples did not thaw after being frozen. On a few occasions, in spite of every effort made, we did not manage to keep the 40-minute time lapse exactly, but exceeded the time frame with 3-5 minutes. Secondly, our sample size is small: 17 cases and 14 controls. In the study from
Ethiopia, 125 TB patients and 45 healthy persons participated(4) and in the study from India 30 cases and 30 controls participated(86). We may not have had enough information to discover a difference between the two groups as the variability among samples increases as the sample size decreases(107). Hence a small study may fail to detect a difference that is real. Thirdly, both patients and controls have enough intake of cysteine to cover the daily requirement which is 13 mg/kg. The mean weight for cases is 49 kg and for controls 56 kg. Required intake is then 637 mg vs 728 mg. Food intake is 1147 mg for the former and 1112 for the latter group; i.e. enough sulphur amino acids to cover daily requirements. It is believed that cysteine is the limiting amino acid for GSH synthesis in humans(73). Therefore, sufficient intake in both groups could contribute to understanding for why the difference in GSH levels in cases and controls was not significantly different.

6.2.3 Selenium and GSH concentrations in relation to nutritional status

In our regression analysis of 34 participants, we found that the mean difference between cases and controls was estimated to be 2.45 kg/m² when age and sex are adjusted for; the controls have on average a BMI status that is 2.45 kg/m² higher than the cases. This difference is significant (P=0.001). This finding is supported by Madebo et al(4) and Kassu et al(50) who found that TB patients with or without HIV infection had lower BMI values than did healthy Ethiopian control subjects.

The mean BMI value in our study is 18.47 kg/m² in the patient group compared to 16.5 kg/m² for HIV-positive TB patients and 16.6 kg/m² for HIV-negative TB patients(4). The other study reported 17.5 kg/m² for HIV-positive TB patients and 18.0 kg/m² for HIV-negative TB patients(50). Hence the BMI values found in our study are slightly higher than those reported from Ethiopia. Regarding the control group, we found a BMI of 21.07 kg/m² which is nearly the same as in the two studies from Ethiopia; 21.6 kg/m²(4) and 21.0 kg/m²(50).

In the present study, bivariate correlation analysis demonstrated that there is a very weak association between selenium and nutritional status in TB patients; the correlation is almost 0 (r=0.05, p=0.831) which indicates that there is almost no association between the two variables. Our results are supported by findings from Ethiopia where no significant association was found between selenium and BMI using regression analysis(50). In the USA, when calculating the risk of developing TB, multivariate analysis suggested that the effect of selenium is independent of body mass status(72). In Malawi, however, mild wasting (BMI 17.0-18.49 kg/m²) and severe wasting (BMI<16.0 kg/m²) was associated with selenium deficiency(3).

For GSH concentrations we found a medium correlation (r=0.41, p=0.097) suggesting a association of medium strength between the two variables; as BMI is increasing, so are the GSH values. This is in contrast to the association between selenium and nutritional status where the correlation was close to 0. Our results correspond with the findings from the study in Ethiopia where BMI was positively correlated with GSH and cysteine(4).

Regarding the controls, we found a small, negative correlation between BMI status and selenium concentrations (r=-.14), suggesting a weak association between the two variables compared to GSH concentrations and BMI where a positive correlation (r=0.55) was found between the two variables. These findings correspond with the findings for cases.

Malnutrition associated with TB is likely caused by a combination of decreased appetite, which leads to a decrease in food intake. This state interacts with increased losses and altered
metabolism as part of the inflammatory and immune responses (88). It is difficult to identify those factors that have the greatest impact on the nutritional status in TB patients. One reason for this is that the wasting phase is rarely observed by the investigator (9). However, researchers have found that the pro-inflammatory cytokine IL-6 was associated with loss of appetite in TB patients (94) and that a greater proportion of ingested amino acids was oxidized rather than being used for protein anabolism (9). PEM is associated with a significant impairment of the cell-mediated immune system (1) and TB patients were found to be low in albumin in studies in Ethiopia (4) and Singapore (88). TB patients had lower values of vitamins (4; 86) and minerals (50; 51; 85) compared with healthy people.

6.2.4 Selenium and GSH concentrations in relation to clinical signs

A majority of the patients experienced cough (90%) and fever (95%). All experienced weight loss (100%). Haemoptysis was experienced by 68%. These findings are similar to the findings from Ecuador (51) and Ethiopia (50). TB has since ancient times been connected with malnutrition (9) and in our study we found that there is a significant difference in BMI status between cases and controls; the BMI status in patients is on average 2.45 kg/m² lower than in the control group. Possible explanations for wasting in TB are discussed under section 6.2.3.

Coughing for more than two weeks was experienced by 68% of the cases and the mean duration of coughing was 7 weeks (+/- 8 weeks SD). Regarding AFB load, 63% had high number of bacilla in their sputum tests (> AFB++).

Bivariate correlation analysis demonstrated that there is a weak association between selenium and number of symptoms (r = -0.26) and between GSH and number of symptoms (r = -0.19). There is also a weak association between selenium and coughing period (r = -0.13) and between GSH and coughing period (r = -0.12). This is what we would expect; that when number of symptoms increases the level of selenium and GSH would decrease and that long period of cough would result in lower levels of selenium and GSH. However, our findings should be handled with care because the correlations are weak and the sample size is small.

Our results for selenium are, though, supported by findings from Ecuador where little evidence was found of associations between micronutrient concentrations and symptoms (51) and from Ethiopia where regression analysis did not show any significant association between micronutrients and clinical signs (50). However, in the study from Ecuador the serum concentrations of selenium were relatively high in patients who had haemoptysis and fever (51). Haemoptysis is often a sign of progressive disease (49), but haemoptysis may also appear early depending on the location of the cavitation within the lung. This can be the case here where the selenium level is still high. Different methods for analysing are used in our study vs. comparing studies; however the trend seems to be pointing in the same direction; that there is little association between selenium concentrations and clinical signs. For the association between GSH concentrations and number of symptoms/coughing period, no comparable information was found in the literature.

Regarding selenium levels and AFB load, a positive, small correlation was found between the two variables (r = .13). This was also the case for GSH concentrations and AFB load (r = .08). This implies that when selenium and GSH concentrations are increasing, so is the AFB load. We would expect the contrary; as the selenium/GSH levels increase the AFB load would decrease and vice versa. We demonstrated that 63% of the patients had a high AFB load which is a sign of advanced disease (112). Madebo et al found increased levels of oxidative stress in TB patients compared to healthy controls (4). On this basis we could suggest that advanced disease would result in higher levels of oxidative stress and increased consumption.
of selenium and GSH. However, the correlation is very weak, close to 0 – which indicates no association. An explanation for not finding what we, based on theory, would expect, may be due to lack of information; i.e. our sample size is too small.

Statistical significance does not necessarily mean clinically important and vice versa; a result that is not statistical significant could be of clinical importance to the patients. A very important aspect for the patients is whether the micronutrient concentrations of selenium and the concentration of the antioxidant GSH can contribute to an improvement in the general health status.

There are three overlapping goals for global TB control: to ease the illness and suffering, to decrease disease transmission and reduce the costs and to help alleviate poverty(48). Rapid diagnosis and treatment is considered one of the most effective means of controlling the transmission and reducing the incidence of pulmonary TB(52). Current recommendations from WHO include checking for TB when a person is experiencing cough that has lasted 2 weeks of more(105).

In a public health context the duration of exposure to a contagious TB patient, type of association and location of exposure are considered important for transmission of the disease(113). Advanced disease with a high bacillary load may also promote the transmission in the society(112). A study done in the USA with the aim of examining the value of network analysis to complement TB contact tracing, found that delayed diagnosis of a highly infectious TB patient was associated with a large outbreak(113).

Our study demonstrates that the majority of the patients have been coughing for more than 2 weeks before treatment was started and that the number of bacilla was high in their sputum tests. This represents a risk for their closest contacts to be exposed for the TB bacilla and thereby being at risk of developing the disease. Therefore, early diagnosis is an important step for controlling TB which is in line with the MDG goal number six; to prevent the spread of HIV, malaria and TB(96). However, public information on the symptoms of TB and when to see a doctor/health centre, is crucial in the fight against the disease.

6.2.5 Socio-economic status in relation to selenium and GSH in cases and controls
Tuberculosis often occurs in populations suffering from poverty, poor housing and economic deprivation(9). In Malawi nearly 42 % of the population is living below the poverty line which is less than 1 dollar a day(19). The UN has through the MDGs addressed extreme poverty; income poverty, hunger, disease and lack of housing in order to save the lives of millions of people and improve the lives of even more persons(96). The link between socio-economic deprivation and the incidence of TB was illustrated in a study from Italy where neighbourhoods that were less well off had higher incidence of TB than did neighbourhoods that had higher socio-economic levels(97).

Our study demonstrates that 74% are classified as poor compared to 53% of the controls. At the same time two persons in the control group were classified as very poor compared to none in the case group. The regression analysis showed no difference in the socio-economic status between the two groups when adjusted for age and sex. Completed school years were the same in both groups but more of the cases reported to be able to read than the controls. The main occupation is subsistent farming and more of the controls reported to having two jobs. Hence the socio-economic status between the two groups is much the same, which may reflect
the overall poverty in the society. The reason could also be that the sample is very small, thus preventing us from being able to detect a real difference.

6.2.6 Food intake of selenium and cysteine versus plasma levels
The major source of selenium intake in TB patients is fish 34% and maize 23%. These are the main sources for controls as well, 33% and 26% respectively. The major source of cysteine intake in TB patients is maize 42% and fish 15% and for the controls maize 51% and fish 15% (data obtained from co-investigator Frode Eick).

Descriptive analysis shows that the food intake of selenium is 50.3 μg for the cases and 56.7 μg for the controls. Recommended daily intake varies around the world; i.e. 55 μg for USA/Canada(66) and WHO/FAO recommends 30-40 μg(65). Based on these figures, both cases and controls get enough selenium to meet the daily requirements which also is the case for cysteine (discussed earlier in this chapter).

Regression analysis on food intake demonstrates that there is no difference in selenium intake (P=0.728) and in cysteine intake (P=0.583) for the two groups. There is a significant difference in plasma selenium concentrations for the two groups (P=0.024) Here the outlier is excluded. For plasma cysteine concentrations, there is no significant difference (P=0.583) between the two groups; to the contrary, cases were found to have 97 mg higher intake than the controls.

Referring to the results for food intake of selenium in our study, the intake is the same in both groups whereas the plasma concentrations are significantly different. It is shown that lipid peroxidation as an expression of oxidative stress is markedly increased in TB patients(86) and especially in TB patients that also are HIV-positive(4). At least 52% of our TB patients are HIV-positive(31) and the percentage could be as high as 66% for males and 77% for females(3). We may therefore infer that a high percentage of our cases are HIV-positive and hence have a dual infection. As TB, HIV infection also increases oxidative stress(114) which may lead to the damage of the body’s major cellular components including lipids, protein, carbohydrates and DNA(59). Selenium concentrations were found to be low in TB patients(3;50;51;85). Selenium concentrations were also found to be low in people with HIV infection and may be a result of true deficiency or as a result of immune activation(114). Our findings should be handled with great care, especially because of the recall bias and the small sample size. However, we can suggest that the lower levels of selenium in TB patients are connected to altered digestion and increased oxidative stress rather than lower food intake in our study population. This finding is supported by our descriptive analysis showing that both groups get enough selenium to cover daily needs, but still the cases have lower plasma levels than the controls.

Regarding food intake of cysteine, we found that cases had a slightly higher intake than the controls and that there was no significant difference in food intake. We did not find any significant difference in the plasma levels of cysteine in the two groups, possible reasons discussed under 6.2.2. The antioxidant GSH concentrations were found to be low in TB patients(4;86) whereas the findings for GSH concentrations in people with HIV infection varies; a significant difference is not always found which could be explained by the severity of the disease(114). It is shown that TB causes increased levels of oxidative stress and lower levels of antioxidant concentrations(4;86). This difference has not been expressed in our findings.
Chapter 7: Summary

7.1 Summary

The main aim of the study was to determine and compare the plasma levels of selenium and GSH in cases and controls. We found that the cases had significantly lower concentrations of selenium than the controls. For the GSH concentrations, we saw that the cases had lower values than the controls, but the difference was not significant.

Regarding food intake of selenium we found no difference between cases and controls. We may therefore suggest that the lower levels of selenium in TB patients are connected to altered digestion and increased oxidative stress rather than lower food intake. For cysteine no differences in plasma levels or in intake levels were noted.

The first specific objective was to describe nutritional status in the two groups and we found the controls to have a significantly higher BMI status than the cases. In the case group no association was found between selenium and nutritional status compared to GSH where a association of medium strength was found. These findings reflect the findings for the control group where a weak association was found between selenium and nutritional status and strong association strength between GSH and BMI status.

The next specific objective was to describe the clinical presentation in TB patients and we found that the majority of the patients experienced cough and fever and that all had lost weight. Nearly three quarters of these experienced haemoptysis. The mean duration of cough was 7 weeks and more than 60% had a high bacilla load in their sputum tests. Little evidence was found between the micronutrient and the antioxidant and clinical presentations.

For the socio-economic status we found no difference between cases and controls.

7.2 Remarks and recommendations

Collecting samples for antioxidant analysis in rural areas with limited accessibilities is a challenging process. The sensitive nature of the antioxidants makes them highly vulnerable to factors such as deadline for preparation, light and temperature. Hence we recommend that the sampling procedure be very carefully planned.

We recommend that the level of oxidative stress be measured in order to better understand the association between antioxidants and markers of oxidative stress. We further recommend analysing other antioxidants like vitamin E to get a broader picture of antioxidant activity. We also recommend analysing glutathione peroxidase to get a better understanding of selenium levels.

In addition, we recommend distinguishing between HIV-positive and HIV-negative participants; also in the control group. This will help us to understand the interaction between TB and HIV in relation to oxidative stress.
Finally, in order to explore if selenium- and GSH’s concentrations can have an impact on the patient’s experience of health, relevant questions related to their general health status is recommended.

Our study demonstrates, in spite of its small size, that there are differences in selenium and GSH concentrations in cases and controls and that the difference for selenium is significant. Whether supplementation with selenium and antioxidants will improve the outcome of TB or has a preventive effect should be examined in future prospective studies.
Reference List


Appendix 1
Ethical Review
Appendix 2
Information and consent form
(English)

Invitation to participate in the study:

“Investigation into plasma levels of Selenium and Glutathione in smear-positive adult tuberculosis patients and healthy controls in Mangochi district, Malawi.”

Tuberculosis is a serious disease that affects an increasing number of people each year in Malawi. Tuberculosis is causing weight loss and poor appetite. Nutritional food is important in order to gain weight and recover from the disease. The trace mineral selenium and the antioxidant glutathione are essential for the immune system and therefore also the healing process.

This study aims to identify the selenium and glutathione status in tuberculosis patients on treatment and in a healthy control group. This information can be used as a basis for studies in the future where supplementary interventions can be carried out.

More specifically, the study will determine and compare blood levels of selenium and glutathione in tuberculosis patients and in the controls. The study will also determine and compare food intake of selenium and sulphur amino acids in tuberculosis patients and in the controls. Information on the food intake will be collected through an interview where the participant will tell all that he/she had to eat and drink the day before.

The study will also compare the blood level of selenium and glutathione with the food intake of Selenium and sulphur amino acids.

Purpose of the study

The purpose of the study is to identify whether tuberculosis patients are deficient in selenium and glutathione. Deficiency of selenium and glutathione may impair the immune system and consequently determine the outcome of disease and treatment. Our findings may provide a basis for future prospective studies where supplementary interventions can be carried out. Whether supplementation will improve tuberculosis outcome or has a preventive effect, can only be definitively concluded through such studies.

Who is invited to participate?

Patients with pulmonary tuberculosis who have started on their tuberculosis medication 2-8 weeks ago. Selected healthy people. All participants should live in the Mangochi district.

Information to the participants:

You ought to know that your participation in this study is voluntarily and without any repercussions. If you agree to participate in this study; please sign in the attached consent form.
You should know that you are free to withdraw from the study at any time. Even if you agree now to be included in this study, you can change your mind later without being asked to give any reason for your decision. Your decision will have no repercussions for you personally.

Your information will be treated confidentially, and in a manner that ensures security.

The results of this study will be presented at group level. However, if you want to know the result of your blood-test, please thick the appropriate box on the consent form. The results can be obtained from the District TB Officer at Mangochi District Hospital. The blood test will be used to analyse the nutritional status related to selenium and glutathione. The blood test will not be used to determine for instance HIV status.

This study has been approved by the Ethical Committee at the University of Oslo, Norway and it has been approved by College of Medicine research and Ethics Committee in Malawi.

If more information is needed please contact Dr Maleta at Mangochi District Hospital Tel 08 232 202 or Stephano Mwaliwa, Tel 09692799.

**Information on the results**

When this study is completed (from August 2006 to June 2007), I will prepare a report containing results and the report will be distributed to the Ministry of Health and other health institutions that are interested in the results. The report will be available for all the participants as well.

University of Oslo  
Faculty of Medicine  
International Health Department
**Declaration of consent for the study of**

“Investigation into plasma levels of Selenium and Glutathione in smear-positive adult tuberculosis patients and healthy controls in Mangochi district, Malawi.”

I am hereby declaring that I have received information on the study “Investigation into plasma levels of selenium and glutathione in smear-positive adult tuberculosis patients and healthy controls in Mangochi district, Malawi.” I have been informed of the purpose of the above mentioned study, and that the information I will provide will be used for this study.

Besides, I have been informed that all the information I will provide will be treated strictly confidentially. Furthermore, I have been informed that the study has been approved by relevant authorities in Norway and Malawi.

I have also been notified that I can later withdraw from this study if I intend to in the future. I also know that I and the information pertaining to me can be contacted and used in similar study in the future.

Based on all that, I am hereby declaring the following:

1. I agree voluntarily and without any repercussions that I will participate in this study.
2. I agree that information I will provide can be used in similar study in the future.
3. I agree that I can be contacted and invited to attend a similar study in the future.

Please cross on any item(s) to which you do not give your consent.

I want to know the result of my blood-test: Yes No

Signature Witness

Date and place Heidi Arntsen
Investigator
Invitation to participate in the study:

“Investigation into plasma levels of Selenium and Glutathione in adults in Mangochi district, Malawi.”

Nutritional food is important in order to maintain a healthy status, gain weight and also to recover from disease. The trace mineral selenium and the antioxidant glutathione are essential for the immune system and therefore also the general health status in people.

This study aims to identify the selenium and glutathione status in adults. This information can be used as a basis for studies in the future where supplementary interventions can be carried out.

More specifically, the study will determine the blood levels of selenium and glutathione. The study will also determine the food intake of selenium and sulphur amino acids in adults. Information on the food intake will be collected through an interview where the participant will tell all what he/she had to eat and drink the day before.

The study will also compare the blood level of selenium and glutathione with the food intake of Selenium and sulphur amino acids.

Purpose of the study
The purpose of the study is to identify whether adults are deficient in selenium and glutathione. Deficiency of selenium and glutathione may impair the immune system and consequently have an impact on the general health status. Our findings may provide a basis for future prospective studies where supplementary interventions can be carried out.

Who is invited to participate?
All people aged 15 to 60 are invited to participate. All participants should live in the Mangochi district.

Information to the participants:
You ought to know that your participation in this study is voluntarily and without any repercussions. If you agree to participate in this study; please sign in the attached consent form.

You should know that you are free to withdraw from the study at any time. Even if you agree now to be included in this study, you can change your mind later without being asked to give any reason for your decision. Your decision will have no repercussions for you personally.

Your information will be treated confidentially, and in a manner that ensures security. The results of this study will be presented at group level. However, if you want to know the result of your blood-test, please thick the appropriate box on the consent form. The results can be obtained from an appointed contact person at your local health centre. The blood test will be used to analyse the nutritional status related to selenium and glutathione. The blood test will not be used to determine for instance HIV status.
This study has been approved by the Ethical Committee at the University of Oslo, Norway and it has been approved by College of Medicine research and Ethics Committee in Malawi.

If more information is needed please contact Dr Maleta at Mangochi District Hospital Tel 08 232 202 or Stephano Mwaliwa, Tel 09692799.

**Information on the results**
When this study is completed (May 2007), the researcher will send a report containing results to the relevant district health officer and other health institutions that are interested. The report will be available for the participants as well.

University of Oslo  
Faculty of Medicine  
International Health Section
(English)

Declaration of consent for the study of

“Investigation into plasma levels of Selenium and Glutathione in adults in Mangochi district, Malawi.”

I am hereby declaring that I have received information on the study “Investigation into plasma levels of selenium and glutathione in adults in Mangochi district, Malawi.” I have been informed of the purpose of the above mentioned study, and that the information I will provide will be used for this study.

Besides, I have been informed that all the information I will provide will be treated strictly confidentially. Furthermore, I have been informed that the study has been approved by relevant authorities in Norway and Malawi.

I have also been notified that I can later withdraw from this study if I intend to in the future. I also know that I and the information pertaining to me can be contacted and used in similar study in the future.

Based on all that, I am hereby declaring the following:

1. I agree voluntarily and without any repercussions that I will participate in this study.
2. I agree that information I will provide can be used in similar study in the future.
3. I agree that I can be contacted and invited to attend a similar study in the future.

Please cross on any item(s) to which you do not give your consent.

I want to know the result of my blood-test:   Yes       No

Signature   Witness

Date and place   Heidi Arntsen
    Investigator


Chulinga cha phunziroli ndikuzindikira selenium ndi glutathione mwa munthu amene akudwala matenda a TB amene akulandira mankhwala ndi a anthu a thanzi. Ndondomeko iyikhoza kugwiritsidwa nthito ngati mfundo ya maphunzirii owonjezerapo amene adzachitikite mtsogolo muno.

Cholinga cha phunziroli

Makamaka phunziroli lidzanena ndi kufanizira mlingo wa magazi a mu selenium ndi glutathione mwa anthu odwala chifuwa cha TB ndi ndi anthu a thanzi. Phunziroli lidzanenanso ndi kufanizira kadyedwe ka chakudya cha selenium ndi sulphur amino acids mwa anthu odwala chifuwa TB ndi ndi anthu a thanzi. Ndondomeko yakadyedwe ka chakudya idzatengedwa kudzera mu mafunso amene otenga mbali adzayankhe atadya chakudya ndi kumwa tsikulo lisanafike.

Phunziroli lidzafanizidwanso pakati pa mlingo wa magazi a mu selenium ndi glutathione ndi kadyedwe ka chakudya mu Selenium ndi mu sulphur amino acids.

Oyenera kutengapo mbali ndani?

Odwala TB onse amene ayamba kulandira thandizo la mankhwala masabata awiri mpakana asanu ndi atatu apatawo. Anthu ena onse osankhika amene ali athanzi. Onse otengapo mbali akhale kuti amachokera m’boma la Mangochi.

Uthenga kwa onse otenga mbali:

Dziwani kuti kutenga kwanu mbali mu phunziroli ndi kofuna nokha komanso opanda kukakamizidwa. Ngati mwagwirizana nazo kuti mutenga mbali mu phunziroli chonde siinani ac hi fomu chomwe alumikizacho.

Dziwani kuti muli omusaka kusiya phunziroli nthawi ili yonse. Ngakhale mungagwirizane nazo kuti mutenge nawo mbali mu phunzilori, mungathe kusintha maganizo ndipo
simudzafunsidwa kupeleka zifukwa zina zili zonse za chiganizo chanu. Chiganizo chanu chilibe vuto lina lili lonse kwa inu ngati munthu.

Maganizo anu adzasungidwa mwa chinsinsi komanso ndi chitetezo.

Zotsatira za phunziroli zidzapelekedwa m’magulu. Komabe, ngati mungafune kudziwa zotsatira za magazi anu chonde chongani pa chifomu chomwe chilli kutsogolocho. Zotsatirazo mukhonza kuzipeza kwa a District TB Officer ku Mangochi District Hospital. Zotsatira za magazi anu zidzawiritsidwa ntchito kupeleka mmene mlingo wa zakudya zopatsa thanzi mogwirizana ndi selenium and glutathione. Zotsatira zamagazi anu sizidzawiritsidwa ntchito kudziwa ngati mwachitsanzo muli kachilombo koyambitsa matenda a Edzi.

Phunziroli ndilovomelezedwa ndi a Ethical Committee ku University ya Oslo, ku Norway komanso yovomerezedwa ndi a College of Medicine Research and Ethics Committee m, Malawi.

A kafukuku alibenso udindo wina uli wonse opeleka chithandizo cha mankhwala kwa anthu otenga nawo mbali. Ngati mungafune kudziwa zambiri mukhonza kupeleka Dr. Maleta ku College of Medicine pa Mangochi District Hospital Tel 08 232 202, Stephano Mwaliwa, Tel 09692799.

Uthenga wa zotsatira
Pamapeto pa phunziroli (Kuyambira mwezi wa August chaka cha 2006 mpakana mu June chaka cha 2007), ndidzalemba ndondomeko yonse yazotsatirazo ndipo idzapelekedwa ku Unduna wa zaumoyo ndi malo ena a zaumoyo omwe angadzakhale osangalatsidwa ndi zotsatirazo. Ndondomekoyo izakhalanso yopezeka kwa onse amene angatenge nawo mbali.

University of Oslo
Faculty of Medicine
International Health Department
Kulengeza za kutenga nawa mbali muphunziro la;

“Kafukufuku wa mlingo wa plasma ya Selenium ndi Glutathione mwa akulu akulu onse amene ali ndi chifuwa cha mmapapo cha TB ndi a thanzi m’boma la Mangochi ku Malawi.”

Ndikunena pano kiti ndalandira ndondomeko ya phunziro “Kafukufuku wa mlingo wa plasma ya Selenium ndi Glutathione mwa akulu akulu onse amene ali ndi chifuwa cha mmapapo cha TB ndi a thanzi m’boma la Mangochi ku Malawi.” Ndauzidwa za chifukwa cha phunziro lomwe latchulidwa mmwambali, ndikuti chili chonse chomwe ndingapeleke chigwiritsidwa ntchito mu phunziroli.

Ndauzidwa kuti chili chonse chomwe ndingapeleke chidzatetezedwa kwambiri. Komanso, ndauzidwa kuti phunziroli ndilovomelezedwa ndi maulendo oyenelera a dziko la Norway ndi Malawi.

Ndauzidwanso kuti ndikhonza kusiya phunziroli ngati ndingafune mtsogolo muno. Ndikudziwanso kuti chillichonse chomwe ndingapeleke chidzagwiritsidwa ntchito mu phunziro ngati lomweli mtsogolo muno.

Malingana ndi mfundozi ndikuvomeleza izi:

1. Ndikuvomeleza kuti mwakufuna kwanga ndidzatengako mbali mu mphunziroli.

2. Ndikuvomeleza kuti zonse zomwe ndidzapeleka zikhonza kudzagwiritsidwa ntchito pa mphunziro lofanana ndi lino mtsogolo.


Chonde dulani mało onse omwe simupelekapo maganizo anu.

Ndikufuna kudziwa zotsatira za magazi anga


Signanture

Mboni

Tsiku ndi malo

Heidi Arntsen

Wankulu wa kafukufuku
Muli kuitanidwa kutengapo mbali pa phunziro la:

“Kafukufuku wa mlingo wa plasma wamu Selenium ndi Glutathione mwa akulu akulu m’boma la Mangochi ku Malawi.”

Chakudya chopatsa thanzi ndi chofunika kwambiri kuti thupi likhale la thanzi komanso kuti munthu achiire ku matenda. Zakuwuka zing’onozoza mnyama za mineral selenium ndi za antioxidant glutathione ndizofunika ka kayandetsedwe ka chitetezo nthupi komanso choncho zimathandizanso kubweletsa thanzi.

Cholinga cha phunziroli ndikuzindikira selenium ndi glutathione mwa akulu akulu. Ndondomeko yikwanza kugwiritsidwa ntchito ngati mfundo ya maphunziro owonjezerapo amene adzachitike mtsogolo muno.

Makamaka phunziroli lidzanena ndi kufanizira mlingo wa magazi a mu selenium ndi glutathione. Phunziroli lidzanenanso ndi kufanizira kadyedwe ka chakudya cha selenium ndi sulphur amino acids mwa akulu akulu. Ndondomeko yakadyedwe ka chakudya idzatengedwa kudzera mu mafunso amene otenga mbali adzayankhe atadya chakudya ndi kumwa tsikulo lisanafike.

Phunziroli lidzafanizidwanso pakati pa mlingo wa magazi a mu selenium ndi glutathione ndi kadyedwe ka chakudya mu Selenium ndi mu sulphur amino acids.

Cholinga cha phunziroli

Cholinga cha phunziroli ndi kuzindikira za kup elewera kwa Selenium ndi glutathione mwa akulu akulu kulili. Kupelewenza kwa selenium ndi glutathione kukhonza ku kufanizira kayandetsedwe ka chitetezo cha nthupi la munthu.

Oyenera kutengapo mbali ndani?
Akulu akulu ena onse oyambira zaka 15 mpakana 60 omwe adzaitanidwe kutengapo mbali. Onse otenga pa akhale kuthela m’boma la Mangochi.

Uthenga kwa onse otenga mbali:
Dziwani kuti kutenga kwanu mbali mu phunziroli ndi kofuna nokha komanso opanda kukakamizidwa. Ngati mwagwirizana nazo kuti mutenga mbali mu phunziroli chonde sainani pa chi fomu chimwe alumikizacho.


Maganizo anu adzasungidwa mwa chinsinsi komanso ndi chitetezo.

Zotsatira za phunziroli zidzapeledwa m’magulu. Komabe, ngati mnagafune kudiwa zotsatira za magazi anu chonde chongani pa chifomu chimwe chiliti kutsogolocho. Zotsatiraro mukhonza ku kuziwa kwa munthu oyenelera ku ma Health centre anu Zotsatira za magazi anu zidzawiritsidwa ntchito kupeza mmene mlingo wa zakudya zopatsa thanzi mogwirizana ndi
selenium and glutathione. Zotsatira zamagazi anu *sizidzagwirtsidwa* nthito kudziwa ngati mwachitsanzo muli kachilombo koyambitsa matenda a Edzi.

Phunziroli ndilovomelezedwa ndi a Ethical Committee ku University ya Oslo, ku Norway komanso yovomerezedwa ndi a College of Medicine Research and Ethics Committee m, Malawi.

Ngati mungafune kudziwa zambiri mukhonza kupeza Dr. Maleta ku College of Medicine pa Mangochi District Hospital Tel 08 232 202, Stephano Mwaliwa, Tel 09692799.

**Uthenga wa zotsatira**

Pamapeto pa phunziroli (Kuyambira mwezi wa August chaka cha 2006 mpakana mu June chaka cha 2007), ndidzalemba ndondomeko yonse yazotsatirazo ndipo idzapelekedwa ku Unduna wa zaumoyo ndi malo ena a zaumoyo omwe angadzakhale osangalatsidwa ndi zotsatirazo. Ndondomekoyo idzakhalanso yopezeka kwa onse amene angatenge nawo mbali.

University of Oslo
Faculty of Medicine
International Health Department
Kulengeza za kutenga nawi mbali muphunziro la;

"Kafukufuku wa mlingo wa plasma wamu Selenium ndi Glutathione mwa akulu akulu m’boma la Mangochi ku Malawi."

Ndikunena pano kuti ndalandira ndondomeko ya phunziro “Kafukufuku wa mlingo wa plasma ya Selenium ndi Glutathione mwa akulu akulu m’boma la Mangochi ku Malawi”. Ndauzidwa za chifukwa cha phunziro lomwe latalami la mawambali, ndikuti chili chonse chomwe ndingapeleke chigwiritsidwa ntchito mu phunziroli.

Ndauzidwa kuti chili chonse chomwe ndingapeleke chidzatetezedwa kwambiri. Komanso, ndauzidwa kuti phunziroli ndilovomelezedwa ndi maudindo oyenelera a dziko la Norway ndi Malawi.

Ndauzidwanso kuti ndikhonza kusiya phunziroli ngati ndingafune mtsogolo muno. Ndikudzidwanso kuti chili chonse chomwe ndingapeleke chidzagwiritsidwa ntchito mu phunziro ngati lomweli mtsogolo muno.

Malingana ndi mfundozi ndikuvomeleza izi:

1. Ndikuvomeleza kuti mwakufuna kwanga ndidzatengako mbali mu mphunziroli.

2. Ndikuvomeleza kuti zonse zomwe ndidzapeleka zikhonza kudzagwiritsidwa ntchito pa mphunziro lofanana ndi lino mtsogolo.


Chonde dulani malo onse omwe simupelekapo maganizo anu.

Ndikufuna kudziwa zotsatira za magazi anga inde ayi

Siginature Mboni

Tsiku ndi malo Heidi Arntsen

Wankulu wa kafukufuku
Akuwilanjidwa kuti ajigale nawi mbali mu majiganyo ga:

“Kawungunya jwa winji wa plasma jwa Selenium ni Glutathione mwa achakulungwa wakuloleka kuthula wapi wale wali wa thanzi ku Mangochi m’Malawi.”

Ulwele wa TB uli ulwele wakogoya m’nope waukamula wandu wajinji chachachili chose m’malawi. TB jikusatendekasya kuthula m’nope kuwa jwaganda nambo soni kusowa chilakolako cha yakulya. Yakulya yakupeleka thanzi ili yakusosekwa kuthula chijimbale soni ikusakamuchisya kulamisya chilu ku ilwele.

Jele Seleniumji ni glutathione ili yakusosekwa pa chitete cha m’chilu nambo soni ikusakamuchisya kulamisya wapi wakulwala.

Chakulinga cha gele majiganyoga ni chakuungunya Selenium ni Glutathione mwavelere mwa wandu wakulwala TB wakupochela mtera ni mwa wandu wali wakukamuchisya kuteteya thanzi m’chilu. Gele majiganyoga mpaka gakamulichisidwe masengo mpala mfundo sya majiganyo gakusogolo patichigatendekhawa majiganyo gane gakonjechesyaya.

Mnopem’nope gele majiganyoga tigalole nikuwanichisya saizi ja katupe ka miyasi ga Selenium ni Glutathione mwa wakulwala TB ni mwa wandu wali wa thanzi. Gele majiganyoga tigalole soni kuwanichisya ya katupe kayakulya ya selenium ni sulphur amino acids mwa wakulwala TB ni wandu walla wathani.

Utenga pakajigale ka yakulya tuchijigalidwa kupitila kwausya muso wakupigala nawi mbali ni tachisala yuso ne yaweje alinkulya ni kumwa lisikuli nkaniliwe.

Majiganyoga tigachiwanichisya katupe ka miyasi ka Selenium ni Glutathione ni yakulya yaweje alinkulya ya Selenium ni amino acids.

Chakulinga cha majiganyoga

Chakulinga cha majiganyoga ni chakuti tumanyilire naga wakulwala TB akulembelwa Selenium ni glutathione. Kupelembelwa kwa Selenium ni Glutathione mpaka kusokonesye chitete cha m’chilu kabe kaulewele soni chikamuchisya cha mtera.

Yatupate pelepa, mpaka ipeleche mfundo sya majiganyo gakusogolo patichigatendekhawa. Chinga majiganyo gakonjechesyago tigachijausya msogolo yakuyichisya ya TB kapena kulola naga pana kapeve kambone, yeleyi mpaka tanyi tulese gele majiganyoga.

Wani waliwakusosedwa kuti ajigale mbali?

Wosope wakulwala TB wakupochera mtera kutendiria ijuma iwiri mpaka ijuma msano ni ituta yipiteyo, Wandu wane wasagulidwe wathangi akuwilanjidwa soni kuti ajigale nawi mbali. Wosope wakujigala nawi mbali awe wakutyochele m’boma ja Mangochi.
Utenga kwa wakujigala mbali.


Nganisyo syaawo tisichiwatengali sya asili soni syakuteteleywa.


Gele majiganyoga gali gakwitichisidwa ni wa Ethical Committee ku University ja Oslo ku Norway soni gali gakwitichisidwa ni wa College of Medicine Research in Ethics Committee ku Malawi. Naga akusaka kumanyinlila jejinji wasimane Dr Maleta ku College ja Medicine ku chipatala chaboma ku Mangochi Tel 08 232 202, Stephano Mwaliwa, Tel 09692799.

Utenga payakuyichisya

Patuchimalisya gele majiganyoga (kutandila August 2006 mpaka mu June 2007), tinjilemba m’ndandanda wayakuyichisya soni m’ndandandawo tuchipelechedwa ku Unduna wa za umoyo ni ku mabungwe gane ga za umoyo gatigachiwa gakusangalala ni yakuyichisayo. M’ndandandawo tuchiwa soni wakusimanikwa kwa wosope wakujigala nawo mbali mumajiganyoga.

University of Oslo
Faculty of Medicine
International Health Department
Kusala yakwitika kutenda nawo majiganyo ga

"Kaungunya jwa katupe kaplasma jwa Selenium ni Glutathione mwa achakulungwa wakuloleka kuti akulwala TB ni kateteye ka thanzi ku Mangochi ku Malawi."

Une apano ngusala kuti mbochele utenga wa majiganyo ga: "Kaungunya jwa katupe kaplasma jwa Selenium ni Glutathione mwa achakulungwa wakuloleka kuti akulwala TB ni kateteye ka thanzi ku Mangochi ku Malawi." Asalire chakulungwa cha majiganyo galembele penanipa, nikuti utenga wutimbeleche tuchikamulichisidwa masengo mu gele majiganyoga.

Kupatula pele, asalire kuti utenga utimbeleche tuchiwa wa asili soni wakuteteyedwa. Nambo soni asalire kuti majiganyoga gajitichisidwe ni achakulungwakulungwa wa amaudindo gawo wa ku Norway ni ku Malawi.

Asalire soni kuti mpaka ngomboleche kuleka gele majiganyoga naga ndili sachile kusogolo kuno.

Payosopeyi, une ngusala ayi:
1. Ngwitika mwakulipeleka kuti tinjigale nawo mbali pa gele majiganyoga soni tipachipawa pangali magongo galigose.
2. Ngwitika kuti utenga utimbeleche komboleka kamulichisya masengo mumajiganyo gakulandana ni gelelga kusogolo kuno.
3. Ngwitika kuti mpaka simanidwe nikuwilanjidwa ku kutenda nawo majiganyo mpala gagaga kusogolo kuno.

Chonde afute malo galigose gankanagasaka.

Ngusaka manyilire yakuyichisya ya miyasi jangu. Elo □ Iai □

Wakusaina: Mboni:
Lisiku ni malo: Saini ja wakafukufuku, Heidi Arntsen
Akuwilanjidwa kuti ajigale nayo mbali mu majiganyo ga:

“Kawungunya jwa winji wa plasma jwa Selenium ni Glutathione mwa achakulungwakulungwanga wa m’boma ja Mangochi m’Malawi.”

Yakulya yakupeleka thanzi ili yakusosekwa kuti umi wambone upelenganye, chilu chijimbale soni ikusakamuchisya kulumisya chilu ku ilwele. Jele Seleniumji ni Sulphur amino acids ili yakusosekwa pa chiteteyo cha m’chilu nambo soni ya umi wambone wa wandu.


Mnopem’nope gele majiganyoga tigalole nikuwanichisya saizi ja katupe ka miyasi ga Selenium ni Glutathione. Utenga pakajigale ka yakulya tuchijigalidwa kupitila kwawsya mausyo wakujigala nayo mbali n tachisala yosope yaweleye alinkulya nyumwa lisikuli nkaniliwe.

Majiganyoga tigachiwanichisya katupe ka miyasi ka Selenium ni Glutathione ni yakulya yaweleye alinkulya ya Selenium ni amino acids.

Chakulinga cha majiganyoga


Wani waliwakusosedwa kuti ajigale mbali?

Achakulungwakulungwanga wakwete yaka kutandira 15 mpaka 60 ali wakuwilanjidwa kuti ajigale mbali.

Utenga kwa wakujigala mbali.

Nganisyo syawo tisichiwa sya asili soni syakuteteyedwa.


Gele majiganyoga gali gakwitichisidwa ni wa Ethical Committee ku University ja Oslo ku Norway soni gali gakwitichisidwa ni wa College of Medicine Research in Ethics Committee ku Malawi.

Naga akusaka kumanyinlila jejinji wasimane Dr Maleta ku College ja Medicine ku chipatala chaboma ku Mangochi Tel 08 232 202, Stephano Mwaliwa, Tel 09692799.

**Utenga payakuichisya**

Patuchimalisya gele majiganyoga (kutandila August 2006 mpaka mu June 2007), tinjilemba m’ndandanda wayakuichisya soni m’ndandandawo tuchipelechedwa ku Unduna wa za umoyo ni ku mabungwe gane ga za umoyo gatigachiwa gakusangalala ni yakuyichisyayo. M’ndandandawo tuchiwa soni wakusimanikwa kwa wosope wakujigala nako mbali mumajiganyoga.

University of Oslo
Faculty of Medicine
International Health Department
Kusala yakwitika kutenda nawo majiganyo ga

“Kawungunya jwa winji wa plasma jwa Selenium ni Glutathione mwa achakulungwa wa m’boma ja Mangochi m’Malawi.”

Une apano ngusala kuti mbochele utenga wa majiganyo ga: “Kawungunya jwa winji wa plasma jwa Selenium ni Glutathione mwa achakulungwa wa m’boma ja Mangochi m’Malawi.” Asalire chakulinga cha majiganyo gagalemedwe penanipa, nikuti utenga wutimbeleche tuchikamulichisidwa masengo mu gele majiganyoga.

Kupatula pele, asalire kuti utenga utimbeleche tuchiwa wa asili soni wakuteteyedwa. Nambo soni asalire kuti majiganyoga gajitichisidwe ni achakulungwakulungwa wa amaudindo gawo wa ku Norway ni ku Malawi.

Asalire soni kuti mpaka ngomboleche kuleka gele majiganyoga naga ndili sachiile kusogolo kuno.

Payosopeyi, une ngusala ayi:
1. Ngwitika mwakulipeleka soni mwangasunga nganisyo sine kuti tinjigale nawo mbali pa gele majiganyoga.
2. Ngwitika kuti utenga utimbeleche komboleka kamulichisya masengo mumajiganyo gakulandana ni gelelga kusogolo kumo.
3. Ngwitika kuti mpaka simanidwe nikuwilanjidwa ku kutenda nawo majiganyo mpala gagaga kusogolo kuno.

Chonde afute malo galigose gankanagasaka.

Ngusaka manyilire yakuyichisya ya miyasi jangu.    Elo □     Iai □

Wakusaina:                                            Mboni:

Lisiku ni malo:                                       Saini ja wakafukufuku, Heidi Arntsen
Appendix 3
Questionnaire to TB patients

ID Number:..............
Tuberculosis patient:.............................
Classification of tuberculosis:.............................

Did you experience any of the following symptoms of tuberculosis?
Kodi munamvapo zina mwa zizindikiro izi za TB?
Pakwete pasimene ni yine nwa ilyosyo ayi ya TB?

Cough  
Yes  No
Kukhosomola
Kosomola

If yes, for how many weeks were you coughing until you got the diagnosis? .....weeks
Ngati munamvapo, mwakhala mukukhosomola kwa masabata angati?
Naga elo, watemi ali nkosomola kwa ijuma ilingwa ali wasimene ni wele ulwelewo?

Fever  
Yes  No
Kutentha thupi
Kolesya chilu moto

Weight loss  
Yes  No
Kuwonda
Ganda

Blood in the sputum  
Yes  No
Magazi m’makhololo
Miyazi m’makololo

How many days did it take from you got the diagnosis until you started treatment? Same day □
Panatenga masiku angati kuyambira pomwe Day after □
anakupezani ndi matendawa kufikira pamene .................. Days
munayamba kulantira chithandizo cha mankhwala? .................. Week(s)
Pajigale masiku galingwa kutandire pawasimene ni wele ulwelewa mpaka pawatardire kupochela chikamuchisyo cha mtre?
Appendix 4
Recording form and questionnaire

<table>
<thead>
<tr>
<th>Time</th>
<th>Place eaten</th>
<th>Food or drink, description and cooking method</th>
<th>Amount eaten</th>
<th>Weight equivalent (g)</th>
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</thead>
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<td>English</td>
<td>Chichewa</td>
<td>Chiyao</td>
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<tr>
<td>Was food intake unusual?</td>
<td>Yes  No</td>
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<tr>
<td>Kodi chakudy a chomwe munadya ndichachilendo?</td>
<td>Chakulya chiwali recho chaliji chachilendo?</td>
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<tr>
<td>If yes, how was it unusual?</td>
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<tr>
<td>Ngati chili chachilendo ndi chachilendo bwanji?</td>
<td>Naga elo, chaliji chachilendo chamtuli?</td>
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<tr>
<td>Were you sick yesterday?</td>
<td>Yes  No</td>
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<td>Kodi munadwala dzulo?</td>
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<tr>
<td>Waliji nkulwala liso?</td>
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<tr>
<td>If yes, did sickness affect the appetite?</td>
<td>Yes  No</td>
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<tr>
<td>Ngati munadwala, chilakolako cha chakudy a chinaonongeka?</td>
<td>Naga elo, kulwalako kwatendekasisye kuti akasachiona kunong'a chakulyacho?</td>
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<tr>
<td>If yes, how?</td>
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<tr>
<td>Ngati inde, chinaonongeka bwanji?</td>
<td>Naga elo, chamtuli?</td>
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<tr>
<td>Was it a feast day?</td>
<td>Yes  No</td>
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<td>Kodi linali tsiku la phwando?</td>
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<tr>
<td>Lyaji lisiku lya chisangalalo?</td>
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<tr>
<td>Did you take any tablets or herbs yesterday?</td>
<td>Yes  No</td>
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<tr>
<td>Munamwa mankhwala amapilisi kapena achikuda ena ali wonse dzulo?</td>
<td>Wamwere mtera wamapilisi kapena wachikuda wine wuli wose liso?</td>
<td></td>
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<tr>
<td>If yes, which kind of tablets or herbs?</td>
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<tr>
<td>Ngati inde, munamwa mankhwala amapilisi kapena achikuda anji?</td>
<td>Naga elo, galiji mapilisi kapena ntelachi wachikuda?</td>
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<tr>
<td>Where did you buy them?</td>
<td></td>
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<tr>
<td>Munagula kuti mankhwalawo?</td>
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<tr>
<td>Wagasumile kwapi mapilisigo?</td>
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</tbody>
</table>
EDUCATION/ RELIGION/ WORK

Do you know how to read?  Yes  No
Kodi mumadziwa kulemba?
Akusakombolaga kuwaranga?

How many years at school have you completed?
Kodi munakhala zaka zingati ku sukulu?
Kusukulu wasomile yaka iliungwa?

Which religious group do you belong to?
Ndinu ampingo wanji?
Wawo dini jawo chi?

What is your occupation?
Mumagwira ntchito yanji?
Akusakamula masengo gachi?

If farmer, do you use fertilizer in your field?
Ngati muli Mlimi, mumagwiritsa ntchito feteleza m'munda mwanu?
Naga mlimi, akusatagagafeteleza mum'gunda mwawo?

If yes, what kind?
Ngati inde, feteleza wake wamtundu wanji?
Naga elo, mtunduchi wafetelezajo?

Did you burn your field to clean it?
Kodi munaotcha m'munda mwanu pososa?
Akusajocha m'gunda wawo pakusaka kulangusya?

When do you usually harvest?
Ndi nthawi yanji yomwe mumakonda kukolola?
Mnopemnope akusagoolaga mwezichi?

When does your crop end?
Ndi mwezi wanji womwe zokolola zanu zimatha?
Yagoola yawo ikusamalaga Mwesichi?

POSESSIONS

Does your household have a blanket?  Yes  No
Kodi pakhomo panu pali bulangete?
Ana mnyumba mwawo wana libulangeti?

Does your household have a radio?  Yes  No
Kodi pakhomo panu pali wailesi?
Ana mnyumba mwawo wana wailesi?

Does your household have a mattress?  Yes  No
Kodi pakhomo panu pali matress?
Ana mnyumba mwawo wana matilesi?

Does your household have a bicycle? Yes  No
Kodi pakhomo panu pali njinga yopalasa?
Ana mnyumba mwawo wana Njinga?

Does your household have a TV? Yes  No
Kodi pakhomo panu pali television kapena kanema?
Ana mnyumba mwawo wana TV?

Does your household have a boat for fishing? Yes  No
Kodi pakhomo panu pali bwato?
Ana mnyumba mwawo wana liboti kapena wato wakulajila somba?

Do you have shoes? Yes  No
Muli ndi nsapato?
Akwete sapato?

Does your family own land? Yes  No
Kodi banja lanu lili ndi malo?
Ana liwasa lyawo lyana malo gawogawope?

Does your household have domestic animals? Yes  No
Kodi banja lanu lili ndi ziweto?
Ana nyumba mwawo wana ilango?

If cows, how many?
If goats, how many?
If chicken, how many?
If guineafowl, how many?

HOUSING CONDITIONS

Where do you get your drinking water from? Tap or borhole
Kodi madzi akumwa mumatunga kuti? Unprotected well/lake
Mesi gakumwa akusatecheraga kwapi?

How long does it take you to get to the watersource? < 15 min
Mumatenga nthawi yaitali bwanji kukafika kotunga madzi? 30 min
Akusajenda ndawi jelewu uli kuti akaiché kwakusatecheraga mesiko? 45 min
> 1 hour
Don't know

What kind of flooring is there in your house? Sand/dung or earth
Kodi pansi pa chimbudzi munazila ndi chani?
Pasi panyumba jawo wasyasyajile nich? Wood or cement

What kind of toilet do you use? Traditional pit latrine
Ndi mtundu wanji wa chimbudzi chomwe mumagwiritsa ntchito?
Akusaka mulisya masengo chimbuzi chachi? Traditional pit latrine with san plat
Flush toilet
None
# Appendix 5

## Picture chart

<table>
<thead>
<tr>
<th>Chimanga</th>
<th>Usipa</th>
<th>Mzama/ Sugama</th>
<th>Therere/ Linyololo</th>
<th>Chainisi/ Liponda Lyachisungu</th>
<th>Anyezi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mapeyala</td>
<td>Mkaka</td>
<td>Mazira/ Mandanda</td>
<td>Nthochi/ Magombo</td>
<td>Chinangwa</td>
<td>Mzimbe/ Muwa</td>
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<tr>
<td>Sikono</td>
<td>Malambe</td>
<td>Suga</td>
<td>Mbatata</td>
<td>Utaka</td>
<td>Nsima/ Ugali</td>
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<tr>
<td>Matimati</td>
<td>Mandasi</td>
<td>Mbwanda</td>
<td>Zakumwa/ Yakumwa</td>
<td>Milamba/ Makambale</td>
<td>Mandimu</td>
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<tr>
<td>Item</td>
<td>Image</td>
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<tr>
<td>Nyama ya Mbuzi/ Nyama ja Mbuzi</td>
<td><img src="image" alt="Goat Image" /></td>
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<td>Chambo</td>
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<td>Mtedza/ Mtesa</td>
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<td>Mbatata zakachewere/ Mbatata syachisungu</td>
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<td>Papaya/ Lipapaya</td>
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<td>Kambuzi/ Kambusi</td>
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<td>Malalanje/ Maolanje</td>
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<td>Nyama ya Ng’ombe/ Nyama ja Ng’ombe</td>
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