COMPARISON OF MICRONUTRIENT- INTAKE OF LACTATING MOTHERS FROM THE HLABISA DISTRICT IN KWAZULU-NATAL USING TWO DIFFERENT DIETARY INTAKE METHODS

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Declaration

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ABSTRACT

INTRODUCTION: The objective of this research study was to analyze previously collected dietary intake data using multiple 24-hour dietary recalls and semi-quantitative food frequency questionnaires (FFQ's) in a group of HIV-positive and HIV-negative breastfeeding women from a rural region in KwaZulu-Natal in order to compare the intake of selective micronutrients obtained with the two instruments. Identifying the pattern of food intake and the contribution of different foods to the micronutrient intake in this population group will contribute to possible recommendations aimed at dietary changes to improve dietary micronutrient intake. This study was designed as a sub-study of a longitudinal prospective cohort study and subjects (N=108) were lactating mothers enrolled in a cohort which investigated the combined effect of HIV-infection and breastfeeding on women's nutritional status.

METHOD: A locally constructed FFQ and 24h-recall were used to collect dietary intake data from 108 subjects on three occasions, (~6 weeks, 14- and 24-weeks post partum). Analysis was done using the Food Finder Program™2. Micronutrients under investigation were iron, zinc, copper, selenium, vitamin A, B6, C, D and E, thiamin, riboflavin and folic acid and were selected on their relevance in HIV (AIDS).

Descriptive statistics was used to determine the consumption of food items as percentage of all food items consumed and to calculate mean, mode, median and range of serving sizes for the ten food items most frequently consumed (measured with the 24h and FFQ respectively). Data was not normally distributed (indicated by the paired t-test and confirmed with a RM ANOVA nonparametric test). The F-value was determined (using Wilcoxon matched pairs test) and the significance of the difference between the micronutrient intakes measured with the two instruments ($p<0.05$) calculated. To investigate the strength of the correlation between the two dietary intake measures, Spearman's correlation coefficients were determined for the nutrients under investigation. The significance level for these measurements was 95% ($p<0.05$).

RESULTS: Both methods identified maize meal and *mahewu*, bread, chicken, dried beans, cabbage, onion, bananas, oranges and green leaves as the foods most often consumed. Bread, dried beans, maas, pilchards, mango and green wild leaves were the foods that contributed the most to the micronutrients under investigation. Although maize meal (in the form of *phutu* or *mahewu)* was the food item most frequently consumed in large portions, it was not in the top ten food items for any micronutrient contribution, except for selenium. Correlation coefficients (unadjusted for energy) in this study were very poor, ranging from 0.038 for vitamin B12 up to 0.48 for iron. All correlations (except vitamin B12) were poor but significant ($p<0.05$).

CONCLUSION: There was some agreement found in the type of foods most frequently consumed and their contribution to the micronutrient intake of this population group, when using three 24h-recalls and FFQ's and therefore in describing the habitual food intake of the population group. There was however no agreement between the micronutrient intake measured with three 24h-recalls and three FFQ's ($p<0.05$). Further analysis of the data and comparisons with the biochemical results reported in another study, is recommended.

OPSOMMING

INLEIDING: Die doel van hierdie navorsing was om voedsel-inname data (vooraf ingesamel met die veelvuldige 24h-heroep metode en Voedsel Frekwensie Vraelyste) te analiseer Die data was ingesamel onder ‘n groep MIV-positiewe en MIV-negatiewe borsvoedende moeders vanuit ‘n plattelandse distrik in Kwa-Zulu Natal met die doel om die inname van selektiewe mikronutriente te vergelyk wanneer dit gemeet word met behulp van ‘n 24h-heroep en met ‘n Voedsel Frekwensie Vraelys (VFV). Die identifisering van die dieetpatroon en die bydrae wat die verskillende voedsels wat geëet word maak tot die mikronutriënt inname van hierdie studiegroep, kan help om aanbevelings te maak in die dieet ter verbetering van die mikronutriënt inname. Die studie was ontwerp as ‘n sub-studie van ‘n groter longitudinale prospektiewe kohort en die proefpersone (N=108) was lakterende moeders wat deel was van ‘n studie wat die gekombineerde effek van MIV-infeksie en borsvoeding op die voedingstatus van vroue, ondersoek het.

METODE: ‘n Plaaslik gekonstruktureerde VFV en ‘n 24h-heroep vraelys was gebruik om dieet-inname data van 108 proefpersone te versamel tydens 3 geleenthede naamlik (~6 weke, 14- 24-weke postpartuum). Analise was gedoen met die *Food Finder Program™2* nutriëntanalise program. Mikronutriente wat ondersoek is, sluit in yster, koper, sink, selenium, vitamiene A, B6, C, D en E, tiamien, riboflavin en foliensuur, almal gekies op grond van hul relevansie in MIV (VIGS).

Beskrywende statistiek is gebruik om die verbruik van voedselitems as persentasie van all voedsel verbruik, uit te druk en om die gemiddeld, modus , mediaan en reikwydte van porsiegroottes vir die tien voedselsoorte wat die meeste verbruik was (soos bepaal deur die 24h-herroep en VFV) te bereken. Data was nie normaal verdeel nie (soos uitgewys deur die gepaarde t-toets en bevestig is met RM ANOVA nonparametriese toetse). Die F-waarde is bepaal (met behulp van die *Wilcoxon* gepaarde toets) en die betekenisvolheid van die verskil tussen die metings soos verkry deur die twee metodes, bereken ($p<0.05$). Korrelasie sterkte tussen die twee

metodes se meting van mikronutrientinname, is ondersoek met behulp van die *Spearman's* korrelasie koeffisiente. Die betekenisvolheidsvlak vir hierdie meetings was 95% ($p<0.05$).

RESULTATE: Beide metodes het mieliemeel en *mahewu*, brood, hoender, droë bone, kool, uie, piesangs, lemoene en groen blaargewasse (van verskillende wilde plante) geidentifiseer as die voedsels wat mees algemeen gebruik word. Brood, droë bone. maas, sardyne, mango en wilde groen blare was die voedsels wat grootliks bygedrae het tot die mikronutriënte wat ondersoek is. Hoewel mieliemeel (in die vorm van *phutu* of *mahewu*) die voedselsoort is wat die hoogste frekwensie van verbruik gehad het en in die grootste hoeveelhede geëet is, was dit nie een van die tien voedselsoorte wat die grootste bydrae gelewer het tot die mikronutriënt inname nie. Korrelasie koëffisiënte (nie aangepas vir energie) verkry in die studie was baie swak en het gestrek vanaf 0.038 vir vitamin B12 tot by 0.48 vir yster. Alle korrelasie, behalwe vit vitamiene B, was baie swak maar betekenisvol ($p<0.05$).

GEVOLGTREKKING: Daar was 'n mate van ooreenstemming tussen die tipe voedsels wat mees algemeen gebruik is en hul bydrae to die mikronutriënt inname onder hierdie populasie groep wanneer die drie 24h-herroepe en VFV met mekaar vergelyk word betreffende hul vermoë om die gewoontelike eetpatroon van hierdie groep te beskryf. Daar was egter geen ooreenstemming in die mikronutriënt inname soos gemeet deur die 24h-herroep en met die VFV ($p<0.05$). Verdere analise van die data en 'n vergelyking van die bevindings met die biochemiese waardes wat in 'n ander studie bepaal is, word aanbeveel.

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LIST OF ABBREVIATIONS

AI	average intake
AIDS	acquired immunodeficiency syndrome
Cu	copper
DRI's	dietary reference intakes
EAR's	estimated average intakes
Fe	iron
FFQ	food frequency questionnaire
HIV	Human Immunodeficiency Virus
HIV/AIDS	refers to HIV infection at any stage of the disease
mg	milligram
µg	microgram
MNS	maternal nutrition study
NAIDS	Nutritionally Acquired Immune Deficiency Syndromes
RDA's	recommended dietary allowances
ROS	reactive oxygen species
SD	standard deviation
Se	selenium
tprot	total protein
UKZN	University of KwaZulu Natal
Vit A	Vitamin A
Vit B12	Vitamin B12
Vit B6	Vitamin B6
Vit C	Vitamin C
Vit E	Vitamin E
yr	years
Zn	zinc

LIST OF DEFINITIONS

AIDS: Acquired immune deficiency syndrome or acquired immunodeficiency syndrome (AIDS or Aids) is a collection of symptoms and infections resulting from the specific damage to the immune system caused by the human immunodeficiency virus (HIV) in humans, and similar viruses in other species. The late stage of the condition leaves individuals prone to opportunistic infections and tumors. Although treatments for AIDS and HIV exist to slow the virus progression, there is no known cure.[1]

HAART: Current treatment for HIV infection consists of highly active antiretroviral therapy, or HAART. Current optimal HAART options consist of combinations (or "cocktails") consisting of at least three drugs belonging to at least two types, or "classes," of anti-retroviral agents. Typical regimens consist of two nucleoside analogue reverse transcriptase inhibitors (NARTIs or NRTIs) plus either a protease inhibitor or a non-nucleoside reverse transcriptase inhibitor (NNRTI).[2]

CD4+-COUNT: AIDS is the most severe acceleration of infection with HIV. HIV is a retrovirus that primarily infects vital organs of the human immune system such as $CD4^+$ T cells (a subset of T cells), macrophages and dendritic cells. It directly and indirectly destroys $CD4^+$ T cells. $CD4^+$ T cells are required for the proper functioning of the immune system. When HIV kills $CD4^+$ T cells so that there are fewer than 200 $CD4^+$ T cells per micro liter (μL) of blood, cellular immunity is lost, leading to the condition known as AIDS. Acute HIV infection progresses over time to clinical latent HIV infection and then to early symptomatic HIV infection and later to

AIDS, which is identified on the basis of the amount of $CD4^+$ T cells in the blood and the presence of certain infections.[3]

WHO DISEASE STAGING SYSTEM FOR HIV INFECTION AND DISEASE*:* Infections and conditions grouped together by the World Health Organization (WHO) in 1990 to introduce a staging system for patients infected with HIV-1.[4]

- *Stage I:* HIV infection is asymptomatic and not categorized as AIDS
- *Stage II:* includes minor mucocutaneous manifestations and recurrent upper respiratory tract infections
- *Stage III:* includes unexplained chronic diarrhea for longer than a month, severe bacterial infections and pulmonary tuberculosis
- *Stage IV:* includes toxoplasmosis of the brain, candidacies of the esophagus, trachea, bronchi or lungs and Kaposi's sarcoma; these diseases are indicators of AIDS.

AFRICA CENTRE FOR HEALTH AND POPULATION STUDIES: The Africa Centre for Health and Population Studies is a joint initiative of the University of KwaZulu-Natal and the South African Medical Research Council, with support from the Wellcome Trust and other international funders, to create a global centre of research excellence in a rural area. The Centre's mission is to conduct in partnership with the community, policy relevant health and population research in an ethical manner, and to enhance the capacity of the people of sub-Saharan Africa to conduct research.

VTS: **VERTICAL TRANSMISSION STUDY:** Africa Centre Vertical Transmission Study (VTS): study to investigate the relationship between exclusive breastfeeding and HIV transmission from mother-to-child.

MNS: **MATERNAL NUTRITION STUDY:** sub-study of the larger VTS, studied body composition changes in HIV-infected South African breastfeeding mothers.

CHIEF INVESTIGATOR OF MNS: Study leader of the Maternal Nutrition Study (MNS)-PhD study conducted by dietitian from University of California Davis, USA who received funding for a study on the body composition changes in HIV infected South African breastfeeding mothers.

INVESTIGATOR OF THIS STUDY (DIETARY INTAKE STUDY): Author of this thesis, South-African registered dietitian employed by the chief investigator of the MNS, in the capacity of research assistant responsible for the dietary intake research of the MNS and supervision of the nutrition assistants working on the MNS.

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CHAPTER 1: INTRODUCTION AND STATEMENT OF PROBLEM

1.1 Introduction

More than 36 million persons were infected with the human immunodeficiency virus (HIV) by the end of 2006, and 5.3 million new cases were identified during the past year.[5] Of the estimated 17 million HIV-infected women aged 15-49 years, 77% reside in sub Saharan Africa. About 95% of HIV infections are found in developing countries; 15-30% of women attending prenatal clinics in urban centers of sub-Saharan Africa, are infected with HIV.[6] Vertical transmission rates of HIV are estimated to be 10% higher in developing countries compared with industrialized countries.[7]

Malnutrition is a cardinal clinical manifestation of acquired immunodeficiency syndrome (AIDS), but even early asymptomatic human immunodeficiency virus type 1 (HIV-1) infection may lead to impaired nutritional status.[8] Malnutrition is not only the result of HIV infection itself but also of the associated complications. Many studies have shown that the development of malnutrition is multi-factorial, and that the relevant pathogenic mechanisms are influenced by disease stage as well as by the nature of specific disease complications, which may lead to alterations in energy intake, nutrient absorption or energy expenditure. (Fig 1.1)[9,10,11]

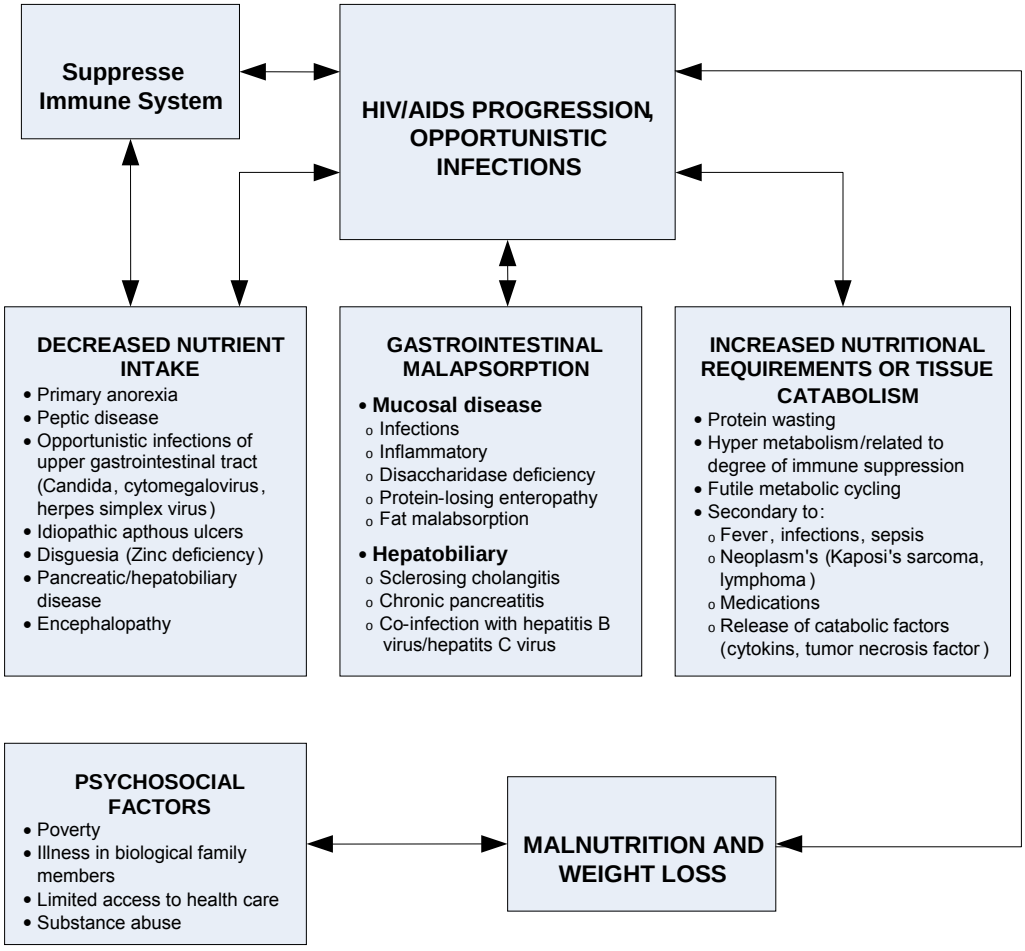


Figure 1.1: Causes of nutritional deficiencies and wasting in HIV/AIDS[12]

Reduction in food intake is believed to be an important cause of the slow and progressive weight loss experienced by people living with HIV/AIDS.[13] Reduction in food intake may be due to sores in the mouth, pharynx, and/or esophagus, fatigue, depression, changes in mental state and other psychological factors that may play a role by affecting appetite and interest in food. Additional factors include personal and family finances that affect food availability and the nutritional quality of the diet as well as side effects from medication, including nausea, vomiting, metallic taste, diarrhea, abdominal cramps and anorexia.[14] Nutrient malabsorption accompanies frequent bouts of diarrhea due to giardia, cryptosporidium and other pathogens affecting people with a compromised immune system and increased intestinal permeability or direct damage of the enterocyte by the infection.[15] Although reduced food intake commonly drives wasting, several metabolic features of AIDS are more consistent with a cachexic type response and may be counter-regulatory. The adaptive reduction in resting energy expenditure seen in reduced food intake is not observed in AIDS wasting. In addition whole body protein is markedly increased, a phenomenon observed in other inflammatory states and this itself may be energy costly.[16]

The effects of an infection are mediated via the acute phase response and localized lesions, leading to reduced intake and absorption and increased utilization and loss of micronutrients (Figure 1.2).[20] The basic nutritional and metabolic disturbances that lead to weight loss and wasting in HIV-infected persons may represent an adaptive response to an inflammatory state.[13,14,15,17] Pro-inflammatory cytokine concentrations are significantly higher in HIV-positive persons than in HIV-negative persons. Elevated concentrations of interleukin 6 and tumor necrosis factor (TNF) have been associated with higher HIV viral loads, and TNF-ά interferon γ can inhibit myosin expression in muscle cells and induce anorexia.[20] Elevated cytokines may also contribute to the chronic oxidative stress observed in HIV-positive persons, which could lead to HIV disease progression through impairment of immune function, enhancement of HIV replication, or both.[17] Nutritional and metabolic disturbances can also lead to altered acute phase response proteins in response to acute or chronic inflammation, which have been observed in persons with advanced HIV disease.

Changes in acute phase response proteins, mainly decreased albumin and elevated C-reactive protein concentrations, have been shown to be associated with low serum concentrations of several micronutrients in HIV-negative persons[18] and with low serum concentrations of vitamin A and selenium in HIV-positive persons not receiving Highly Active Antiretroviral Therapy (HAART).[19]

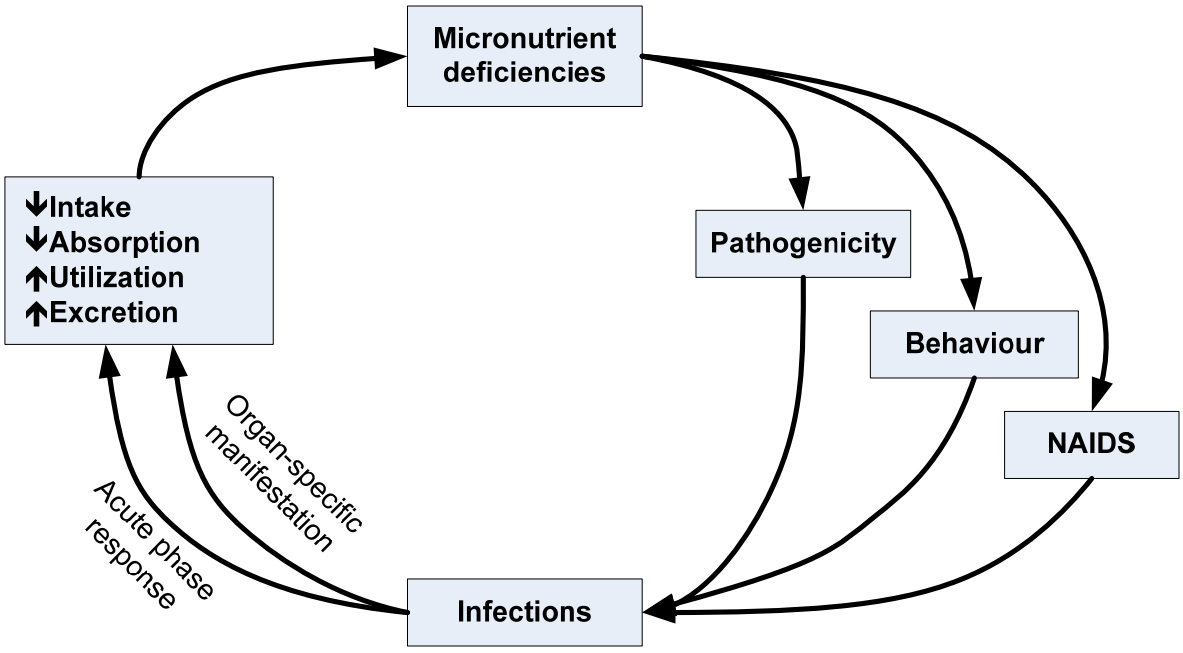


Figure 1.2: The two-way relationship between micronutrient deficiencies and infections.[20]

1.1.2 Effects of HIV infection on micronutrient status.

HIV infection is characterized by an acute syndrome accompanying the primary infection, followed by a prolonged asymptomatic state eventually leading to advanced HIV disease. During the asymptomatic period the viral load slowly increases and the CD4+ count declines. After a number of years, opportunistic and other infections become increasingly frequent. The length of the asymptomatic period and the type, timing and frequency of the subsequent infections may vary depending on general health and exposure to pathogens.[20]

Little acute phase response occurs during the long asymptomatic stage of HIV

infection, but viral replication occurs continuously. Changes in the structure and function of the intestinal tract seem to occur relatively early in HIV infection. An HIV enteropathy characterized by villous atrophy and crypt hyperplasia, accompanied by malabsorption, have been described in HIV-positive individuals. Reduced absorption likely leads to impaired micronutrient status at this stage, which may be important because of the stage's long duration. During symptomatic HIV infection, the effects of HIV in the gastrointestinal tract are more severe. The increasingly frequent enteric and other infections result in both acute phase and localized lesions which further exacerbate an impaired micronutrient status.[20,21]

A micronutrient deficiency may affect the risk of infection with a specific agent as well as the severity of the infectious disease morbidity.[20] These effects are mediated via pathogenicity of the infectious agent, host risk behavior or the host defense and may be either synergistic or antagonistic.[20] A synergistic relationship exists when a specific micronutrient deficiency increases infectious disease morbidity, in which case either improved micronutrient intake or treatment of the infection will break the vicious cycle. An antagonistic relationship exists when a specific micronutrient deficiency reduces - or increased intake increases - infectious disease morbidity.[9,10] A micronutrient may also act synergistically in moderate doses but antagonistically in high doses.

Micronutrient deficiencies induce a wide array of immunologic alterations resulting in the progressive development of opportunistic infections and malignancy, which results in AIDS.[21,22] Micronutrient deficiencies are prevalent in many HIV-infected populations, and studies have reported that these deficiencies impair immune responses, weaken epithelial integrity, and are associated with rapid disease progression and mortality and may affect transmission as well as clinical course of HIV infection.[23,26] These effects may be mediated through effects on immune-function, as well as on viral replication and pathogenicity.[8] Of the mechanisms contributing to this progression, oxidative stress induced by the production of reactive oxygen species (ROS) may play a critical role in the stimulation of HIV replication and the development of immunodeficiency.[22]

Protective relationships between micronutrient status and HIV vertical transmission have been reported in a number of epidemiologic studies.[21,23,25] A randomized trial of multivitamin supplements and HIV disease progression in Tanzania significantly delayed the progression of disease among HIV-infected pregnant women, as reflected by the reduced relative risk of progression to WHO stage 4 or death from AIDS-related causes. In this study, multivitamin supplementation also resulted in significantly higher CD4+ and CD8+ cell counts and significantly lowers viral loads.[24] Earlier studies in Tanzania indicated that multivitamin supplementation also resulted in a significant reduction in risk of fetal loss (39%) and low birth weight (40%).[25]

Zinc, selenium, iron, copper, vitamins A, C, E, pyridoxine and folic acid all have important roles in the immune system and immune responses.[26] Vitamin A plays an essential role in vision and various systemic functions, including normal cell differentiation and cell recognition, growth and development, bone development, immune function and reproduction. Considerable evidence shows that vitamin A enhances phagocytosis and cell-mediated killing. Vitamin A deficiency is associated with decrease in the in-vitro proliferate response of splenic lymphocytes to mitogens as well as reduction in the delayed-type hypersensitivity. Provitamin A carotenoids enhances T- and B-cell immunity by acting as antioxidant or by conversion to vitamin A. Low blood levels of Vitamin A are associated with accelerated disease progression and increased mortality in HIV-infected adults.[8,27,28] In addition low concentrations of vitamin A may be associated with increased mother-to-child transmission of HIV, higher infant mortality and child growth failure In a prospective study among HIV-infected men in the United States, a U-shaped relationship was however observed between dietary vitamin A intake and the risk of progression to AIDS and mortality, which suggests that men with higher or lower levels of intake were at higher risk than men who consumed moderate amounts of vitamin A.[40] It was suggested that vitamin A supplements may increase the risk of transmission of HIV-1 by enhancing the differentiation of myeloid and lymphoid cells, which is associated with an increased expression of CCR5 receptors that increase the susceptibility to HIV-1 infection.[29] Despite the biological plausibility of a role for vitamin A in epithelial integrity, no data

support the hypothesis that increased vitamin A intake or status reduces susceptibility to infection through sexual transmission.[20] Although low concentrations of vitamin A may be associated with increased mother-to-child transmission of HIV, higher infant mortality and child growth failure, maternal vitamin A supplementation during pregnancy and postpartum was not found to decrease mother-to-child transmission in two trials, one in South-Africa and the other in Malawi.[30,31] Furthermore in a Tanzanian trial, vitamin A supplementation increased risk of HIV-shedding in cervico vaginal lavage and mother-to-child HIV-transmission.[25] Despite the lack of a positive effect of vitamin A supplementation in adults, the situation seems to be different in children who are more likely to have sub-optimal vitamin A status. Regular mega doses of vitamin A to HIV-positive children under 5 years of age have shown to reduce diarrheal morbidity and AIDS-specific mortality and all-cause mortality.[32]

An increase in oxidative stress due to a weakened antioxidant defense system has been reported in HIV positive patients.[22] The antioxidant system depends first on the integrity of an enzymatic system that requires adequate intake of trace minerals such as selenium, copper zinc and manganese, and second on adequate concentrations of vitamin E, A and C, and β-carotene in the cytoplasm and lipid membrane of the cells.[22] Vitamin E is the most important lipid-soluble in the cell membranes and is an important component of the cellular antioxidant defense system, which involves other enzymes, many of which depend upon adequate levels of other antioxidants Therefore the antioxidant function of vitamin E can be affected by the levels of other nutrients including zinc, selenium, copper and vitamin C.[8] Apart from its antioxidant role, vitamin E also influences the function of T cells, B cells and phagocytic cells and may protect immune effector cells against oxidative stress. Several immune parameters correlate with vitamin E deficiency, causing the host to be susceptible to opportunistic infections and development of tumors.[38] In vitro experiments have demonstrated that the production of reactive oxygen species can specifically activate the transcription factor nuclear factor (NF)-ϰB to induce the expression and replication of HIV.[33] Vitamin C is the major water-soluble antioxidant and act as first defense against ROS in whole blood and plasma.[34] In addition a cooperative interaction exists

Vitamin B6 (pyridoxine) is a coenzyme in numerous enzyme reactions particularly amino acid transport and metabolism and has a direct effect on immune system through its role in protein and nucleic acid synthesis. A study in HIV positive patients reported that vitamin B6 deficiency is associated with a reduced lymphocytic response to mitogens and natural killer cell cytotoxicity, but lymphocyte counts and serum antibody concentrations did not vary by vitamin B6 status.[23] Vitamin B6 deficiency was shown to be common in CDC stage III HIV infected persons with adequate nutrition.[36] In a study done by Montero-Atienza et al.[37] overt vitamin B6 deficiency was documented in 35% of the HIV infected subjects while an additional 12% had a marginal vitamin B6 status. When the relationship between vitamin B6 status and immune function was examined, a significant correlation between deficient vitamin B6 status and numbers of both CD4 and CD8 cells as well as the CD4/CD8 ratio, were seen. In this same study, normalization of vitamin B6 status resulted in a significant improvement in both CD4 cell number and in the functional parameters of immunity such as response to mitogens. Vitamin B12 is a coenzyme involved in transmethylation from methyfolate to homocysteine; released unmethylated folate becomes available for nucleic acid synthesis. Deficiency of vitamin B12 is common in HIV infection and its prevalence varies between 10 and 35%, depending on the stage of the disease.[21] As with other micronutrient deficiencies in HIV infected patients, vitamin B12 deficiency could result from decreased intake or possible malabsorption due to direct infection of the ileum.[38] Decreased vitamin B12 serum levels cause metabolic and clinical disturbances including lowered hemoglobin, leukocytes, and the ratio of CD4/CD8 lymphocyte counts in HIV infected patients compared to those with normal serum vitamin B12 levels.[38] Neutrophil function was also reduced in

clinical studies of vitamin B12 deficiency and vitamin B12 supplementation improved antibody immunity and mitogenic responses in animal and in vitro studies. Riboflavin (B2) deficiency impairs the ability to generate humoral antibodies in response to antigens.[23]

Copper (Cu) can work as a passive virus inhibitor by blocking the intracellular activation of essential protein-splitting enzymes such as HIV protease. HIV-1 intergrase is required for the integration of a double-stranded DNA copy of the viral DNA genome into a host chromosome and for HIV replication. The enzyme for both integration and disintegration can be inhibited by cuprous complexes in a non-competitive fashion with respect to substrate DNA.[38] The concentration of micronutrients like copper (Cu) and zinc (Zn) normalizes after supplementation, yet the amounts required to maintain normal serum concentrations suggest a persistent intracellular deficiency, possibly correlating to poor absorption, low dietary intake, vomiting, diarrhea or even sequestrating by the human immunodeficiency virus. Zinc deficiency has been shown to impair a variety of immune functions including decrease in lymphocyte counts, loss of T-helper cell function, decrease T-lymphocyte killer activities, delayed zinc dermal hypersensitivity responses and decreased humoral and cell-mediated immunity.[39] Zinc also inhibits the production of tumor necrosis factor, which is implicated in the pathophysiology of cachexia and wasting in acquired immune deficiency syndrome.[39] Observational studies that examine the relations between zinc status and HIV-related outcomes provided conflicting results. Higher levels of zinc intake were associated with significantly faster disease progression and higher mortality among men in a prospective cohort study of asymptomatic HIV-infected men in the United States.[40] In another U.S. study however, plasma levels of zinc were inversely associated with mortality. Evidence to date indicates that adequate amounts of zinc are essential to maintain the integrity of the immune system in HIV-infected individuals who are a population particularly susceptible to zinc deficiency. On the other hand, excessive zinc supplementation may stimulate HIV-1.[36]

Selenium has a major function as part of glutathione peroxidase which reduces cellular peroxides to H_2O and alcohol and prevents oxidative damage to proteins, lipid, lipoproteins and DNA. Selenium deficiency inhibits neutrophil function, the cytoxicity of T-lymphocytes and natural killer cell, lymphocyte proliferation in response to mitogens, the DTH response, antibody production and resistance to pathogens.[23,40,41] An adequate selenium status may support humoral and cell-mediated immunity.[41] In a prospective study among 949 HIV-1 infected pregnant women in Tanzania, Kupa et al.[41] examined the association between plasma selenium levels and survival and CD4 counts over time. In this study lower plasma selenium levels were significantly associated with an increased risk of mortality. Recent studies have demonstrated that not only is the host immune response affected by the deficient diet, but the viral pathogen itself can be altered.[42] Dietary deficiencies that lead to oxidative stress in the host, e.g. selenium deficiency, can alter a viral genome such that a normally benign or mildly pathogenic virus becomes highly virulent in the deficient, oxidative stressed host. Once the viral mutations occur, even hosts with normal nutrient intake can be affected by the newly pathogenic strain.[43] A deficiency of selenium in China was found to lead to a cardiomyopathy characterized by necrotic lesions throughout the myocardium with varying degrees of cellular infiltration and calcification, known as Keshan disease.[44] The discovery that this juvenile cardiomyopathy disease likely has a dual etiology that involves both a nutritional deficiency of selenium as well as an infection with an enterovirus, provided the impetus for additional studies of relationships between nutrition and viral infection.[43] These studies shown an amyocarditic strain of coxsackievirus, converted to virulence when it was inoculated into selenium deficient mice. The conversion was accompanied by changes in the in the viral genome in a segment previously thought to be relatively stable.[43] The importance of this essential micronutrient in HIV-1-related survival suggests that selenium supplementation may be immunorestorative and of therapeutic benefit in persons with HIV infection or AIDS, particularly when antiretroviral treatment are not readily available.[45] High levels of antioxidants however may interfere with the host's oxidative processes and must be carefully

monitored.[46]

1.1.3 Effect of antiretroviral therapy on micronutrients

With the transition to more universal access to highly active antiretrovirals (HAART), a better understanding of micronutrient deficiencies and the role of micronutrient supplements in HIV-persons receiving HAART, has become a priority. A small number of observational studies have suggested that some, but not all, micronutrients may become replete after HAART initiation, and few intervention studies have found that certain micronutrients may be beneficial adjunct to HAART.[47-48] Although HAART has been shown to be associated with a decreased prevalence of opportunistic gastrointestinal diseases[49] and incidence of malnutrition,[50] gastrointestinal infections and severe gastroenteritis, which alters micronutrient absorption, may persist after HAART initiation.[51] Several HIV medications can inhibit the replication of mitochondrial DNA and cause vomiting and diarrhea that can reduce the absorption or increase the losses of several micronutrients.[52] Mitochondrial dysfunction may be responsible for HAART – associated lipodystrophy.[53] Individuals receiving antiretroviral therapy for the treatment of HIV-1 infection have also experienced peripheral lipoatrophy, gain in visceral fat, hyperlipidemia and insulin resistance.[54]

Nutritional status should be assessed at regular intervals as part of management HIV-infection. Measurement of dietary intake is part of the comprehensive nutritional assessment needed in HIV, including anthropometric measurements of body composition, biochemical measurements of metabolic parameters and clinical assessment of altered nutritional requirements.[55]

1.1.4 Dietary assessment

As is apparent in historical and recent literature, the measurement of dietary intake to determine nutritional status is an activity that is fraught with difficulties. Over the past decade there have been some spirited exchanges between scientists over the use of food frequency questionnaires in nutritional epidemiological research.[56-57] It is now recognized that errors are inherent in any assessment of dietary status, and that no

measure of diet will convey the truth about any individual, household, or national food consumption or nutrient intake. There is perhaps no other epidemiologic discipline that has attracted as much public attention and, at the same time, as much scientific criticism as has dietary epidemiology. That is because the exposure is both of immediate interest to the public and notoriously difficult to measure.[58]

Dietary assessment instruments however are important tools in epidemiological studies investigating the relationship of diet and disease, dietary intervention trials, evaluation of supplemental food programs and a variety of other research areas, including studies on the effect of HIV and HIV treatment on groups within a population.[59,60] Three types of information may be provided with dietary assessment methods; the estimate of individual intakes, the ranking of individuals on the basis of their food and/or nutrient intakes within a group and the average intake of the group.[61]

Methods available for dietary assessment range from quantitative approaches that usually involve the weighing of food consumed, to qualitative approaches such as dietary histories. Instruments used most frequently include the dietary record approach where recorded foods are weighed and measured for two or more days, dietary history, 24-hour dietary recall and food frequency questionnaires. The 24-hour recall and food record methods are based on foods and amounts actually consumed by an individual on one or more specific days, while food frequency questionnaires (FFQ's) and diet histories, are based on an individual's perceptions of usual intake over a less precisely defined period of time.[62]

No ideal standard method for evaluating dietary intake of a population has been found. Use of the method that best suits the purpose and objectives of the study is acceptable.[62] The selection of the dietary intake instrument to use, depends on the objectives of the study, the foods or nutrients of primary interest, the need for group versus individual data, the need for absolute versus relative intake estimations, characteristics of the population (for instance age, sex, education/literacy, motivation, socio-cultural diversity), the time frame of interest, the level of specificity needed for describing foods and available resources.[57] Each assessment instrument used should

be validated by some method to minimize errors of reported dietary data.

The advantages and disadvantages (Table 1.1) of the different dietary intake instruments should be considered together with all the above mentioned aspects.[62]

Table 1.1: The advantages and disadvantages of the different dietary intake instruments

Instrument	Advantages	Disadvantages
Food record	Intake quantified Could enhance self monitoring for weight control	High investigator cost High respondent burden Extensive respondent training and motivation required Many days needed to capture individual's usual intake Affects eating behavior Intake often underreported Reports of intake decrease with time May lead to substantial bias
24-Hour recall	Intake quantified Appropriate for most populations: low sample bias Relatively low respondent burden Does not affect eating behavior	High investigator cost Many days needed to capture individual's intake Intake often underreported
Food Frequency Questionnaire	Usual individual intake asked Information on total diet obtained Does not affect eating behavior Low investigator burden Administration by non-professionals Relatively inexpensive Relatively low respondent burden	Not quantifiable precise Difficult cognitive task for respondent Intake often misreported
Brief instruments	Usual individual intake often asked Low investigator cost Low respondent burden Does not affect eating behavior	Not quantifiable precise Assessment limited to small number of nutrients/foods Intake often misreported
Diet History	Usual individual intake asked Information on total diet obtained Information often available on foods consumed by meal	Not quantifiable precise Difficult cognitive task for respondent Intake often misreported

	Can have low investigator cost Does not affect eating behavior	Can have high investigator burden

The semi-quantitative food frequency questionnaire has emerged as a useful tool to assess diet in large scale epidemiologic studies and of all methods the FFQ is most frequently used. The underlying principle of the food frequency approach is that average long-term diet is conceptually an important exposure rather than intake on a few specific days.[63] Although subject to important limitations and debate about their appropriate use, food-frequency questionnaires are commonly used in epidemiological research on diet and disease to assess the usual food or nutrient intakes of individuals. In contrast, the 24-hour dietary recall does not characterize an individual's usual diet and tends to underestimate intakes while the FFQ method provides an estimate of the usual intake of an individual over a given period. It may be used to rank individuals according to usual intake within the population.[56,64] Various other studies also report that FFQ's can reliably and accurately measure usual intake of nutrients amongst a group from a population.[65,66]

The 24-hour dietary recall usually involves a face-to-face interview using directed and open-ended questions. Due to intra-individual variability, a single 24-hour recall does not represent the usual individual intake but it characterizes the average intake of a group or population.[70] The interviewer's skill and experience contributes to the reliability of the collected data. There is no literacy requirement of the respondent and the respondent burden is relatively small. The interview is open-ended and the procedure does not alter food intake pattern. The principal limitation of the 24-hour dietary recall is that it does not provide a reliable estimate of an individual's intake due to day-to-day variation. A single 24-hour dietary recall from each subject cannot be used to rank subjects reliably.[57]

1.1.5 Validation of dietary assessment

Validity is an expression of the degree to which a measurement is a true and accurate measure of what it proposes to measure. Establishing validity requires a true external reference measure, an absolute standard, against which the measurement can be

compared. In nutrition no such reference measure exists. Every measurement of dietary intake includes an element of bias therefore only the relative validity of a measurement can be assessed by comparing the results obtained with what are believed to be more accurate measures of food or nutrient intake.[67]

In contrast to the potential for correlated errors associated with the different dietary assessment methods, errors in the estimation of nutrient status from dietary and biochemical measurers are much more likely to be independent. Comparing such dissimilar methods does not however allow for direct validation, one method measures intake and the other measures circulating concentrations that are influenced not only by intake but also by a number of physiological and environmental factors.[68] The use of biochemical markers to validate a measure of intake is based on the assumption that the biochemical indicators are responsive to intake in a dose-dependant manner. Biological measures of nutrients in tissues may not accurately and reliably reflect dietary intake because of complex mechanisms that regulate or enhance absorption of nutrients in circulation.[68] The effect of inflammation on micronutrient status has been recognized for many decades and the characteristics of the biochemical and immunological response to infection are now reasonable well characterized and described as the acute phase response.[69] Low levels of micronutrients are frequently described also in sub-clinical infection, as seen on many studies on HIV subjects.[68]

1.1.6 Comparison of FFQ and 24-hour recall

Diet records represent the best comparison method as they are open-ended, do not depend on memory and allow direct assessment of portion sizes. If literacy levels or subject co-operation mean that dietary record keeping is not feasible, multiple 24-hour recalls are the second choice.[65] The advantages of using the FFQ as dietary assessment method in a large rural population were discussed earlier.

Previous comparisons of the Food Frequency Questionnaire (FFQ) and the 24-hour dietary recall have shown conflicting results. Several studies in specific populations

have also shown that the FFQ obtain higher energy and nutrient estimates than the 24-hour dietary recall, whereas studies in other populations have shown the opposite. Still other studies have shown no real differences between methods and demonstrated reasonable levels of validity for FFQ's developed for specific groups.[70-71]

There are limitations to comparing a FFQ with multiple 24-hour dietary recalls. The FFQ captures fewer frequently eaten foods; however, portion size categories are limited. In contrast, the 24-hour dietary recall is less prone to response modification bias, but it only reflects the time period during which the recalls are collected, and is dependant on memory.[64]

1.2 Rationale and Significance of the Study

There is wide consensus that an inadequate dietary intake may contribute to the poor micronutrient status prevalent in HIV disease, improving nutritional status may be a cost-effective prophylactic and treatment modality for HIV-infected persons.[72] HIV-infected women from developing countries are particularly vulnerable to nutrient deficiencies because of inadequate dietary intake and likely increased nutrient requirements associated with HIV.[73] Despite concerns about the HIV transmission risk to the infant from breastfeeding and the possible effect of breastfeeding on the health and nutrition of HIV-infected mothers, use of replacement milk is largely considered unacceptable, unaffordable or unsafe in the developing world. Therefore it is likely that breastfeeding will remain the norm for HIV-infected mothers in most of Africa, irrespective of the impact of lactation on the health of the HIV-infected mother.[74]

During lactation the maternal requirements for vitamins A, B6 and C, riboflavin, pantothenic acid, protein, and the minerals zinc and iodine are 40-90% higher than before pregnancy. The requirements for thiamin, niacin, folate, vitamin E and selenium are about 25% higher.[75.] Adequate maternal micronutrient status is especially critical during pregnancy and lactation. The main cause of multiple micronutrient deficiencies is a poor quality diet, often due to an inadequate intake of

animal source foods. [75a] Several micronutrient deficiencies are well established to be contributors to abnormal prenatal development and/or pregnancy outcome, these include folate, iron and iodine status.[75a] Anemia postpartum is another neglected problem and is associated with increased risk of postpartum depression.[75b]

The documented benefits of exclusive breast-feeding for the first six months of life on infant health and survival emphasize the importance of paying attention to the nutritional status of lactating women. Maternal micronutrient deficiencies during lactation can cause a major reduction in the concentration of some of these nutrients in breast milk, with subsequent infant depletion.[75a]

In a study done by Papathakis et al.[78] serum albumin, pre-albumin, vitamin B12, folate, retinol, retinol (A), α-tocopherol (E), ferritin, and zinc concentrations were compared at different stages postpartum, in 92 HIV-positive and 52 HIV-negative mothers. The results of this study indicated that a large proportion of breastfeeding women in rural South Africa, have multiple nutrient deficiencies which can affect both their health and that of their infant. In this study a higher proportion of HIV-positive mothers had albumin concentrations <35 gm/L at 14 and 24 weeks postpartum. (HIV positive, 17.2% and 16.9% respectively versus HIV-negative 0% and 2.5%, $p<0.05$). More than 20% of all mothers were deficient in vitamin B12 (<150 µmol/L) or folate (<6.8 µmol/L) and >45% had marginal values (<210 µmol/L B12 and <14.0 µmol/L folate). At 24 weeks postpartum, a higher proportion of HIV-positive mothers had marginal vitamin B12 status (70.5% vs 46.2%, $p< 0.02$). Mean serum retinol was significantly lower in HIV-positive mothers consistently, even after controlling for acute phase response. At 24 weeks, 70% of both groups had α-tocopherol concentrations <11.6 µmol/L, with no difference in mean concentration by HIV status. Iron deficiency was common; 25% of all mothers had low serum ferritin (<12.9 µg/L). Zinc deficiency (<10.2 µmol/L) was more common in HIV-positive mothers (45.0% vs 25.0%, $p=0.04$).[78]

Micronutrient malnutrition is associated with inadequate dietary intake. Dietary surveys in developing countries have consistently shown that multiple micronutrient

deficiencies rather than a single deficiency is common and that low dietary intake and poor bio-availability of micronutrients account for the high prevalence of these multiple deficiencies.[76] A dietary strategy to combat micronutrient deficiencies will be most successful if preceded by assessing dietary intake and obtaining information on local food preparation and availability.[76]

The diverse clinical spectrum of HIV/AIDS and its effects on nutritional status, underscores the need for valid tools to assess nutritional status on individual and population level.[77] The lack of available biochemical indicators for assessing micronutrient status in populations burdened with a high prevalence of infection that may lead to an underestimate of some micronutrient deficiencies (iron for example) and an overestimate of others (zinc for example),[67] together with the expense and difficulty of collecting and analyzing blood samples in remote rural areas, created the need for an fairly easy yet reliable method to evaluate the micronutrient intake of a group of people. The development of relatively simple methods of assessing micronutrient intake and status from dietary intake are needed to identify nutritionally at risk individuals and groups.

Several large studies are currently being conducted through the Africa Center for Health and Population Studies, located in the Hlabisa district. In a longitudinal prospective cohort sub-study, called the Maternal Nutrition Study (MNS), information was collected to investigate the combined effect of HIV-infection and breastfeeding on women's nutritional status by comparing HIV-infected and HIV-uninfected lactating mothers. This study aimed to measure HIV-infected and HIV-uninfected breastfeeding mothers at 3 points postpartum, the points of visit were at 6 weeks, 14 weeks and 24 weeks. The outcome variables measured included:

- body composition measured by anthropometry and bio-impedance spectrometry (not within scope of this study reported elsewhere [78])
- serum micronutrients and proteins (reported elsewhere[78])
- dietary intake evaluated by locally constructed and validated FFQ and 24-hour

dietary recall methods

During the MNS, data collection for dietary intake was completed at the end of July 2004. The purpose of this research study was to become a sub-study of the MNS to analyze and explore the dietary intake data collected with the two instruments used, in order to describe the habitual dietary pattern of this community and to estimate the dietary micronutrient intake of this population. Identifying the pattern of food intake and the contribution of different foods to the micronutrient intake in this population group will contribute to possible recommendations aimed at dietary changes to improve dietary micronutrient intake.

Comparison of the dietary intake data obtained with the two instruments, a 24-hour recall and a Food Frequency Questionnaire, will identify the usefulness of the FFQ instrument in relation to its ability to measure dietary intake adequacy and to serve as a proxy for adequate micronutrient intake in the larger community, without the need for biochemical analysis. A valid FFQ for investigating micronutrient status in the community will be a valuable contribution to new studies on dietary intake and nutritional status in anticipation of antiretroviral program planned by the government and the Africa Center, in this community.

CHAPTER 2: METHODOLOGY

2.1 Study Aim

The aim of the study was to compare the micronutrient intake of lactating HIV-positive and HIV-negative mothers from the Hlabisa District in KwaZulu-Natal as obtained by the FFQ and 24-hour recall dietary questionnaire instruments.

2.1.1 Objectives

a) To evaluate and analyze previously collected but not analyzed dietary intake data using multiple 24-hour dietary recalls and semi-quantitative food frequency questionnaires (FFQ's) in a group of HIV-positive and HIV-negative breastfeeding women from a rural region in KwaZulu-Natal, South Africa in order to validate the FFQ for its ability to classify individuals into quintiles for intakes of selected micronutrients.

b) To describe the habitual dietary intake of all breastfeeding women in the study, irrespective of HIV status, using the dietary intake data collected with the FFQ.

c) To identify and compare the foods most commonly consumed on a regular basis in this population by using dietary intake data collected with multiple 24-hour dietary recalls and the semi-quantitative FFQ.

d) To identify and compare the major micronutrient rich food sources in this population using data collected with 24-hour recalls and FFQ's.

e) To compare the data collected on dietary intake using the FFQ with the data collected using the 24-hour recall in order to validate the FFQ for its ability to measure dietary adequacy and to serve as a proxy for micronutrient intake.

2.2 Study Design

This study was designed and registered as a sub-study of a larger longitudinal prospective cohort study, the Maternal Nutrition Study (MNS).

The MNS was conducted at the Africa Centre for Health and Population Studies in the

northern KwaZulu-Natal Province, South-Africa. The study site was largely rural, and the residents were mostly of Zulu ethnic origin. The population was characterized by a high prevalence of HIV (36.5% of women attending antenatal clinics in 2002)[92] unemployment (54%), poor access to clean water (87%), and high infant mortality (79/1000 live births).[79]

Mothers were enrolled at three rural clinics (conveniently selected for easy access) in the Hlabisa District. Most of the mothers enrolled in the MNS were simultaneously participating in the Africa Centre Vertical Transmission Study (VTS) to investigate the relationship between exclusive breastfeeding and HIV transmission from mother-to-child.[80]

The aim of the MNS was to evaluate the nutritional status in HIV-positive and HIV-negative breastfeeding mothers between 6 and 24 weeks postpartum, in a rural region of South Africa, with a focus on body composition and micronutrient status and to characterize the changes during the first six months of lactation.[78]

Study participants were enrolled at ~6 weeks post partum and had subsequent study visits at 14- and 24-weeks post partum, timed to coincide with their infants' routine immunization schedule and clinic visits.

Sample size for the MNS was determined to be 51 per group in order to be able to detect 0.5 standard deviation unit differences between groups for body composition and serum micronutrient variables, with a 0.05 level of significance and 80% power.[6]

The chief investigator for the MNS, from the United States of America, recruited and employed two South African registered dietitians (the investigator of this study and a registered dietitian from the Zulu ethnic group) as well as three Zulu nutrition assistants (Diploma in Food and Nutrition, University of Zululand, KwaZulu-Natal) to assist with the MNS. The dietary intake tools were developed by the investigator of this study, who was also to be responsible for co-ordination of data collection and analysis of dietary intake data. Dietary intake data was collected from each participant, on three occasions at two points namely during a clinic visit and during a

home visit. During each of the three clinic visits (~6 ~14- and ~24 weeks post partum) a FFQ was administered by the nutrition assistant working in that clinic and during each of the three home visits with a 24-hour recall administered by the dietitian (from Zulu ethnic group). The clinic and home visits for each participant were conducted 1-3 days apart, preferably in the same week depending on the subject's circumstances. Bio-impedance measurements and anthropometric measurements were also done by the registered dietitian during home visits. Changes in body composition and biochemical analysis were reported in another study.[78]

2.3 Study Population

Study participants for the MNS were enrolled at ~6 weeks post partum and had subsequent study visits at ~14- and ~24 weeks post partum, timed to coincide with their infants' routine immunization schedule and clinic visits.[78] One hundred and forty-four mothers were enrolled, of whom 92 were HIV-positive and 52 were HIV-negative; Staff members at the clinics were blinded to the HIV status of mothers, resulting in more HIV-pos mothers being enrolled by chance.

2.3.1 Inclusion Criteria

The MNS was designed as a sub-study of the larger VTS and therefore the inclusion criteria used for recruiting subjects for the VTS, applied. Subjects were recruited at the antenatal clinics and pregnant women between the ages of 16 and 50 years of age, due to give birth within 4 weeks or longer, were approached. The objectives of the research were explained to them and consent forms, translated into isiZulu had to be signed.

2.4 Development of Dietary Intake Instruments

2.4.1 The semi-quantitative food frequency questionnaire

The semi-quantitative food frequency questionnaire (FFQ) was designed by the investigator of this study on the request of the chief investigator of the MNS, to suit specific requirements for the MNS. The requirements for the FFQ to be used in the MNS were the following:

- The foods included on the FFQ should include the foods and drinks most frequently consumed by the members of this community.
- The foods should have a substantial content of the nutrients of interest (specified micronutrients). The FFQ was chosen as a possible tool for detection of intake of specific micronutrients known to have a possible effect on the immune system. The micronutrients decided upon on the basis of the scientific literature were zinc, selenium, iron, copper, vitamins A, C, E, pyridoxine (B6), riboflavin, vitamin B12 and folic acid.
- The questionnaire should be easily administered by a trained nutrition assistant in a clinic setting and should be easy to code and computerize.
- The questions in the questionnaire should be easily understood by the respondent. And the questionnaire should not take more than fifteen minutes to complete.

The following procedure was followed in developing the FFQ:

- Two months prior to the start of the parent study, breastfeeding mothers visiting two clinics with their infants for immunization and who were willing and available to be interviewed, were questioned on their food intake the previous day with the help of an interpreter (the trained nutrition assistant). They were also asked to indicate the amount or portion sizes (large, medium or small) of the foods they consumed. Bowls of three different sizes, different size spoons,

different size cups and a ruler were used to establish the size of the indicated portions. The list of foods compiled during the interviews with the women at the clinics, was further discussed in a workshop at the Africa Centre with six female Zulu employees (of grade 12 minimum qualification and aged between 20 and 40 years), originating from and living in the area. The three isiZulu-speaking nutrition assistants the two registered dietitians recruited for the MNS study (investigator of this study and the isiZulu-speaking dietitian) also formed part of the workshop convened by the chief investigator of the MNS. One of the requirements for the nutrition assistants was that they should be originally from the study and would therefore be familiar with the food and serving equipment available and used in this community. The list of food items that was compiled after the interviews and discussions during the workshop was used to develop the food frequency questionnaire. The different serving sizes that were indicated were used to establish a population specific small, medium or large serving where relevant.

- The food list compiled was also compared with similar lists for the type of food items, which were developed by other investigators in other studies of dietary intake and food production, in KwaZulu-Natal[81] as well as on the FFQ used in the National Food Consumption Survey.[82]

The final FFQ was tested on a further 10 women (conveniently selected) visiting the clinics, for layout, logical flow and duration of its administration. Layout was developed to suit the requirements for the scanning process and data capturing at the Africa Centre. The FFQ was designed to measure the intake of the specific nutrients targeted in the MNS study (iron, zinc, copper, selenium, riboflavin, folic acid, vitamin B6, vitamin B12, vitamin C, vitamin A and vitamin E). Food items with a zero contribution to the intake of these micronutrients, such as sugar, black coffee/tea and jam, were omitted. The decision on which foods to omit based on level of micronutrient content, was done by the chief investigator of the MNS.

- The final format of the semi-quantitative FFQ was approved by the chief

investigator of the MNS and could be used for assessing frequency of intakes of these food items over the preceding month. The time period of evaluation was one month retrospective. An open ended question at the end of each section, provided the opportunity to record any other food that was consumed on a regular basis by a participant but was not listed in the FFQ.

- Questions about fruit and vegetables grown in home gardens and the intent for their primary use (i.e. income generation, consumption), were included. The inclusion of these few questions could be used to verify answers given in previous sections of the questionnaire and could be used in future intervention programs.

The final format of the FFQ (Appendix 1) provided information that could be captured into the database using the Teleform[83] software program.

2.4.2 Development of the 24h-recall

This questionnaire was developed by the investigator of this study. An open ended questionnaire format was used for the 24h-recall (Appendix 2). It was designed to be used by a trained interviewer (the isiZulu speaking dietitian employed for this task). Most people from this rural area did not speak English and it was therefore important for the dietitian to be fluent in isiZulu. The 24h-recall was administered during the home visits.

The following aspects were accommodated in the 24h-recall:

- Adequate space to document types and amount of foods consumed the previous day, cooking methods and main ingredients for stews and other combined dishes.

- The type of bread (brown/white/whole-wheat) and type of maize meal eaten.

- Questions on the day of the week, whether it was a normal day or not, the subject's health, namely if the subject was sick and did not eat normally due to

any disease or other reason.

- A question on the use of supplements was also included and recorded.

The form for recording the 24-hour intake and the FFQ were however not translated into isiZulu but their administration was in isiZulu.

The 24-hour recall was designed to be used as an open ended questionnaire. The date, day of the week, study number of the subject and name of the interviewer appeared on the front page. An additional question on the “type” of day that was being reported was included. The subject had to indicate whether it was a normal day, whether she was ill or visiting someone. The rest of the questionnaire consisted of three lined pages divided into two columns each, one column for the description of the food item and preparation method, the second for the quantity consumed.

2.4.3 Training of interviewers

A period of ten weeks was dedicated to prepare and train the nutrition assistants for their role in the MNS, including the assessment of the dietary intake by the FFQ and 24h-recall. The training was done by the chief investigator of the MNS and the investigator of this study. Training on the collection of dietary intake with the FFQ and 24-hour recall was conducted by the investigator of this study over a two week period as part of the ten week training. No inter-observer error assessment of variation was conducted because the 24-h recalls were done by the same person and the FFQ was a set of questions with only one correct choice for each question. Role-playing was used to teach interviewing skills to the nutrition assistants and to help them gain confidence in administering the questionnaire. The correct method to complete the answer fields on the questionnaire were taught, the importance of proper number formatting when completing fields and the importance of double checking for completeness before sending forms to the Africa Center to be scanned were emphasized.

2.4.3 Composition of Foods Table

Food Finder Program™2 was the Composition of Foods Table chosen for the analysis of the dietary intake data.[84] The Food Finder Program was used for analysis based on the fact that it is a locally developed instrument with a data base compiled from South African food items.

On examination of the existing recipes in the Food Finder program, it was found that they did not reflect the recipes of commonly prepared dishes in this community. Therefore recipes for the Food Finder database that reflects commonly consumed recipes according to local customs and preferences were developed. To do this, the nutrition assistants prepared recipes of commonly consumed mixed foods. The ingredients of the recipes were weighed and the averaged amount per serving was used in the recipe that was added to the Food Finder Program (Appendix 3).

The nutrition assistants also prepared the different dishes made from maize meal, *phutu, stiff* pap*, porridge and mahewu* from the commonly used maize meal brands in order to determine the proportion of maize meal and water for each dish. The commonly used maize meal brands were identified by the nutrition assistants and through visits by the dietitians and nutrition assistants to the popular local supermarkets. The maize meal used in this area was classified in order of refinement as *special white*, *super white*, *sifted and enriched*. Both questionnaires (FFQ and 24-h recall) recorded the brand of the maize meal used. The nutritional values of the different types of maize meal (requested from the manufacturer if not labeled) were added to the Food Finder database. Fortification of staple foods became mandatory in the last two months of data collection therefore the possible effect of fortification on the micronutrient intake was not considered in this study.

2.5 Ethics

A research protocol for this study was submitted to and approved by the Human Research Committee of the Health Science Faculty of the University of Stellenbosch, Tygerberg, South Africa (N04/11/185).

The scope of the sub-study fell within the ethics approval already obtained for the main study as submitted to and approved by the University of California at Davis and the University of KwaZulu-Natal (Appendix 4).

2.6 Data Collection

2.6.1 Collection of dietary information for the Maternal Nutrition Study

2.6.1.1 Data collection using the FFQ

The MNS was based at the Africa Centre where each member of the team had an office space and workstation. The three clinics selected for the recruitment of subjects for the MNS were within a radius of 50km from the Africa Centre. On certain designated days of the week, each of the nutrition assistants went to their clinics with the research team of the VTS. The nutrition assistants were assigned to specific clinics and were responsible for the administration and records of the participants from that clinic. The nutrition assistants stayed at the clinic for the day with the VTS team. On arrival mothers usually first took the infant to the primary health care sister for immunization and general health check and then moved over to the section where the VTS and MNS studies were based.

The nutrition assistant completed the FFQ with each participant who consented to participate and signed an informed consent form. The assistants weighed and measured the participants and in addition assisted with some of the tasks of the VTS. Each nutrition assistant took a set of equipment with her to be used for determining portion sizes. The set included different sizes bowls, cups spoons and a ruler. They had to make an appointment with the participant on behalf of the dietitian responsible for home visits to follow the next day, or as soon as possible. These appointments were then communicated to the dietitian responsible for home visits back at the Africa Centre, in order for the dietitian to plan her home visits. Before returning to the Africa Centre after the day's activities, the nutrition assistants had to check the completed FFQ to make sure that all fields were filled in correctly and completely and verify the study code numbers and the appointments made.

At the Africa Centre, the completed and verified FFQ's were handed over to the investigator of this study, who again controlled and verified all information on the completed FFQ's. No fields were allowed to be empty, correct codes had to be used for fields that were not applicable (if for example a subject did not take any milk, a certain code had to be filled in). After the investigator of the study was satisfied that all fields were completed, the study numbers and dates were correct and all the numbers were filled in properly without touching the lines of the blocks, the FFQ's were taken (usually in bundles of ten) to the data centre at the Africa Centre to be scanned. A log book was used to sign bundles in and out from the data centre. Once the forms were scanned successfully by the data capturer, the data centre informed the investigator of this study who then collected the forms from the data centre. The investigator of this study had access to the program (Teleform)[83] used for scanning (capturing) the data and was able to verify the scanned data on the database from her workstation. Only after the investigator of this study was satisfied that all fields were scanned correctly, could the data be committed to the MNS database on the server at the Africa Centre. A form could not be committed if there was any open spaces, duplicates or if any numbers were not clear. The data was stored with all the other data from the MNS until the data collection was completed. The hard copies of the FFQ's were filed together with the other information on each participant.

2.6.1.2 Data collection using the 24h-recall

During a subjects' visit to the clinic at 6,12 and 24 weeks, an appointment was made with the subject for a follow-up home visit within the following week. The home visits were done by the Zulu-speaking dietitian who had a four-wheel drive vehicle belonging to the MNS, at her disposal. The 24h-recall was then conducted at the homestead (she also collected other data for the MNS as reported elsewhere). The presence of the dietitian at the homestead of the subject made it easier to determine portion sizes and quantities and the preparation methods as well as to observe brand names of staple foods used. To improve the assessment of portions, the dietitian measured the volume of cups, bowls and plates from which the meals were consumed in the home. The 24h-recall was an open ended questionnaire with

sufficient space for making notes and recording quantities. No coding was done at this stage.

On return to the Africa Centre, the 24h-recall was coded and checked by the dietitian who conducted it before handing it over to the investigator of this study who then proceeded with entering the data into Food Finder. Verification of the data collected with the 24h-recall was more effective if coding and capturing were done while the visit was still fresh in the mind of the dietitian who administered the questionnaire. Although the Food Finder database was saved on a workstation PC, backups were automatically created and stored on the main server of the Africa Centre.

The additional programming that was done on the Food Finder program indicated a warning or error if any values were higher than the cut-off values set by the dietitians involved in research at the Africa Centre (Appendix 6). The cut-off values were not chosen on any scientific basis, they were included as a practical measure to warn the researcher when extreme values were reported. A warning would not exclude the data from being part of the analysis, it just prompted the researcher to take note of this value and to verify it with the original data (original 24h-recall) that was captured. This feature assisted the cleaning of data and limited the errors made during capturing of the data. The investigator of this study monitored the Food Finder database throughout the study period by printing reports (overviews) of the captured data on a regular basis (after the addition of 5-10 records). The creation of reports in a format that summarize all the data and nutrient analysis captured up to a certain point, was part of the modifications done in Food Finder. (Appendix 7)

2.6.2 Data analysis

2.6.2.1 FFQ

The FFQ was designed to measure intake one month retrospectively. The sequence of questions on the FFQ was as follow (bananas as example): *Banana: yes/no; eaten on days per week (0-7)?; days per month (0-4)?; times per day (1-10)?; amount of serving*. Information of these frequencies of consumption was used to calculate an amount per month, week or day.

To analyze the data collected using the FFQ for micronutrient intake, the data was converted to a similar format to that of the data collected by the 24h-recall and therefore expressed as an amount of food per day. To achieve this aim, the total amount of each item on the FFQ was calculated using the portion size that was indicated by the subject (small, medium, large) and multiply that with the frequency of consumption per month (calculated by multiplying the amount per week by four or multiplying with the days per month). This was then also multiplied with the times per day. Dividing this total of food item consumed per month by 30, a theoretical amount per 24 hours was established. (Example: If a subject reported on the FFQ that she had consumed bread 5 times a week, 2 times per day and usually eats 2 slices (45g each) at a time, her total consumption of bread per month was calculated as (5 x 4 (weeks per month), therefore 20*2*2*45g=3600g per month or 120g per day (3600g/30). If she did not eat bread at least once every week, the “weeks per month” was zero and only the “times per month” was used. (Example: If a subject reported on the FFQ that she had consumed chicken only 3 times twice a day during the past month (120g per meal), the total consumption of chicken per month was calculated as (3x2)*120g=720g per month and theoretically 720g/30=24g per day (Appendix 5). This procedure was done for each of the 3 FFQ’s and the average of the 3 was used as intake per day, measured with the FFQ.

2.6.2.2 *24h-recall*

Data collected with the 24h-recall was captured and analyzed with the Food Finder

Program. The average intake of the three 24h-recalls for each participant, was used to determine nutrient intake.

2.6.3 Statistical analysis

Statistical analysis was done by the Department of Statistical Support at the University of Stellenbosch.

To investigate the types of food consumed by this community, descriptive statistics was used to determine the consumption of a food item as a percentage of all the food items consumed. This was done for both the two dietary intake measures (FFQ and 24h-recall).

All the different types of food items reported in the combined number of 24h-recalls (n=324) were listed as well as the number of times a food item was consumed. This procedure was repeated with the FFQ. These two sets of data were used to determine the frequency of consumption of different food items measured with the 24h-recall and with the FFQ, the total amount (gram) of each food item consumed and the mean, mode, median and range of serving sizes for the ten food items most frequently consumed (measured with the 24h and FFQ respectively).

The FFQ data was also used to establish the number of subjects that consumed a food type 1-7 times per week. This frequency analysis was only done using the FFQ data, a 24hr-recall can not be used to establish the number of times per week a food type is consumed.

The contribution of the foods mostly consumed to the intake of the specific micronutrients under investigation, was demonstrated in a graphical format. This was done for both the FFQ and the 24h-recall.

For analysis on micronutrient intake, the average of the three food records from each participant was used for both the 24h-recall and the FFQ. The foods that were consumed most often and in the largest quantities, were analyzed to determine their contribution (in percentage) to the total micronutrient intake for the FFQ and 24h-

recall.

The micronutrient intake measured with the two methods was compared in terms of mean intake, correlations, quintile rankings and percent below the EAR or AI of the DRI. The paired t-test indicated that the data was not normally distributed and therefore a nonparametric test (RM ANOVA) was done to confirm this. The Wilcoxon matched pairs test was used to determine if the mean of the individual differences for each nutrient was significantly different than zero and to determine the F-value. The F-value was used to determine the significance of the difference between the micronutrient intakes as measured with the two instruments ($p<0.05$). To investigate the strength of the correlation between the two dietary intake measures, the Spearman's correlation coefficients were determined for the nutrients under investigation ($p<0.05$).

Percent of subjects with micronutrient intakes below the EAR as assessed by the two methods, was compared and the percentage of subjects with micronutrient intakes less than the EAR, established.

The ability of the FFQ's to rank an individual in the same quintiles as the 24h-recall understood was determined and the percentage of agreement calculated (CI=95%).

CHAPTER 3: RESULTS

3.1 Description of Sample

Of the total of one hundred and forty-four mothers enrolled, twenty-four dropped out before the 6-month measurement thus leaving a total of 120 women who were included in the study. Reasons for the subjects dropping out included moved out of the area (n = 8), study withdrawal (n = 7), infant death (n = 7) and return to work (n =2). The mothers who dropped out were not different from those who continued with the study in terms of body composition, number of previous pregnancies, CD4 t-cell count, HIV viral load, or demographic indicators. The only differences were that mothers who dropped out were younger (23.3y ±5.0 vs 26.0y ±7.3, p=0.03) and were HIV+ (p=0.02).

3.2 Description of Data Collected

Three 24h-recalls and three FFQ's were available for one hundred and eight of the one hundred and twenty subjects who were included in the study. Subjects who did not complete a 24h-recall and FFQ for each of the first three visits, were excluded. Subjects who missed a second or third visit, or were not available for a home visit at any stage fell into this category. Reasons for missing a visit were visiting partner/relatives/friends (n=7), in hospital/sick (n=3), not found at home after three appointments/visits (n=2).

The 24-hour recall dietary intake and the FFQ data were collected from the beginning of May 2002 until the end of July 2004.

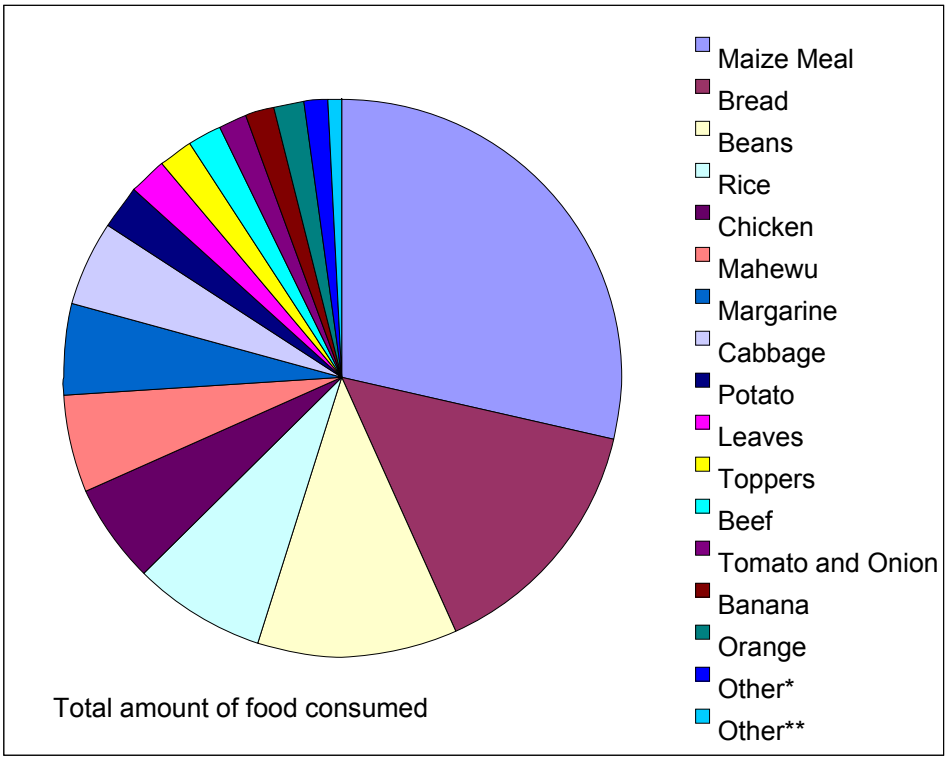
3.3 Study Outcomes in Terms of Objectives of the Study

3.3.1 Description of foods consumed using the 24-hour recall and FFQ

3.3.1.1 *24h-recall*

Combining the intake of the three 24h-recalls per subject (n = 108) the frequency of consumption of different food items for the total number of 24h-recalls (n=324) was calculated. A total of 55 different types of food were eaten on 1897 occasions. Maize meal was eaten 473 times and contributed to 24.9% of all items reported. Subjects often reported that they ate maize meal more than once a day, some even three times a day. It was consumed in different forms for example porridge in the morning and phutu in the evening.

The fifteen most frequently consumed food items according to the 24-hour recalls were maize meal 24.9% (473 times), bread (white and brown) 12.9% (246 times) times), dried beans 9.9% (188 times), rice 6.8% (130 times), chicken 4.9% (96 times), mahewu 4.6% (94 times), margarine 4.6% (88 times), cabbage 4.3% (81 times), potato 2.2% (41 times) and green leaves 2.2% (39 times), toppers 1.7% (33 times), beef 1.6% (31 times), tomato and onions 1.5% (28 times), banana 1.4% (27 times) and orange 1.4% (26 times). The 15 most frequently consumed foods contributed 85.5% of all the food consumed (Figure 3.1), Vetkoek 1.4% (26 times), apple 1.3% (24 times), egg 1.1% (20 times), spinach 1.1% (20 times), maas 1% (19 times), pilchards 0.95% (18 times), polony 0.9% (17 times), fish 0.9% (17 times) and mango 0.6% (14 times), contributed another ten percent. Samp and beans, peanut butter, peanuts, mutton, avocado, milk, sweet, potato, tomato, sausage and lemon juice made up the last five percent of the items and contributed between 0.85%.and 0.6% each to the total amount consumed. Ten of the 55 different food items reported (dairy-fruit juice mix, biscuits, mealie, paw-paw, mixed vegetables, pasta, pumpkin, savory snacks, niknaks, naartjie and breakfast cereal) were eaten only twice (0.11%). Food items that were consumed only once (0.05%), included oats, peas, guava, liver, grapes, onion (excluding onions in food preparation), meat pie, sweets and milk jelly.



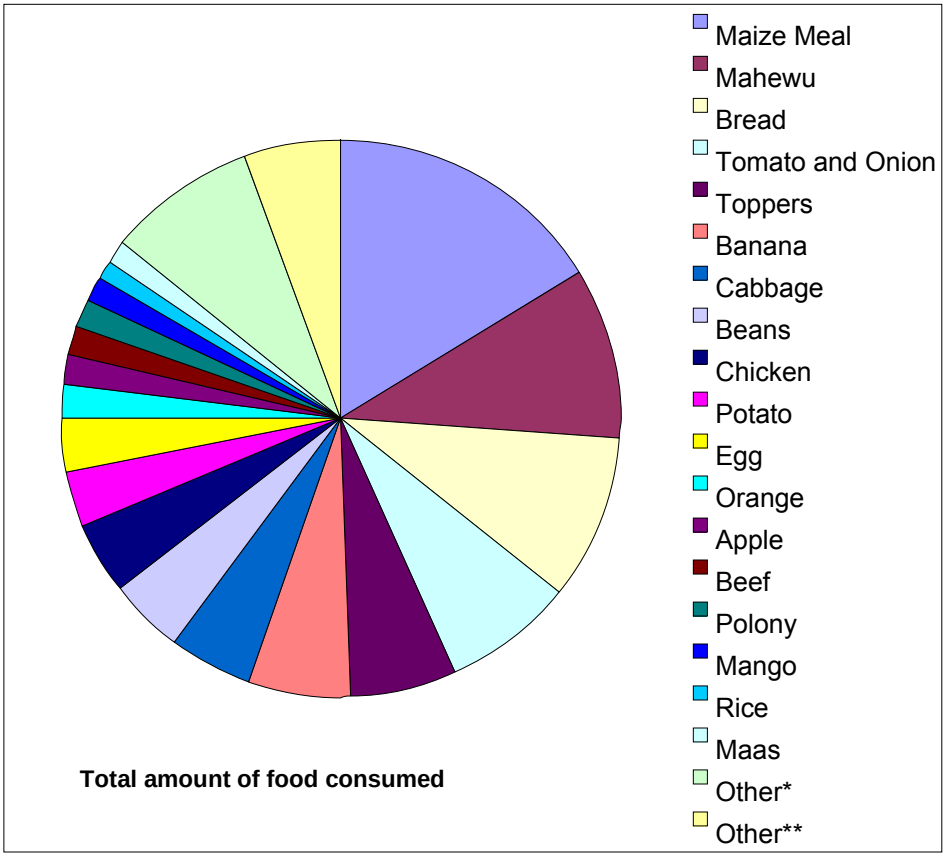
* Vetkoek, Apple, Egg, Spinach, Maas, Pilchards, Polony, Fish, Mango

** Peanut Butter, Peanuts, Mutton, Avocado, Milk, Offal, Sweet Potato, Tomato, Sausage, Lemon Juice, Dairy-fruit Juice Mix, Biscuits, Mealie, Paw-paw, Mixed Vegetables, Pasta, Pumpkin, Savoury snacks, Niknaks, Naartjie, Breakfast Cereal, Oats, Peas, Guava, Liver, Grapes, Onion, Meat Pie Sweets, Milk Jelly , Carrot

Figure 3.1: Contribution of different food items expressed as percentage of total number of food consumed, determined with a 24h-recall

3.3.1.2 *FFQ*

Using the FFQ, it was determined that 48 different types of foods were consumed on 2733 occasions (24h-recall: 55 different items on 1897 occasions) The fifteen most frequently consumed food items used according to the FFQ, were maize meal 16,5% (452 times), mahewu 9.7% (265 times), bread (white and brown) 9.5% (271 times), tomato and onion 7.4% (204 times), Toppers 6.2% (169 times), banana 5.9% (161 times), cabbage 4.6 % (161 times), dried beans 4.5 % (124 times), chicken 4% (110 times), potato 3.5% (97 times), egg 2.9% (79 times), orange 2.1% (58 times), apple 1.7% (46 times), beef 1.6% (45 times) and polony 1.6% (44 times). The 15 most frequently consumed foods contributed 82.5% of all the food consumed (Figure 3.2). Guava 1.17% (32 times), samp and beans 1.1% (30 times), leaves (variety of wild green plants) 1.1% (30 times), spinach 1.1% (30 times), fish 1.06% (29 times), pilchards 1.03% (28 times), paw-paw 0.77% (21 times), peach 0.77% (21 times) and carrot 0.7% (19 times) and milk 0.6% (16 times) contributed another ten percent. Peanuts, beetroot, avocado, pumpkin, sausage, offal, mealie, pineapple, liver, sweet potato, cheese, breakfast cereal, samp (on its own), grapes, Vienna, goat, lemon, tomato, pear and dairy fruit mix, contributed each between and 0.6%.and 0.04% to the total amount of foods consumed. Five of the 48 different food items reported (meat pie sweets, milk jelly, and carrot) were only consumed once.



***** Guava, Samp And Beans, Leaves, Spinach, Fish, Pilchard, Paw-paw, Peach Carrot, Milk

**Peanuts, Beetroot, Avocado, Pumpkin, Sausage, Offal, Mealie, Pineapple, Liver, Sweet Potato, Cheese, Breakfast Cereal, Samp, Grapes, Vienna, Goat, Lemon, Tomato, Pear, Dairy fruit mix

Figure 3.2: Contribution of different food items expressed as percentage of total number of food consumed, determined with a FFQ

A summary of the total amount (in gram) of the ten food items most frequently consumed, indicated a consumption of 147,398 gram maize meal, (excluding the 61,791 grams of *mahewu*) 39,152 gram bread, 29,208 gram of dried beans, 30.948 gram rice and 10,533 gram of chicken on three occasions when measured with the 24h-recall. Mean, mode, median and range of intake for the ten foods most often consumed were summarized (Table 3.1).

Table 3.1: Summary of total amount of food item, average serving portion, standard deviation, median, mode and range of serving portions of ten foods most frequently consumed as measured with the 24h-recall and FFQ

Food item and intake measurement 24h=24h-recall F=FFQ	Total amount per day (gram)	Mean serving portion per day (gram)	STD DEV	Median serving portion (gram)	Mode serving portion (gram)	Range for servings (gram)
maize meal (24h)	147398	312	182.18	258	290	50-1050
maize meal (F)	176455	426	194	345	345	40-1046
bread (24h)	39152	159	67.3	120	120	60-360
bread (F)	47867	30	30	30	30	30
beans (24h)	29208	155	76.85	128	128	45-560
beans (F)	92176	89.3	111.4	64	64	30-896
rice (24h)	30948	238	99.43	239	120	90-560
rice (F)	14575	193.2	113.65	184	123	12-490
chicken (24h)	10533	109	56.91	97	80	33-290
chicken (F)	20600	20.4	22.96	80	60	40-120
mahewu (24h)	61791	657	296.27	700	1000	250-2000
mahewu (F)	86506	848.5	1012.3	533	2800	12-3500
banana (24h)	6635	255	107	213	150	100-450
banana (F)	11932	49.5	58.6	75	75	30-560
cabbage (24h)	8754	108	52.01	102	102	51-33
cabbage (F)	49201	156	46.18	160	160	80-900
potato (24h)	4465	109	55.12	80	90	30-320
potato (F)	800.37	244.8	284.94	160	160	20-900
green leaves (24h)	4170	107	53.42	115	115	29-257
green leaves (F)	7138	38.4	54.83	21	21	12-480

3.3.1.3 Frequency of consumption per week as measured with the FFQ

With the FFQ it could also be determined what number and percentage of subjects consumed a food item at a specific frequency, namely times per week. Maize meal (in the form of phutu, a stiff porridge) was consumed by 33% (36 subjects, n=108) seven times a week. (Figure 3.3) *Mahewu* (a fermented drink made of maize meal) was an additional source of maize meal consumption with a further 34 subjects (31%, n=108) indicating that they consumed *mahewu* every day. Bread was also consumed on a daily basis (seven times a week) by 48 subjects (43%, n=108). Tomato, mainly in stews or cooked with onions, was the only vegetable that was consumed on a daily basis by more than a quarter of subjects (27 subjects). Eleven subjects indicated that they ate oranges 7 times a week but other fruit was seldom consumed on a daily basis. Chicken was consumed by 4 subjects seven times a week and dried beans by 6 subjects. Other protein sources that were consumed on a daily basis were eggs (7 subjects), cold meat (3 subjects) and cow's milk (6 subjects).

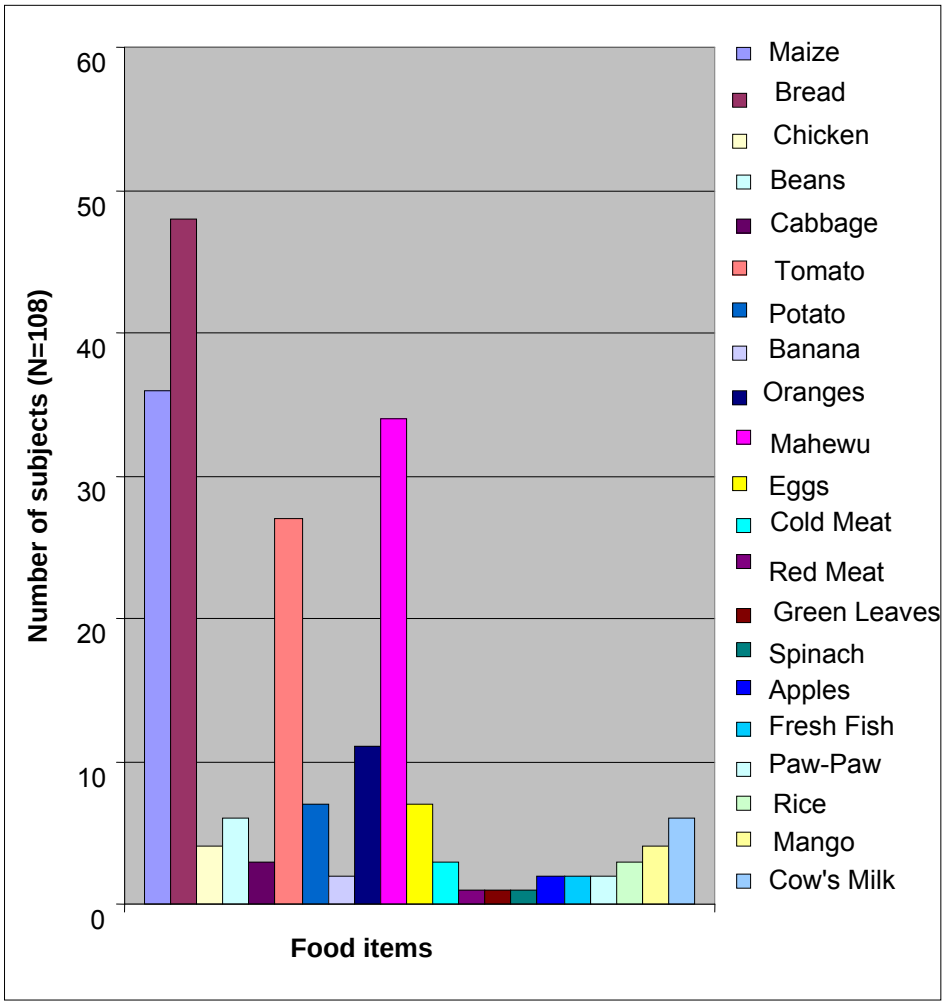


Figure 3.3: Number of subjects who consumed a food item 7 times a week as determined by the Food Frequency Questionnaire

Bread and maize meal were consumed by 19 (bread) and 12 (maize meal) subjects at a frequency of four to six times a week (Figure 3.4). Frequency analysis of protein sources indicated that 12 subjects (11%) consumed beans and eggs four to six times a week and 8 subjects (11%) consumed chicken four to six times a week. Cold meat (*polony*), red meat, canned and fresh fish and maas were all consumed by less than 5 subjects four to six times a week.

Oranges were consumed between three and six times per week by 5 subjects while 4 subjects indicated that they ate apples and bananas four to six times a week.

Cabbage (5 subjects), tomato (11 subjects) and green leaves (8 subjects) were the vegetables consumed four to six times a week.

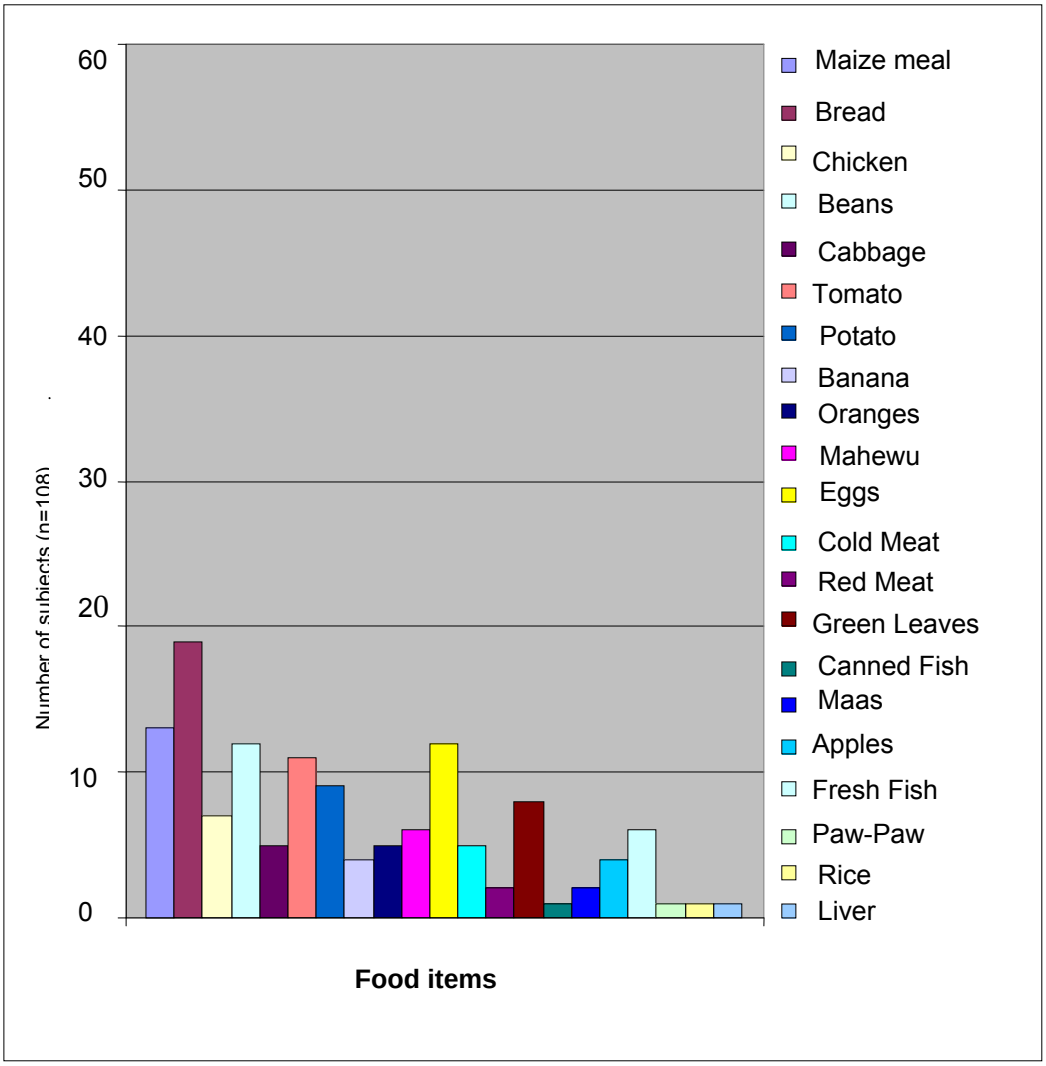


Figure 3.4: Number of subjects who consumed a food item 4-6 times per week as determined by the Food Frequency Questionnaire

Chicken was consumed by 71 subjects (68%) and beans by 61 subjects (56%) at a frequency of one to three times per week but other protein sources such as red meat and eggs were only consumed by 39 (red meat) and 38 (eggs) subjects at a frequency of one to three times a week (Figure 3.5). Seven subjects indicated that they ate liver one to three times a week. Cabbage, tomato, green leaves (form a variety of wild plants), spinach, potato, bananas and oranges were the fruit and vegetables that were consumed at least by 30 subjects, one to three times a week.

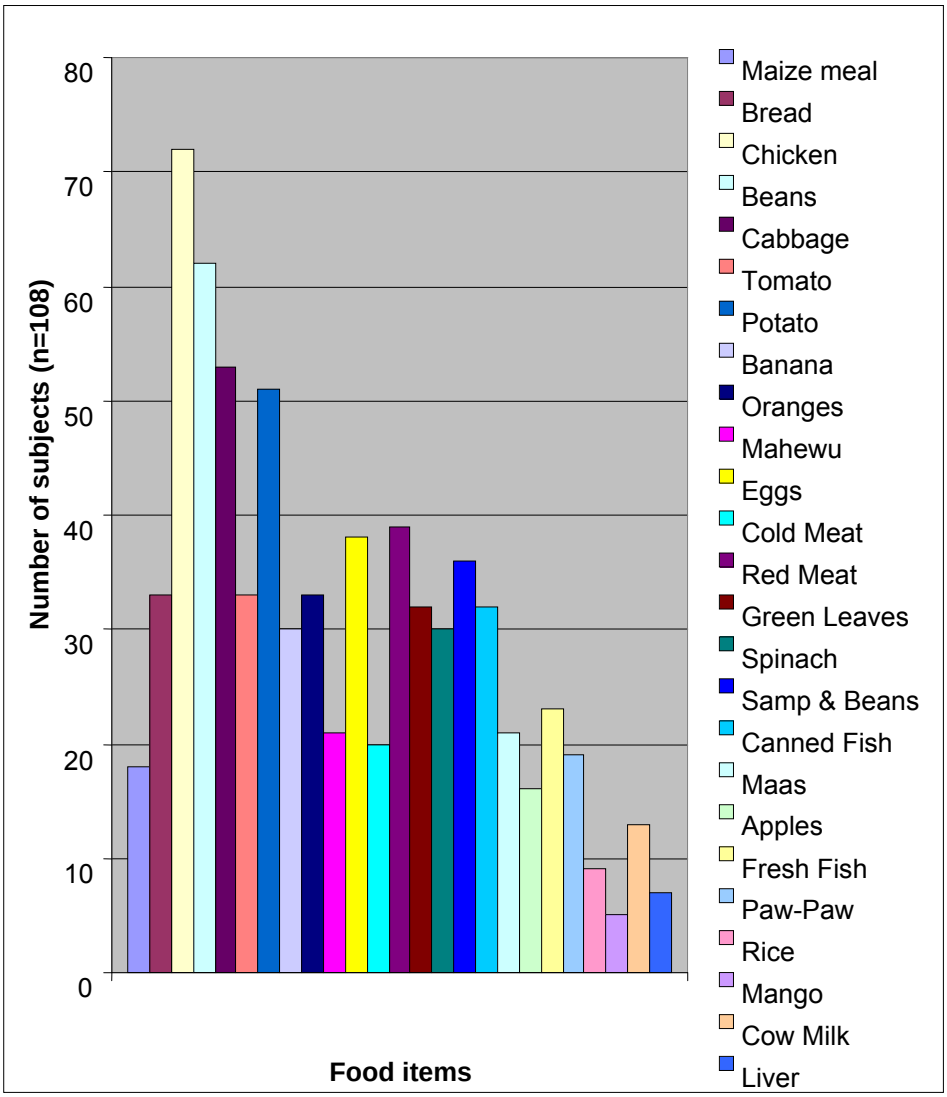


Figure 3.5: Number of subjects who consumed a food item 1-3 times per week as determined by the Food Frequency Questionnaire

3.3.2 The major sources of micronutrients consumed in this population, as measured with the 24h-recall and FFQ

To identify the food sources contributing mostly to the intake of iron, zinc, copper, selenium, vitamin A, riboflavin, folate, vitamin C, vitamin B6, vitamin B12 and vitamin E, the contribution of each food item to the total amount of each of these micronutrients was determined (Appendix 8) as a percentage of the total micronutrients consumed from different food sources.

For each of the micronutrients under investigation, the top twenty food items that made the largest contribution to the micronutrient intake of the total group (as measured with the 24h recall and with the FFQ) were identified. Although all the data were analysed only zinc, vitamin A, riboflavin, vitamin B6, folic acid, vitamin D and vitamin E are presented here, they have been selected for reasons of importance in terms of inadequate intake (Figures 3.6 - 3.17). The rest of the micronutrients analysed have been appended (Appendix 9).

Both dietary intake methods indicated bread to be the food item that contributed the most to the iron, selenium and copper intake and the second most to the zinc intake. Bread was further in the top five food items contributing mostly to the riboflavin and folate intakes, when measured with both dietary intake methods. Dried beans were in the top 5 food items contributing to iron, zinc, copper and folate intake for both dietary intake methods. Both dietary intake methods identified maas (sour milk), to be the food item that contributed the most to the riboflavin intake, and in the top 5 food items that contributed to the zinc, selenium, vitamin A and vitamin B12 intake.

Considering the fruit and vegetables that contributed the most to the micronutrient intakes measured with both dietary intake methods, mango was the fruit item that contributed the most to the copper, vitamin A, riboflavin, vitamin B6, folate and vitamin C intakes, followed by orange (copper, folate and vitamin C). Green leaves (from variety of wild plants), spinach and cabbage were the only vegetables in the top 5 foods that contributed the most to the iron, vitamin A, folate, vitamin C and vitamin

E intake. Other food items that were identified in the top 5 food items contributing to the micronutrient intake for both dietary intake methods, were chicken (iron and selenium), beef (zinc, selenium, vitamin B6 and vitamin B12), rice (zinc, selenium, vitamin B6), egg (selenium, riboflavin, vitamin B12 and vitamin E), pilchards and fish (selenium, riboflavin, vitamin B12 and vitamin E), peanuts and peanut butter (folate and copper) and tomato (vitamin C). Maize meal was identified only once as one of the top 5 food items that contributed to the micronutrient intake (selenium) when measured with both dietary intake methods.

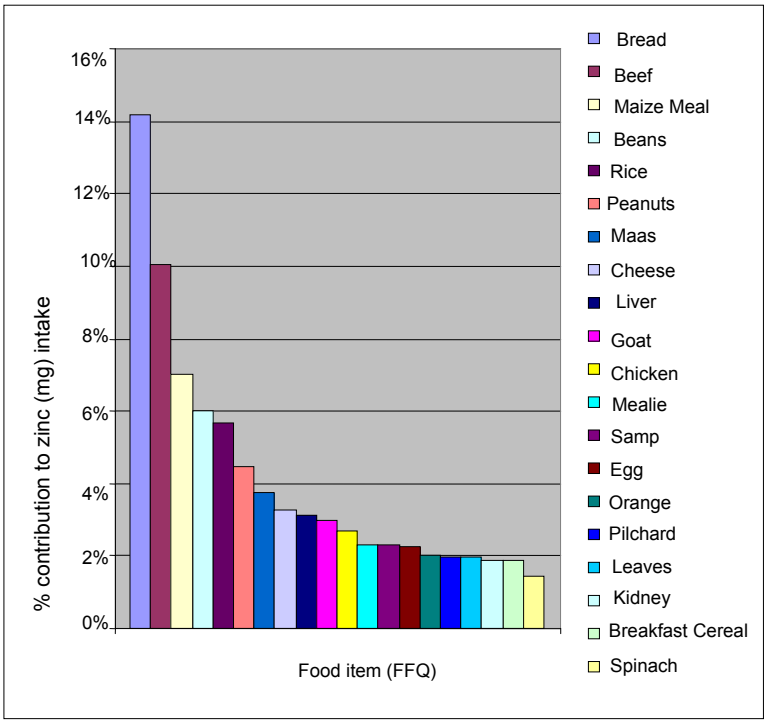


Figure 3.6: Top twenty food items that made the largest contribution to the zinc intake of the total group as measured with the FFQ

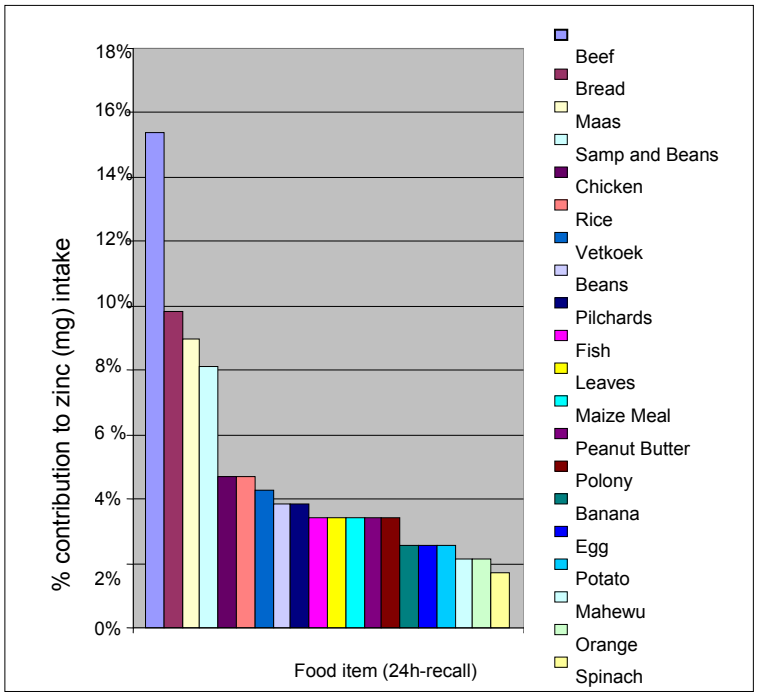


Figure 3.7: Top twenty food items that made the largest contribution to the zinc intake of the total group as measured with the 24h-recall

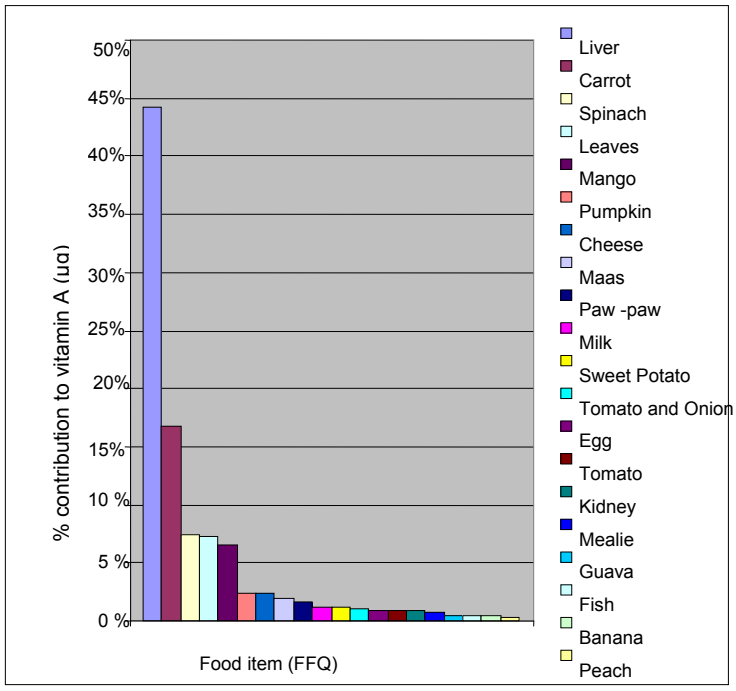


Figure 3.8: Top twenty food items that made the largest contribution to the vitamin A intake of the total group as measured with the FFQ

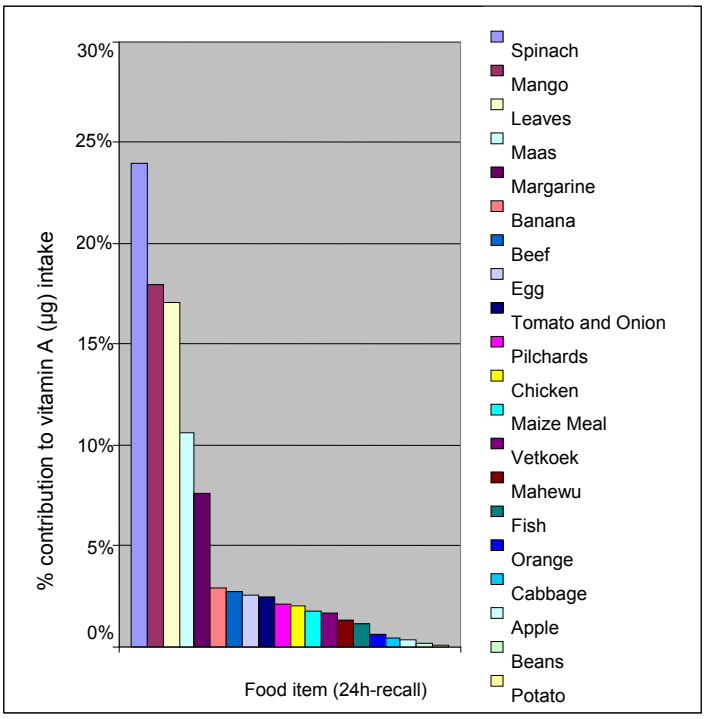


Figure 3.9: Top twenty food items that made the largest contribution to the vitamin A intake of the total group as measured with the 24h recall

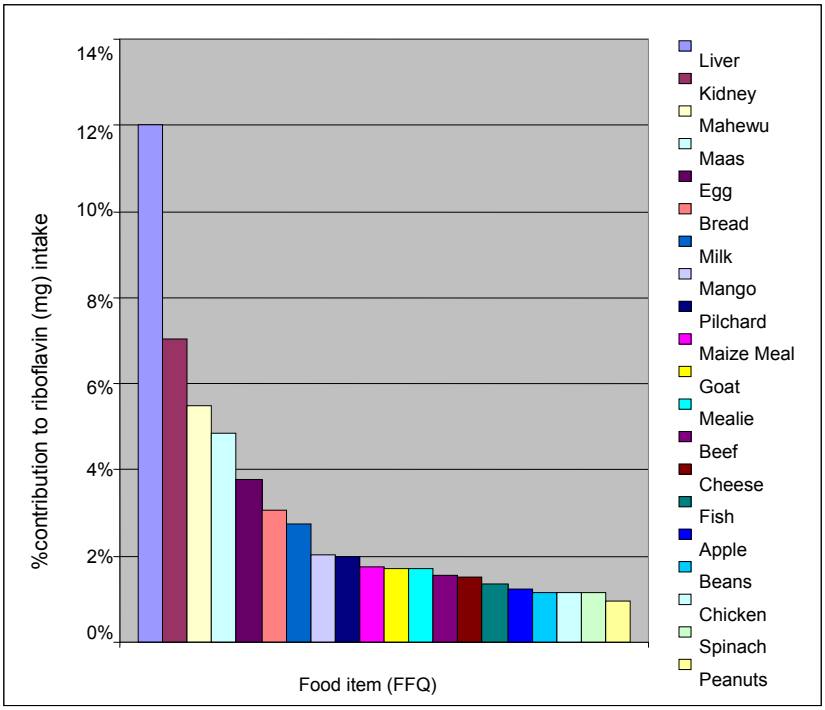


Figure 3.10: Top twenty food items that made the largest contribution to the riboflavin intake of the total group as measured with the FFQ

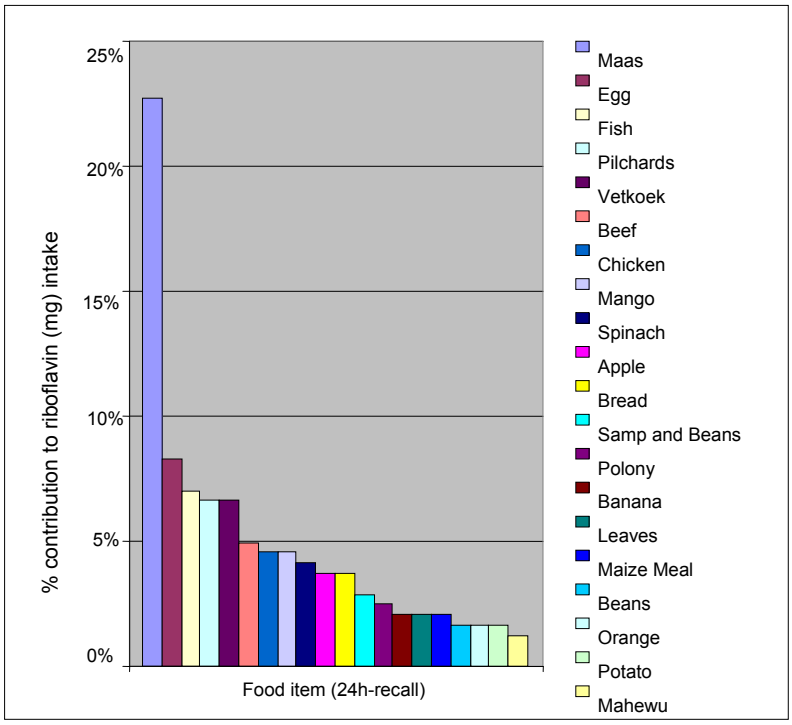


Figure 3.11: Top twenty food items that made the largest contribution to the riboflavin intake of the total group as measured with the 24h recall

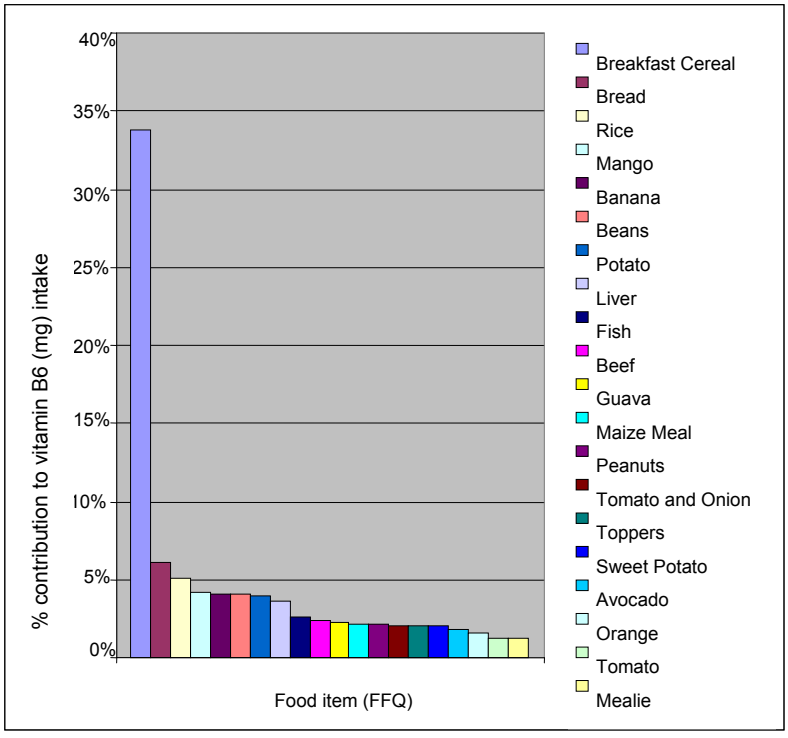


Figure 3.12: Top twenty food items that made the largest contribution to the vitamin B6 intake of the total group as measured with the FFQ

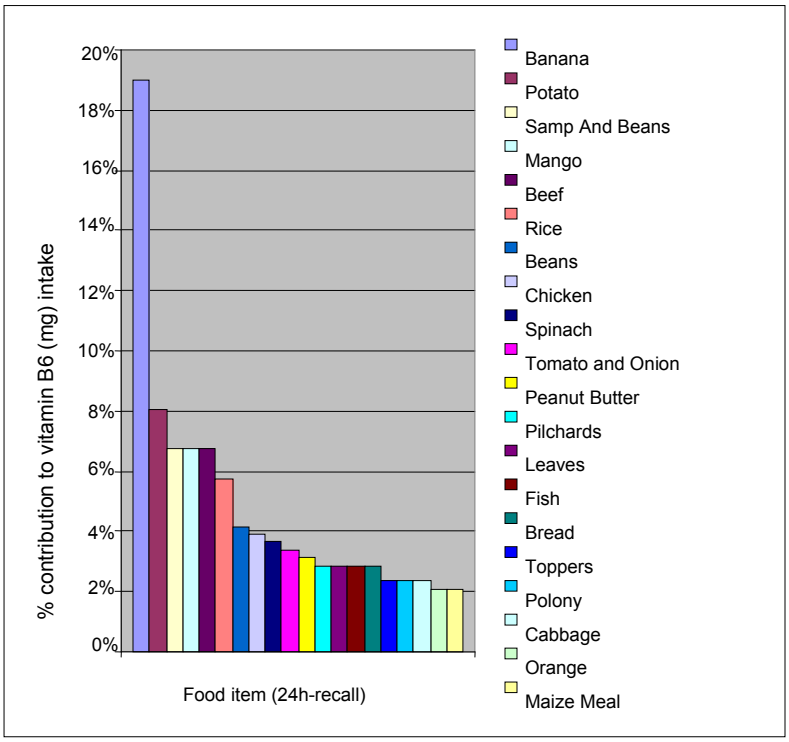


Figure 3.13: Top twenty food items that made the largest contribution to the vitamin B6 intake of the total group as measured with the 24h recall

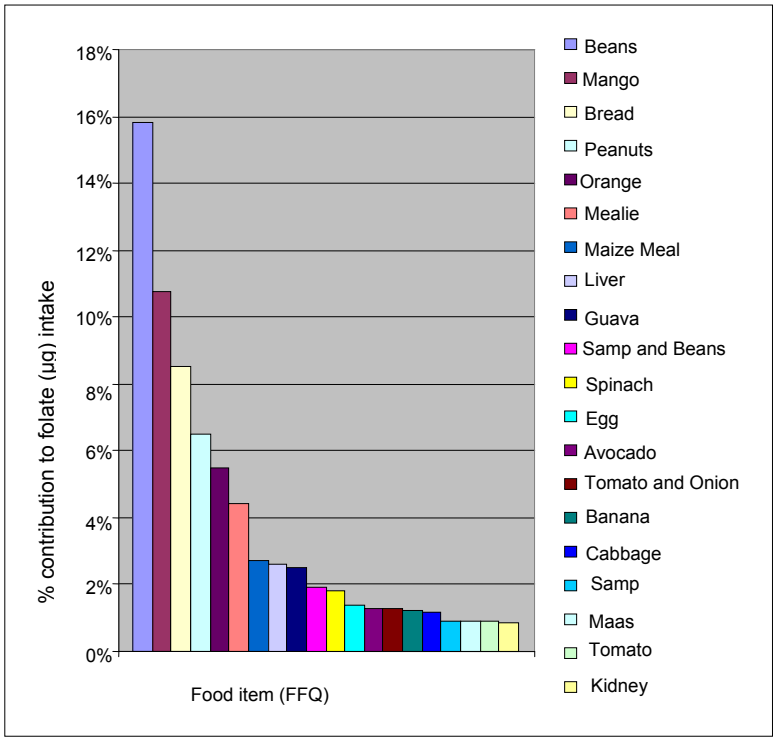


Figure 3.14: Top twenty food items that made the largest contribution to the folate intake of the total group as measured with the FFQ

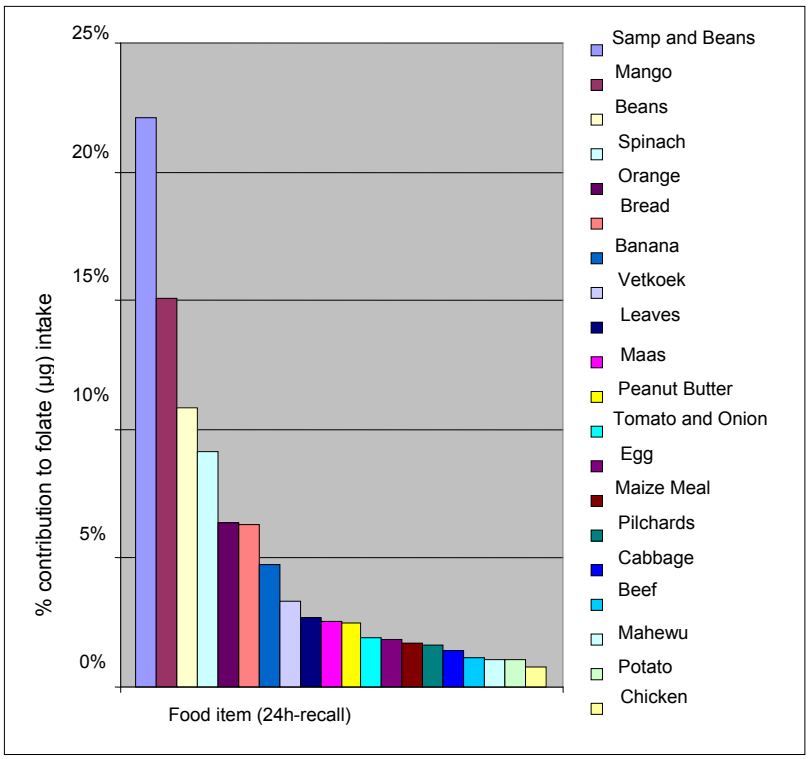


Figure 3.15: Top twenty food items that made the largest contribution to the folate intake of the total group as measured with the 24h recall

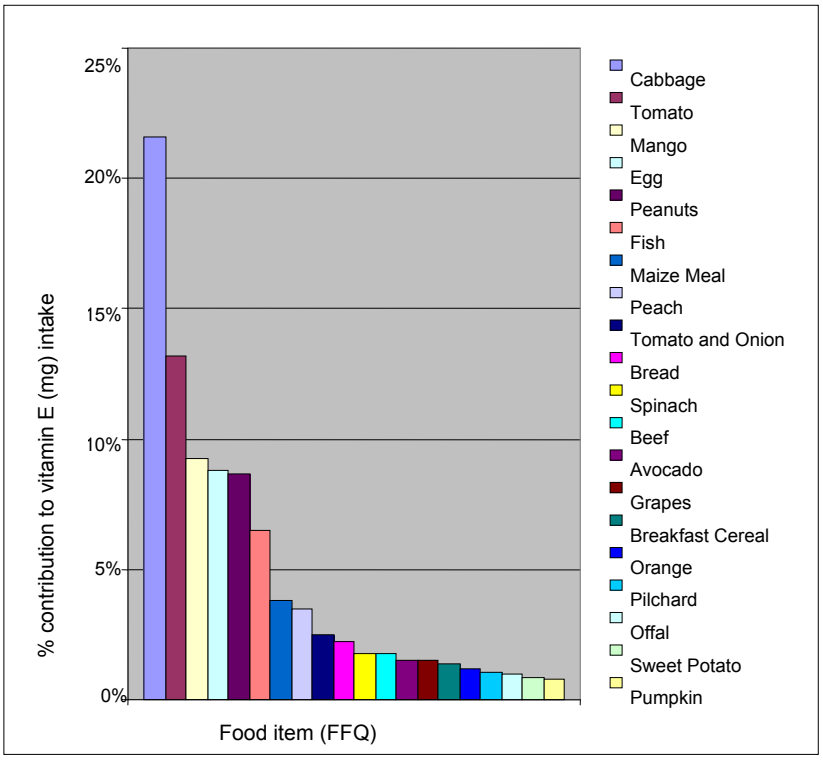


Figure 3.16: Top twenty food items that made the largest contribution to the vitamin E intake of the total group as measured with the FFQ

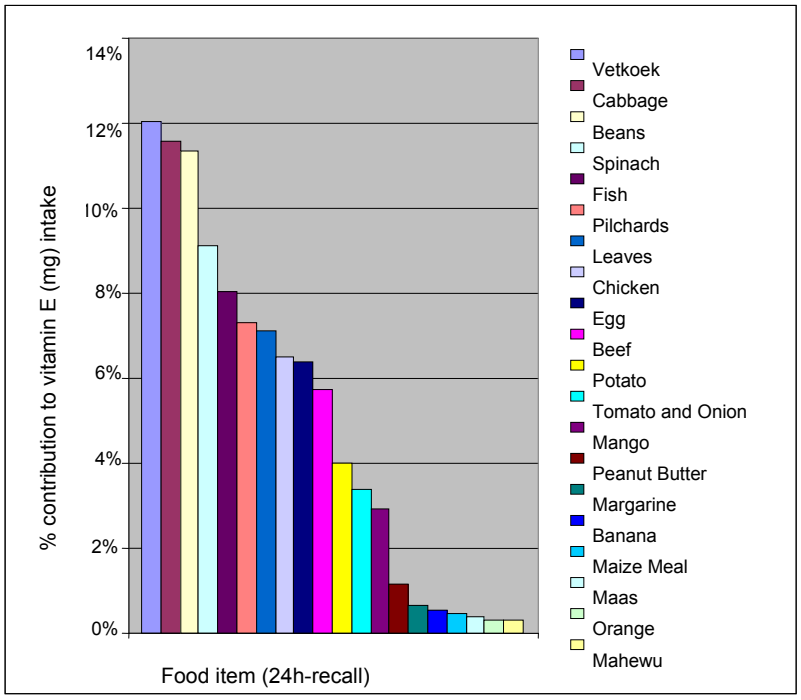


Figure 3.17: Top twenty food items that made the largest contribution to the vitamin E intake of the total group as measured with the 24h-recall

3.3.3 Mean daily micronutrient intake of subjects when using the dietary data collected with the 24h and with the FFQ

The mean micronutrient intake from the three FFQ records and the mean micronutrient intake of the three 24h-recall records for each subject, were calculated and used to determine the mean intake of nutrients for the group (n=108). The Estimated Average Requirement (EAR) for this population group was used as reference for adequacy of nutrient intake. Where no EAR was established, the Average Intake (AI) was used. In the case of copper the Recommended Dietary Allowance (RDA) was used as reference. (Table 3.2)

When the mean daily micronutrient intake as measured with the 24h-recall, was compared with the EAR, the intake of iron (101% of EAR), magnesium (102% of EAR) and selenium (101%of EAR) were the same as the EAR for this group. Thiamin (76% of EAR) and niacin (69% of EAR) intakes were within the recommended range (≥ 67%) of the EAR for thiamin and niacin respectively. When using the 24h-recall to

determine daily mean micronutrient intake, the intake of zinc (53% of EAR), copper (65% of EAR), vitamin A (24% of EAR), riboflavin (35% of EAR), vitamin B6 (44% of EAR), folic acid (47% of EAR), vitamin B12 (58% of EAR), vitamin C (45% of EAR), and vitamin D (25% of EAR) were all below the recommended (≥ 67%) of intake. Vitamin E intake (154% of EAR was the highest when measured with the 24h-recall.

Comparing the mean daily micronutrient intake as measured with the FFQ, with the EAR for each of the micronutrients under discussion, the intakes of iron (179%), magnesium (133%), selenium (127%), thiamin (129%), niacin (129%), vitamin B12 (220%), vitamin C (179%), zinc (77%), copper (94%), vitamin B6 (72%), folic acid (76%), and vitamin E (67%) were all in the acceptable range of the EAR for the specific micronutrient. Vitamin A, vitamin D and riboflavin (57%, 45% and 46% of EAR respectively) were the only micronutrients with daily mean intakes less than the recommended 67%, when measured with the FFQ.

Table 3.2: Comparison of mean intake of selected micronutrients as measured with the 24h-recall and FFQ, with the DRI's for this population group.

Micronutrient	DRI (EAR or AI)	Mean 24h recall (n=108)	Std Dev	% of EAR/AI	Mean FFQ (n=108)	Std Dev	% of EAR /AI
Iron (mg)	6.5 – 7 (EAR)	7.5	3.6	101	12.5	8.1	179
Magnesium (mg)	255-300 (EAR)*	286	133	102	400	400	133
Zinc (mg)	**10.4 – 11.6 (EAR)**	**5.9**	**2.9**	**53**	**8.5**	**5.2**	**77**
Copper (mg)	1.5 (RDA)	0.98	0.5	65	1.4	1.03	94
Selenium (µg)	59 (EAR)	59.4	36.4	101	74.7	58	127
Vitamin A (µg)	**880 – 900 (EAR)****	**209.3**	**541.5**	**24**	**507.6**	**739.5**	**57**
Thiamin (mg)	1.2 (EAR)	0.91	0.45	76	1.55	1.10	129
Riboflavin (mg)	**1.3 (EAR)**	**0.45**	**0.45**	**35**	**0.85**	**0.74**	**45**
Vitamin B6 (mg)	**1.7 (EAR)**	**0.74**	**0.45**	**44**	**1.23**	**0.95**	**72**
Niacin (mg)	13 (EAR)	8.9	5.78	69	16.7	11.4	129
Folic acid (µg)	**450 (EAR)**	**211**	**146**	**47**	**343**	**306**	**76**
Vitamin B12 (µg)	2.4 (EAR)	1.38	5.41	58	5.3	9.31	220
Vitamin C (mg)	96-100 (EAR)***	42.7	61.2	45	178.5	376.14	179
Vitamin D (µg)	**5 (AI)**	**1.26**	**2.14**	**25**	**2.3**	**3.23**	**46**
Vitamin E (mg)	**16 (EAR)**	**24.6**	**14.6**	**154**	**10.5**	**9.49**	**66**

* > 19yr - 300mg, 19-50yr - 255mg
**> 19yr - 880mg, 19-50yr - 900mg
*** > 19yr – 96mg , 19-50yr - 100mg

3.3.4 Agreement and correlation between FFQ and 24h-recall when used to determine micronutrient intake

The micronutrient intake data collected with the FFQ and 24h-recall was not normally distributed and a nonparametric comparison (Wilcoxon Matched Pairs Test) of the two variables (FFQ and 24h-recall) was used to compare the agreement of the two methods for measuring specified micronutrient intake.

Nutrients considered in this analysis were iron, zinc, copper, selenium, vitamin A, thiamin, riboflavin, niacin, vitamin B12, vitamin B6, folic acid, vitamin C, vitamin D and vitamin E. There was no significant agreement between any of the values (means) obtained by the 24h-recall when compared with the values (Table 3.3) obtained by the FFQ ($p>0.05$)

Table 3.3: Agreement between 24h-recall and FFQ as determined with the Wilcoxon Matched Pairs test ($p<0.05$) and RM ANOVA ($p<0.05$).

Nutrient (N=108)	F-value	P-value
Iron	108.9	P=0.0000
Zinc	57.07	P=0.0000
Copper	36.33	P=0.0000
Selenium	16.43	P=0.0001
Vitamin A	26.07	P=0.0000
Thiamin	114.21	P=0.0000
Riboflavin	60.5	P=0.0000
Vitamin B6	63.68	P=0.0000
Niacin	107.45	P=0.0000
Vitamin B12	30.82	P=0.0000
Folate	51.19	P=0.0000
Vitamin C	38.02	P=0.0000
Vitamin D	18.1	P=0.00005
Vitamin E	187.84	P=0.0000

To investigate the correlation between the two methods in obtaining values for selected micronutrients, Spearman's correlation coefficients were calculated (Table 3.4). All correlations were significant ($p<0.05$) but poor ranging from $r=0.40$ (thiamine) to the lowest $r=0.14$ (vitamin B12).

Table 3.4: Correlation between 24h-recall and FFQ as determined with the Spearman Correlation coefficient ($p<0.05$) and RM ANOVA ($p<0.05$)

Nutrient (N=108)	Spearman Correlation coefficients (r)	P-value
Iron	0.33	p=0.000
Zinc	0.35	p=0.000
Copper	0.27	p=0.000
Selenium	0.31	p=0.000
Vitamin A	0.25	p=0.01
Thiamin	0.40	p=0.000
Riboflavin	0.22	p=0.01
Vitamin B6	0.28	p=0.0035
Niacin	0.37	p=0.0000
Vitamin B12	0.14	p=0.14
Folate	0.29	p=0.000
Vitamin C	0.34	p=0.08
Vitamin D	0.21	p=0.000
Vitamin E	0.32	p=0.000

3.3.5 Agreement between 24h-recall and FFQ when ranking subjects according to intake of selected micronutrients.

The percentage intake from the EAR for each nutrient was determined for each subject when using data from the 24h-recall and from the FFQ. For each individual micronutrient, subjects were then ranked into one of five quintiles of intake for each of the data-collection methods and the percentage of subjects that were classified in the same quintile with the two methods was calculated. (Table 3.5)

The Confidence Interval (CI) was determined in each case by calculating the Lower and Upper Confidence Levels (LCL,UCL). The agreement between classifying subjects into quintiles according to percentage of EAR consumed, between the two different methods, were significant for all selected micronutrients, but reflect the poor agreement between the two instruments.

Table 3.5: Classification of individuals according to daily micronutrient intake into quintiles; agreement between 24h-recall and FFQ

Nutrient	Q1	Q2	Q3	Q4	Q5	% Agreement	CI $(P)_{0.95}$= LCL-UCL
Fe	4.63	3.7	3.7	2.78	9.26	24.07	0.2407 =0.160 - 0.321
Zn	4.63	3.7	4.63	2.78	3.7	19.44	0.1944=0.120 - 0.269
Cu	6.48	3.7	3.7	5.56	4.63	24.07	0.2407 =0.160 -0.321
Se	0.93	2.78	3.7	5.56	4.63	17.6	0.1760=0.104 - 0.248
Vit B6	2.78	3.7	2.78	2.78	2.78	14.82	0.1482=0.812 – 0.215
Riboflavin	5.56	0.93	2.78	2.78	3.7	15.75	0.1575=0.089 - 0.226
Vit B12	3.7	6.48	2.78	8.33	1.85	23.14	0.2314=0.152 – 0.311
Vit C	2.78	4.63	1.85	3.7	3.7	16.66	0.1667=0.096 – 0.237
Vit E	4.63	2.78	3.7	4.63	6.48	22.22	0.222=0.144 – 0.300
Folate	3.7	3.7	2.78	1.85	1.85	13.88	0.1388=0.074 – 0.204

CHAPTER 4: DISCUSSION

4.1 Findings of the Study

4.1.1 Habitual diets of study population

The findings of the present study indicate that the habitual diet of lactating mothers in the rural areas of KwaZulu-Natal consists mainly of phutu, bread, rice, dried beans, cabbage and chicken stew (often chicken feet) with onion and tomato. Beef and tripe are consumed at special occasions. Other animal protein sources included tinned fish (pilchards) as well as fresh fish. Reported fruit consumption seems to be very low, bananas and oranges are consumed by 80% of subjects (FFQ) only once or twice a week.

Food Frequency Questionnaires have been used widely to collect information on the habitual diets of population groups and to describe the type and frequency of food items typically consumed by a population group. Information collected with Food Frequency Questionnaires can also be used to identify the contribution of commonly consumed food items, to specific nutrient intakes of population groups.[85-100]

Multiple 24-hour recalls, rather than one singular 24-hour recall are better suited for the purpose of estimating food intake over a longer period, in this study three 24-h recalls were done 6, 8 and 14 weeks apart. Although the purpose of 24-hour recalls was not to determine the frequency of consumption of food items, the frequency of consumption as estimated by the 24-hour recall was included in order to strengthen the findings of the FFQ. Information collected with the 24h-recall indicated that only 10 different food items contribute to 80% of the foods most frequently consumed. These food items were maize meal, bread, beans, rice, chicken, mahewu, cabbage, potato and green leaves (from a variety of wild plants). Both methods identified maize meal, bread, chicken, dried beans, cabbage, tomato and onion, bananas, oranges and green leaves as the most frequently consumed food items. In a study done by Steyn et al,[86] maize porridge (78%/848 g), white sugar (77%/27 g), tea (68%/456 g), brown bread (55%/165 g), white bread (28%/163 g), non-dairy creamer (25%/6 g), brick margarine (21%/19 g), chicken meat (19%/111 g), full-cream milk (19%/204 g)

and green leaves (17%/182 g) were the food items most commonly consumed by the South African adult population. High frequencies of consumption of maize meal, bread, chicken meat and green leaves were reported in a number of other studies.[85,87]

Applying the FFQ it was determined that maize meal (in the form of phutu, a stiff porridge) forms the staple food in the diet of this population group; 83% of all subjects (n=108) consume maize meal between four and seven times a week. Mahewu (a fermented drink made of maize meal) was an additional source of maize meal consumption with 56% of subjects consuming it at least once a week and 31% indicating that they consumed mahewu every day. The average consumption of maize meal in this population group (phutu and mahewu) is about 811g when measured with a FFQ and 475g (mahewu and phutu) per day when using a 24h-recall. Bread was also consumed on a daily basis by 43% of subjects, 98% indicating that they ate bread at least once a week. Although 97% of subjects indicated that they ate chicken at least once a week, only 10% consumed chicken more than three times a week. Beans were consumed by 64% of subjects at a frequency of one to three times per week but other protein sources such as red meat and eggs were only consumed by 36% (red meat) and 35% (eggs) at a frequency of one to three times a week. (Figure 3.5)

4.1.2 Foods contributed the most to the micronutrient intake of subjects

To identify the food sources contributing mostly to the intake of iron, zinc, copper, selenium, vitamin A, riboflavin, folate, vitamin C, vitamin B6, vitamin B12 and vitamin E, the total contribution of each food item to the total amount of each of these micronutrients, as measured with the two methods, were determined.

Bread, beans, maas, pilchards, mango and green wild leaves were the foods that contributed the most to the micronutrients under investigation. Although maize meal (in the form of *phutu* or *mahewu)* was the food item most frequently consumed in large portions, it was not in the top ten food items for any micronutrient contribution,

except for selenium. Fortification of staple foods became mandatory in the last two months of data collection therefore the possible effect of fortification on the micronutrient intake was not considered in this study.

The energy intake associated with the large amount of maize meal and bread consumed, should be investigated. Nutrient adequacy was strongly correlated with energy intake in a number of studies done on dietary variety where adjusting for energy intake substantially reduced associations of food group intakes and variety counts with nutrient adequacy. Thus, any guidance to a community that promotes dietary variety should emphasize that the goal is to alter variety within the context of a diet that maintains appropriate energy balance.[88,89]

Although a question on supplements was included on the FFQ and 24h-recall, less than five subjects indicated that they consumed supplements on a regular basis. Contribution of supplements to the micronutrient intake in the diet, where therefore not investigated in this population.

4.1.3 Estimated nutrient intake of subjects

Food frequency questionnaires are commonly used to assess habitual food intake. Although food frequency questionnaires are known to produce measurement error, the amount of error and effectiveness of correction methods are poorly understood.[90] Most large cohort studies have used a food frequency questionnaire for assessing dietary intake and to place individuals into broad categories along a distribution of nutrient intake. Because of severe attenuation reported in most studies, the FFQ cannot be recommended as an instrument for evaluating relations between absolute intake of energy or protein and disease. The utility of either the 24hour-recall or the FFQ for detecting important but moderate relative risks even for energy-adjusted dietary factors is questionable.[91]

4.1.4 Percentages consumed less than the EAR when using the FFQ to determine intake of selected nutrients versus using the 24h recall

The estimated intake of all micronutrients was measured higher with the FFQ than with the 24h-recall, except for vitamin E. A possible explanation for this could be in the way the nutrient analyses were done. In the analyses of the 24h-recall, recipes were adjusted to accommodate a larger amount of sunflower oil in the cooking process. In converting the FFQ information to a format that can be used for nutrient analyses with Food Finder, only single food items were analyzed and not recipes, e.g. chicken meat (FFQ) versus chicken stew (24h-recall).

The DRI used in this analysis, was the EAR (Estimated Average Intake) for this population group, which is defined as the intake that meets the estimated needs of a nutrient of 50% of individuals in a specific gender group, at the given life-stage.[92]

In this study (using the 24h-recall), low mean intakes of zinc (5.9mg, 53% of EAR), vitamin A (209 µg, 24% of EAR) riboflavin (0.45mg, 35% of EAR), vitamin B6 (0.74mg, 44% of EAR), folic acid (211 µg, 47% of EAR), vitamin C (42.7mg, 45% of EAR) and vitamin D (1.26µg, 25% of EAR) were found. Low intake of micronutrients, specifically folic acid, zinc, vitamin B12 and vitamin C was reported in earlier studies that investigated the micronutrient status of South-Africans.[93] In the South African Nutritional Survey which investigated the nutrient intake of South Africans, the mean intake of zinc for this population group was 7.5mg (SD 5.8mg), riboflavin 1.9mg (SD 0.9mg), vitamin B6 0.6mg (SD 0.6), folic acid 229 µg (SD 193 µg) and vitamin D 33.3 µg (33.3 µg).[94] The low intake of the specified micronutrients in this population group, regardless of the method used to establish micronutrient intake, reflects the findings of the above mentioned studies.

Validity, based on comparisons with multiple-day diet records or 24-hour recalls, was generally not good. Correlations between FFQ-and recall-derived nutrients are often <0.4 and rarely >0.6 with a wide range of correlation coefficients reported in previous studies, ranging from 0.1-0.7.[82,95] Findings of poor validity usually look better

following statistical adjustment for total energy or day-to-day variability.[95]

The correlation coefficients (unadjusted for energy) in this study were likewise very poor, ranging from 0.038 for vitamin B12 up to 0.48 for iron. All correlations (except vitamin B12) were poor but significant ($p<0.05$). The difference between the two measurements (with the 24h-recall and FFQ) for vitamin B12 could be partially explained by the high Standard Deviation for the average estimated intake of vitamin B12, when measured with the FFQ (average intake 5.3 µg, SD=9.31) and the 24h-recall (average intake 1.38 µg, SD=5.41). The higher consumption of pilchards and liver reported on the FFQ, rare mainly responsible for extreme intake levels. The same is true for the very low correlation coefficient of Vitamin A (average intake 209.3 µg, SD 541.5) with the 24h-recall recorded and for the FFQ (average intake 507.6 µg, SD=739.5).

4.1.5 Comparison between 24h-recall and FFQ when ranking subjects according to intake of selected micronutrients.

The purpose of the comparison of the quintile distributions of nutrient intake as obtained by the FFQ and the 24h-recall was to determine the proportion of subjects classified into similar quintiles by the two methods employed in this study. Statistical significant agreement has been shown for a high proportion of subjects classified into the same quintiles by both methods (CI=0.95). The range of agreement is from 13.9% to 24.1% with the mean 19.1%. This agreement is for subjects classified in the same quintiles by both methods and does not include the percentage being classified into adjacent quintiles. (Percentage of agreement for ranking into adjacent quintiles was not done)

CHAPTER 5: CONCLUSION AND RECOMMENDATIONS

5.1 Conclusions

In the field of dietary epidemiology a rapidly growing number of studies are using variations of the food frequency method of dietary assessment and, at the same time, other studies continue to critically examine the validity of that very same method. Most large cohort studies have used a food frequency questionnaire for assessing dietary intake. There has been concern about not only random measurement errors in food frequency reports but also biases in reporting diet related to disease or diseased-linked factors. Because of severe attenuation reported in most studies, the FFQ cannot be recommended as an instrument for evaluating relations between absolute intake of energy or protein and disease. The utility of either the 24hour-recall or the FFQ for detecting important but moderate relative risks even for energy-adjusted dietary factors is questionable.[95]

Studies and commentaries by Subar et al.[96] Willett[97] and Block,[98] concluded that food frequency methods are sufficiently valid for etiological studies and that there may be a "ceiling of validity" for food frequency questionnaires. Further refinements and improvements in dietary questionnaires may not substantially improve our current capabilities using existing questionnaires.[58]

The FFQ in this study however was specifically developed to describe the habitual dietary patterns of rural lactating mothers in KwaZulu-Natal, to measure the estimated intake of specific micronutrients that play an important role in the immune system and to compare the extend of correlation between the findings of the two dietary methods used. No attempt will be made to contribute any relative risk for disease on the findings in this study.

The dietary pattern of the population in this study can be described as mainly maize meal, bread, beans, spinach/cabbage, green leaves and chicken stew. The intake of fruit and dairy products were extremely low. The low intake of specified micronutrients is alarming.

The importance of consuming a diverse diet to maximize nutrient intake, came under

the spotlight recently.[60] Promoting small changes in eating patterns and awareness of the importance of micronutrient-rich foods in a population with very little economical resources could be a step in the right direction.

5.2 Recommendations

More studies are needed to identify which nutrients are likely to be consumed in inadequate amounts and their relationship to micronutrient status among women of reproductive age in areas of high HIV prevalence, as in the rural areas of KwaZulu-Natal. The previously collected quantitative and qualitative dietary intake data (repeated 24-hour recalls and FFQ's) could be analyzed deeper and compared with average nutrient intake by HIV status, socio-economic status, location, clinical and viral parameters and seasonality.

The study reported here, was a sub-study from the Maternal Nutrition Study[6]. During the MNS, biochemical measures of nutritional status (Vitamin B12, folic acid, tocopherol, retinol, ferritin, zinc and selenium) were collected. Further studies should investigate the relationship between nutrient intake and biochemical measures comparing by HIV-status and controlling for potentially confounding variables.

The results of this study and possible further analysis on the data previously collected, may help in identifying food pattern predictors of micronutrient intake. This information can be used to conduct focus group discussions in the community to investigate perceptions and barriers regarding possible recommended changes in dietary intake.

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LIST OF APPENDICES

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Page 1

Africa Centre for Health and Populations Studies

Maternal Nutrition Study

Food Frequency Questionnaire

1.Form completion details

Place	Field worker	Form completion date	VTS Number	MNS Number
		2 0 0 / m m / d d		

2.Visit

○ 1st ○ 2nd ○ 3rd ○ 4th ○ 5th

3.Fruit: Have you consumed any of these in the past month?

			Days per week(0-7)	Days per month(0-10)	Times per day(1-4)	Amount of serving
Bananas	○ Yes	○ No				. each
Oranges/ Nartjies	○ Yes	○ No				. each
Peaches	○ Yes	○ No				. each
Mangoes	○ Yes	○ No				. each
Pawpaw	○ Yes	○ No				. each
Pineapple	○ Yes	○ No				. each
Guavas	○ Yes	○ No				. each
Avocados	○ Yes	○ No				. each
Other fruit	○ Yes	○ No				
						. each
						. each

Do you have fruit trees or access to fruit trees close to you? ○ Yes ○ No

If yes, is fruit used for: ☐ Self ☐ Family ☐ Income

If yes, which ones

- ☐ Bananas
- ☐ Oranges
- ☐ Peaches
- ☐ Mangoes
- ☐ Pawpaw
- ☐ Pineapple
- ☐ Guava
- ☐ Avocado
- ☐ Other fruit

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Africa Centre for Health and Populations Studies
Maternal Nutrition Study
Food Frequency Questionnaire

MNS Number

4.Visit

○ 1st ○ 2nd ○ 3rd ○ 4th ○ 5th

5.Vegetables: Have you consumed any of these in the past month?

			Days per week (0-7)	Days per month (0-10)	Times per day (1-4)	Amount of serving	
Cabbage	○ Yes	○ No					ml
Amaranthi	○ Yes	○ No					ml
Spinach/ Imifino	○ Yes	○ No					ml
Pumpkin	○ Yes	○ No					ml
Tomatoes	○ Yes	○ No				.	each
Carrots	○ Yes	○ No				.	each
Mealies/ sweetcorn	○ Yes	○ No					each
Beetroot	○ Yes	○ No					ml
Potatoes	○ Yes	○ No					ml
Sweet potatoes	○ Yes	○ No				.	each
Other vegetable	○ Yes	○ No					
							ml/each

Do you have a vegetable garden at home or access to a vegetable garden close to you? ○ Yes ○ No

If yes, are vegetable used for: ☐ Self ☐ Family ☐ Income

If yes, which ones
- ☐ Cabbage
- ☐ Amaranthi
- ☐ Spinach
- ☐ Pumpkin
- ☐ Tomatoes
- ☐ Carrots
- ☐ Mealies
- ☐ Beetroot
- ☐ Potatoes
- ☐ Sweetpotato
- ☐ Other vegetable

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Africa Centre for Health and Populations Studies

Maternal Nutrition Study

Food Frequency Questionnaire

MNS Number

6. Visit

○ 1st ○ 2nd ○ 3rd ○ 4th ○ 5th

7. Protein Foods: Have you Consumed any of these in the past month?

			Days per week (1-7)	Days per month (0-10)	Times per day (1-4)	Amount of serving		
Chicken, roasted, plain, stew	○ Yes	○ No				○ Small	○ Medium	○ Large
Red meat, roasted, plain, stew	○ Yes	○ No				○ Small	○ Medium	○ Large
Liver: Ox/ sheep/ chicken	○ Yes	○ No				○ Small	○ Medium	○ Large
Kidney: Ox/ sheep/ chicken	○ Yes	○ No				○ Small	○ Medium	○ Large
Tripe/ other parts of intestines	○ Yes	○ No				○ Small	○ Medium	○ Large
Goat	○ Yes	○ No				○ Small	○ Medium	○ Large
Sausages/ frankfurters/ viennas	○ Yes	○ No				○ Small	○ Medium	○ Large
Cold meat-polony/ ham	○ Yes	○ No				○ Small	○ Medium	○ Large
Canned meats, e.g. bully beef	○ Yes	○ No				○ Small	○ Medium	○ Large
Fish, fresh, fried, steamed	○ Yes	○ No				○ Small	○ Medium	○ Large
Canned fish: pichards, sardines, pickled	○ Yes	○ No				○ Small	○ Medium	○ Large
Samp with beans	○ Yes	○ No				○ Small	○ Medium	○ Large
Other proteins e.g wild animals	○ Yes	○ No				○ Small	○ Medium	○ Large
Legumes :Beans, split peas, lentils	○ Yes	○ No				ml		
Eggs	○ Yes	○ No				each		
Peanuts	○ Yes	○ No				ml		
Soya products e.g. Toppers, Imana	○ Yes	○ No				ml		

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Africa Centre for Health and Populations Studies

Maternal Nutrition Study

Food Frequency Questionnaire

8.Visit

○ 1st ○ 2nd ○ 3rd ○ 4th ○ 5th

MNS Number

9.Starches: Have you consumed any of these in the past month?

			Days per week (0-7)	Days per month (0-10)	Times per day (1-4)	Amount per serving	
Maize meal porridge	○ Yes	○ No					ml
Phutu/ stiff pap	○ Yes	○ No					ml
Morvite	○ Yes	○ No					ml
Other type of porridge/ cereal	○ Yes	○ No					ml
Bread	○ Yes	○ No					cm
Samp (plain)	○ Yes	○ No					ml
Other type of bread, starch (specify)	○ Yes	○ No					
							ml/cm

10.Spreads: Have you consumed any of these in the past month?

			Days per week (0-7)	Days per month (0-10)	Times per day (1-4)	Amount per serving	
Peanut butter	○ Yes	○ No					ml
Cheese	○ Yes	○ No					slices

11.Milk and other drinks: Have you consumed any of these in the past month?

			Days per week (0-7)	Days per month (0-10)	Times per day (1-4)	Amount per serving	
Fresh cows milk	○ Yes	○ No					ml
Powder milk	○ Yes	○ No					ml
Fresh goat milk	○ Yes	○ No					ml
Maas	○ Yes	○ No					ml
Amahewu	○ Yes	○ No					ml
Fruit juice (specify)	○ Yes	○ No					
							ml
Vitamin/Mineral Supplements	○ Yes	○ No					each

How many times per day do you usually eat,including snacks? ○ 1 ○ 2 ○ 3 ○ 4 ○ >4 ○ Don't Know

APPENDIX 2

DIETARY INTAKE QUESTIONNAIRE: 24-HOUR RECALL

				2	0	0	2

Subject Number:						

Interviewer: _____

1. Day of the week recalled	1 Mon	2 Tue	3 Wed	4 Thu	5 Fri	6 Sat	7 Sun
2. Was yesterday typical/routine for you?	YES	NO IF NOT, WHY					

Please answer the following:

What type of bread do you usually eat?	Brown	White	Whole wheat	Other	Do not know

How often do you buy enriched bread?	Always	1 x week	2 x month	1 x month	Seldom/never	Do not know

What brand of mealie meal do you usually buy? _____

TIME OF DAY	FOOD ITEM/ DESCRIPTION	QUANTITY (INDICATE G/ML)

APPENDIX 3:

List of recipes added to Food Finder

(Updated: 25 August 2004)

1. Beef Curry
2. Beef stew mns with onion and Imana
3. Beef stew with vegetables mns
4. Boerewors stew
5. Cabbage mns sautéed with onion and tomato
6. Cabbage mns with onion
7. Chicken curry mns with onion
8. Chicken curry mns with potato and onion
9. Chicken stew mns plain
10. Chicken stew mns plain with potato and onion
11. Chicken stew mns with Imana
12. Chicken stew mns with onion and tomato
13. Chicken stew with vegetables mns
14. Fish fried in batter mns
15. Fried egg in oil mns
16. Mahewu made with maize meal, special, white
17. Mahewu made with maize meal, super, white
18. Mahewu made with sifted maize meal
19. Mahewu made with Ace
20. Mahewu made with Induna maize meal
21. Mahewu made with fortified maize meal special white
22. Mutton curry mns
23. Pasta and cheese dish
24. Peanut Soup
25. Phutu made with maize meal, special white
26. Phutu made with maize meal, super white
27. Phutu made with maize meal, sifted
28. Phutu made with Induna maize meal
29. Phutu made with Ace
30. Phutu made with Ace fortified
31. Phutu made with fortified maize meal, special white
32. Porridge made with maize meal, special white
33. Porridge made with fortified maize meal, special white
34. Porridge made with maize meal, super white
35. Porridge made with Ace
36. Porridge made with Induna maize meal
37. Potato Curry
38. Sauteed Amaranti mns
39. Sauteed Spinach
40. Stewed Pilchards with vegetables
41. Stewed pilchards with vegetables
42. Stiff Pap (Ace)
43. Stiff pap (Induna)
44. Stiff pap, maize special
45. Stiff pap made with fortified maize meal special white
46. Sugar beans with oil and onion
47. Sugar beans with onion & tomato
48. Tomato and onion in oil/stew

APPENDIX 4:

Ethics Approval

Form 310 Page 1 of 1

OMB No. 9999-0020 Approved for use through 7/31/94

Protection of Human Subjects
Assurance Identification / Certification / Declaration
(Common Federal Rule)

POLICY: Research activities involving human subjects may not be conducted or supported by the Departments and Agencies adopting the Common Rule (56FR28003, June 18, 1991) unless the activities are exempt from or approved in accordance with the common rule. See Section 101 (b) of the common rule for exemptions. Institutions submitting applications or proposals for support must submit certification of appropriate Institutional Review Board (IRB) review and approval to the Department or Agency in accordance with the common rule.

Institutions with an assurance of compliance that covers the research to be conducted on file with the Department, Agency, or the Department of Health and Human Services (HHS) should submit certification of IRB review and approval with each application or proposal unless otherwise advised by the Department or Agency. Institutions which do not have such an assurance must submit an assurance and certification of IRB review and approval within 30 days of a written request from the Department or Agency.

1. Request Type: ☑ Original ☐ Follow Up ☐ Exemption

2. Type of Mechanism: ☐ Grant ☑ Contract ☐ Fellowship ☐ Cooperative Agreement ☐ Other: _____

3. Application or Proposal Identification No. *(if known)*:

5. Name of Principal Investigator, Program Director, Fellow, or Other
Peggy C. Papathakis

4. Title of Application or Activity:
Nutritional Status Of Lactating Mothers: A Cohort Study To Assess The Impact Of HIV During The First 6 Months Of Lactation

6. Assurance Status of this Project *(Respond to one of the following)*:

☑ This Assurance, on file with the Department of Health and Human Services, covers this activity:
Assurance identification no. M-1325 . . . . . IRB identification no. 01

☐ This Assurance, on file with *(agency / dept.)* _____, covers this activity.
Assurance identification no. _____ . . . . . IRB identification no. _____ *(if applicable)*

☐ No assurance has been filed for this project. This institution declares that it will provide an Assurance and Certification or IRB review and approval upon request.

☐ *Exemption Status:* Human subjects are involved, but this activity qualifies for an exemption under Section 101 (b), paragraph ____

7. Certification of IRB Review *(Respond to one of the following IF you have an Assurance on file)*:

☑ This activity has been reviewed and approved by the IRB in accordance with the common rule and any other governing regulations or subparts on (date) 2/20/02 by: ☑ Full IRB review or ☐ Expedited Review.

☐ This activity contains multiple projects, some of which have not been reviewed. The IRB has granted approval on condition that all projects covered by the common rule will be reviewed and approved before they are initiated and that appropriate further certification will be submitted.

8. Comments:
UCD Human Subjects Reference Number 994142
The following study documents were approved by the IRB on the date referenced above: consent forms(s), description of study, sponsor's protocol and/or sponsor amendments (if applicable), advertisement for the recruitment of subjects (if applicable). Contact the PI for copies of the approved documents.

9. The official signing below certifies that the information provided above is correct and that, as required, future reviews will be performed and certification will be provided.

10. Name and Address of Institution:
University of California, Davis Medical Center
Office of the Vice Chancellor for Research
Office of Human Research Protection
Ambulatory Care Center, Suite 3870
4860 Y Street, Sacramento, CA 95817

11. Phone No. *(with area code)*:
(916) 734-6897

12. Fax No. *(with area code)*:
(916) 734-6872

13. Name of Official:
David Holt, JD

14. Title:
Director, Office of Human Research Protection

15. Signature:

16. Date:
3/7/02

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10 May 2002

Ms P Papathakis,
Fax 035 550 1674

Dear Ms Papathakis

PROTOCOL: Nutritional status of lactating mothers: A cohort study to assess the impact of HIV during the first six months of lactation. P Papathakis Africa Centre **Ref E009/02**

The Research Ethics Committee considered the abovementioned application and made various recommendations. These recommendations have been addressed and the protocol was approved on 9 May 2002.

Yours sincerely

PROFESSOR J MOODLEY
Chairman – Research Ethics Committee
Hdeg/Papathakis.11

APPENDIX 5

Example of calculations on the FFQ

A	B	C	D	E	F	G	H	I	J	K
MNSNO1	DATECOMP	CHICKENW	nrpm	CHICKENM	CHICKEND	small/40	med/80	large/120	g/month	g/day
501	14-May-2002	1	4	0	1	40			160	160/30
510	14-May-2002	1	4	0	1		80		320	320/30
510	09-Jul-2002	0	0	3	1	40			120	120/30
510	03-Sep-2002	1	4	0	1	40			160	
510	07-Jan-2003	1	4	0	1	40			160	
520	04-Jun-2002	3	12	0	1	40			480	
520	27-Aug-2002	3	12	0	1	40			480	
520	28-Oct-2002	2	8	0	1	40			320	
520	15-Apr-2003	3	12	0	1	40			480	

FORMULA for calculation: { [((C*4) + E) *F] } * portion size (G/H or I

A: Identification code
B: Date captured
C: Times per week consumed (eg chicken)
D: Times per month (C *4) if value in C = 0, use E)
E: Times per month (If less than once a week)
F: Times per day
G/H/I: portion size (use G or H or I)
J: Amount (in gram) consumed in last month (theoretically)
K: Amount per day

APPENDIX 6

Nutrient value limits

The following values were used in Food Finder to indicate a “warning” :

Energy (kJ):	< 3 000
	> 15 000
Total Protein:	> 100 g
Zinc:	> 20 mg
Selenium	> 150 µg
Riboflavin:	> 4.5 mg
Folic acid:	> 1000 µg
Vitamin B12:	> 7.5 µg
Vitamin C:	> 500 mg
Vitamin E:	> 45mg
Fe: error when	> 25mg

APPENDIX 7

Example of Data displayed with adapted Food Finder output

Record Type	Last Name	Date of Birth	Meal Date	energy_kj	ca_mg	fe_mg	zn_mg	se_ug	vita_ug	riboflavin_mg	folate_ug	vitb12_ug	vitc_mg
R	Mkhwanazi	1963-11-15	2002-05-15	10371	635	12.1	7.59	129.4	268	0.67	259	10.0	17
R	Myeni	1987-10-29	2002-05-16	5089	131	4.4	4.46	40.4	127	0.35	36	1.5	14
R	Myeni	1987-10-29	2002-07-10	8407	282	6.1	4.90	55.0	119	0.42	219	0.3	141
R	Myeni	1987-10-29	2002-09-04	7261	177	5.8	4.01	47.6	24	0.33	156	0.5	6
R	Myeni	1987-10-29	2003-01-10	5698	128	3.6	3.18	36.4	13	0.16	136	0.0	29
R	Thabede	1976-08-24	2002-06-06	6690	96	3.8	3.10	45.9	259	0.39	105	0.8	25
R	Thabede	1976-08-24	2002-08-29	7713	257	4.8	3.59	40.6	470	0.32	123	5.9	125
R	Thabede	1976-08-24	2002-10-29	9545	309	6.9	6.43	101.2	72	0.52	148	5.8	38
R	Thabede	1976-08-24	2003-04-17	7586	177	4.9	5.21	66.2	107	0.37	150	0.1	159
R	Mfekayi	1982-05-13	2002-06-07	10741	114	5.7	4.92	32.1	23	1.00	247	0.0	50
R	Mfekayi	1982-05-13	2002-09-10	10103	166	7.0	5.66	55.6	18	0.87	239	0.0	41
R	Dlamini	1959-01-12	2002-06-18	9163	170	7.1	4.79	51.4	265	0.22	338	0.0	6
R	Dlamini	1959-01-12	2002-08-29	7604	269	6.7	4.91	64.6	317	0.24	269	0.0	50
R	Dlamini	1959-01-12	2002-10-17	7294	363	9.4	5.32	81.1	460	0.24	264	0.0	3
R	Masinga	1964-05-12	2002-06-25	4209	120	3.2	1.32	13.0	6	0.09	55	0.0	58
R	Masinga	1964-05-12	2002-08-27	9015	456	14.5	5.68	32.8	693	0.25	244	0.0	22

APPENDIX 8: The major sources of micronutrients consumed in this population, as measured with the 24h-recall and FFQ

Contribution of different food items to total micronutrient intake (In percentage)

Example: (See Tables A8.1 and A8.2)

FFQ: Contribution of beans to the intake of Fe

1) Total amount of Fe consumed by 108 subjects on three occasions, measured with FFQ and analyzed with Food Finder: **1366.53 mg**
2) Total amount of Fe (mg) provided by total amount of beans consumed (g) , measured with the FFQ = **83.90 mg**
3) Percentage of Fe contributed by beans to total Fe –intake = **6.14%**

The same procedure was followed for all micronutrients and for the two different methods.

Itemtype	N	energy kj Means	tpro g Means	fe mg Means	zn mg Means	cu mg Means	se ug Means	vita ug Means	riboflavin mg Means	vitb6 mg Means	folate ug Means	vitb12 ug Means	vitc mg Means	vite mg Means
Maize Meal	518	2452	12.9	1.0	1.10	0.11	9.12	0.0	0.05	0.08	21.2	0.00	0.0	0.86
Chicken	302	217	6.3	0.2	0.42	0.02	3.45	2.9	0.04	0.04	1.4	0.06	0.0	0.10
Bread	301	1721	13.7	2.4	2.23	0.30	40.23	0.0	0.10	0.22	66.8	0.00	0.0	0.51
Beans	290	546	6.3	1.9	0.95	0.27	1.07	0.1	0.04	0.15	124.2	0.00	0.8	0.13
Cabbage	255	389	0.8	0.3	0.14	0.01	0.50	1.6	0.01	0.04	9.3	0.00	16.4	4.87
Tomato and Onion	247	91	0.7	0.2	0.15	0.07	0.32	23.9	0.01	0.07	10.1	0.00	13.9	0.56
Potato	242	227	1.2	0.5	0.18	0.06	0.39	0.0	0.01	0.14	2.0	0.00	13.4	0.04
Banana	241	190	0.7	0.3	0.11	0.03	0.54	8.0	0.01	0.15	9.5	0.00	5.9	0.14
Orange	212	327	1.1	0.4	0.32	0.14	0.71	5.7	0.03	0.06	43.0	0.00	76.0	0.27
Leaves	186	43	0.7	1.8	0.31	0.06	0.11	155.6	0.02	0.02	2.0	0.00	0.3	0.10
Egg	185	249	3.9	0.6	0.36	0.04	9.57	20.5	0.12	0.01	10.9	0.48	0.0	1.98
Beef	180	225	5.2	0.5	1.58	0.02	1.81	0.4	0.05	0.09	2.9	0.45	0.5	0.40
Mahewu	171	1349	6.8	2.5	0.00	0.00	0.00	0.0	0.17	0.00	0.0	0.00	0.0	0.00
Samp And Beans	161	117	1.0	0.3	0.13	0.03	0.00	0.0	0.00	0.02	15.3	0.00	0.0	0.02
Fish	136	253	6.8	0.3	0.15	0.04	0.00	8.6	0.04	0.09	1.4	1.42	0.1	1.46
Maas	134	270	3.3	0.1	0.59	0.01	2.20	40.0	0.15	0.02	7.0	0.40	0.9	0.13
Paw Paw	132	63	0.1	0.0	0.01	0.02	0.00	34.0	0.00	0.00	4.8	0.00	29.4	0.00
Pilchard	132	104	3.7	0.6	0.31	0.04	6.61	5.5	0.06	0.02	3.8	2.62	0.0	0.24
Toppers	128	89	1.2	0.8	0.19	0.07	0.00	0.0	0.02	0.07	0.0	0.01	2.1	0.00
Spinach	113	54	1.1	1.0	0.22	0.04	1.19	160.2	0.04	0.02	14.3	0.00	2.7	0.40
Apple	109	183	0.1	0.2	0.06	0.05	0.21	2.1	0.04	0.02	0.6	0.00	3.3	0.11
Polony	98	94	0.8	0.1	0.14	0.00	0.82	0.0	0.01	0.01	0.2	0.09	0.0	0.02
Mango	71	639	1.3	0.4	0.15	0.15	0.20	139.1	0.06	0.15	84.3	0.00	65.4	2.09
Sausage	67	111	1.0	0.1	0.13	0.00	0.99	0.0	0.01	0.00	0.0	0.01	0.0	0.02
Peanuts	66	541	5.5	1.0	0.70	0.24	1.54	0.0	0.03	0.07	51.2	0.00	0.0	1.95
Kidney	63	40	1.8	0.7	0.29	0.04	9.94	18.0	0.22	0.02	6.5	4.86	0.3	0.02
Liver	59	80	2.4	0.6	0.49	0.43	5.13	950.9	0.37	0.13	20.6	9.92	2.1	0.06
Rice	59	1026	5.2	0.4	0.89	0.12	14.50	0.0	0.02	0.18	5.8	0.00	0.0	0.08
Pumpkin	58	23	0.2	0.1	0.03	0.02	0.01	52.3	0.00	0.01	0.7	0.00	1.1	0.17
Mealie	54	397	2.5	0.5	0.36	0.04	0.59	17.1	0.05	0.05	34.9	0.00	5.0	0.07
Guava	50	302	0.8	0.4	0.16	0.16	0.00	10.4	0.02	0.08	19.8	0.00	361.4	0.15
Milk	48	142	1.7	0.1	0.21	0.00	1.08	25.4	0.09	0.02	2.6	0.21	0.4	0.06
Carrot	47	20	0.1	0.1	0.05	0.00	0.07	361.5	0.00	0.01	0.6	0.00	0.4	0.05
Sweet Potato	47	284	0.9	0.3	0.11	0.07	0.46	24.9	0.01	0.07	5.5	0.00	6.5	0.20
Avocado	43	353	0.6	0.1	0.20	0.10	0.16	1.0	0.01	0.07	10.1	0.00	4.8	0.35
Pineapple	42	21	0.0	0.0	0.00	0.00	0.04	0.3	0.00	0.00	1.3	0.00	3.6	0.00
Peach	42	221	0.8	0.3	0.10	0.09	0.46	6.8	0.02	0.01	2.2	0.00	8.9	0.78
Beetroot	33	34	0.3	0.1	0.07	0.02	0.00	0.7	0.00	0.00	2.7	0.00	0.3	0.00

Table A8.1 Micronutrient intake from different food items consumed, measured with the FFQ

Itemtype	N	energy kj Means	tpro g Means	fe mg Means	zn mg Means	cu mg Means	se ug Means	vita ug Means	riboflavin mg Means	vitb6 mg Means	folate ug Means	vitb12 ug Means	vitc mg Means	vite mg Means
Maize Meal	473	1530	8.1	0.7	0.8	0.08	7.2	24.5	0.05	0.08	17.10	0.00	0.0	0.57
Bread	246	1762	15.5	2.4	2.3	0.39	41.8	1.3	0.09	0.11	62.63	0.00	0.0	0.33
Beans	188	1431	5.6	1.7	0.9	0.27	1.1	2.3	0.04	0.16	107.47	0.00	4.0	14.37
Rice	130	1264	6.4	0.5	1.1	0.14	17.8	0.0	0.02	0.22	7.12	0.00	0.0	0.10
Chicken	96	1147	15.9	1.0	1.1	0.10	8.4	27.9	0.11	0.15	7.80	0.17	4.7	8.22
Mahewu	94	999	5.3	0.5	0.5	0.05	4.6	18.7	0.03	0.06	10.67	0.00	0.0	0.37
Margarine	88	441	0.0	0.0	0.0	0.00	0.0	104.6	0.01	0.00	0.01	0.00	0.0	0.85
Cabbage	81	1073	1.3	0.3	0.2	0.05	0.8	6.2	0.02	0.09	13.88	0.00	22.5	14.65
Potato	41	891	3.6	2.1	0.6	0.22	0.7	1.6	0.04	0.31	10.37	0.03	20.2	5.06
Leaves	39	691	1.9	2.9	0.8	0.15	0.5	234.4	0.05	0.11	26.46	0.00	22.1	9.03
Toppers	33	111	1.5	1.0	0.2	0.09	0.0	0.0	0.03	0.09	0.00	0.02	2.6	0.00
Beef	31	1037	12.1	1.6	3.6	0.11	4.1	38.0	0.12	0.26	11.10	0.95	9.1	7.27
Tomato and Onion	28	447	1.4	1.4	0.4	0.13	1.0	33.7	0.02	0.13	19.04	0.00	18.4	4.28
Banana	27	949	3.2	1.5	0.6	0.13	2.7	39.8	0.05	0.73	47.07	0.00	29.8	0.67
Vetkoek	26	2488	11.7	1.9	1.0	0.16	3.8	22.8	0.16	0.04	32.65	0.65	0.0	15.25
Orange	26	479	1.7	0.6	0.5	0.21	1.1	8.3	0.04	0.08	63.00	0.00	111.2	0.40
Apple	24	430	0.3	0.4	0.2	0.12	0.4	4.5	0.09	0.05	1.96	0.00	8.6	0.24
Spinach	20	791	1.7	1.4	0.4	0.11	1.9	328.6	0.10	0.14	90.55	0.00	18.6	11.57
Egg	20	726	6.6	0.9	0.6	0.06	16.2	35.3	0.20	0.02	18.10	0.86	0.0	8.08
Maas	19	984	12.0	0.4	2.1	0.04	8.0	145.8	0.55	0.06	25.32	1.45	3.7	0.48
Pilchards	18	898	9.8	1.5	0.9	0.14	19.9	28.8	0.16	0.11	16.33	5.81	5.9	9.25
Polony	17	514	5.3	0.5	0.8	0.03	5.5	0.0	0.06	0.09	1.65	0.51	0.0	0.09
Fish	17	1137	17.2	1.4	0.8	0.12	0.5	16.0	0.17	0.11	6.41	5.81	0.9	10.18
Mango	14	1131	2.2	0.8	0.3	0.26	0.4	246.4	0.11	0.26	149.29	0.00	115.6	3.69
Samp And Beans	12	1685	14.9	3.6	1.9	0.50	0.0	0.0	0.07	0.26	218.42	0.00	0.0	0.30
Peanut Butter	11	810	7.6	0.5	0.8	0.17	0.0	0.0	0.03	0.12	24.27	0.00	0.0	1.45

Table A8.2: Micronutrient intake from different food items consumed, measured with the 24h-recall

APPENDIX 9

Graphs not included in main paper, indicating food items contribution to micronutrients when measured with 24h and FFQ.

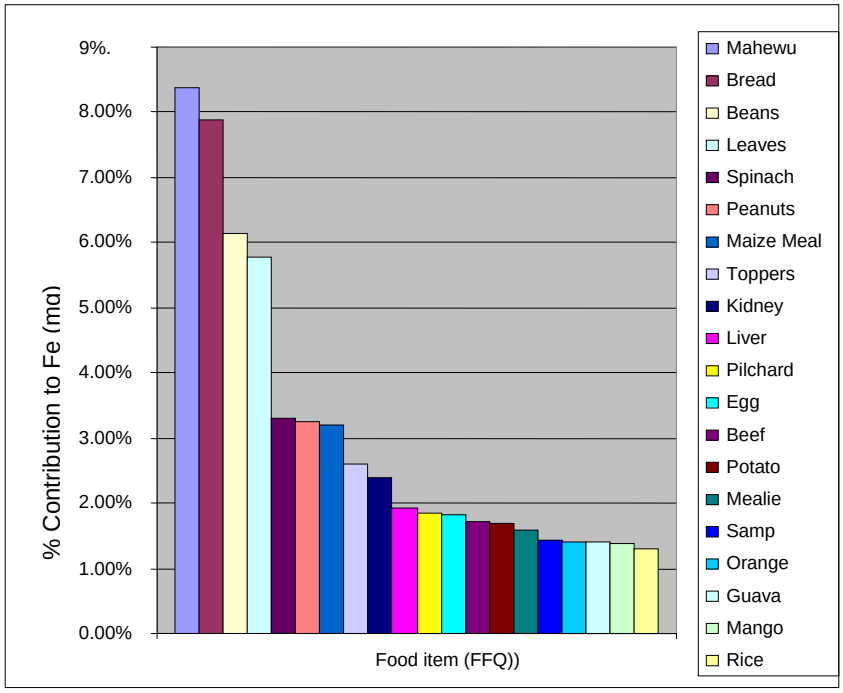

Fig 9.1: Top twenty food items that made the largest contribution to the Fe intake of the total group as measured with the FFQ

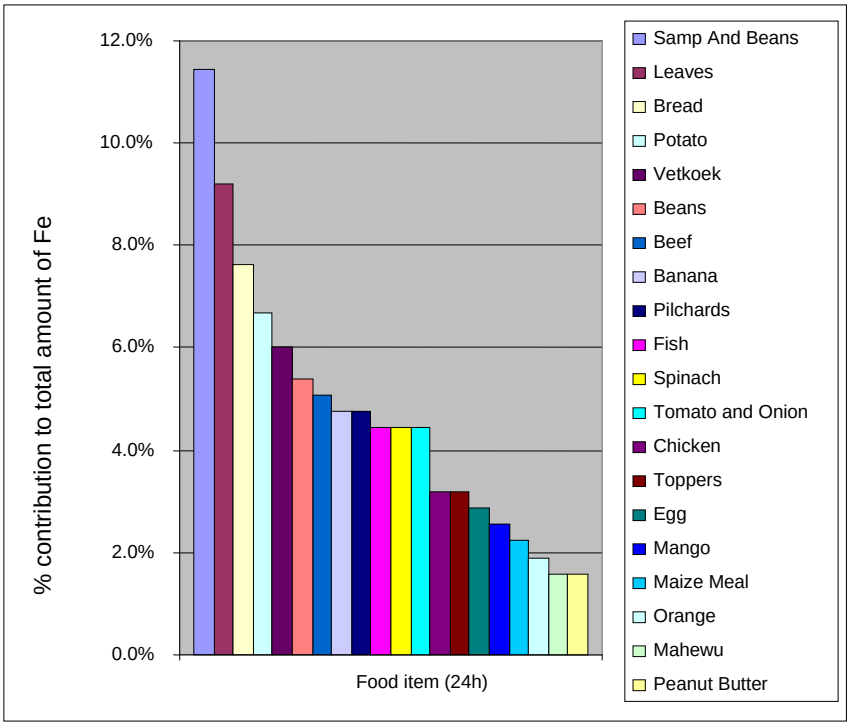

Figure 9.2: Top twenty food items that made the largest contribution to the iron intake of the total group as measured with the 24h recall

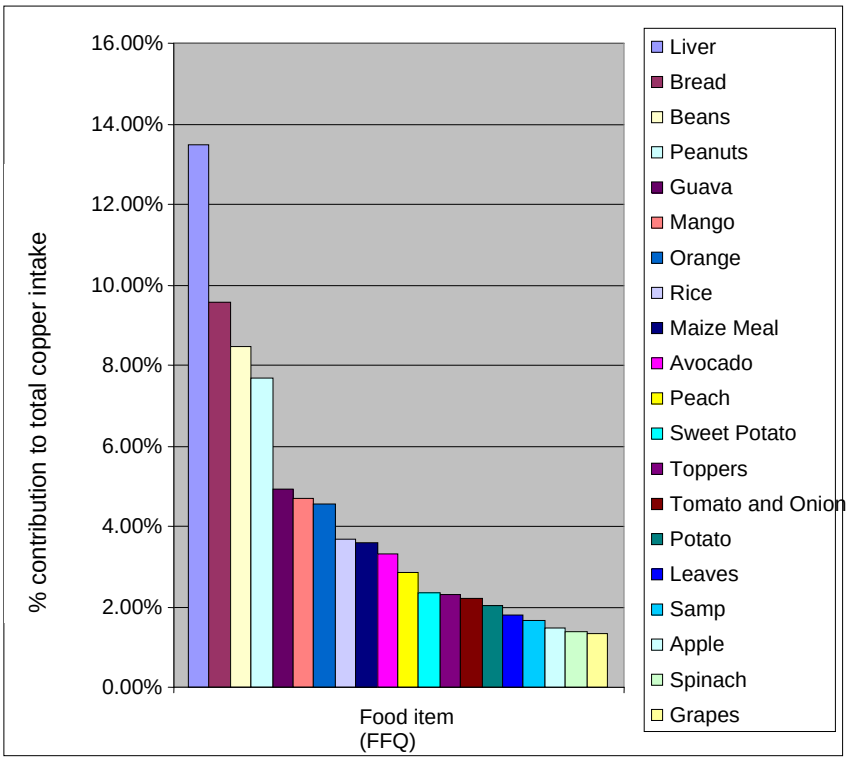

Figure 9.3: Top twenty food items that made the largest contribution to the copper intake of the total group as measured with the FFQ

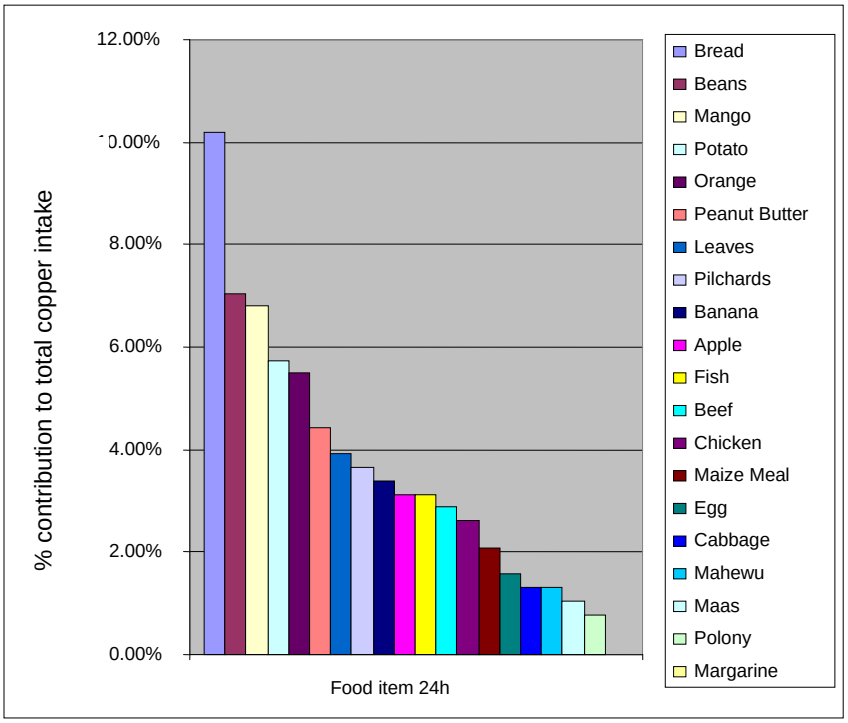

Fig 9.4: Top twenty food items that made the largest contribution to the Cu intake of the total group as measured with the 24h recall

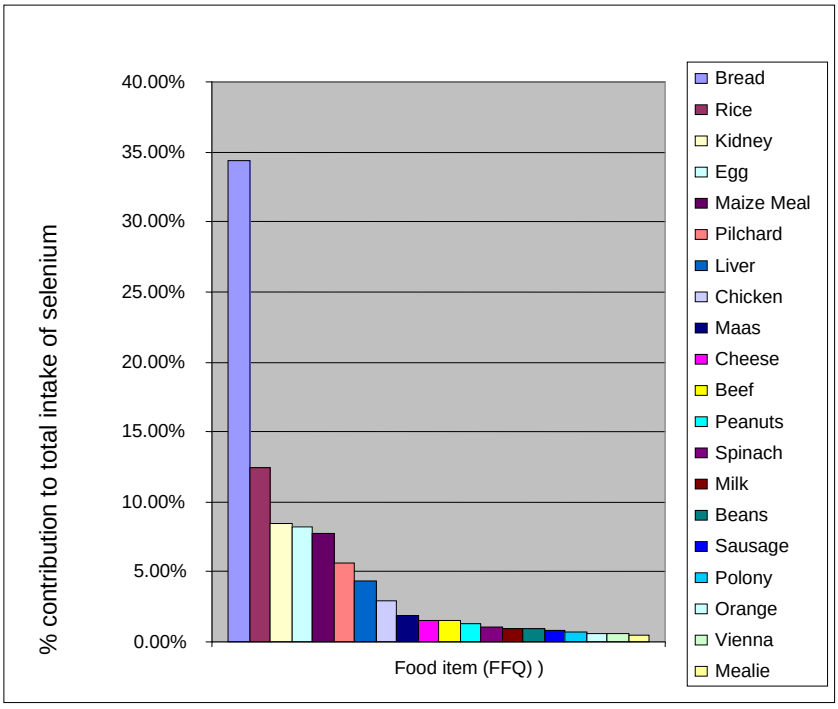

Fig 9.5: Top twenty food items that made the largest contribution to the selenium intake of the total group as measured with the FFQ

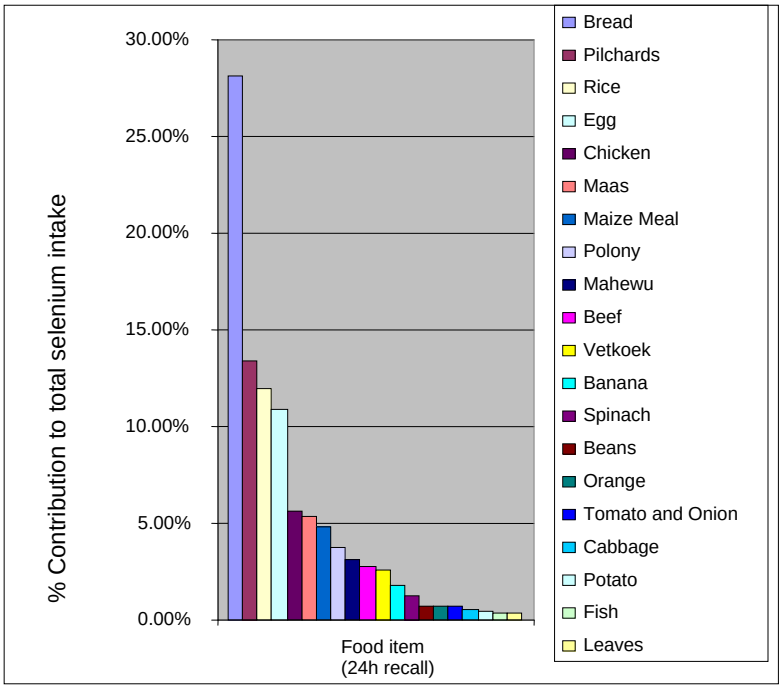

Figure 9.6: Top twenty food items that made the largest contribution to the selenium intake of the total group as measured with the 24h recall

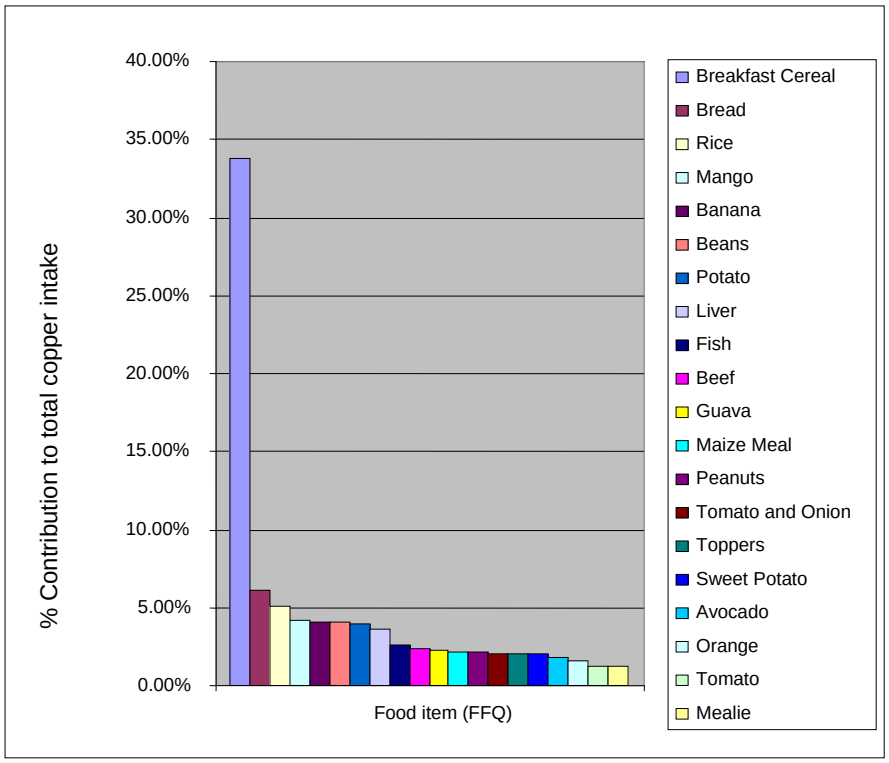

Figure 9.7: Top twenty food items that made the largest contribution to the copper intake of the total group as measured with the FFQ

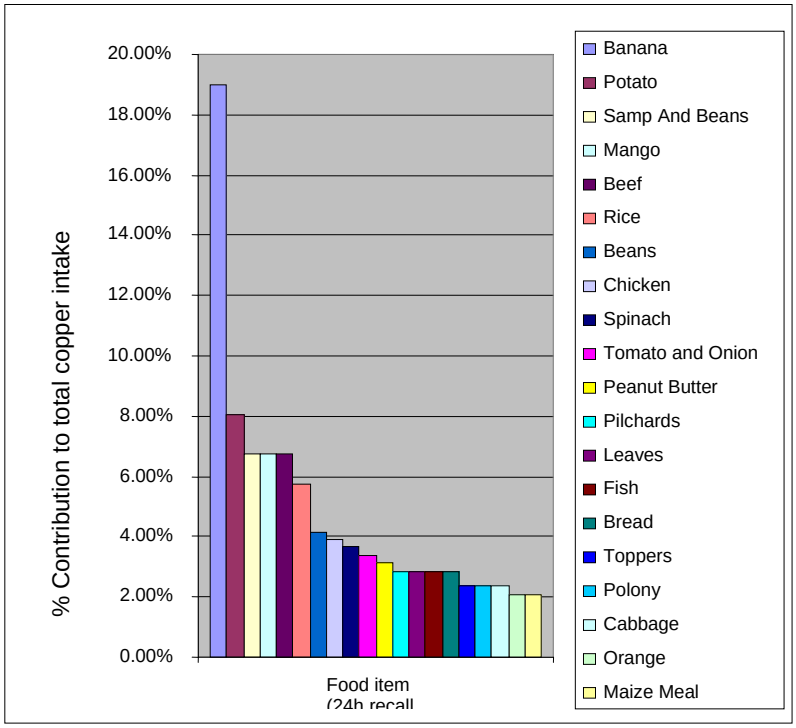

Figure 9.8: Top twenty food items that made the largest contribution to the Copper intake of the total group as measured with the 24h recall

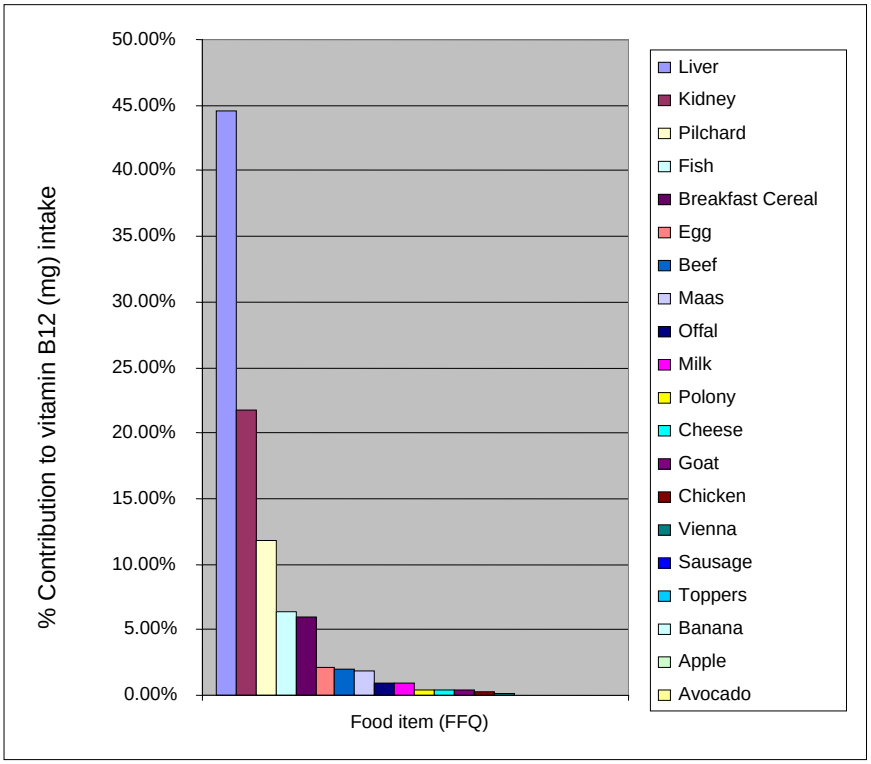

Figure 9.9: Top twenty food items that made the largest contribution to the vitamin B12 intake of the total group as measured with the FFQ

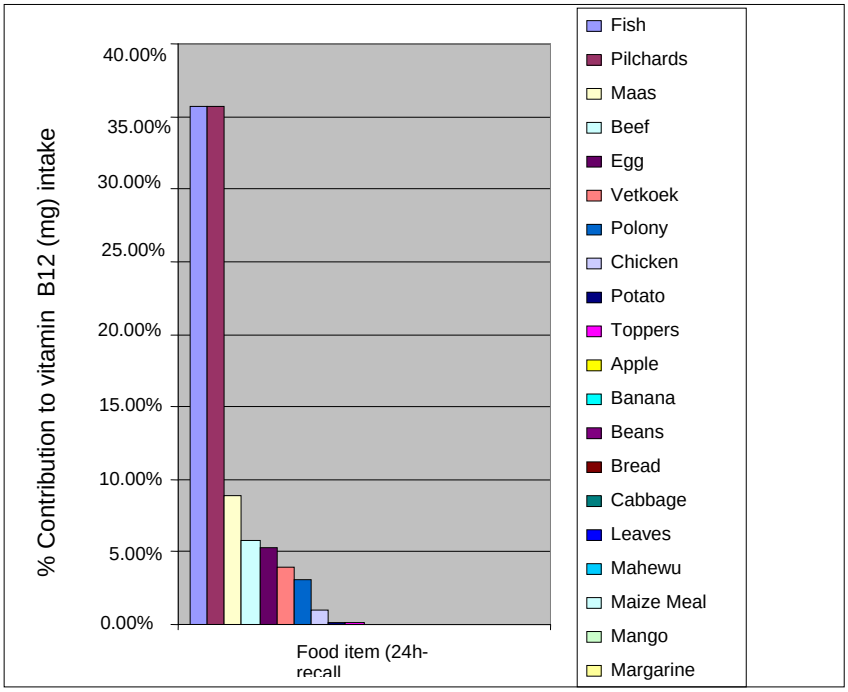

Figure 9.10: Top twenty food items that made the largest contribution to the vitamin B12 intake of the total group as measured with the 24h recall

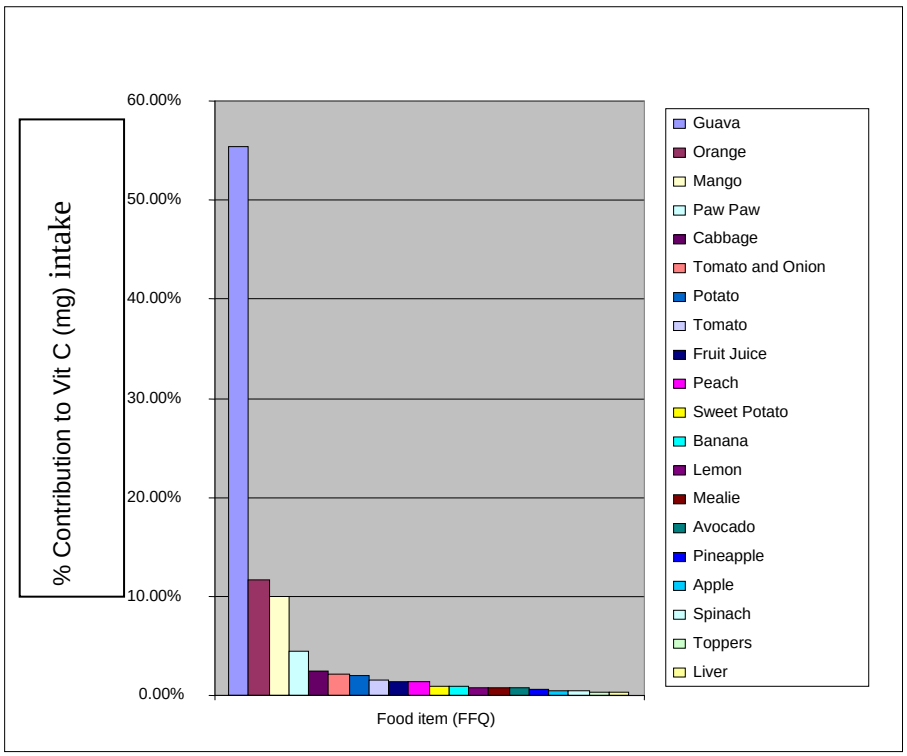

Figure 9.11: Top twenty food items that made the largest contribution to the vitamin C intake of the total group as measured with the FFQ

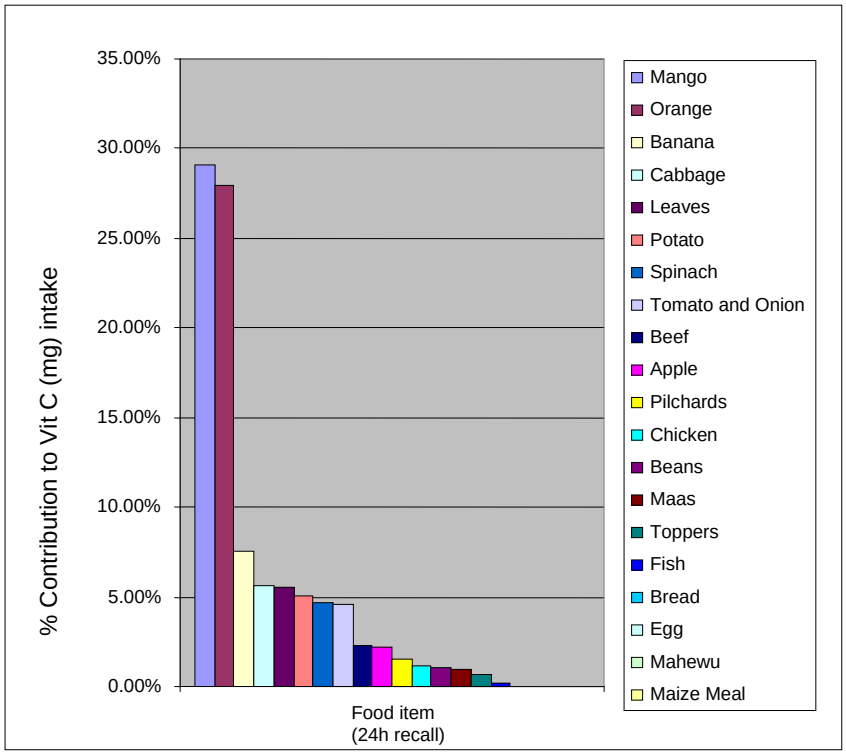

Figure 9.12: Top twenty food items that made the largest contribution to the vitamin C intake of the total group as measured with the 24h recall