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EFFECT OF NUTRITIONAL STATUS AND NUTRITIONAL SUPPLEMENTATION ON CLINICAL, NUTRITIONAL AND IMMUNOLOGICAL OUTCOMES AMONG HIV INFECTED ADULTS IN UGANDA

**A dissertation submitted in partial fulfilment of the
requirements for the Degree of Doctor of Philosophy**

By

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DECLARATION

I Dr. Bernard Kikaire declare that this thesis has not previously been submitted as an exercise for a degree at the University of Dublin, Trinity College, or any other university, and is entirely the product of my own work.

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SUMMARY

Background

Malnutrition is a major complication of HIV disease. In Uganda, up to 25% of adults initiating antiretroviral treatment were found to be malnourished. Malnutrition is one of the leading causes of immunosuppression. It impairs many aspects of both the innate and adaptive immune system and has been associated with poor clinical outcomes including an increased risk of mortality among HIV infected patients.

Malnutrition in HIV-infected individuals is mainly managed using nutritional supplements such as ready to use therapeutic foods (RUTF). The effect of nutritional supplementation on various outcomes in malnourished HIV infected adults has been studied with contradictory results. Commonly studied outcomes include body mass index, CD4 cell counts, viral loads changes and mortality. There are hardly any studies that have used multiple methods to more comprehensively assess nutritional status or described the clinical, nutritional and immunological characteristics of malnourished HIV infected adults and evaluated the effects of nutritional supplementation on these outcomes in the same study.

The NOURISH study aimed to describe the effects of nutritional status and nutritional supplementation on clinical, nutritional and immunological outcomes in HIV infected adults in Uganda.

Methods

This was a mixed methods study in which both quantitative and qualitative study designs were employed. The quantitative study was divided into two: a randomized study in which moderately malnourished HIV infected participants were randomized to receive or not to receive RUTF (the intervention). One group in this study was ART naïve, while the other was ART experienced, each group was randomized separately. The second study was a non-randomized prospective interventional study in which all the severely malnourished participants were given a nutritional supplement (the intervention). All participants received nutritional counselling as a standard of care and were followed every four weeks for 12 weeks. The nutritional supplement used was Plumpy'Nut, a ready to use therapeutic food

(RUTF). The participants in the interventional arm received two (2) sachets of Plumpy'Nut per day, which were equivalent to 1,000 kcal of energy. The outcomes were median differences of the clinical, nutritional and immunological parameters between the interventional and control arms for the randomized study, and comparison of the baseline and 12-week medians of all the parameters that were assessed for the non-randomized studies.

The clinical parameters that were assessed were clinically relevant laboratory measurements which included CD4⁺ T cell counts, changes in viral load, hemoglobin, serum albumin, liver enzymes and creatinine. The nutritional parameters were: anthropometry, biomarkers of nutrition and 24-hour nutrient intake. The immunological parameters were phenotypes of circulating lymphocytes and capacity for cytolytic degranulation and cytokine production upon stimulation.

The qualitative study used focus group discussions to evaluate the acceptability, challenges and benefits of using RUTF among malnourished HIV infected adults.

Outcomes were compared using the Mann-Whitney test or the Wilcoxon signed rank test and significance was measured at 5%. Analysis was done using Stata 13.0 IC version for the clinical and nutritional outcomes, or GraphPad prism 7.04 for the immunological outcomes. The qualitative data were analyzed using NVivo 12.0

Results

A total of 102 HIV positive participants were enrolled into the study, of whom 32 (31.4%) were moderately malnourished ART naïve (MAM), 18 (17.7%) were severely malnourished ART naïve (SAM), 27 (26.5%) were malnourished and ART experienced (ART) and 25 (24.5%) were well-nourished and ART naïve (WN). 53 (52.0%) women and 49 (48.0%) men were enrolled into the study. The SAM participants had the most advanced HIV disease with 15 (83.3%) of them reporting WHO stage IV disease, and the lowest median CD4⁺ cell count of 64 (IQR 10-133) cells/μl of blood. At baseline, the overall median body mass index for the study participants was 17.9 (IQR 16.4-18.9). The BMI for the SAM participants was 16.3 (IQR 15.9 – 16.4) and for the MAM 18.1 (17.4 – 18.3). 56% of the SAM and 73% of the MAM participants had less than normal percent fat mass. The median values for all the serum biomarker of nutrition were within normal ranges except for high

density lipoproteins (HDL), indeed more than 90% of all the ART naïve participants (SAM, MAM and WN) had less than normal levels of HDL. The median energy intake for the study population was 1871.7 kcal (iqr 1422.2 -2421.8), the SAM participants had the lowest energy intake. 74% of all study participants had more than the recommended daily intake of carbohydrates, but only 29% had adequate protein and 58.6% had adequate fat intake at baseline. 82% of the ART experienced participants had less than the recommended daily allowance of protein. There was a low intake of all micronutrients except iron and vitamin B6.

The ART naïve HIV infected participants had lesser frequencies of the circulating B cells, NK cells, CD4⁺ T cells, double negative T cells, Vδ2 T cells, iNK T cells, and CD56⁺ T cells compared to the HIV negative controls. The frequencies of these cells were lesser in malnourished participants compared to the well-nourished participants HIV infected participants. Their circulating frequencies of the total T cells and Vδ3 T cells of the HIV infected participants were not different with the ones of the HIV negative controls, while the frequencies of CD8⁺ T cells, double positive and Vδ1 T cells were higher among the HIV infected compared to the HIV negative controls, and among the malnourished HIV infected adults compared to the well-nourished adults.

After ex-vivo stimulation, the CD8⁺ T cells of the WN participants had better degranulation than the CD8⁺ T cells of the SAM participants and the Vδ1 T cells of the MAM participants had better degranulation than those of the SAM participants.

The WN also had more CD4⁺ T cells and CD8⁺ T cells producing IFN-γ than the MAM and SAM. However, the SAM participants had more CD8⁺ T cells producing IL-4 than the WN participants, and the MAM participants had more Vδ1 cells producing IL-17 than the WN cells.

After 12 weeks, RUTF and ART led to higher absolute and proportional increases in in all the assessed parameters for clinical, nutritional and immunological outcomes. Additionally, the proportional increases in weight; BMI; fat mass; percent fat mass and MUAC were higher in the participants that received ART and RUTF compared to those that received ART only (no RUTF group). Similarly, there higher

increases in hemoglobin, albumin and the lipids in the participants that received RUTF and ART, but not in the other biomarkers of nutrition. In the ART naïve MAM participants, there were significant differences in fat intake, and all assessed micronutrients, but not in proteins between the randomization arms. The participants that received RUTF had a reduced intake of carbohydrates. These changes were also observed in the ART experienced participants except the differences in fat intake were not statistically significant.

Although the RUTF group had higher proportional changes in all the parameters from baseline.

There were significant differences in all the circulating lymphocytes between the RUTF and No RUTF groups in the MAM participants initiating ART, and the ART experienced participants. However, the proportion of CD4⁺ T cells, CD8⁺ T cells, NK cells and Vδ1 cells exhibiting cytotoxic degranulation were higher in the participants that received RUTF, although the only significant difference was seen in the Vδ1 cells. There were no differences in cytokine production between the RUTF and No RUTF participants.

Conclusion

The NOURISH study showed that the malnourished participants had lower clinical, nutritional and immunological parameters. RUTF led to bigger proportional increases in in most parameters in the participants that received RUTF and ART compared to those that received ART only. Statistical significance was found in nutrient intake, and increased cytotoxic degranulation, but not in, circulating lymphocytes or cytokine production.

These findings show that RUTF is beneficial in malnourished HIV infected participants.

To Kirabo, Kuteesa, Kudzie and Kusiima, the challenge is yours.

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PHD OUTPUTS AND SKILLS ACQUIRED

Courses attended:

- 1- Basic immunology course at Trinity College Dublin (Feb – May 2014)
- 2- Advanced Epidemiology and Biostatistics course organized by London School of Hygiene and Tropical Medicine, and the MRC Research Unit in Uganda (August 2014)
- 3- Principles of flow cytometry and Training in the use of Facs Canto II (Becton Dickinson) at Trinity College Dublin (April 2014)
- 4- Pharmacokinetics and Pharmacokinetics modelling using NONMEN (June 2014)

Skills gained

1. Basic flow cytometry
2. Extracellular and intracellular staining of PBMCs with flouorochromes
3. Development and validation of drug extraction methodologies using liquid chromatography and mass spectrometry

Presentations

1. Presentation of PhD proposal to:
 - a. Department of Immunology at Trinity College Dublin (May 2014)
 - b. Infectious Diseases Institute at Makerere university (Oct 2014)
 - c. MRC/UVRI Research Unit on AIDS in Uganda (Jan 2015).

2. Provisional Results

- a. Infectious Diseases institute at Makerere university, July 2015
- b. Department of Immunology at Trinity College Dublin, March 2016

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LIST OF ACRONYMS

3TC-Lamivudine

ABC- Abacavir

ACD-Acid citrate dextrose

AE- Adverse events

AIDS-Acquired Immune Deficiency Syndrome.

AOR- Adjusted odds ratio

ART-Anti retroviral Therapy.

BMD- Bone mineral density

CD- Cluster of differentiation

CD4+- Cluster of Differentiation 4

CD8+- Cluster of Differentiation 8

CDLS – Clinical Diagnostic Laboratories

CI- Confidence interval

COR- Crudes odds ratio

CRP-C reactive protein

CSB – Corn Soy Blend

CTX-Cotrimoxazole

DNA-Deoxyribonucleic acid

DNCB-2-4-di-nitro-chlorobenzene

DTHR- Delayed type hypersensitivity response

EDTA- Ethylenediaminetetraacetic acid

EFV- Efavirenz

FBC-Full blood count

FCT- Food composition tables

FCS- Fetal calf serum

GC-Gas chromatography

GFATM-Global Fund to fight AIDS, Tuberculosis and Malaria

HAART-Highly Active Antiretroviral Therapy

HAZ- Height for age z-score

HIV-Human Immunodeficiency Virus

HPLC- high-performance liquid chromatography

IFN- γ - Interferon gamma

Ig- Immunoglobulin

IL- Interleukin

iNKT- Invariant natural killer T cells

INR-immunological none responders

IRIS-Immune reconstitution Inflammatory Syndrome

IRB: Institutional review board

LFT's-Liver function Tests

LT- Leukotrienes

LRSs-Low resource settings

MAM: Moderately acute malnutrition

MDG's- Millennium Development goals

NK- Natural killer cells

IMAM: Integrated management of acute malnutrition

IMCI: Integrated management of childhood illnesses

MOH- Ministry of Health

MRC: Medical research council

MUK- Makerere University

NAIDS- Nutritional acquired immunodeficiency syndrome

NVP-Nevirapine

OI's-Opportunistic Infections

PBS- Phosphate buffered saline

PCR-Polymerase chain reaction

PHA- Phyto-hemagglutinin

PhD- Doctor of Philosophy

RFTs-Renal function Tests

RFS- Re-feeding syndrome

RNA- Ribonucleic acid

RPMI- Roswell Park Memorial Institute medium

RUTF- Ready to Use Therapeutic Food

SAE- Serious/severe adverse events

SD- standard deviation

TB- Tuberculosis

TLC- Total lymphocyte count

TNF α - Tumor necrosis factor alpha

UNAIDS-United Nations program against AIDS

UNAIS-Uganda Aids Indicator Survey

UNICEF-United Nations International Children's emergency fund

WBC-White blood cells

WHO-World Health Organization

CHAPTER ONE:

INTRODUCTION AND BACKGROUND

1.1. INTRODUCTION

1.1.1. HIV: The Global and Sub-Saharan Africa Burden

HIV/AIDS remains a global challenge with the number of people living with HIV estimated to be 36.7 million by the end of 2016 worldwide (1,2) . Although the burden of the disease differs across regions, Sub-Saharan Africa continues to be most affected with 4.2% of the adults living with HIV and accounting for almost two thirds of the people living with HIV globally(2). Approximately 19.4 million people living with HIV live in East and Southern Africa, which is more than half of the world's population living with HIV (2).

Globally, there is a reduction in the number of new HIV infections, it is estimated that there were 1.8 million new HIV infections in 2016, with 790,000 of these coming from East and Southern Africa. However, this remains above the fast track goals of having less than 500,000 new infections per year by 2020 (2).

In Uganda, the prevalence of HIV among adults stands at 6.2% in the general population, with females having a higher prevalence (7.6%) than men (4.7%) (3). Prevalence is highest among males aged 45 to 49 at 14%, and in women aged 35 to 39 years, at 12.9%(3). Across Uganda, HIV is more prevalent among the population living in urban areas (7.5%) compared to those in rural areas (5.8%). In fact, amongst the females, HIV prevalence in urban areas is 9.8% versus 6.7% in rural areas(3). In 2016, the incidence of HIV in Uganda stood at 1.2 per 1000 population, translating into approximately 52,000 new infections in that year(2). This was a reduction from more than 100,000 new infections observed in 2005(2). This reduction is mainly attributed to the introduction of antiretroviral therapy as observed by Ron Gray and others(4).

1.1.2. HIV Virology

The HIV virus is a member of the retrovirus family and genus lentiviruses (5–7), and is classified into two types (type 1 and 2) (8) on the basis of genetic differences. HIV-2 is predominantly found in West Africa(9)(10) and not as virulent as HIV-1(11). HIV-1 is then subdivided according to genetic heterogeneity into three groups M (Main), N (Non-M/Non-O) and O (Outlier)(12–14). Another group, was identified in a Cameroonian residing in France and was named group P ‘pending the identification of further human cases’(15). The group responsible for the majority of infections worldwide is then subdivided into 10 subtypes or clades (A-K) and many other recombinant forms (16,17). Figure 1.01 below is a map showing the geographic distribution of HIV-1 in the world, and Figure 1.02 shows the HIV-1 subtypes.

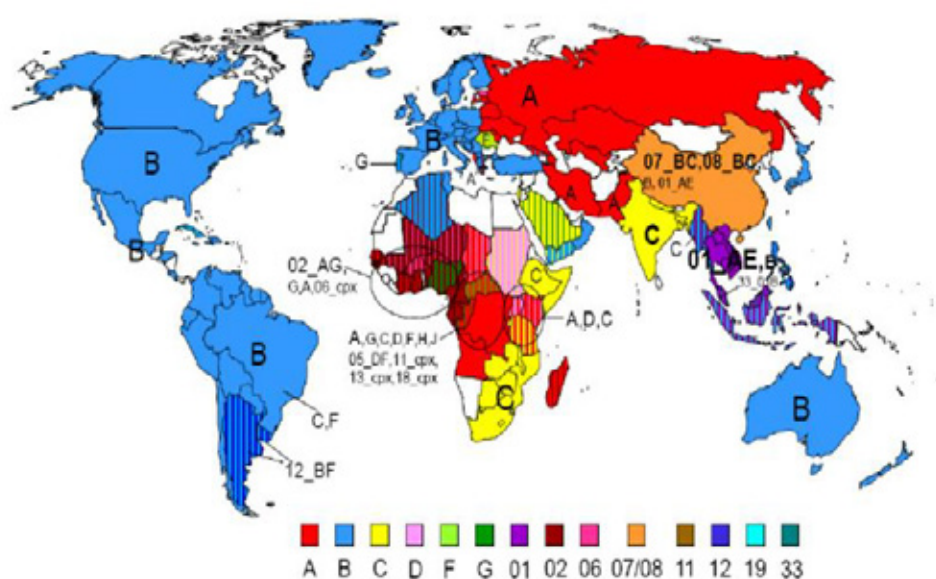


Figure 1.01: Geographical distribution of HIV-1 subtypes:

The American region, Western Europe and Australia are predominated by subtype B, Eastern Europe is mainly subtype A. The Indian subcontinent, and Southern Africa have subtype C, while most of Sub-Saharan Africa has a mixture of different subtypes. Uganda is predominantly A and D. Adapted from WHO-UNAIDS HIV Vaccine Initiative

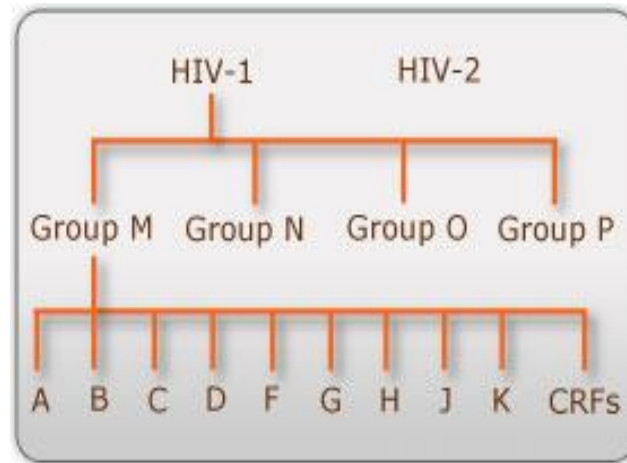
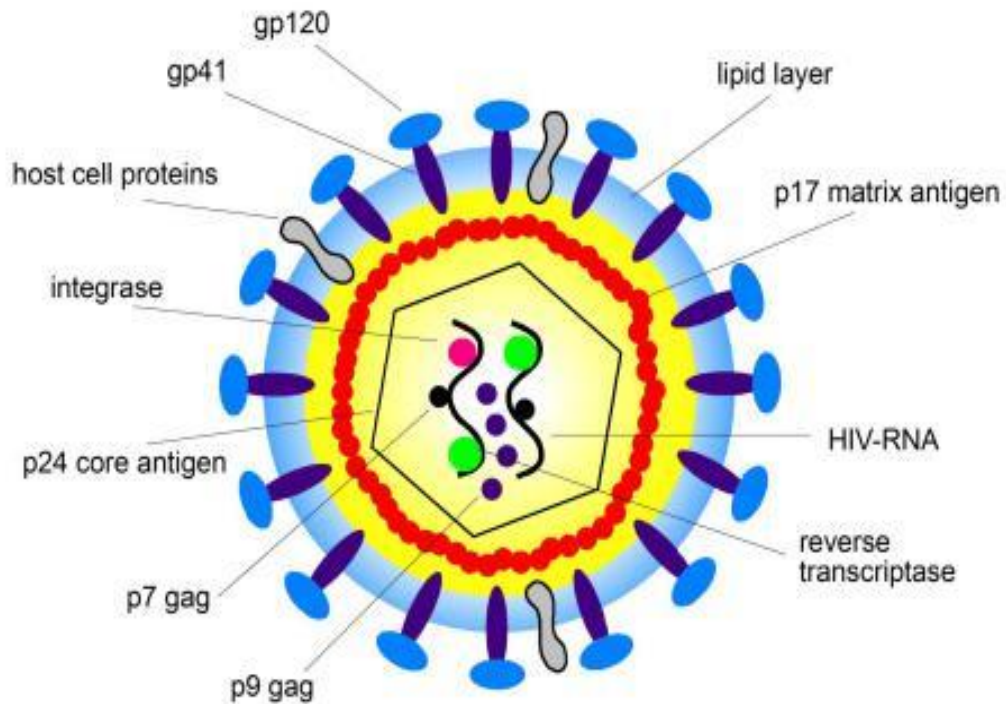


Figure 1.02: HIV groups, types and subtypes
(378–380)

HIV is divided into HIV type 1 and type 2. HIV type 1 is then subdivided into four groups M, N, O and P. Group M is further subdivided into 10 subtypes of clades A-K, and the circulating recombinant forms (CRFs).)

The HIV-1 viral particles are about 100nm in diameter and enclosed in a lipoprotein membrane (18). Each viral particle has 72 glycoprotein complexes that are integrated in the lipoprotein membrane (18). The glycoproteins each have an external glycoprotein gp120, and a transmembrane glycoprotein gp41. The viral particle also has a matrix protein P-17 which is attached to the inside of the lipoprotein membrane. The P-24 core antigen contains the two copies of HIV-1 RNA. The RNA strands are part of the protein nucleic acid complex which is made up of nucleoprotein P7 and the reverse transcriptase enzyme. Each viral particle has the reverse transcriptase, the integrase and the protease enzymes, which it uses for replication (18).

The genetic structure of HIV is made up of two single strands of RNA molecules enclosed in the core of the virus particle. Figure 1.03 is a representation of the HIV particle.



1.1.3. The HIV Replication cycle

The HIV virus specifically infects a variety of immune cells through specific target cell receptors; in macrophages and CD4 T cells, an interaction between viral surface glycoprotein gp120 and chemokine receptor CCR5, CCR4 and CD4 molecules aid entry of the virus into the cells(20–22). Individuals with homozygous 32 base pair deletion in the CCR5 gene are resistant to HIV (23). After virus binding to the cell, the HIV virus uses the envelop protein to aid fusion of the virus cell membrane with the cell membrane of the CD4 T cells (24), and this establishes the infection of the immune cells. Once in the cell, the HIV virus uses the reverse transcriptase enzyme to change its genome from RNA to DNA (25), and then uses the integrase enzymes to integrate its DNA into the host cell DNA (26). The proviral DNA is then replicated together with the cellular DNA during the cell division (26) to create longer chains of viral proteins. These viral proteins are then assembled together near the surface of the host cell and released as non-infectious HIV virions. Using the protease enzyme, it breaks up the long chains into smaller infectious viral proteins ready to infect another cells. (27). Figure 1.04 is a representation of the 7 steps involved in the HIV replication cycles

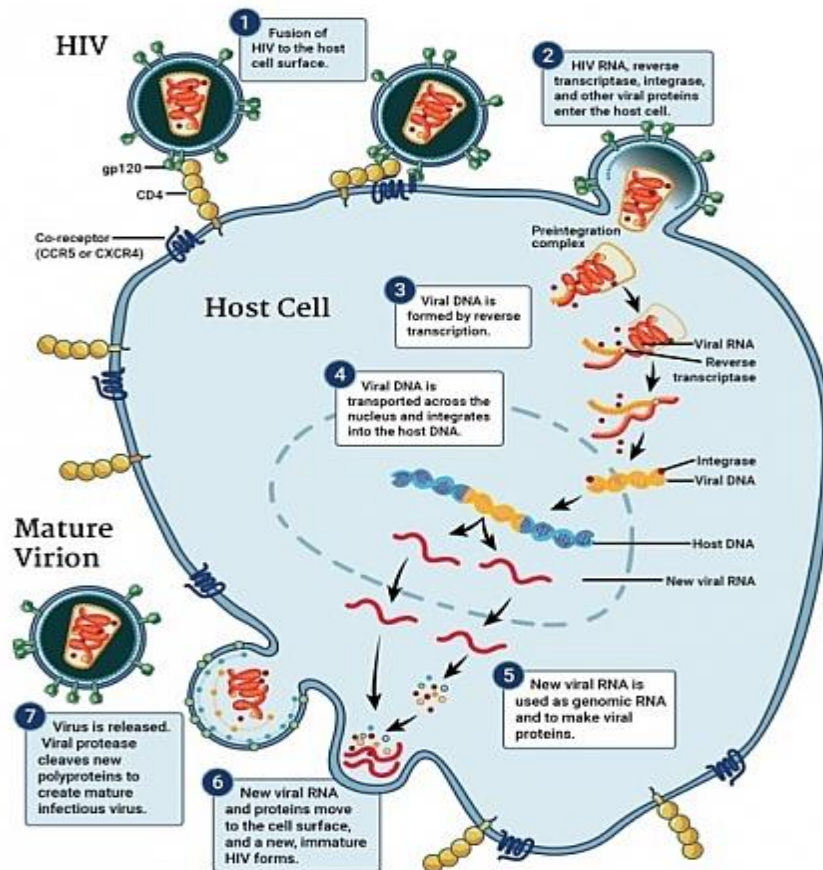


Figure I.04: HIV replication cycle (27)

Binding, in which the HIV virus attaches to the surface of the CD4 cells by the gp120 attaching to the CCR5 or the CXCR4. The virus then fuses its envelope with the host cell membrane thereby gaining entry into the cell and releasing its genetic material into the host cell. Using the reverse transcriptase enzyme, the HIV converts its single stranded RNA material into double stranded DNA material, and then integrates it into the host DNA using the integrase enzyme. This integrated DNA is called the proviral DNA and remains latent for long periods without producing any new virus. Upon receiving an activation signal, HIV uses the host cells RNA polymerase to create several copies of HIV genomic material and shorter chains of mRNA which it uses to make longer chains of viral proteins. Using the polymerase enzyme, it then cuts the long chain proteins into shorter chains of protein materials and these join together to form the new virus. The newly assembled virus will then bud off the host cell taking with it part of the host cell membrane which is used to form its membrane glycoproteins that will be used to attach to the next CD4 cell.

Initial infection is usually followed by a period of latency during which the virus undergoes dormancy for a varying period in different individuals and remains undetected with no expression of symptoms of infection. When the virus withdraws from latency, it utilizes host resources to transcribe new viral genomes as RNA and accompanying viral proteins that get packaged and released from the host cell as new virions to carry on new cycles of infection(21,22,28).

1.1.4. Immune response to HIV

1.1.4.1. The Cellular Immune Response to HIV Infection

The immune response to HIV infection mediated in part by cytotoxic T-lymphocytes (CTL)(29,30). This CTL response increases gradually from early infection reaching a peak, at which point there is reduction in viral load (31). Figure 1.05 is an illustration of the CTL and viral load changes from the time of infection with HIV.

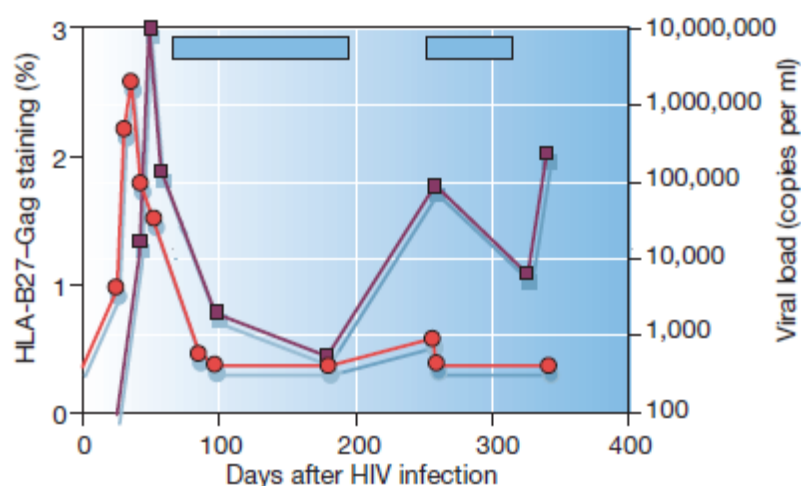


Figure 1.05: Percentage changes in HIV-specific CD8⁺ T-cells (stained with HLA-B27-Gag) (purple line) and viral load (red line) during acute infection. The CTLs rapidly increase, as the viral load increases, reaching a peak in less than 100 days' post infection. At this point, the viral load begins to reduce. Further changes in the figure are due to introduction of ART, which is represented by the blue bars (31).

a. CD8⁺ T Cell response to acute HIV infection

As mentioned earlier, there is a large CD8⁺ T cell response to acute HIV infection. Up to 10% of all the T cells with the CD8 surface protein may be involved in this initial response (32,33). Most of these HIV-specific CD8⁺ cells seen in early

infection are restricted to a small number of clones (34), being monoclonal in some patients. This is an a poor prognostic indicator (34) due to risk of exhaustion from over expansion and the risk of the virus escaping them with single epitope mutations (35). Studies have shown that the initial CD8⁺ T cell response is ineffective in inhibiting viral replication and is predictive of the viral set point and chronic infection with HIV (36–38).

b. CD8⁺ T Cell response to chronic HIV infection

In chronic HIV infection, the frequency of the expanded HIV-specific CD8⁺Tcells remains high at about 1-2% of the total circulating CD8⁺ T cells in the blood and lymph nodes. This high level of expansion is maintained by the presence of HIV antigen, and a reduction in viral load due to introduction of ART leads to a reduction in these cells. Without treatment, these chronically expanded HIV-specific CD8⁺ T cells persist into advanced HIV disease (Figure 1.06).

The CTL response controls HIV infection is through recognition of virus peptides presented by HLA class I molecules (39)(40). The type of HLA that presents the peptide has an influence on the rate of HIV disease progression, with some types such as HLA-B27 and HLA-B57 associated with slow progress, while HLA-B35 has been associated with rapid progress (39,40).

HIV specific CTLs control the virus through production of cytokines such as interferon- γ (IFN- γ) and TNF- α which inhibit viral replication (41–43). These CTLs also produce CC chemokines MIP-1 α and MIP-1 β , as well as RANTES, which compete or down regulate the expression of CCR5 receptors, thereby inhibiting replication (44–46). CTL also kill virus-infected cells, thereby controlling viral replication, by the release of perforin and granzymes (47), and by the induction of apoptosis through Fas ligand ligation (48).

Despite, all these viral control mechanisms, the HIV virus manages to wear down the immune system and persist in the body of the infected person for prolonged periods.

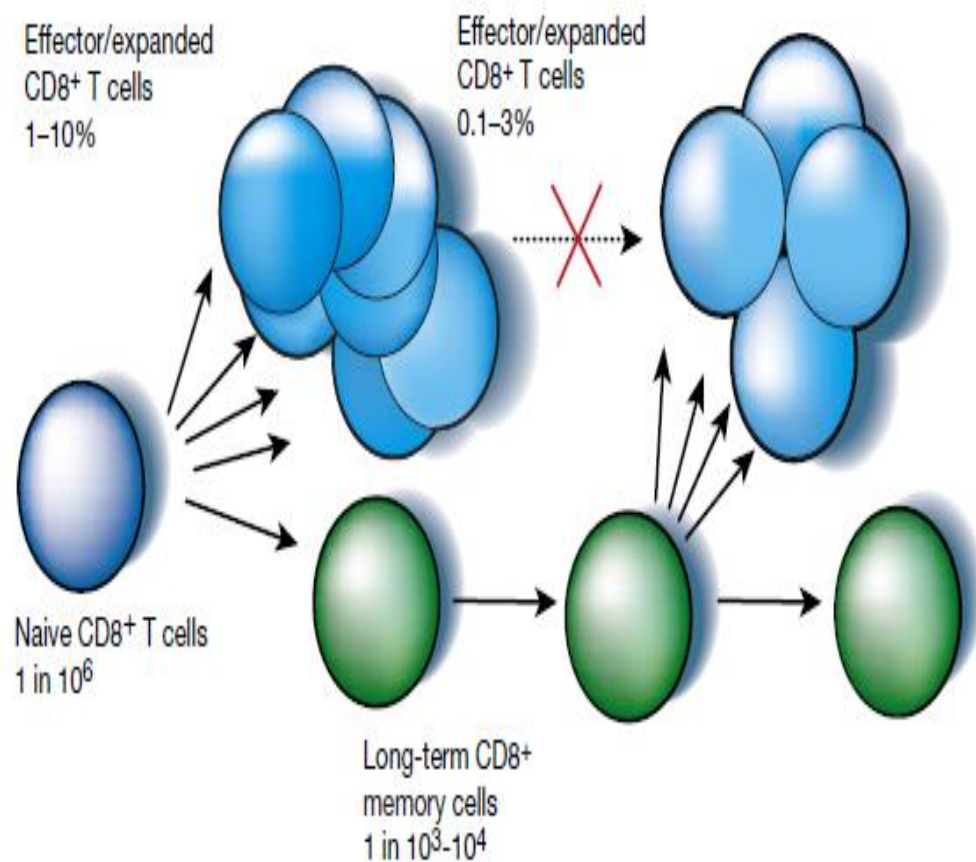


Figure 1.06: Expansion of HIV-specific CD8⁺ T-cells in acute and chronic infection (381).

The naïve T-cell is stimulated to divide into expanded effector cells, and a smaller percentage of long-term memory cells. The effector T-cells are maintained by continued presence of HIV antigen, and in the absence of this antigen, they die off through apoptosis and programmed cell death (red X shows failure of persistence by the effector cells due to absence of antigen). The memory cells divide further to maintain long-term memory and in presence of antigen can expand rapidly into effector cells.

HIV-specific CD8⁺ T cells have been found to be less effective killers than other CD8⁺ T cells because they produce less than 15% of the perforins needed for lysis (49,50). The continued activation of HIV-specific CD8⁺ cells due to presence of HIV antigens also leads to functional exhaustion (38), associated with the expression of the negative regulators of T cell activation, programmed death-1 (PD-1), cytotoxic

T cell antigen-4 (CTLA-4) and lymphocyte activation gene 3 (LAG-3) (51). Figure I.07 is an illustration of the loss of CD8⁺ T-cell function during chronic viral infection.

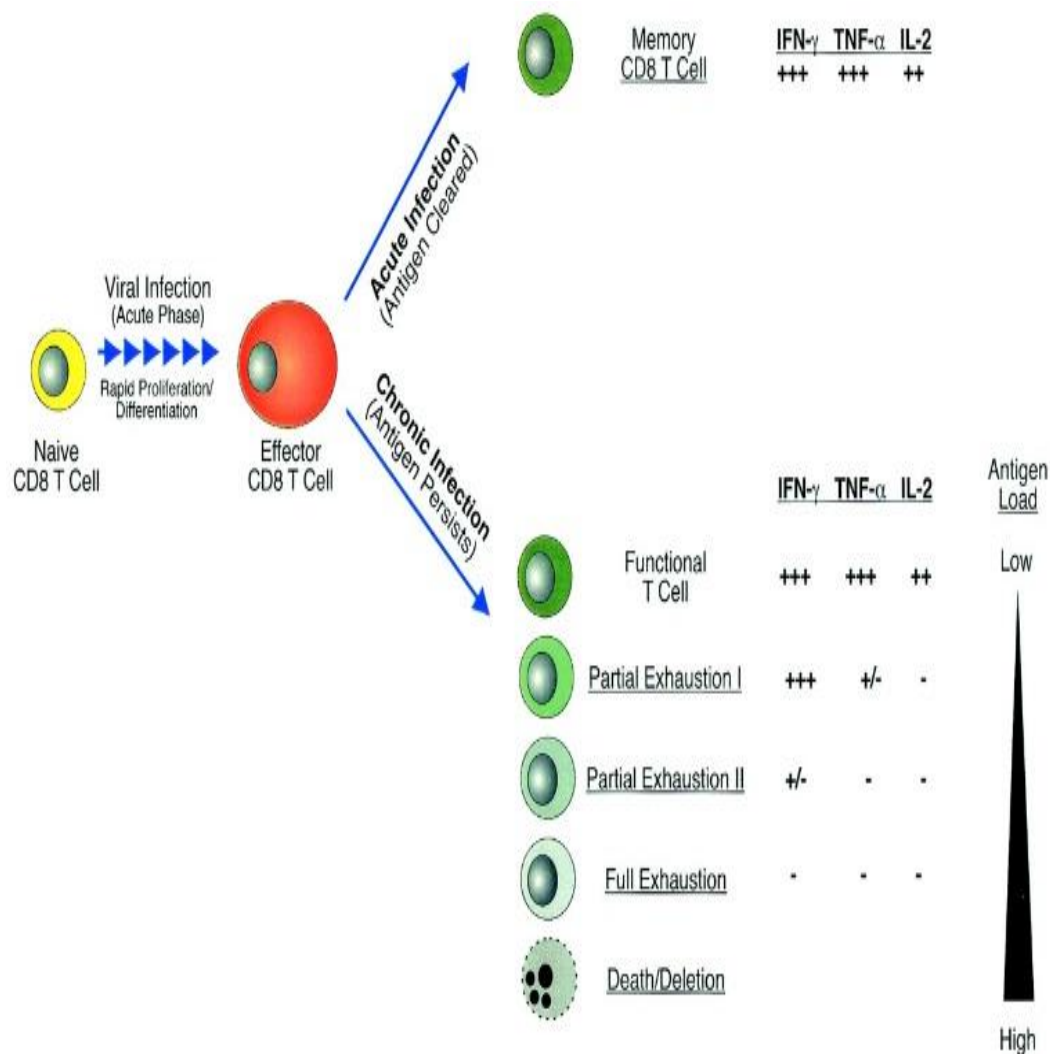


Figure I.07: Gradual loss of function of CD8⁺ T cells during chronic viral infections (38). Memory cells that remain after an acute viral illness are functional and can produce the cytokines IFN- γ , TNF- α , and IL-2, upon viral antigen re-stimulation (Top). Persistent viral antigen stimulation lead to CD8 T cells with various levels of function (bottom). Partial Exhaustion I represents the stage when there is impaired production of IL-2 and TNF- α but production of IFN- γ is preserved. Partial Exhaustion II is a stage when there is no more cytokine production. Full Exhaustion is the stage of total loss of all effector functions. Finally, deletion of epitope-specific CD8 T cells occurs when viral antigen stimulation is sustained.

1.1.4.2. Humoral immune responses to HIV (B cells)

B-lymphocytes are responsible for the humoral function of the immune system and act through secretion of antibodies. Their mode of action involves engagement of the B cell receptor (BCR), the immunoglobulin molecule that is expressed on their cell surface. Engagement of the BCR with antigen through specific binding triggers the initiation of antibody production and secretion. Secreted antibodies play a pivotal role in control and management of viral infections by the immune system through either binding to antigens and marking them for destruction by other cells, or neutralizing pathogens and rendering them biologically inactive, as has been demonstrated by antibodies specific for diphtheria antitoxin, influenza and HIV.

An immunological role and therapeutic potential of B cells in HIV infection has been demonstrated in studies that have shown that some patients develop broadly neutralizing antibodies that target multiple variants of HIV (52). Broadly neutralizing antibodies (bNAbs) have the peculiar ability to bind to various viral strains, having binding sites that are significantly longer than most antibodies, hence increasing their binding repertoires to accommodate various viral strains (53).

HIV infection affects B cells by causing hyperactivation, which is characterized by hypergammaglobulinemia and polyclonal activation among other features (54,55). There is also increased immature transitional B cells in the peripheral blood of HIV infected persons which is mediated by an increase in IL-7 (56) which occurs in patients with increased viremia and a depletion of CD4⁺ T cells (57). There is also a reduction in the percentage of the mature B cells in the peripheral circulation (58). The hyperactivation and increased immature B cells lead to loss of function of the B cells leading to the ineffective antibody response seen in HIV infection (59). ART reverses most of the effects of HIV on the B cells. There is increase in the B cell counts and reduced hyperactivation of immature B cells in the peripheral circulation (55,60,61).

1.1.5. HIV induced Immune deterioration

HIV impairs the body's immune system through depletion of the CD4⁺ T cells (62–68). This impairment of the immune system is a gradual process that takes a period of 5-10 years (67). If left untreated, HIV gradually takes a toll on the immune system affecting and weakening it, leaving the host with limited defenses and hence the onset of the Acquired Immune Deficiency Syndrome (AIDS) (69), which further increases susceptibility to other infections such as tuberculosis and meningococcal infections(70).

At the time of infection when the CD4⁺ T cells are adequate, the immune system is virtually normal(71) however, with time and increased depletion of the CD4⁺ T cells, the immunity weakens (72). During the acute phase of HIV infection, there is an abundance of CD4⁺ T lymphocytes expressing the CCR5 chemokine receptor, which aids the entry of the HIV into the CD4⁺ T-cell (23,73–75). This results in an increased efficiency of viral transmission from infected cells to uninfected cells, leading to a massive depletion of CD4⁺ T cells (76). Numerous studies have demonstrated a rapid depletion of CD4⁺ T lymphocytes in the lamina propria and mucosal epithelia in the acute phase of the infection especially the first few weeks of infection (77). The acute phase of infection is also characterized by an almost irrevocable alteration of the gastrointestinal mucosa including loss of the immune cell function such as IFN- γ , IL-17– and IL-22–producing cells coupled to changes in the gene expression of epithelia (62,63)

The chronic phase of HIV infection is characterized by proliferation, differentiation and death of CD4⁺ T lymphocytes (65). This chronic immune profile is also linked with an increase in expression of pro-inflammatory cytokines such as IFN- γ and TNF- α as well as T cell exhaustion and depletion (65). This chronic and persistent immune activation state has been associated with HIV progression with increased CD4⁺ T cell depletion, increased viral replication and progress of HIV disease (64,78). The depletion of CD4⁺ cells is critical because, they are the helper T cells that are needed for the priming, proper functioning and maintaining memory by the cytotoxic T-cells(79,80). With a depleted CD4⁺ T helper cell pool, the cytotoxic response of the cellular immune system is unable to function, and HIV virus is able

to replicate unabated (67). This results in an increased susceptibility to opportunistic infections during advanced HIV disease stage also known as Acquired Immune Deficiency Syndrome (AIDS) and eventually death, if untreated (81). Figure I.08 is a graphic representation of the life cycle of HIV showing changes in CD4+ T cells and viral load from the time of infection until death due to AIDS.

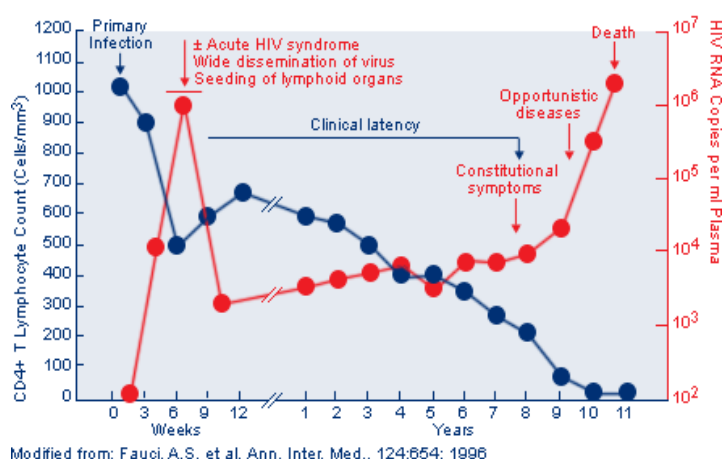


Figure I.08: The Stages of HIV Infection (382,383)

During primary infection, the number of CD4⁺ T cells is still high (blue graph), while the viral load (red graph) is low. As the disease progresses, the CD4⁺ T cells reduce as the viral load increases until about 4-6 weeks when there is reversal with the viral load reaching a higher level than the CD4 cells. At this point, there is an acute HIV seroconversion illness. However, the body mounts an immune response which subsequently controls the infection leading to a period of clinical latency where there are no clinical signs and symptoms of HIV infection. During this period, there is continued infection and destruction of the CD4 cells by the HIV virus. After about 5-10 years, the remaining CD4 cells are not able to mount a good immune response and this leads to a syndrome of immune deficiency (Acquired Immune Deficiency Syndrome - AIDS) which is characterized by a plethora of opportunistic infections such as tuberculosis, and if ART is not introduced, it eventually leads to death.

I.1.5.1. Antiretroviral therapy reverses the HIV related immune deterioration.

Antiretroviral therapy (ART) has been shown to improve the immunity of HIV infected persons through reversing of immune activation and inhibiting HIV replication (82). Use of antiretroviral treatment leads to suppression of viral replication leading to lower viremia amongst infected individuals (83). This greatly

reduces the amount of virus available to infect new CD4⁺ T cells, and this leads to an increase in the CD4⁺ T cells (84,85), and the earlier the ART is started, the better the effect (77). ART has also been shown to reduce systemic immune activation, which also leads to reduced death of CD4⁺ T cells (83,86). This increase in CD4⁺ T cell count consequently leads to better functioning of CD8⁺ cytotoxic T cells, which also augment the reduction in viral load (79,80). Because of the reduced viral load, the cytotoxic T-cells also reduce in number, as shown in figure 5.1

ART also slows the progression of HIV disease, improves quality of life and reduces HIV-related deaths (87,88). Antiretroviral drugs suppress viral replication and reduce plasma viral load leading to the reconstitution of the immune system as measured by increase in CD4⁺ cell counts (89,90). These drugs act through interference with the different parts of the HIV life cycle where they inhibit the enzymes that the HIV virus uses to replicate (91), and are classified according to their site of action. The drugs which prevent the binding of HIV to the CD4⁺ cells and entry into the cells (92), these include fusion inhibitors, attachment inhibitors and chemokine receptor antagonists. After entry into the cell, another class of drugs act to prevent the actions of the reverse transcriptase (RT) enzyme (93). These are called RT inhibitors and they include nucleoside/nucleotide RT inhibitors (NRTIs) (94) and non-nucleoside RT inhibitors (NNRTIs) (94,95). The integrase inhibitors (96–98) act by preventing the integration of viral DNA into the host cell DNA, and the protease inhibitors act by inhibiting the protease enzymes which facilitates the assembly of newly formed viral proteins (99). In the treatment of HIV, at least three different classes of drugs are combined and used as ART (100–102). In Uganda, the first line treatment for HIV contains two (2) NRTIs such as Lamivudine and Tenofovir, and an NNRTI such as Nevirapine or Efavirenz (101).

In summary, in early HIV infection, there is a rapid increase in cytotoxic T cells leading to inhibition of viral replication. There is also steady reduction in CD4⁺ cells due to increased death from viral infection and immune activation. In chronic infection, there is a continuous expansion of cytotoxic T cells, but with reduced function, due to exhaustion and the reduction in the CD4⁺ helper T cells. With the

introduction of ART, there is a reversal of events with a reduction in viraemia, increase in CD4+ cells, improved function of cytotoxic T cells, and a gradual decline in the cytotoxic T cells due to the reduction in viraemia (antigen stimulation).

1.2. MALNUTRITION: THE GLOBAL AND SUB-SAHARAN AFRICA PERSPECTIVE

. Malnutrition has been defined severally by different authorities. The British association of parenteral and enteral nutrition (BAPEN) define it as ‘ a state of nutrition in which a deficiency or excess (imbalance) of energy, protein and other nutrients causes measurable adverse effects on tissue or body form, function and clinical outcome (103). The American society of parenteral and enteric nutrition recommend the use of 6 characteristics to diagnose malnutrition (104). These include; reduced energy intake, weight loss, loss of muscle mass, loss of subcutaneous fat, localized or generalized fluid accumulation and diminished functional status as measured by hand grip strength (104). Malnutrition (undernutrition) may be due to reduced nutrient intake, excessive loss of nutrient like in chronic diarrhea, malabsorption, increased demand of nutrients like in oxidative stress states due to underlying disease (105) The three subgroups of malnutrition are: Under nutrition which includes wasting, stunting and being underweight; micronutrient related malnutrition- which includes micronutrient deficiencies or excesses; and overweight, obesity and diet-related non-communicable diseases (106,107).

Globally in 2014, 1.9 billion adults were found to be overweight or obese while 462 million were underweight(106,108,109). In 2016, 52 million children below 5 years of age worldwide were found to be wasted, 17 million were severely wasted, 155 million were stunted and 41 million were overweight or obese(109).

According to the Institute for Health Metrics and Evaluation (IHME) global health data, the worldwide prevalence of nutritional deficiencies across age groups was nearly 24% in 2016 (110). Globally, the prevalence of nutritional deficiencies varies

inversely with income status across the different regions. In the high-income countries, nutritional deficiencies are less prevalent with an estimated prevalence of 5.9%, in the middle-income countries, prevalence of nutritional deficiencies is higher than in the high-income countries i.e. about 23% in South East Asia, 24.75% in North Africa and Middle East, etc. (110).

In the low-income countries, there are some differences in the data on malnutrition, although all the results highlight the fact that nutritional deficiencies remain a significant problem. In 2016, the prevalence of nutritional deficiencies for all age groups and sexes in Sub-Saharan Africa was found to be about 37.6%. The prevalence of nutritional deficiencies was found to be 42% in Tanzania, 41% in Kenya, 26% in South Africa and 35% in Nigeria (110). In Uganda, IHME estimated a 39% prevalence of nutritional deficiencies including protein-energy malnutrition, iron deficiency, vitamin A deficiency and others, in 2016 (110). A previous assessment indicated a 12% prevalence of Chronic Energy Deficiency (CED) among women of child bearing age in Uganda, with this prevalence increasing with age and among HIV infected women (111).

1.2.1. Assessment of nutritional status

Assessment of nutritional status of individuals can be done using different methods. The methods most commonly used are: Anthropometry, Biomarkers of nutrition, Clinical assessment and Dietary assessment (ABCD) (112). Each method has its own strengths and weaknesses, and most studies employ at least two or three methods for nutritional assessment (113–117).

1.2.1.1. Anthropometry

Anthropometry refers to external morphological traits which can be measured. In nutrition, it refers to the measurement of the changes in physical dimensions of the human body at different ages and levels of nutrition(118–120). Anthropometry is a quick and cheap method for assessing nutritional status of individuals. The most commonly used anthropometric measures in adults are weight and height (121), which may be combined into body mass index (BMI). Other anthropometric measurements include hip circumference; waist circumference and mid upper arm

circumference (MUAC). These measurements may be combined into anthropometric indices such as BMI which is the ratio of weight in kilograms to the square of the height in meters, and the waist: hip ratio among others. Anthropometry, as a measure of nutritional status is of value if there are clear cut off points that can be used as indicators of either normal nutrition status, malnutrition or over nutrition.

BMI is a measure of weight adjusted for height and is used to estimate the amount of body fat that an individual has, however, it is a surrogate measure since it estimates excess weight and not fat. The following cut offs are used as indicators of varying levels of malnutrition: <16.5 is severely malnourished; $16.5-18.5$ is moderately malnourished (122–124) and $>18.5-25$ is well nourished; $25-32$ is overweight and >32 is obese (125,126). BMI has been widely used in the assessment of nutritional status because it is a non-invasive, simple and cheap to use method and has been recommended by the World Health Organization (WHO) (121). Studies have shown that BMI accurately correlates with body fat (127)(128) and future health risks of morbidity and mortality (129). However, using BMI is not without limitations like misclassifying some malnourished individuals as well-nourished; this is common among short individuals and those with heavy bones and muscle build such as trained athletes (130). BMI has also been reported to underestimate obesity and overweight (126) individuals since it is a surrogate measure of fat (131). Studies have shown that using BMI to classify nutritional status varies by ethnicity, for example Asians are will be classified as obese at a BMI of $\geq 23\%$ (132)(133)(. BMI has been used to assess nutritional status and predict prognosis of HIV infected adults (114,134,135), with a low BMI being a predictor of early mortality among HIV infected adults initiating antiretroviral therapy.

Mid-upper arm circumference (MUAC) and upper arm skinfold thickness have been invariably used to assess for malnutrition among children and adults and has been found to correlate with BMI (124)(136)(137), body fat (138) and body weight (139). However, the use of MUAC as a stand-alone anthropometric measurement has been limited to conditions in which the weight or height of the patient cannot be measured. There are hardly any studies that have assessed nutritional status of HIV

infected adults using MUAC, but one study showed that a low baseline MUAC (<20 cm) was associated with increased mortality at 3 months among HIV infected adults initiating ART (134). There are conflicting cut off indices for MUAC that indicate malnutrition with one study (140) considering patients below the 5th percentile of the criteria developed by Bishop (141) as malnourished, while other studies have found a MUAC between 22 cm and 25 cm to be diagnostic of malnutrition, with the women having a slightly lesser value than the men (142).

Waist-hip ratio is another anthropometric index that has been used to assess nutritional status, especially as a measure of obesity (143) and the related risk of cardiovascular disease and type II diabetes mellitus (144). However, its use is limited by the lack of clear cut offs for the different levels of obesity and the many varied points at which waist circumference can be measured (145), but WHO recommends a cut off of 1.0 and 0.85 for men and women, respectively, as the cut off beyond which the risk of type II diabetes and cardiovascular disease increase (145).

Total fat mass, total lean muscle mass and other such body parameters have frequently been used to assess the nutritional status of individuals (125,127,140). Body composition can be analyzed using bioelectric impedance analysis (BIA); dual energy x-ray absorptiometry (DEXA) scan, body density; total body water estimates (129,143,144) and many others. Studies of body composition among HIV infected adults have mainly used BIA (146–148) and DEXA scanning (149–151) to assess effects of different exposures to the body composition of this population. Nutritional studies have mainly used BIA while morphological studies (due to effect of antiretroviral therapy or HIV infection) have preferentially used DEXA scans. BIA is an indirect method of measuring body composition (152) and uses the body's resistance to the conduction of a small electrical current (50 KHz) through the body to produce estimates of total body water, fat mass and fat-free mass (153). The main challenge in the use of BIA is the large predictive error that is inherent to all indirect methods of body composition analysis (154) which is brought about the equations used in the estimation of the different body compartments and other factors such as weight status, age, ethnicity and variations in the time of

measurement. Subsequently BIA is more useful in estimating body composition of groups of people, or one individual over a long period of time, than with a onetime measurement (152). However, the tetrapolar BIA devices have improved the performance of BIA for public and clinical use. Additionally, BIA is not sensitive in estimating body fat among the obese (155,156).

1.2.1.2. Clinical Assessment

Clinical assessment for malnutrition includes a detailed history and examination of the patient. Most clinical signs and symptoms are non-specific. The most frequently reported symptoms include: unintended weight loss, changes in dietary intake (loss of appetite), gastrointestinal symptoms, medications that may affect dietary intake, edema, use of alcohol (146,157). The features of malnutrition include: edema of the extremities; decreased subcutaneous fat; angular stomatitis; cheilosis; thinning of hair; sparse thin or brittle hair, hair color changes to dull brown or red and fissured or ridged nails (158). Other features include demonstrable weight loss, pallor of mucous membranes and muscle wasting (159). Clinical assessment has been found to correlate well with other measures used in the assessment of malnutrition (159). Specific nutritional deficiencies present with specific signs and symptoms and these have been detailed elsewhere (158).

1.2.1.3. Biomarkers of nutrition

Several studies have used different biomarkers to assess for nutrient status of individuals (160–162) but most of these will fall under the categories of serum protein, micronutrients, serum lipids, hematological parameters like hemoglobin and mean corpuscular volume, and some trace elements like magnesium (146). When most of these biomarkers are below the normal reference ranges, it is an indicator of some form of malnutrition. It is worth noting that none of these biomarkers can be used individually or as a group to diagnose a patient with malnutrition, but they are used as a compliment to clinical assessment and anthropometric measurements. However, studies have indicated that most HIV infected patients would have normal ranges of most biomarkers except for hemoglobin (162), and low serum phosphates (160–162)

1.2.1.4. Dietary Intake

Inadequate nutritional intake is one of the many causes of malnutrition, therefore dietary intake is a key aspect in the assessment of nutritional status as evidenced by the many studies that have used it (161–164). Different studies have used different methods to assess dietary intake. Some of the methods used include: the 24-hour dietary recall (165,166); specifically developed methodology to assess nutritional intake over a longer period of time (164); use of both the 24-hour recall and food frequency questionnaires (166); and the food frequency questionnaire only (167). Although food frequency questionnaires are often used in large epidemiological studies and the 24-hour dietary recalls used in small studies, both methods have been found to be highly reliable and valid (167)(168). The 24-hour recall has a better inter-individual variability and thus is better used in assessing group level dietary intake compared to the food frequency questionnaire.

1.3. MALNUTRITION AMONG HIV INFECTED PATIENTS: THE GLOBAL AND SUB-SAHARAN AFRICA PERSPECTIVE.

HIV infected patients are at an increased risk of malnutrition (169), indeed the early description of HIV as ‘slim’ disease indicates that the disease was associated with severe weight loss (170). In a multi-country study across continents, more than 80% of the HIV positive ART naïve patients had at least one micronutrient deficiency (171); more than 32% of the participants had more than three micronutrient deficiencies.

The prevalence of malnutrition among HIV infected persons varies in different countries with a bigger burden in the developing countries (172–174). Salomon and others observe that malnutrition in HIV is still a challenge in developing countries and needs to be actively managed (175). In sub-Saharan Africa, studies have shown the prevalence of malnutrition among HIV infected adults to range between 15-40%(58,)(177).

There are no major differences in prevalence of malnutrition between ART naïve and HAART experienced HIV infected persons. The ATARAO study in Mali estimated that 36% of HIV infected patients initiating HAART were malnourished

(178), while a retrospective analysis of data of the patients in a South African HIV clinic cohort initiating ART, found that 22% of them were malnourished (179), and a study among Senegalese HIV infected patients who had been on HAART for about 3 years in two different cities found the prevalence of malnutrition to be 19.2% and 26.3% respectively (180). Two studies done in Ethiopia, a country with the second highest rate of malnutrition in Sub-Saharan Africa, found that the prevalence of malnutrition was above 40% among ART treated HIV infected adults(181) ,(182).

In Uganda, up to 25% of adults initiating antiretroviral treatment were found to be malnourished (111), and the prevalence was highest in rural areas. Micronutrient deficiencies are also high in HIV infected patients and 36.8% of HIV infected ART naïve patients were found with sub-optimal vitamin B12 levels(183).

1.4. THE CAUSES AND EFFECTS OF MALNUTRITION IN HIV

Malnutrition in HIV has several causes which may be related to opportunistic infections, HIV infection, and or other factors(146,184). These include malabsorption which may be due to chronic diarrhea or destruction of the gut absorptive mucous membrane by the HIV virus, reduced intake of nutrients due to loss of appetite and opportunistic infections in the mouth like oro-esophageal candida or increased metabolic demands due to the increased oxidative state coupled with reduced intake. Other factors that may lead to malnutrition are poor dietary diversity and food insecurity (184). All the above mechanisms work synergistically to lead to malnutrition in HIV. Figure 1.09 is a diagrammatic representation of the causes and effects of malnutrition in HIV.

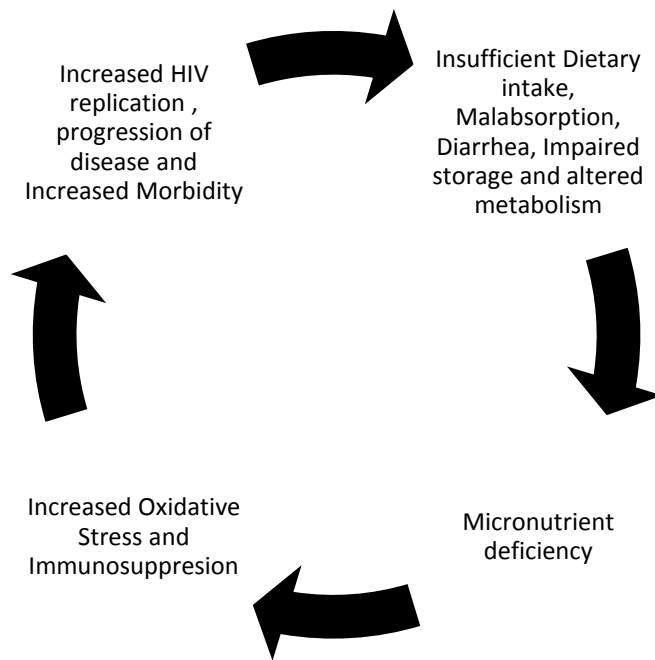


Figure 1.09: Causes and effects of malnutrition in HIV (185)

1.5. EFFECT OF MALNUTRITION ON THE IMMUNE SYSTEM

The nutrient status of an individual plays a pivotal role in the functioning of the immune system. This ranges from the effects of nutrition on the integrity of physical barriers, including mucosal barriers of epithelia, and extends to the innate and adaptive cellular components of the immune system. Nutritional status also influences the composition of the microbiome of an individual which eventually has downstream effects on how the immune system responds to infection and disease (186,187)

Micronutrient deficiencies make individuals vulnerable to several infections including HIV(188)(189). Vitamin A influences growth and development of T and B cells, preserves the epithelial lining and modulates immune responses(189–193). Zinc mediates maturation and function of neutrophils, T and B lymphocytes, natural killer cells and macrophages (194). Vitamin E enhances the functioning of immune effector cells and also shields them against oxidative stress (195). Vitamin B6 affects both the cell-mediated and humoral immune functions; vitamin B6 deficiency leads to impairment of antibody production and affects differentiation and maturation of

lymphocytes(196); selenium supplementation leads to increased lymphocyte counts and plays an active role in activation, differentiation and proliferation of immune cells, and folate affects the T lymphocyte responses to certain mitogens (197–199).

The increased oxidative stress and immunosuppression caused by the micronutrient deficiencies, in turn lead to increased morbidity and mortality among the HIV infected patients. For example, low levels of Vitamin A have been found to contribute to HIV disease progression (200).

Malnutrition has been shown to be one of the leading causes of immunosuppression worldwide (169,201–203) It impairs many aspects of both the innate and adaptive immune system.

In the innate immune system, malnutrition leads to a reduction in the activity of complement system especially C3, reducing its ability to opsonise and kill pathogens(203,204). The functioning of antigen presenting cells (both B cells and macrophages) is reduced in malnutrition, this is mainly due to atrophy of the lymphoid organs which leads to reduced production of B and T cells, as well as derangement in hematopoiesis leading to reduction in the production of monocytes which later become macrophages (205). Natural killer cells have been shown to be regulated by nutritional status among the elderly (206). Malnutrition also affects innate immune activation by altering the mucosal barrier and pro-inflammatory cytokine expression and has been demonstrated to lower IgA secretion and alter the distribution of eosinophils (187). Studies by Elia (68) and Farmery (207) have demonstrated that malnutrition affects the distribution of microbiota in the gastrointestinal tract leading to inflammation and susceptibility to pathogenic microbes.

In the adaptive immune system, malnutrition mainly affects the cell mediated immune system(169,208). In children, malnutrition, especially protein energy malnutrition (PEM) has been shown to lead to thymus and bone marrow atrophy as well as atrophy of other lymphoid organs like the spleen and lymph nodes (209). This leads to reduction in the numbers of T cell subpopulations, with atrophy of the bone marrow, there is increased numbers of immature T cells in peripheral

blood and reduced neutrophils. (210). Malnutrition has also been found to be associated with reduced activation and proliferation of T cells due to low serum potassium levels which are critical in the activation of this cell population (211). The reduced activation and proliferation of T cells leads to reduction in production of cytokines which form part of the main drivers of the immune system (211). The epithelial immune defenses are also affected in malnutrition with atrophy of microvilli in the gut, reduced lymphocyte count in the Peyer's patches and reduced secretion of IgA by the epithelial cells(212)(213). The Peyer's patches are small masses of lymphatic tissue that are found in the small intestines. Their main function is to prevent pathogenic growth of bacteria in the intestines. Although a study in malnourished Ghanaian children showed an increase percentage of T cell subpopulations among malnourished children compared to normal ones and an increase in IgA levels, all the other parameters they studied were depressed in the malnourished children (214).

Malnutrition has been found to be associated with an increased susceptibility to infections. Indeed, Chandra and colleagues observed that malnutrition increases susceptibility to infections such as bacterial pneumonias and viral diarrheas, but may have mild effects on infections such as tetanus and viral encephalitis (202). Vitamin A deficiency leads to lymphoid atrophy, impaired cellular immunity and impaired IgG responses (169). Zinc deficiency has been associated with decreased CD4⁺ and CD8⁺ cells(215).

Both HIV and malnutrition are known to cause immunosuppression especially the cell mediated immunity (169) and thus increase susceptibility to infections. Interventions with nutritional supplementation are aimed at breaking this cycle of events and achieving the best outcome possible with regards to clinical, nutritional and immunological outcomes.

Combined effect of HIV and Malnutrition on immunity

HIV and malnutrition form a vicious cycle which ultimately impairs the immunity of the patient through the depletion of both CD4⁺ and CD8⁺ T cells among other mechanisms.(169,216).

Table I.I below shows the effects on HIV and malnutrition on different immune parameters. Effects of malnutrition on the immune system are almost similar effects of HIV on the immune system. Therefore, malnourished HIV infected patients have dual impact on their immune system, which increases their vulnerability to opportunistic infections.

Table I.I Affected lines of immunity by HIV and Malnutrition (169)

Immunological Parameter	Effect of HIV Infection	Effect of Malnutrition	Nutrient Deficiency
Total Lymphocytes	Decreased	Decreased	PEM
T- Lymphocytes	Decreased	Decreased	PEM
CD4 T Lymphocytes	Decreased	Decreased	PEM
CD8 T lymphocyte	Decreased	Relatively Maintained	
CD4:CD8 T cell Ratio	Inverted	Reversed	PEM
Lymphocyte responsiveness to mitogens	Reduced	Reduced	PEM, VitA &E, Zinc, Iron
Cell Mediated Immunity	Compromised	Compromised	PEM, essential Amino acids
B- Lymphocytes	Polyclonal activation	Generally maintained	
Immunoglobulin levels	IgA & IgG Increased	IgA, IgG & IgM reduced	PEM, Amino Acids, Vit B complex
Secretory IgA	Increased	Decreased	PEM
B-Cell Activity	Reduced	Reduced	PEM
Primary antibody response	Reduced	Reduced	PEM
Antibody Affinity	Decreases with progression	Reduced	PEM
NK Cell Activity	Increased	Reduced	Vi A,C, Zinc, Iron, Selenium
Serum Compliment	Increased	Reduced	PEM, essential Amino acids
Serum- β microglobulin	Increased	Increased	PEM
IFN- γ	Increased	Reduced	Amino Acids, Essential Fatty acids, Iron
TNF- α , IL6	Increased	Increased	PEM
Anti-inflammatory Cytokines	Reduced	Increased	PEM
Soluble IL-2 receptors	Increased	Increased	PEM
Antioxidants	Reduced	Reduced	Arginine, Selenium, Zinc, Vit A,C,E
C-Reactive Protein	Increased	Increased	PEM

I.6. NUTRITIONAL SUPPLEMENTS USED IN REHABILITATION OF MALNOURISHED HIV INFECTED PATIENTS

There are many foods that can be used for nutritional supplementation. The World Food Program uses various foods for supplementation in their different programs across the world, among them fortified blended foods (FBFs) including Corn Soy Blend, high energy biscuits, micronutrient powder, compressed food bars, ready to use foods such as Plumpy'Nut(107,217).

Fortified blended foods are partially precooked cereals, soya, beans which are cooked as porridge, and are fortified with micronutrients i.e. vitamins A, C, B12, D, E, K, B6, thiamine, riboflavin, niacin, pantothenic acid, folic acid plus zinc, iron, calcium and potassium. The FBFs include Corn Soy Blend and Wheat Soya Blend. Corn Soy Blend is rich in energy, carbohydrates, protein, fat and micronutrients(107,218).

High energy biscuits are wheat-based biscuits which contain hydrogenate vegetable shortening, sugar, soy flour, invert syrup, high fructose, corn syrup, skimmed milk powder, sodium and ammonium, bicarbonates, salt. They are fortified with calcium, magnesium, iron, iodine, folic acid, pantothenic acid, vitamins B1, B2, B6, B12b C,D,E, niacin, vitamin A-retinol (107,217). Micronutrient powder is a powder containing the recommended daily intake of 16 vitamins and minerals, which is sprinkled onto cooked food before eating (107,217).

Compressed food bars are made of baked wheat flour, vegetable fat, sugars, soya protein concentrate and malt extract and can be eaten as bars or used to make porridge. They are fortified with vitamins A, D3,E, C, B1, B2, B6, B12, niacin, folic acid, pantothenic acid, biotin, calcium, phosphorus, magnesium, iron, zinc, potassium, sodium, copper, selenium and iodine (107,217).

Ready to use therapeutic foods such as Plumpy'Nut is specifically designed for the management of severe acute malnutrition. It is a peanut paste containing skimmed milk, vegetable fat, whey, maltodextrines, sugar which is fortified with micronutrients i.e. vitamins A, E, B1, B2, niacin, pantothenic acid, vitamin C, B6,

B12, calcium, magnesium, selenium, zinc, iron, iodine, copper, phosphorus, potassium, manganese and folic acid (107). Plumpy'Nut has been widely used in many nutritional studies among HIV infected adults including studies in Malawi by Ciliberto and others (219,220), and in Kenya and Uganda by the FANTA project although with varied results(111).. Plumpy'Nut does not need any preparation or cooking; it can be stored and consumed even with high outside temperatures(221)(222). Plumpy'Nut is easy to handle, because it contains no water, therefore has a long shelf life and is resistant to bacterial contamination. Being conveniently packaged in 92g sachets makes it easy to handle and prevent contamination of product between feeds (223). The prevalence of peanut related allergies in the developing world is low. (224). Table 1.2 shows the contents of Plumpy'Nut.

Table 1.2 The contents of Plumpy'Nut^R are detailed in the table below: (223,224)

Vitamins			Minerals			Nutritional Value		
	/100g	/92g		/100g	/92g		/100g	/92g
A (mg)	0.91	0.84	Calcium(mg)	320	294	Protein (g)	13.6	12.5
D(µg)	16	14.7	Phosphorus (mg)	394	362	Energy (Kcal)	545	500
E(mg)	20	18.4	Potassium (mg)	1111	1022	Lipids(g)	35.7	32.8
K (µg)	21	19	Magnesium (mg)	92	85			
C(mg)	53	48.8	Sodium (mg)	189	174			
B1	0.6	0.55	Iron (mg)	11.5	10.6			
B2 (mg)	1.8	1.65	Zinc(mg)	14	12.9			
B6 (mg)	0.6	0.55	Copper (mg)	1.78	1.63			
B12 µg	0.53	0.48	Iodine (µg)	110	101			
Folate (µg)	210	193	Selenium (µg)	3µg	2.76			
Niacin (mg)	5.3	4.8						
Biotin (µg)	65	60						
Ca-D-Pantothenate (mg)	3.1	2.8						

1.7. THE EFFECT OF NUTRITIONAL SUPPLEMENTATION ON SELECTED OUTCOMES AMONG HIV-INFECTED ADULTS

There is a large body of literature on nutritional supplementation in malnourished HIV infected persons. However majority of these are observational studies and very few interventional studies and the few studies found varied results on the effect of nutritional interventions on several outcomes in malnourished HIV infected adults much less in Sub-Saharan Africa. A Cochrane review on nutritional supplementation in HIV in 2013 identified only 14 interventional studies involving 1725 HIV infected adults and 271 HIV infected children in this area (227). Of the 14 studies included in the review, only 4 were conducted in Africa, and all were before 2005 except for the Kenya study which was conducted in 2011, but was not published. Another review by Hong (228) identified only 6 randomized studies and 4 retrospective cohort observations. Majority of the nutritional supplementation studies have explored the effect of micronutrient supplementation on HIV clinical outcomes such as change in CD4 count, mortality or viral load (160,174,229–232). The few interventional studies involving macronutrient supplementation have used different types of supplements, although most have used lipid dense supplements, and the comparators varied from placebo, to standard of care; to alternative nutritional supplements (117,135,233). The duration of supplementation also varied between 4 weeks to 12 months with the majority of studies offering the nutritional supplement for 3-6 months. A number of the interventional studies did not use a placebo control as the comparator but rather used another nutritional supplement deemed inferior to the interventional supplements. This was mainly due to the ethical dilemma of randomizing malnourished patients to no supplement at all.

Majority of studies explored only the effect of nutritional supplementation on nutritional outcomes such as anthropometry (mainly weight and BMI), and clinical outcomes such as CD4⁺ T cells and viral load (135,206,229,230,234). Few studies have evaluated the effect of nutritional supplementation on biomarkers of nutrition (114,117,160,161) or nutrient intake in malnourished HIV infected adults (114)(235). Additionally, there is paucity of data on the effect of nutritional

supplementation on immune responses among HIV infected adults. It should also be noted that most studies did not find a statistically significant difference between the intervention and control arms. This may be attributed to either low sample sizes, (many studies had small sample size) or a placebo effect or lack of a difference in the comparison arms due to the control arm receiving a supplement almost similar to the intervention.

Several studies show that nutritional supplementation improves body weight and BMI, although the effects might not be sustained (236). Ndekha and colleagues (135) found that ready to use therapeutic food (RUTF) marginally increased patients' BMI more than Corn Soy Blend (CSB) after 3 months of supplementation (19.0 Vs 18.5 $P<0.001$) They compared the differences in BMI and fat free mass increase among wasted HIV infected adults receiving either RUTF or CSB after 14 weeks of supplementation found that patients receiving RUTF have higher increases in BMI and fat-free mass. Fat-free mass contributed to 2.9 kg (51.8%) of the 5.6 kg weight gain in the fortified spread group and 2.2 kg (51.2%) of the 4.3 kg weight gain in the Corn Soy Blend group. There were no significant differences in survival, HIV viral load, CD4 count, or quality of life between the groups. However, this study had a high loss to follow-up >15%, and the comparator in the study was another nutrient supplement (Corn Soy Blend) and both are energy dense supplements. This could have diminished any significant effects of RUTF if they existed since the intervention and control participants were receiving a nutritional supplement. A follow-up study comparing these results (RUTF arm and CSB arm) to a historical control group without supplementation, found that 12 weeks after stopping supplementation, there were no differences in weight and body mass index (BMI) between the groups. There was also no difference in survival(237). Historical comparisons present challenges to such studies since a number of factors that affect the outcome may have changed and cannot be controlled for.

A clinical trial among Senegalese HIV infected patients in whom RUTF was given versus standard diet alone after 9 weeks of home-based therapy; body weight (+11%; $p=0.033$), BMI, fat-free mass (+11.8; $p=0.033$) and fat mass increased in the RUTF arm and anemia decreased significantly compared to the control group. In

the RUTF arm, fat-free mass increased significantly more in those on HAART compared to those not on HAART. Zinc status remained unchanged. The mean plasma zinc concentrations were similar to the respective baseline values in both the RUTF ARM and the control arm (114). Study was conducted in hospitalized patient, although study duration quoted is 9 weeks. These weeks were post hospitalization, but average duration in hospital is not stated. Thus duration of supplementation may have been longer than stated. Dietary data was taken from a selected number of patients involved in the study, and this could have introduced selection bias. The authors do not show the comparison of demographics of the participants that had dietary data with those that did not. Therefore the dietary comparisons may not be a true representation of the whole study.

In a cohort of HIV infected adults initiating ART in rural Haiti, there was less weight loss among those receiving food supplementation in the first 6 months of the program (-0.20 vs. -0.66, $P = 0.012$) and significant weight gain at 12 months (+0.22 vs. -0.67, $P = 0.002$) (115). The Haiti study was a program evaluation and inclusion into the study was food insecurity rather than biological nutritional status criteria such as BMI or weight. It was not clear how food insecurity was assessed, and thus selection bias was a possibility in this study. In addition to BMI, nutrition supplementation has been shown to improve immunological outcomes in HIV patients as well as overall AIDS-related morbidity and mortality. In the NUSTART trial conducted among HIV infected ART naïve patients in Tanzania and Zambia, high dose vitamin and mineral supplementation in a lipid based nutritional supplement, improved CD4 counts in this patient group, but had no effect on mortality and severe adverse events (230). The NUSTART study had a very high mortality, although the participants that completed the study provided enough power to make the study comparisons and outcomes. The investigators hypothesized that addition of a vitamin and mineral would improve appetite, thus increase intake and have an effect on mortality. Given that the comparator was the same supplement but without the vitamin and mineral mix, the comparison groups (intervention and control) were probably similar with respect to the intervention. Therefore the lack of difference in outcomes is not unexpected. In a trial among HIV infected ART naïve patients in Botswana, 24-month micronutrient

supplementation with multivitamins and selenium slowed HIV disease progression and reduced AIDS-related morbidity and death (116). This study was conducted in ART naïve patients, and inclusion criteria was not based on nutritional status but rather on not being eligible to initiate ART at the moment (CD4 <350 cells/ μ l). However, it was a well-designed study with adequate power to answer the study question. Total loss to follow-up in the study was upto 17.5% which was higher than the 15% that was accounted for in the sample size calculation. This affected the generalizability of the study. With the change in policy to test and treat, the findings of this study are no longer relevant. Among the HIV infected ART naïve patients in South Africa, initiating ART with FutureLife porridge nutritional supplement led to increased percentage change in body weight, increased BMI, higher CD4 counts, raised hemoglobin levels and greater mean percentage fat-free mass (117). Participants included in this study were not malnourished (median BMI>18.5) but had self-reported weight loss. This potentially introduced selection bias into this study. Secondly, the study had a very small sample size, and high loss to follow-up (28%). The results of the 26 participants included in the analysis may be chance findings.

In Uganda and Kenya, a retrospective cohort analysis of HIV infected malnourished patients enrolled into the nutrition program showed that simultaneously giving ART and RUTF improves the chances of nutritional recovery (220). This was a cohort study that evaluated a program and did not control for any confounding.

Some studies (238–240) on nutrition supplementation in HIV infected patients have showed no effect on either nutritional or immunological outcomes. A pilot study conducted by Cantrell and others using food supplementation to improve adherence to ART among food insecure adults in Lusaka found no differences in weight gain or CD4 count among patients receiving and not receiving food supplementation. The adjusted mean weight gain among those on supplements was 5.4 kg compared to 5.1 kg at 6 months (238). Cantrell also conducted a program evaluation that did not control for confounding. Swaminathan and colleagues found that macronutrient supplementation did not have significant effect on weight gain and had inconclusive effect on immune recovery among HIV infected adults in India

(239). This study was a non-randomized study and had more than 40% loss to follow-up with most of the participants lost to follow-up belonging to the control arm.

This literature review highlights the fact that there are not many well designed interventional studies that have been conducted in the area of nutritional supplementation among malnourished HIV infected adults. The small sample sizes and high loss to follow-up reported by many of the studies coupled with different comparators used in the control arms highlight the challenges in designing such studies.

1.8. STUDY RATIONALE, QUESTIONS AND OBJECTIVES

1.8.1. Study rationale

In light of the inconclusive studies describing the effects of malnutrition and nutritional supplementation on different outcomes among HIV infected adult individuals, there remains a need to evaluate the effect of nutritional supplementation on clinical, nutritional and immunological outcomes in HIV infected adults. Additionally, there is need to characterize the clinical and nutritional parameters as well as immune responses of malnourished HIV infected adults.

A limitation of most of the studies exploring the effect of nutritional supplementation on immune responses among HIV infected adults is that they have mainly described the effect on CD4 and CD8 cells and not T cell sub-populations as well as the functionality of these cells. These studies also describe the effect of nutritional status on mortality, and anthropometry only. There are hardly any studies that describe other nutritional outcomes like dietary intake, body composition or detailed biomarkers of nutrition. Additionally, many of the studies are not current, and were conducted in the times before the widespread use of antiretroviral therapy. These studies did not characterize the study participants by their level of malnutrition. As a result, the effect of the level of malnutrition on the different outcomes are not described by the studies. This might explain the lack of consensus on the effect of nutritional supplementation on the various outcomes that were studied. Finally, very few studies used more than two methods in determining the nutritional status of the study participants.

The NOURISH study set out to examine the effect of nutritional status and nutritional supplementation on clinical, immunological and nutritional outcomes among HIV infected adults in peri-urban Uganda. The study planned to categorize participants by level of malnutrition and used four different methods to assess their nutritional status. The study also conducted detailed immunological assays to describe the immune responses among the study participants.

I.8.2. Study Objective

I.8.2.1. The aim of the study

The aim of the study was to characterize malnourished HIV infected adults with respect to immunological, clinical and nutritional status, and to describe the effects of nutritional supplementation on these outcomes.

I.8.2.2. Primary Objectives

The Primary objectives of the study were:

1. To describe the influence of nutritional status on clinical characteristics and immune responses of HIV infected adults in Uganda.
2. To describe the effect of nutritional supplementation on clinical outcomes, nutritional status and immune responses among HIV infected adults in Uganda.

I.8.2.3. Secondary objectives

The secondary objectives of the study were:

1. To determine the acceptability and challenges associated with the use of ready to use therapeutic foods by HIV infected adults in Uganda.
2. To establish the effect of nutritional supplementation on the quality of life among HIV infected adults in Uganda.

I.8.3. Study questions

The NOURISH study set out to answer the following questions:

1. What is the effect of nutritional supplementation on clinical outcomes among malnourished HIV infected adults in Uganda?
2. What is the effect of nutritional supplementation on nutritional parameters among malnourished HIV infected adults in Uganda?
3. What is the effect of nutritional supplementation on immune responses among malnourished HIV infected adults in Uganda?

I.9. THE NOURISH PROJECT

NOURISH (Nutrition and treatment outcomes: development of a Ugandan-Irish HIV/Nutrition Research Cluster) aimed at increasing the understanding of the complex interactions between food security (i.e. when all people at all times have access to sufficient, safe, nutritious food to maintain a healthy life), HIV/AIDS and socio-economic factors, to impact intervention programs at national level and benefit the poor in Uganda. NOURISH created an HIV/Nutrition research cluster of academics, clinicians and policy contributors whose purpose was to build research and teaching capacity in Irish and Ugandan institutions.

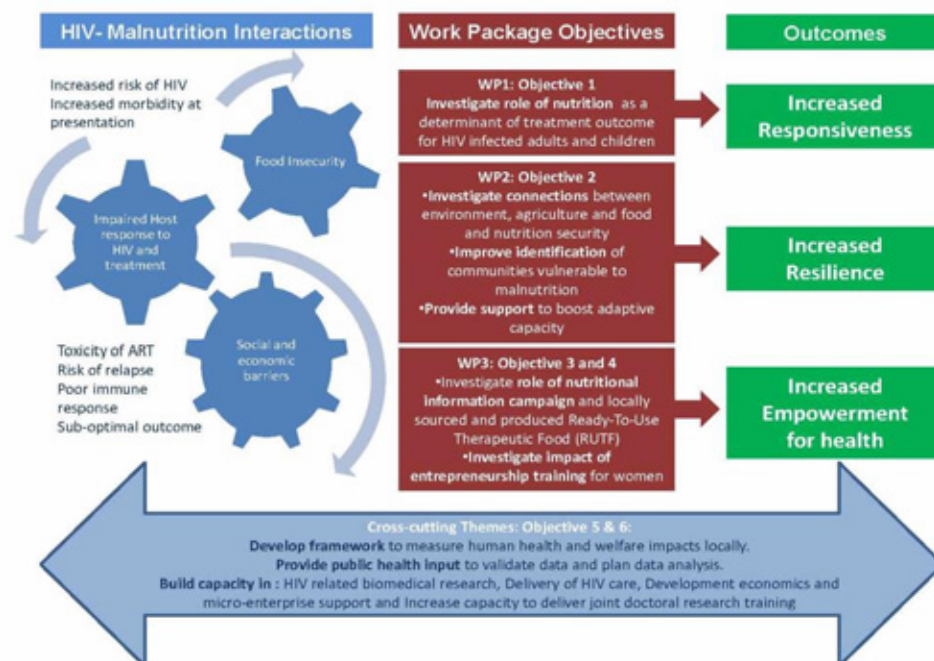


Figure I.10: The NOURISH Project

The cluster was to be led by Trinity College Dublin (TCD) with key partners Makerere University Kampala (MUK) and the Infectious Diseases Institute at MUK, Gulu University and the Joint Clinical Research Centre (JCRC), along with supporting partners; University College Dublin (UCD) and Kings College London. Researchers with expertise in health sciences, natural sciences and economics designed and delivered interventions to determine the impact of environmental, health and economic factors on the experience and outcomes of Ugandans living with HIV/AIDS.

NOURISH had three broad aims relating to HIV and nutrition in Uganda:

1. To build capacity in Ireland and Uganda to engage in cross-disciplinary and demand-led research linking HIV and nutrition.
2. To increase understanding of the complex interactions between food insecurity, livelihood, nutritional deficiencies, immunosuppression, antiretroviral therapy, nutritional supplementation and HIV/AIDS outcomes.
3. To develop ways of integrating HIV/AIDS programs with activities relating to hunger reduction, food security and fighting under-nutrition.

NOURISH was funded under the Program of Strategic Cooperation between Irish Aid and Higher Education and Research Institutes, Round 3. The NOURISH proposal was coordinated by the Trinity International Development Initiative (TIDI) and involved researchers from the Schools of Medicine, Social Sciences and Philosophy, Natural Sciences and the Institute for International Integration Studies (IIS) at TCD.

The NOURISH project was led by Prof Martina Hennessey as the principal investigator, and had 4 work packages (WPs). This PhD thesis was part of Work Package I (WPI).

1.9.1. Work Package I

WPI focused on the role of nutrition as a determinant of treatment outcome for HIV infected adults and children in Uganda, by examining clinical, pharmacological and immunological responses to food supplementation in patients with and without malnutrition. WPI took place primarily in IDI and Entebbe at the MRC/UVRI research unit on AIDS.

Specifically, WPI focused on a study to characterize a group of moderately and severely malnourished HIV positive children and adults with respect to immunological, clinical, nutritional, pharmacological and dietary intake status. The study described the effect of malnutrition on immunological and pharmacological

factors relevant to initiating ART, and the effect of short-term food supplementation on safety, adverse effects and efficacy of ART including early morbidity and mortality.

The work package was delivered by two PhD students who received a PhD in the areas of immunology, pharmacology and nutrition. Each student set up and implemented a study of a nutritional supplement in adults and children who were receiving antiretroviral therapy for HIV infection.

Both PhD research positions were full-time and fully funded and commenced late 2013. Students were registered in Trinity College Dublin (TCD) through the INDIGO program which is Trinity's International Doctoral program in Global Health. As part of this program students spent at least one year in TCD, but undertook the majority of their field work in Uganda. TCD PhD regulation were applied.

This thesis is for NOURISH study that was conducted among the HIV infected adults in Uganda.

I.10. PhD TIMELINES

	Oct-Dec 2013	Jan-June 2014	July-Dec 2014	Jan-Dec 2015	Jan-June 2016	July - Dec 2016	Jan-June 2017	July '17 - June 2018	Jun-18
Enrolment and registration									
Initial Period at TCD									
Proposal Development									
Training in Immunology Techniques									
Training in pharmacology techniques									
Lectures in Immunology and Pharmacology									
Proposal writing and Ethical Approvals									
Field work and data collection									
Second period to TCD									
Immunology experiments									
Pharmacology experiments									
Data management									
Statistical analysis									
Write up									
Submission									

I.1.1. OUTLINE OF THESIS

I.1.1.1. Chapter two: Methodology

The study site and study population are fully described here. Details of the mixed methods study design which was used are elaborated, including the qualitative and quantitative data collection methods. Details of the use of Plumpy'Nut are given. Also included here, are specifics of study subject selection, randomization and allocation of study arm. The study procedures which were conducted as well as how data was managed and analyzed are included. Finally, the ethical considerations of the study including ethics committee approvals and confidentiality are given.

I.1.1.2. Chapter three: Description of the study population, quality of life and acceptability of RUTF

This chapter describes the study population by detailing the demographic and baseline characteristics of the study population. The quality of life of the study participants and how it was influenced by ART and RUTF is also described in this chapter. The chapter also contains the results of the focus group discussions on acceptability, challenges and benefits of using RUTF as a nutritional supplement among HIV infected adults in Uganda. Adherence to RUTF and ART in the NOURISH study are also described in this chapter.

I.1.1.3. Chapter four: Effect of nutritional supplementation on nutritional outcomes

In chapter 4, the median differences in anthropometric, nutrient intake and biomarkers of nutrition among the different study groups are described. The median differences in the baseline and follow-up (12 weeks) of anthropometric, nutrient intake and biomarkers of nutrition are evaluated.

I.II.4. Chapter five: Effect of nutritional supplementation on immune responses

In this section, we looked at the median differences in circulating lymphocyte subset frequencies and absolute numbers in the study participants. The median differences between the baseline and follow-up (week 12) in an intervention trial using Plumpy'Nut were investigated. We also evaluated median differences in the functionality (cytokine production and cytotoxicity) of selected lymphocyte populations in the study groups.

I.II.5. Chapter six: General discussion

This chapter is a review of the main study findings of the thesis and the implication to patient care and policy.

CHAPTER TWO

GENERAL METHODOLOGY

2.1 STUDY SITE AND STUDY POPULATION

The study was conducted in Uganda at the Infectious Diseases Institute of Makerere University in Mulago, with field work and data collection done at MRC/UVRI Research Unit on AIDS HIV Care Research Program (HCP) clinic, in Entebbe. Participants were recruited from the HCP clinic, the Entebbe Hospital HIV/ART clinic, The AIDS Support Organization (TASO) Entebbe clinic and Kisubi Hospital HIV/ART clinic. These clinics are located in Entebbe which is a peninsula in Lake Victoria in Central Uganda. All clinical laboratory tests were done at the MRC/UVRI Research Unit on AIDS Clinical Diagnostic Laboratories (CDLS), and the PBMCs for the immunology studies were isolated in the immunology category three laboratories at the same institution. All immunology laboratory experiments were conducted at the Trinity Translational Medicine Institute, Trinity College Dublin at St. James' Hospital in Dublin Ireland.

2.1.1 Study population

The study population was HIV infected adults attending any of the above clinics and were either well-nourished and ART naïve, or malnourished and ART naïve, or malnourished but had been receiving ART for treatment of HIV for at least one year at the time of joining the study. The majority of the patients were from the fishing villages along the shores of Lake Victoria. These usually report with advanced HIV disease and thus are likely to be malnourished due to either poverty and food insecurity or the complications of advanced HIV disease.

2.1.2 Study period

The PhD commenced in October 2013, but proposal writing and obtaining of all ethical and regulatory approvals were not obtained until January 2015. Data were collected between February and December 2015, and laboratory experiments were

carried out between January 2016 and July 2016. Data analysis and write up commenced in July 2016 up to the time of submission.

2.2 STUDY DESIGN

This was a mixed methods study employing both qualitative and quantitative methods for data collection. Initially, the study was to include only ART naïve moderately malnourished and severely malnourished participants. However, when enrolment began, it was realised that there were not adequate numbers of these participants. The study team decided to amend the proposal to include well-nourished participants and ART experienced malnourished participants.

2.2.1 Quantitative Methods

Two methodologies were employed as follows:

1. Two randomization clinical trials which included one in which moderately malnourished ART naïve HIV infected participants randomized to receive both nutritional supplementation and nutrition counselling (the interventional arm) or nutrition counselling only (the control arm). The second trial involved the moderately malnourished ART experienced participants that were randomized to similar interventions as the moderately malnourished ART naïve participants.
2. A non-randomized interventional study design in which severely malnourished participants were enrolled and given nutritional supplementation and nutritional counseling. It was standard of care in Uganda to give nutritional supplement to severely malnourished HIV infected adults, therefore it would be unethical to randomize these participants.

The well-nourished participants were also studied using a prospective interventional study design. These were given nutritional counseling only since they were well-nourished and provided a control that would be used to assess the effects of ART only on the study outcomes.

All participants were followed up for 12 weeks.

All study participants were grouped according to their level of malnutrition into the following groups (Figure 2.1):

Group A- comprised of moderately malnourished HIV infected adults initiating ART (MAM) that had a BMI less than 18.5 but greater than 16.5 and were ART naïve.

Group B- comprised of severely malnourished HIV infected adults initiating ART (SAM) that had a BMI less than 16.5 and were ART naïve

Group C- comprised of moderately malnourished ART-experienced HIV infected adults and had a BMI less than 18.5

Group D- comprised of well-nourished HIV infected adults initiating ART (WN) that had a BMI greater than 18.5 and were ART naïve.

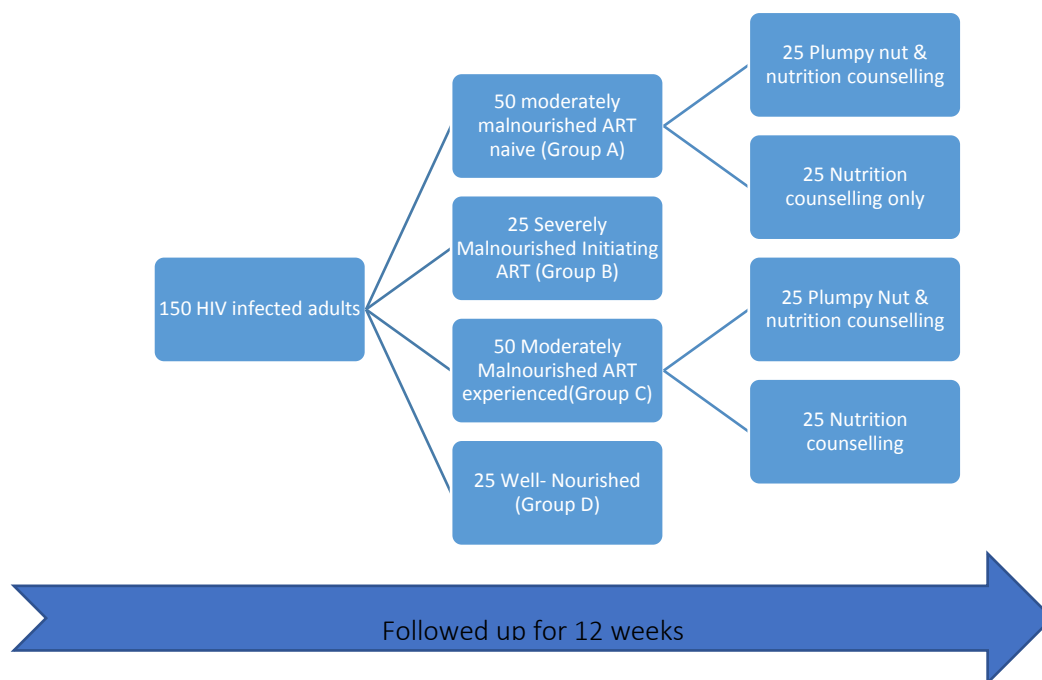


Figure 2.1. NOURISH STUDY SCHEMA: Recruited participants were divided into four groups depending on their baseline BMI and history of ART use. Group A were the moderately malnourished ART naïve, group B were severely malnourished ART naïve, group C were moderately malnourished ART experienced and group D were well nourished. The group A and D were randomized to either receive nutritional supplementation and nutritional counselling or nutritional counselling only. While all the group B participants received nutritional supplementation, and group D participants received no nutritional supplementation. All participants were followed up for 12 weeks.

HIV uninfected adults were included in the immunology studies and these acted as negative controls. Since there was no data on the immunological outcomes among HIV uninfected adults in Africa, the results of the negative controls were used to represent the distribution in the HIV uninfected population in Uganda.

2.2.2 Exposure of interest

The exposure of interest was a nutritional supplement called Plumpy’Nut, a ready to use therapeutic food (RUTF) recommended by WHO for treating malnutrition

in HIV infected persons. RUTF is fully described in chapter one, and its composition is summarized in Table 2.1.

2.2.3 Dosage

Among the participants that received the nutritional supplement, each was given 2 sachets of Plumpy'Nut per day which is equivalent to 56 sachets per month. Each sachet weighs 92g and is equivalent to 500 kilocalories of energy, thus each participant received 1000 kilocalories of energy per day.

Rationale for the 1000 kilocalories per day

The dosage of RUTF given was estimated using total energy intake that would be obtained from each 92g sachet of plump nut. This is because using the energy content provided a straight forward variable that could be used to calculate the number of sachets needed compared to using the other contents of plumpy nut which would need calculations and conversion factors to estimate number of sachets given.

To estimate the total energy intake required for the study participants, the following considerations were used:

The daily recommended calorie intake is 2000-2500 Kcal (241). The average energy intake in Uganda is about 1500 Kcal (242), this means the average Ugandan takes in 1000 kcal less than the recommended daily calorie intake. It was assumed that the study participants were a representation of a typical Ugandan, and thus they would need an extra 1000 kcal to meet the recommended daily intake of energy. However, the study participants were HIV infected, and the Ugandan statistic was from the general population. There could be differences in the energy intake between the Ugandan general population, and the HIV infected patients since HIV infection causes loss of appetite; leads to an increased oxidative stress and is associated with reduced intake of food due to opportunistic infections in the gastrointestinal tract. This means that the HIV infected patients might have a lower energy intake compared to the Uganda general population. However, Mupere et al, found that energy intake among tuberculosis patients in Uganda varied between

3000 Kcal among those with mild disease to 1400 Kcal among those with wasting (165). Among the HIV infected patients in Mupere's study, the average energy intake for the men was 2029 Kcal and 1438 kcal for women. Since more than 50% of Mupere's study participants were HIV infected, it was assumed that the NOURISH study participants were similar to the participants in Mupere's study. Therefore, it was assumed that the lowest average energy intake of the NOURISH study participants was 1400 kcal per day, which was the lowest energy intake by the HIV infected persons in Mupere's study.

It was decided that an extra energy intake of 1000 kcal would be adequate for the NOURISH study participants to have an intake equivalent to the recommended daily energy intake of 2000 -2500 kcal.

Energy intake estimations were not done at individual level due to logistical reasons. Because procurement of plumpy nut had to be done before enrolment of participants, estimation of how much plumpy nut to buy had to be based on how much each participant would receive. The need for advance procurement was to avoid any stock outs of the RUTF which would affect the study timelines that were already constrained. Additionally, the lead time for the procurement were long, and this would also affect the progress of enrolment and study follow-up.

.

2.3 OUTCOMES OF INTEREST

The outcomes of interest in this were the median differences in the baseline and 12-week measurements of the clinical, nutritional and immunological parameters of the study participants. These outcomes were analyzed by study groups.

2.3.1 Clinical parameters

The clinical parameters that were studied were:

- I. Viral load; CD4 count; and hemoglobin.
- II. Quality of life linear score.

2.3.1 Nutritional parameters

The nutritional parameters studied were:

- I. Anthropometric measurements (weight, BMI, MUAC),
- II. Body mass composition parameters (total fat mass, percent fat mass and total fat-free mass).
- III. 24 – Hour dietary nutrient intake.
- IV. Biomarkers of nutrition which were serum levels of high density lipoproteins, low density lipoproteins, triglycerides; trace elements like phosphates, potassium and sodium. Others included vitamin B12 and folate levels.

2.3.2 Immune responses

The immunological parameters studied were:

- I. 12 immunophenotypes namely: T cells, B cells, NK cells, CD4⁺ T cells, CD8⁺ T cells, CD4⁺ CD8⁺ T cells, Vd1 T cells, Vd2 T cells, Vd3 T cells, iNK T cells and CD56⁺ T cells.
- II. The cytolytic activity of CD4⁺ T cells, CD8⁺ T cells, NK cells and Vd1 cells.

- III. Production of cytokines (IFN-gamma, IL-4 and IL-17) by CD4⁺ T cells, CD8⁺ T cells and VDI T cells.

2.4 MEASUREMENT OF OUTCOME

2.4.1 Nutritional outcomes

2.4.1.1 Nutrient Intake

The nutrient intake of the study population was measured using a number of methods as shown below:

2.4.1.1.1 24- Hour dietary recall

The 24-hour dietary recall method was used to collect dietary history of the study participants for the immediate 24-hour period preceding the study visit. The PhD researcher was trained by an expert nutritionist on how to conduct a 24-hour dietary recall. This entailed administering a 15 to 30-minutes' (depending on the number of food items and meals a participant had) interactive structured interview to the study participants.

Participants were asked to mention all the foods they had consumed in the last 24 hours and the characteristics captured were: the meal (breakfast, lunch or dinner), the time of food consumption, type of food or drink, method of preparation, amount or portion measured in terms of spoons, cups, weights or cost. Details of the food description obtained during the interview included: specific food item, specific part of food item (e.g. chicken leg, with skin), fresh or dried state (e.g. fresh or dried peas, beans), processing state (e.g. refined verses whole grain, smoked, salted), stage of maturity or ripeness (ripe mango or raw mango), raw or cooked; if cooked, the cooking method used (e.g., boiled, steamed, roasted, fried: shallow or deep fried). Every participant was interviewed once at the baseline and 12 weeks' visit.

2.4.1.1.2 Estimation of portion sizes

Portion sizes of the food items eaten were estimated using the following methods:

- a) For most solid foods, participants were asked to estimate the amount of food eaten using a set of 3 standard dietary cups that had different colour codes according to their sizes. The sizes used in the study were one (1) cup, half ($\frac{1}{2}$) cup, quarter ($\frac{1}{4}$) cup and an eighth ($\frac{1}{8}$) of a cup. For this project, the standard cup used measured 236.64ml, $\frac{1}{2}$ cup measured 118.32ml, $\frac{1}{4}$ a cup measured 59.16ml and $\frac{1}{8}$ of a cup measured 29.58 ml.

The cups were placed on the table and the participant was asked to indicate which cup best estimated the amount of the food they had eaten for each food item.

- b) Estimating the portion size of snacks such as samosa, chapatti, mandazi, boiled eggs or fruits such as bananas, mangoes; was done using the cost of the food item, the size of the snack eaten i.e. small, medium or large.
- c) Drinks and beverages were estimated in volume using the commonly used utensils like cups, bottles and glasses. The most commonly used utensils were the plastic cups that measured 500 ml of liquid, the drinking glass was estimated to measure 150 ml of liquid. The table spoon was considered to measure 15 ml and the tea spoon 5 ml. The volume of bottled drinks varied but the most commonly used were the soda in the 120 ml plastic bottle, the 500 ml plastic bottle and the 350 ml glass bottle. Beers were reported in 500 ml glass bottles.

The estimated food portions were converted to weight in grams using the HarvestPlus spreadsheets or were weighed directly using a kitchen weighing scale with a precision of 0.01 kg.

2.4.1.1.3 HarvestPlus spreadsheets

We used food composition and conversion tables developed by HarvestPlus Uganda 2014 (243) for the volume to weight conversion as well as nutrient composition analysis of the food items reported in the 24-hour dietary recall by each participant. HarvestPlus Uganda Food Composition Tables (FCT) are a compilation of existing and imputed food composition data for foods commonly consumed in Central and Eastern Uganda. Most of the primary nutrient composition data in the HarvestPlus spreadsheets for Eastern and Central Uganda was generated from the United States Department of Agriculture (USDA) National Nutrient Database for Standard Reference. The HarvestPlus spreadsheets contains 3 excel worksheets; the volume to weight conversion worksheet, the food nutrient content worksheet and the recipe worksheet. The spreadsheets have a special code that is used to identify each food item by food type, method of preparation (raw, steamed, boiled, fried, roasted etc.), food state (ripe, raw, dry, fresh, smoked, salted etc.) or by recipes. (Table 2.1)

A unique code of 888888 was generated for Plumpy’Nut and added to the spreadsheets. The nutritional content of Plumpy’Nut was obtained from the product insert of Plumpy’Nut that is provided by the manufacturers and was added to the food nutrient content worksheet. Table 2.1 is a snapshot of the HarvestPlus FCT.

Table 2.1 snapshot of the HarvestPlus FCT.

A	B	Percent nutrient content per 100g of food			
		D	E	F	G
food_code	food_description	Energ_y_kcal	Protein_g	Lipids_g	Carbs_g
1001	MAIZE GRAIN,WHITE VARIETY,FRESH,RAW	172	4.4	2.2	34.9
1002	MAIZE GRAIN,WHITE VARIETY,FRESH,BOILED/STEAMED	207	5.3	2.6	42.0
1003	MAIZE GRAIN,WHITE VARIETY,FRESH,ROASTED	224	5.7	2.9	45.5
888888	PLUMPY NUT	500	12.5	32.8	0.0

2.4.1.1.4 Methodology for weighing food items

A list of all the food that the study participants had eaten was generated as well as the method of preparation. All the listed foods were obtained and prepared using the same method as the participants had indicated. Standard measures (cups) of those foods e.g. 1 full standard cup of steamed and mashed matooke, boiled rice, were weighed using a kitchen scale. For food items that could not be measured in standard cups e.g. samosa and chapatti, three (3) snacks of each size (large, medium or small) were weighed and the average weight for each size was used. The snacks were not weighed by price because they are priced according to size and thus the size weight estimation approximated the price weight estimation. The snacks were also broken up and filled into a standard cup, and their weight measured using a kitchen scale.

Fruits were weighed whole as would be eaten by a participant; those that necessitated a covering to be removed before eating would be weighed after the covering had been removed. The fruits were also measured by size and an average weight of three (3) fruits per size was used. Some fruits were filled in a standard cup and then the weighed.

Weight measurements for all food items were done as follows; the food item to be measured was placed in a transparent polythene bag of known weight and then measured using a digital kitchen weighing machine with a precision of 0.01 kg. The weight of the food item was obtained by subtracting the obtained weight of the food in the polythene bag from the weight of the polythene bag.

All data was entered into an excel spreadsheet and was used to then calculate the amount in grams for the different food items the participants reported to have eaten in the 24-hour preceding the study visit.

2.4.1.1.5 Calculating the weight of the portion sizes

Each food consumed was assigned a food code from the HarvestPlus Uganda FCTs depending on the type of food and method of food preparation. The codes for composite dishes such as katogo were obtained from the recipe worksheet which

also contained the codes for the individual food items that made up the composite sheet.

To estimate the weight of the portion sizes, the following was done:

1. The food portions generated using the standard cups or spoons had a volume to weight conversion done using the HarvestPlus food conversion spreadsheet. The volume weight conversion factor for each food item was identified using the unique code (from the HarvestPlus spreadsheets) of the food item.
2. Food portions estimated by size (small, medium or large) were weighed in sets of three (3) for each size using a kitchen scale. The average weight for each size was calculated and this was used to estimate the weight of foods in this category.
3. All composite dishes such as katogo had a volume to weight conversion as in 1 above. The weight of each ingredient was calculated by multiplying the proportion of the ingredient with the total weight of the composite dish. The proportion of each ingredient was obtained from the HarvestPlus recipe spreadsheet.
4. For drinks or beverages such as milk and juices, the conversion factors provided by HarvestPlus were used to convert the estimated volumes into grams. Care was taken to consider dilutions when estimating the weights of items in this category.
5. Volume to weight conversion of foods that were not listed in the HarvestPlus volume to weight conversion spreadsheet was done using the information generated from weighing these foods as detailed above.

The information obtained from calculating the portion weights for each participant was entered into a spreadsheet that contained all the participants' demographic details. The spreadsheet also contained the HarvestPlus code for each food item eaten by the participant, and calculated weight for each food item.

2.4.1.1.6 Determination of dietary nutrient intake

An excel spreadsheet (calculated nutrient intake spreadsheet) (table 2.2), was generated by adding the following columns to the HarvestPlus FCT.

- 1- Column **(H)** containing all the food codes as those in the HarvestPlus Food Composition Tables and arranged in the same order and sequence.
- 2- Column **I (amount)**, to contain the total amount of each food item eaten by the participant.
- 3- Column **J (per 100 grams)** to contain amount of food item eaten expressed per 100 grams.
- 4- Column K to the end of the spreadsheet which contained the same headings as the different nutrient content columns on the HarvestPlus Food Composition Table.

The last row of the spreadsheet contained the total intake of each nutrient in a given column.

Table 2.2: Snapshot of calculated nutrient intake spreadsheet

A	B	C	D	E	F	H	I	J	K	L	M	N
food_code	food_description	Ener kcal	Prot g	Lipids g	Carbs g	Food_code	Amt eaten	Amt /100 g	Ener kcal	Prot g	Lipid g	Carb g
1001	MAIZE GRAIN,WHITE VARIETY,FRESH,RAW	172	4.4	2.2	34.9	1001		0	0	0	0	0
1002	MAIZE GRAIN,WHITE VARIETY,FRESH,BOILED/STEAMED	207	5.3	2.6	42.0	1002		0	0	0	0	0
1003	MAIZE GRAIN,WHITE VARIETY,FRESH,ROASTED	224	5.7	2.9	45.5	1003		0	0	0	0	0
888888	PLUMPY NUT	500	12.5	32.8	0.0	888888		0	0	0	0	0
						Total Intake		0	0	0	0	0

HarvestPlus FCT

Added columns to the HarvestPlus FCT

The amount of a given food eaten by the participant was entered column I of the spreadsheet, in the cell of the corresponding food code in the Harvestplus FCT. The amount of food eaten per 100 grams (column J) was calculated by dividing the total amount of food eaten (column I) by 100.

As shown in table 2.3, the amounts of each nutrient contained in a given food eaten by a participants (column K to end), were calculated by multiplying the total amount of the food type eat per 100 grams (column J) by the proportion of the corresponding nutrient in the HarvestPlus Food Composition. For example, the protein content in food item 1001, would be calculated as follows: If a participant ate 50g of food item 1001, which is 0.5 of the food per 100g, the amount of protein contained in the 50g of 1001 (cell L2) is obtained by multiplying 0.5 (cell J2), by 4.4 (cell D2) (the proportion of protein contained in 100g of food item 1001).

Table 2.3: Example of calculated nutrient intake of a participants that ate 2 kinds of food plus Plumpy'nut

A	B	C	D	E	F	H	I	J	K	L	M
food_code	food_description	Ener kcal	Prot g	Lipids g	Carbs g	Amt eaten	Amt /100g	Ener kcal	Prot g	Lipid g	Carb g
1001	MAIZE GRAIN,WHITE VARIETY,FRESH,RAW	172	4.4	2.2	34.9	50	0.5	86	2.2	1.1	17.5
1002	MAIZE GRAIN,WHITE VARIETY,FRESH,BOILED/ STEAMED	207	5.3	2.6	42.0	0	0	0	0	0	0
1003	MAIZE GRAIN,WHITE VARIETY,FRESH,	224	5.7	2.9	45.5	20	0.2	44.8	1.14	0.58	9.1
888888	PLUMPY NUT	500	12.5	32.8	0.0	92	0.92	501.4	12.51	32.84	0
								632.2	15.85	34.52	26.6

The total amount of a given nutrient taken by a participant was summed in the last cell of the given row. E.g. For the example in table 2.3 above, this particular patient would have taken in a total of 15.85g of protein (Cell L6).

2.4.1.2 Anthropometry

Anthropometry was determined using the methods below:

2.4.1.2.1 Measurement of height using the stadiometer

Height in this study was measured using a Leicester stadiometer, and the procedure below details how this was done:

- I. Check that the stadiometer has been assembled correctly.
- II. If required, assemble the stadiometer. Slot white upright sections together. Slide on the two white stabilizers and blue measuring arm. Position stabilizers as required, ensuring they are clear of the joints. Attach upright sections to base.
- III. Place the stadiometer base on a flat surface with stabilizers resting against a wall/door to give rigidity.
- IV. Ask the participant to remove their shoes. Raise the head-plate of the stadiometer to allow sufficient room for the participant to stand underneath it.
- V. Ask the participant to remove any hair ornaments (e.g. bands, hats, turbans) if interfering with the measurement. If the participant is unable to remove the hair piece, for instance due to religious reasons (e.g. turbans), measure height with turban, then take the measurement of the height of the turban. Subtract height of the turban from total height. Enter this deviation from normal procedure in the clinical examination questionnaire under question 3, height comment.
- VI. Check that the participant is standing with their feet flat on the center of the base plate, heels together and against the rod, arms to the side, legs straight, shoulders relaxed, and head in the Frankfort horizontal plane. This plane is represented by an imaginary line between the lowest point on the margin of the orbit (the bony socket of the eye) and the trignon (the notch above the tragus, the cartilaginous projection just anterior to the external opening of the ear - see Figure 2.2).

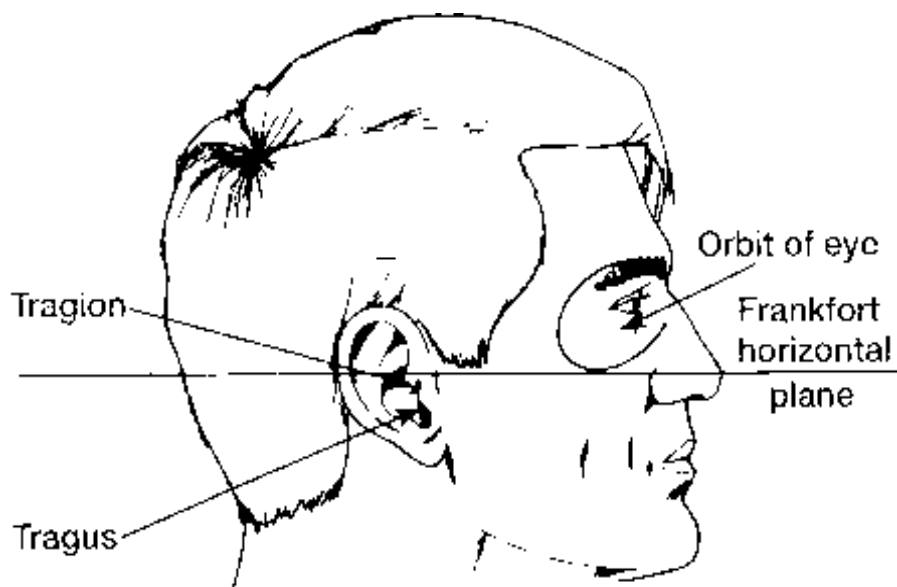


Fig 2.2: Frankfort horizontal plane

- VII. Ensure that heels, buttocks, scapulae (shoulder blades), and back of the head are against the vertical surface of the stadiometer. Some people may not be able to touch all four points against the stadiometer due of obesity or curvature of the spine. If this occurs, have the participant touching two or three of the four points of the vertical surface of the stadiometer.
- VIII. Instruct the participant to keep their eyes focused on a point straight ahead and to maintain an erect posture (to stand as upright/tall as he/she can). Lower the head-plate to the highest point of the head with enough pressure to compress the hair.
- IX. Ask the participant to step away from the stadiometer, ensuring that the head-plate does not move when reading off the measurement. Record to the nearest 0.1 cm on the participant's clinical notes.

2.4.1.2.2 Body Mass Composition

Body mass composition of all study participants was done using the TANITA^R body composition analyzer BC-418 (TANITA Corporation, Japan) Figure 2.3. The

TANITA BC-418 calculates regional body composition using 8 polar electrodes and calculates body fat ratio, fat-free mass, estimated muscle mass and basal metabolic rate using data derived by the dual energy X-ray absorptiometry (DXA) method using Bioelectric Impedance Analysis (BIA). It is an FDA cleared body composition analyzer and has been widely used in research and clinical settings.

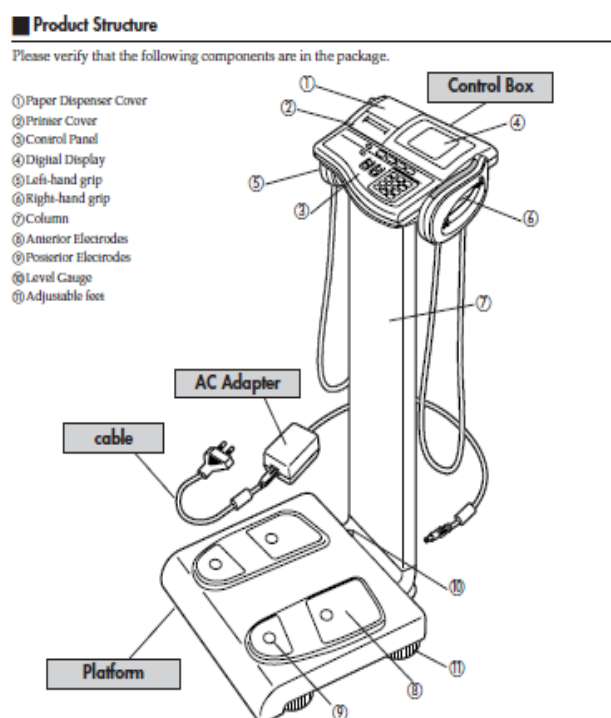


Fig 2.3: TANITA Body composition analyzer BC-418

Participants were requested to remove their shoes and stand on the TANITA machine with the plantar aspect touching the electrodes accordingly. They were asked to firmly hold the hand grips on the machine, place the hands on the sides of the body and the stand upright (figure 2.4). The Body composition analyzer would then analyze the different segments of the body using the flow of electric current through the different body compartments. In order for the machine to do this analysis, the following demographic information had to be entered into the machine: gender; height, date of birth, type of body (athletic or normal). The machine would then start analyzing once the start button is pressed and would give a print out of the measurements of the different compartments. The measurements given included: weight, BMI, Basal metabolic rate, total body water, percent fat mass and

total fat mass, percent fat free mass and total fat free mass, and a complete segmental analysis of the limbs and the trunk.

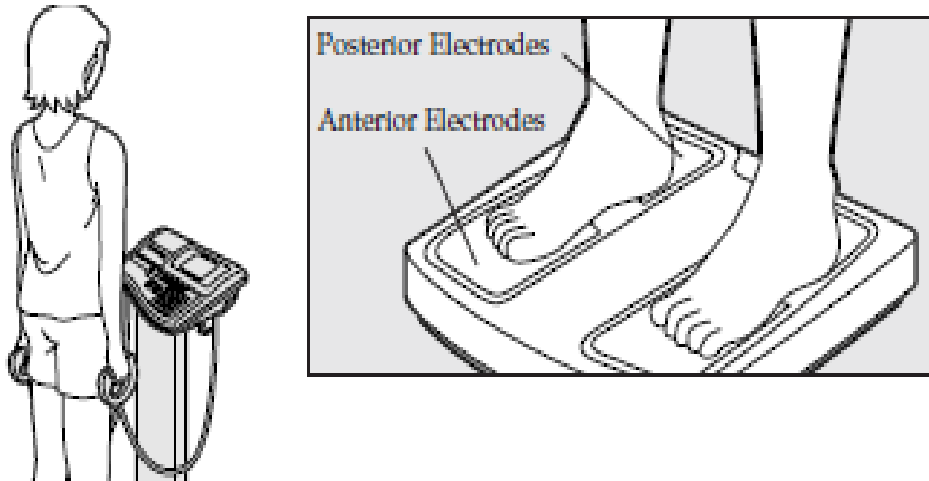


Fig 2.4: How to hold the hand grips and step on the electrodes of the BC-418

2.4.1.2.3 Measurement of mid upper arm circumference (MUAC)

MUAC was measured using the following steps (see figure 2.5 below):

1. Bend the left arm, find and mark with a pen the olecranon process and acromion.
2. Mark the mid-point between these two marks.
3. With the arm hanging straight down, wrap a MUAC tape around the arm at the midpoint mark.
4. Measure to the nearest 1 mm.

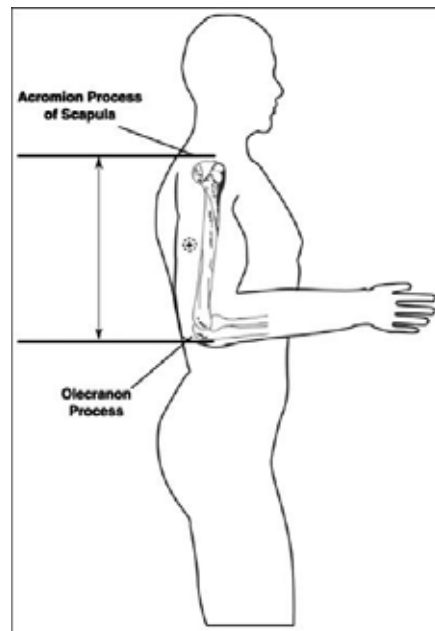


Fig 2.5: How to measure mid upper arm circumference.

2.4.1.3 Measurement of biomarkers of nutrition

The biomarkers of nutrition were done at the MRC/UVRI Research Unit on AIDS Clinical Diagnostic Laboratories (CDLS). The tests done include: Vitamin B12, trace elements like phosphorous and magnesium as well as electrolytes such as potassium and sodium. The CDLS laboratory is a fully accredited laboratory, and all the technicians are GLP certified. The biomarkers of nutrition were done using the COBAS INTEGRA 400 PLUS clinical chemistry analyzer. The tests done included: lipid profile such as High Density Lipoproteins (HDL), Low Density Lipoprotein (LDL) and triglycerides as well as total cholesterol. Others included, C-reactive protein, vitamin B12, and minerals and trace elements like Sodium, potassium, magnesium, phosphates, folate, iron.

The hematology outcomes including the lymphocyte subsets used in routine clinical care such as CD4 cell counts were done using the Ac. T 5diff hematology analyzer (Beckman Coulter-UK) from whole blood collected using the EDTA bottle. PCR for viral loads was done using the Cobas^R AmpliPrep (Roche molecular diagnostics)

Table 2.4 details the type of samples taken and the test done from each sample:

Need to include measurements of the immunological and clinical outcomes here

2.5 SUBJECT SELECTION (INCLUSION/EXCLUSION CRITERIA)

The study enrolled participants that were above 18 years of age and consented to participate in the study, had documented HIV infection and were eligible to initiate antiretroviral treatment (groups A, B and D) or had been on antiretroviral treatment for at least one year (group C). The participants had to be willing and able to take Plumpy'Nut as a nutritional supplement. Participants were not enrolled into the study if they were receiving any kind of nutritional supplement, were critically ill or had been recently diagnosed with tuberculosis. They would also be excluded from the study if they were participating in another study, were either pregnant or breast feeding (women) and had allergies to any of the components of Plumpy'Nut.

2.6 RANDOMIZATION CRITERIA

Only Moderately malnourished (MAM) participants were randomized to either receiving RUTF (Plumpy'Nut) and nutritional counseling, or receiving nutritional counseling only.

Table 2.4: Number and type of samples collected and tests done on each sample

specimen	Type of collection tube	Tests conducted on sample	Tests conducted on stored samples
Blood	2 EDTA tubes (4 ml per tube)	Full blood count and differential counts	N/A
		Lymphocyte subset counts	N/A
		Storage of Plasma and red cell pellet	Efavirenz (EFV) drug level analysis Extraction of DNA and RNA for pharmacogenetics studies
	2 SST tubes (4 ml each) for serum	Renal and liver function tests, Lipid profiles Elements such as Na^{2+} , K^{2+} , PO_4^{2-} Vitamin B 12 and folate	EFV drug level analysis Estimation of CYP3A4 enzyme activity.
	3 CPT tubes (8 ml each)	PBMC Isolation and storage	Enumeration of circulating lymphocytes. Cytotoxic degranulation and cytokine production by lymphocytes after ex vivo stimulation
	1 NaFl (4mls)	Fasting blood glucose	
Urine	General purpose bottle	Urine pregnancy test	Urine metabolomics

2.7 TREATMENT OF SUBJECTS

All participants that were randomized to receive RUTF were given two sachets of Plumpy'Nut (each 92g) per day which is equivalent to 1000 Kcal/day of energy intake.

All ART naïve participants were initiated on ART with an Efavirenz (EFV) based regimen (recommended national first line regimen), on the same day of joining the study or within two weeks of joining the study. Efavirenz, Lamivudine (3TC) and Tenofovir (TDF) was the commonest drug combination used by the participants.

2.7.1 Allocation to Treatment/Group

This was an unblinded study that used simple randomization method with randomization log generated using STATA 12.1. There was no stratification, and allocation of randomization arm was done by use of concealed envelopes which contained the study arm. Study numbers were assigned to participants at the time of randomization.

A participant number (PID) contained one letter and 3 digits. All participant numbers began with letter N which represents NOURISH and the three were serial numbers representing the order of recruitment.

2.8 STUDY PROCEDURES

2.8.1 Screening

This took place 14-30 days before a patient was initiated on ART. Potential participants were given information about the study and those interested to join received further explanation of the study from the PhD student. Participants who consented to join the study would sign a consent form and were assigned a screening number. Consented participants were then screened for eligibility and those found eligible had a detailed medical history and examination done, have their nutritional status assessed using anthropometry, body composition analysis using the TANITA bioelectric analyzer and blood was collected for various tests including full blood count, detailed biochemistry, estimation of viral load and preparation of

PBMCs as well as storage of serum and plasma for other tests that were to be done at Trinity college in Dublin. Urine for pregnancy (women of child bearing age) and for estimation of urine metabolomics was also collected.

2.8.2 Week 0/Baseline

This visit took place on the day the participant was to be initiated on ART. The participant went to the study office after being initiated on ART and was reassessed for readiness to join the study. If they were found ready, the participant was enrolled (randomized if necessary) and have the following procedures done. They would be assigned a study number, given nutritional counselling and educated on how to use Plumpy'Nut (for those receiving Plumpy'Nut). A dietary history (24-hour dietary recall) was collected and blood for fasting blood sugar as well as lipid profile analysis was collected (participants were requested to come in after an overnight fast). Participants that were to receive nutritional supplementation were given one month's supply of Plumpy'Nut.

2.8.2.1 Follow-up Visits

After the screening and baseline visit (enrolment visit), participants were followed up at four-week intervals until week 12. During these monthly visits, adherence to Plumpy'Nut and ART were assessed (details below) and a symptom check list was completed. Participants would be supplied with ART and Plumpy'Nut for another month.

Other procedures done during these visits included collection of a medical history and focused medical examination, body composition analysis using TANITA bioelectric analyser and dietary assessment using 24-hour dietary recall.

On the last visit (week 12), participants would have all the above procedures done, and a repeat of all the baseline blood samples collected and tests done. Table 2.5 is a matrix of the timelines for different study procedures.

Table 2.5: Timelines for the different procedures in the NOURISH study

Study Activity	Screen Visit one	Visit two	Week 1	Week 4	Week 8	Week 12
Informed Consent	x	x				
Enrolment		x				
General Medical History and Physical Examination		x	x	x	x	x
Nutritional assessment	x	x	x	x	x	x
24-hour dietary recall		x	x	x	x	x
Nutritional counseling		x	x	x	x	x
Plumpy'Nut education		x	x	x	x	x
Laboratory Procedures						
Hematology	x					x
Blood Chemistry	x					x
Urinalysis	x					x
Immunology tests	x					x
Pharmacology tests	x					x
Fasting Blood sugar		x				
Lipid Profile		x				
Pregnancy test	x					
Plumpy'Nut		x	x	x	x	x
Adherence assessment			x	x	x	x
Quality of life			x	x	x	x

2.8.3 Assessment of adherence to Plumpy’Nut

Adherence to Plumpy’Nut was assessed every four weeks for all the participants that received Plumpy’Nut. This was done using two methods:

1. Patients were also requested to return all the supplied sachets of Plumpy’Nut. A physical count of used (empty) and unused (unopened) sachets was done on every visit. The total number of sachets returned was also recorded.
2. Objectively, adherence to Plumpy’Nut was done by comparing the changes in serum folate levels of the participants that received RUTF with the changes of the participants that did not receive RUTF.

2.8.4 Loss to Follow-up and subject withdrawal

Study participants may withdraw from the study at any time at their own request, or they may be withdrawn at any time at the discretion of the investigator or sponsor for safety reasons. If a participant did not return for a scheduled visit, every effort was made to contact the subject. This was done through telephone contacts and home visits. If the participant withdrew consent from the study, no further evaluations could be performed, and no additional data could be collected. The sponsor would retain and continue to use any data collected before such withdrawal of consent.

In the NOURISH study, we assessed nutritional status using all the four methods (anthropometry, biochemical analysis, clinical markers and assessment of dietary intake).

2.9 DATA MANAGEMENT AND ANALYSIS

2.9.1 Sample Size Determination

The sample size of the study was calculated using the moderately malnourished participants. Initially, it was planned to conduct a randomized study among the ART naïve moderately malnourished HIV infected participants, and a non-randomized interventional study for the severely malnourished participants.

Assuming there was to be a 2.2 Kg/M² increase in BMI among the participants that would receive RUTF, then a sample size 240 participants would enable the study to detect a 0.7 difference in the week 12 BMIs between the two comparison groups, at a power of 80% and 5% significance level after allowing for 15% rate of attrition

However, it was not feasible to enroll 240 participants given the time and funds available to successfully complete the study. Therefore, convenient sample of 150 was used to answer the study questions. The 150 participants were to be divided into 100 moderately malnourished and 50 severely malnourished. The 100 MAM participants would give a power of 50% to detect 0.7 difference in the median BMI of the comparison groups. This is very low power from a statistical perspective. However, it was believed that this number would be adequate to demonstrate a clinically significant difference in the the study outcomes.

Sample size was not calculated using immunological outcomes because the unit of analysis was to be cell frequencies and absolute counts of cells and cytokines, and it is difficult to determine how many cells or cytokines one individual would have.

2.9.2 Data management

Data was double entered using fluid survey online data entry software. Data were then downloaded and exported to MS Excel for cleaning and management. Data cleaning was done after all data had been entered by cross checking where discrepancies existed with the source documents. Consistency and range checks were used to ensure accuracy of the data. Each variable was given a unique identifier and assigned a code to indicate whether it was a baseline or follow-up variable. After data cleaning, data was then exported to Stata 13, where analysis was done. As will be described in chapter 5, immunological data was downloaded from the flow cytometer, and transferred to FlowJo (flow cytometer data analysis software) where enumeration of the different phenotypes was done. After enumeration of the different phenotypes, data was transferred to GraphPad Prism version 7.0 (244) for statistical analysis.

In Stata, data transformations were done such as some continuous variables were transformed into categorical variables before analysis was done.

Data was anonymized using a unique identifier for each study participant and the data was stored in a database that was password locked and could not be accessed without permission.

2.9.3 Data analysis

Data was analyzed per as follows: The randomized studies were analyzed using per protocol analysis while the not randomized study and the observational studies were analyzed using the pre and post analysis. It was not possible to use intention to treat analysis given that each participants had two data points (baseline and weeks 12). Therefore, there would be no other data to use in the intention to treat since there was only baseline data available for the participants lost to follow-up and it was not possible to impute their data.

The first objective was to characterize the study population in terms of their clinical, nutritional and immunological status.

For this analysis, medians were compared using the t-test for all normally distributed variables, while the Wilcoxon rank sum test was used to compare the medians of variables that were not normally distributed. To compare the study groups, analysis of variance was used for the normally distributed variables and the Kruskal Wallis test was used to for the variables that were not normally distributed. All categorical variables were compared using the chi-square test.

To study the effect of nutritional supplementation on clinical and nutritional outcomes, the following comparisons were done:

For the moderately malnourished ART naïve group and the ART experienced group, comparisons of medians (IQR) were done between the randomization arms, i.e. those that received Plumpy’Nut were compared with those that did not receive Plumpy’Nut. In this analysis, the Wilcoxon rank sum test was used for the variables that were not normally distributed. A difference in difference analysis between the participants that received PlumpyNut and those that did not was done to explore the additional effect of PlumpyNut compared to ART alone on the clinical and nutritional outcomes.

A before and after comparison was also done for participants in both groups and for the severely malnourished participants to assess the effect of RUTF on clinical outcomes in each of the groups.

Analysis for assessing adherence to Plumpy’Nut was done using the number of days that the participant did not take Plumpy’Nut, and comparison of the before and after median serum folate levels for the ART experienced participants.

The number of days that participants did not take Plumpy’Nut was calculated by dividing the number of unused sachets that were returned by two. Participants were then grouped in categories depending on the number of days missed; no days missed; 1-3 days missed and more than 3 days missed.

The baseline and week 12 median serum folate levels for the ART experienced participants were compared using the Wilcoxon rank sum test.

Details of the analysis methods for the nutritional and immunological characterization of the study population will be described in the respective chapters, this section describes the analysis methods for the characterization of the clinical outcomes.

2.10 ETHICS

2.10.1 Institutional Review Board (IRB)

The study was reviewed and approved by the school of Biomedical Sciences Higher Degrees Research Ethics committee at the Makerere College of Health Sciences and the Faculty of Health Sciences Research Ethics Committee at Trinity College Dublin in Ireland. It was also reviewed and approved by the Uganda National Council of Science and Technology (UNCST).

2.10.2 Ethical Conduct of the Study

The study was conducted in accordance with legal and regulatory requirements, as well as the general principles set forth in the International Ethical Guidelines for Biomedical Research Involving Human Subjects and the Declaration of Helsinki (245).

In addition, the study was conducted in accordance with the protocol, GCP guidelines, and applicable local regulatory requirements and laws.

2.10.3 Participant Information and Consent

The study was introduced to the patients in the clinics through general announcements and health talks. Interested participants were asked to speak to the clinic doctors or counselors who gave them information sheets (written in the language they understand) to read at home.

Study information sheets were also placed at the clinic information desk for patients to pick and read at their leisure. When they returned to the clinic, they were referred to the study clinic where the study was explained to them and had their questions and concerns answered. The patients that agreed to join the study were requested to give written consent by signing the consent form.

For participants that could not read, the study information was read to them in the presence of an independent witness (someone not associated with the study and may be chosen by the potential participant). They were then requested to append their thumb print on the consent form and the independent witness signed on the consent form.

Patient information was given both in English and Luganda, the local language used in Kampala.

2.10.4 Confidentiality

Participants' confidentiality was protected using study numbers instead of including personal names on any study forms, publications or any other disclosures.

2.11 QUALITATIVE STUDY DESIGN

Qualitative study to assess acceptability of, and challenges of using Plumpy'Nut among malnourished HIV infected adults initiating art in Uganda.

2.11.1 Introduction

One of the secondary objectives of the NOURISH project was to assess the acceptability of and challenges in using Plumpy'Nut among malnourished HIV

infected Adults initiating ART in Uganda. The specific research questions under this objective were as follows:

1. What is the acceptability of Plumpy’Nut among malnourished HIV infected adults initiating ART in Uganda?
2. What are the challenges associated with using Plumpy’Nut among malnourished HIV adults initiating ART in Uganda?

Focus Group Discussions (FGDs) were used as the primary method of carrying out this assessment. Krueger and Casey define focus group research as:

“A carefully planned series of discussions designed to obtain perceptions on a defined area of interest in a permissive, non-threatening environment.” (246).

We used Focus Group Discussions because the topic under study had a potential for social desirability bias which could lead to false conclusions, which can be minimized with the open discussions that foster open and candid deliberations. According to Hennink, one of the uses of focus groups is to provide explanations for certain beliefs, attitudes or behavior amongst a target population (247). Thus, the Focus Group Discussions were used to explain the study population’s behavior with regard to the acceptability and challenges of using Plumpy’Nut use.

The Focus Group Discussions were used to supplement the quantitative research and may uncover important influences, linkages or contextual information needed to fully understand the effect of Plumpy’Nut on the different outcomes outlined in the quantitative section.

2.11.2 Study Population, Sampling Procedure, and Sample Size

The study population for the qualitative study was the same as that of the quantitative research and has been described earlier.

. All participants that received Plumpy’Nut were invited to participate in the Focus Group Discussions. This is because there were very few numbers of participants that received RUTF, and any other method of sampling would lead to a very small sample which would lead to improper conclusions due to lack of saturation of the themes. They were divided up into three FGDs comprising of between 8-10

persons each. In comprising membership of the FGDs, particular attention was given to ensure that participants within a similar age group were in the same FGD, with the following categories: the younger (18-29) and the older (30+). However, gender was not used as a factor in determining group membership.

2.11.3 Data Collection and Management of Group Discussions

2.11.3.1 Location

The group discussions were conducted at Entebbe Hospital. The study participants were familiar and comfortable with these premises. Study participants frequently visit these premises to seek treatment and support, and it is in this context that they have disclosed their status and are able to openly discuss their experiences with HIV amongst peers. The discussions took place in the patients' seating area with the group discussion participants seated in a circle, to make the setting interactive, informal and relaxed.

2.11.3.2 Management of the Focus Group Discussions

All focus groups were managed by a two-man team comprised of a moderator and a note taker. The PhD student was the moderator and was responsible for facilitating all the group discussions. The essential role of the moderator was to manage each group discussion so that the information gained provides a greater understanding of the research issues. As the moderator, the PhD student was responsible for creating a comfortable environment within each group to put participants at ease, ensuring that all members shared their views, encouraging debate between participants, probing for depth and clarity in the issues, listening to contributions and asking follow-up questions, monitoring the reactions of participants, remembering earlier points, anticipating the next topic of discussion and remaining aware of the timing and pacing of the discussion. The moderator also managed the group dynamics to ensure the full and meaningful participation of all group members. The moderator was also responsible for attending to ethical issues such as consent and confidentiality during the discussion.

The moderation of the group discussions took into cognizance the five stages of focus groups highlighted by Hennink (246). These stages are given as: pre-discussion, introduction, central discussion, closing and post-discussion.

- 1) The pre-discussion is the period before the group discussion, where the moderator and note taker wait for the participants to arrive. This is a useful phase during which rapport was built with the focus group participants, through engaging in small talk around other topics, other than the research questions. During this period, the seating arrangement was checked for comfortability and ease, and recording equipment are set up and tested. Participants who arrived early were served refreshments.
- 2) During the introduction the PhD student/moderator provided an overview of the research topic, indicated how the group discussion would proceed, and provided guidelines for the conduct of the discussion. Ethical issues specifically, group consent to record the discussion was sought.
- 3) The moderator directed the discussion towards the key research issues during the central discussion stage. The moderator adopted a directive style of moderation (246), where he played an active role in facilitating contributions and encouraging debate in the discussion, through probing and follow-up questions to the group. Directive moderation is used when the aim of the discussion is to elicit participants' opinions and experiences around quite specific, pre-determined issues to gain depth and detail. At this stage, the moderator used probing as a key technique. Probing is a technique used by the moderator to gain further clarity, depth and detail from participant's responses (246). A probe is used to stimulate further discussion or to focus the discussion to explore specific issues in depth. The moderator continually assessed whether the information from the discussion would be sufficient to answer the research questions. Throughout the central discussion, the moderator would provide positive encouragement for participants to share their views, through empathetic listening, eye contact with speakers, leaning forward and active listening.

- 4) During the closing stages the moderator summarized the main issues covered during the discussion and sought clarification on ambiguous issues. The moderator also thanked participants for their contributions, reconfirmed the value of the information received and responded to any final queries.
- 5) The moderator and note taker held a debriefing session after each group discussion to share impressions about the information received. The debriefing session was used to review the effectiveness of the group procedures, including the main themes discussed, difficulties encountered, moderator's technique, group dynamics, and suitability of the location and so on. Reflections were useful in modifying subsequent group discussions.

2.11.3.3 Recording

Focus groups were recorded using audio digital recorders after obtaining consent from the participants. The moderator provided sufficient information to participants about the reasons for requesting the recording, how the information was to be used, and how participants' anonymity and confidentiality was to be safeguarded. Although the group discussions were recorded using digital technology, the note-taker wrote down the key issues raised in the discussion in as much detail as possible. The note-taker recorded the proceedings objectively and in verbatim to the extent possible and did not write their judgment/conclusions of the discussion.

The focus group guide is found in annex 3

2.11.4 Data Management and Analysis

Analysis of qualitative data began at the stage of data collection and was an ongoing activity.

2.11.4.1 Transcribing of Data

All recorded data was transcribed and written transcripts of each group discussion derived. The transcripts were processed using the MS Word program. A verbatim transcript was generated for each group discussion. All transcripts were read by

the PhD student, who also had the responsibility of cleaning, labeling and anonymizing them. For group discussions that were conducted in Luganda, the verbatim transcript was translated to English.

2.11.4.2 Identification and Generation of Themes

Major themes from the transcripts were identified and analyzed to determine what meanings they construed for the participants, and how they shaped the participants' acceptability and use of Plumpy'Nut. Krueger and Casey (246) describe themes as topics, issues, concepts, influences, explanations, events, ideas or other things which mark the central focus of a part of the discussion. Themes were also derived from explicit topic areas in the discussion guide, and from the data itself. Each transcript was reviewed to identify the major emerging themes. Themes derived from the transcripts, were identified using principles of Grounded Theory (248). This approach enabled the identification of issues of importance to the participants. While reviewing the transcripts to identify themes, we adopted an analytical and reflexive stance, to interrogate the data using questions such as: What is happening? To whom does it happen? How or why is it happening? Who is involved? Searching the data for the responses to these questions helped to focus a theme or identify a concept in the data (249). As data analysis progressed, further themes were added or modified, with some themes being merged and others split. Flexibility in dealing with the themes was maintained until we reached a point of saturation (248) when no newer issues or concepts were identified in the data.

2.11.4.3 Coding

Nvivo software program was used to aid the data analysis process.

2.11.4.4 Descriptive Analysis and Theory Development

Descriptive analysis was the first level of data analysis and involved an examination of the specific issues in the group discussion and describing the context of each issue (77). Theme labels were used to identify all segments of text related to a specific theme and examine the discussion of each theme across the entire datasets.

Descriptive analyses were also used to identify patterns in the data, across the different population segments and attach meaning to these patterns.

The second level of analysis developed explanations, theories or conceptual frameworks from the data. An inductive approach was used to allow theoretical explanations to be derived from the data analysis. Patterns in the data were identified and relationships between themes and explanations for these patterns were sought and linked all these together into a conceptual framework that contributed to a greater understanding of the group discussion.

CHAPTER THREE

EFFECT OF NUTRITIONAL STATUS AND NUTRITIONAL SUPPLEMENTATION ON CLINICAL OUTCOMES AND QUALITY OF LIFE OF HIV INFECTED ADULTS IN UGANDA

3.1 INTRODUCTION

3.1.1 Burden of HIV

HIV/AIDS remains a global challenge with the number of people living with HIV estimated to be 36.7million by the end of 2016 worldwide (1) . Although the burden of the disease differs across regions, Sub-Saharan Africa continues to be most affected with 4.2% of the adults living with HIV and accounting for almost two thirds of the people living with HIV globally (2) . Approximately 19.4million people living with HIV live in East and Southern Africa, which is more than half of the world's population living with HIV (2).

In Uganda, the prevalence of HIV among adults stands at 6.2% in the general population, with females having a higher prevalence (7.6%) than men (4.7%)(3). Prevalence is highest among males aged 45 to 49 years at 14%, and in women aged 35 to 39 years, at 12.9%(3). Across Uganda, HIV is more prevalent among the population living in urban areas (7.5%) compared to those in rural areas (5.8%). In fact, amongst the females, HIV prevalence in urban areas is 9.8% versus 6.7% in rural areas(3). In 2016, the incidence of HIV in Uganda stood at 1.2 per 1000 population, translating into approximately 52,000 new infections in that year(4). This was a reduction from more than 100,000 new infections observed in 1990(4). This reduction is mainly attributed the introduction of antiretroviral therapy as observed by Ron Gray and others (4).

3.1.2 HIV and malnutrition

One of the known complications of HIV disease is malnutrition (169). Several studies have described this complication especially among HIV infected persons with advanced disease (171–174). In a multi-country study across continents, more than 80% of the HIV positive ART naïve patients had at least one micronutrient

deficiency (171); more than 32% of the participants had more than three micronutrient deficiencies.

The prevalence of malnutrition among HIV infected persons varies in different countries with a bigger burden in the developing countries (172–174). Salomon and others observe that malnutrition in HIV infected persons is still a challenge in developing countries and needs to be actively managed (175). In sub-Saharan Africa, studies have shown the prevalence of malnutrition among HIV infected adults to range between 15-40% among HIV infected adults (176,177).

The ATARAO study in Mali found that 36% of HIV infected patients initiating HAART were malnourished (178), while a retrospective analysis of data of the patients in a South African HIV clinic cohort initiating ART, found that 22% of them were malnourished (179). A study among Senegalese HIV infected patients who had been on HAART for about 3 years in two different cities found the prevalence of malnutrition to be between 19.2% and 26.3% (180). Two studies done in Ethiopia, a country with the second highest rate of malnutrition in Sub-Saharan Africa, found that the prevalence of malnutrition was above 40% among ART treated HIV infected adults (181)(182). In Uganda, up to 25% of adults initiating antiretroviral treatment were found to be malnourished (111), and the prevalence was highest in rural areas. Malnutrition has been associated with poor clinical outcomes including mortality, increased viral load and reduction in CD4 cell count among HIV infected adults (136,180,237). Anemia and low serum creatinine have also been reported among HIV infected persons with malnutrition.(162).

3.1.3 Quality of life among HIV infected adults

A low quality of life has been reported among HIV infected adults and has been associated with advanced HIV disease and opportunistic infections (250), but not low CD4 counts or high viral loads (251). Poor quality of life in this group of people has also been associated with food insecurity and low dietary diversity (252), and malnutrition (253). All of these studies employed cross-sectional study designs and described the quality of life of the participants at one point in time. Additionally, there are few studies that have explored the effect of nutritional supplementation

on the quality of life of malnourished HIV infected adults. A single study in Malawi showed no effect of nutritional supplementation on the quality of life of the malnourished adults with HIV infection (135). Prospective randomized studies are needed to describe the effects of nutritional supplementation on the quality of life of malnourished adults infected with HIV.

3.1.4 Effect of nutritional supplementation on clinical outcomes and quality of life of malnourished HIV infected adults

A number of studies have examined the effect of nutritional supplementation on clinical outcomes, mainly CD4 cell counts, viral load and mortality and have found that nutritional supplementation did not significantly affect changes in CD4 or viral load as well as mortality (135,237). These studies were conducted at the time when ART was not readily available and most participants were not on ART. The mortality comparisons were done with historical controls making this data liable to many confounders and bias. A longitudinal survey conducted in Uganda and Kenya found no significant differences in CD4 counts between the participants that were cured or not cured of malnutrition after being on a nutritional program for at least four months (220). These data from longitudinal national programs present a number of challenges including bias and confounding which weakens their strength of evidence. Micronutrient supplementation did not lead to significant changes among HIV infected adults in a randomized study in Thailand (231). This study reported up to 5% mortality rate, although mortality was lower in the supplemented arm. They also had a very high loss to follow-up (16%) and this might have affected the results. A randomized study to examine the effect of nutritional supplementation among HIV infected adults starting ART in Ethiopia found no differences in suppression of viral load and marginally better increases in CD4 cell counts between the participants that initiated the nutritional supplement early and those that delayed initiation of the supplement.

In the NUSTART trial conducted among HIV infected ART naïve patients in Tanzania and Zambia, high dose vitamin and mineral supplementation in a lipid based nutritional supplement improved CD4 counts in this patient group, but had no effect on mortality and severe adverse events. (230). In a trial among HIV infected

ART naïve patients in Botswana, 24-month micronutrient supplementation with multivitamins and selenium slowed HIV disease progression and reduced AIDS-related morbidity and death (116). Among the HIV infected ART naïve patients in South Africa, initiating ART and FutureLife porridge as a nutritional supplement, there were observed increases in CD4 counts and raised hemoglobin levels (117). Jiamton (231) showed that multiple micronutrient supplementation reduced mortality among HIV infected adults but had no impact on CD4 count. Most of these studies were non-randomized interventional studies, while most of the randomized studies used micronutrient supplementation as the intervention.

3.1.5 Acceptability of RUTF among malnourished HIV infected adults

Despite the plethora of studies and programs using RUTF as a nutritional supplement, there is scanty data on its acceptability among HIV infected adults. This is coupled with the fact that most studies on nutritional supplementation have shown less than desired adherence to the supplement provided (135,229,230,254). This low adherence may be due to non-acceptability of the nutritional supplements used in the studies. A qualitative investigation into the adherence to RUTF found low adherence and acceptability of RUTF due to the taste and complications related to HIV among HIV infected adults in Kenya (254). A study in Malawi (255) found high level of acceptability to a home-made RUTF nutritional supplement. However, the results of this conclusion are not supported by any results in the paper making it difficult to verify.

3.1.6 Rationale of the Clinical study

There is continued use of nutritional supplementation such as RUTF in the treatment of malnutrition in adults infected with HIV. However, most of the studies conducted to describe the effect of nutritional supplementation on clinical outcomes have been observational studies (220), while some of the randomized studies used micronutrients as the supplementation (231). The large studies that studied RUTF, had a comparator as Corn Soy Blend (CSB), which is similar to RUTF in content (135). This made the comparison group similar to the intervention group therefore the observed differences could not be attributed to the intervention. A study in a similar population used historical controls to describe the effect of

malnutrition on mortality (237). Additionally, there are very few studies that have described the acceptability of RUTF as a nutritional supplement among adults infected with HIV, and the effect of nutritional supplementation on the quality of life in this population.

3.1.7 Aim of the clinical chapter

The aim of this chapter was to describe the study population in terms of the nutritional status, the distribution of the various nutritional parameters, quality of life and clinically relevant laboratory measures among the study groups. The chapter also investigates the effect of nutritional supplementation on clinically relevant laboratory parameters and quality of life among malnourished HIV infected adults, and the acceptability of RUTF as a nutritional supplement among malnourished HIV infected adults.

3.1.8 Objectives:

1. To describe the study population by detailing the baseline characteristics including quality of life, by study group.
2. To describe the effects of nutritional supplementation on clinically relevant laboratory parameters and quality of life among malnourished HIV infected adults in Uganda.
3. To describe the acceptability, challenges and benefits of RUTF among malnourished HIV infected adults in Uganda.

3.2 METHODOLOGY

3.2.1 Study Design

The NOURISH study was a mixed method design that employed both quantitative and qualitative methods. The qualitative methods were focus group discussions and were used to answer the objective of acceptability, challenges and benefits of RUTF among HIV malnourished adults in Uganda. The quantitative methods included two randomized clinical trials and a non-randomized interventional trial. The randomized trials included one which malnourished ART naïve HIV infected adults initiating ART, and another in which malnourished ART experienced HIV infected adults were all randomized to either receive or not receive RUTF. The non-randomized interventional trial involved severely malnourished HIV infected adults were given RUTF, and well-nourished participants that received no intervention at all. All participants received nutritional counselling and were followed up for 12 weeks. It was deemed unethical to randomize severely malnourished participants to not receive a nutritional supplement, therefore a non-randomized interventional design was considered most appropriate for this group of participants.

Details of the methodology are described in chapter two.

3.2.2 Measurement of quality of life

Health related quality of life was measured using three questions which included: number of days of work lost due to illness and/or number of days admitted to hospital in the previous four weeks preceding the study visit. Participants were also requested to estimate their quality of life (how well they felt) using a Linear Analogue Self-Assessment (LASA) quality of life scale (256). The scale had numbers from 0 to 100, calibrated at intervals of 10, and participants were requested to indicate how well they felt by choosing a number on the scale. The LASA quality of life scale was chosen because it was the tool used to assess quality of life in the clinic where data collection for the NOURISH study was done.

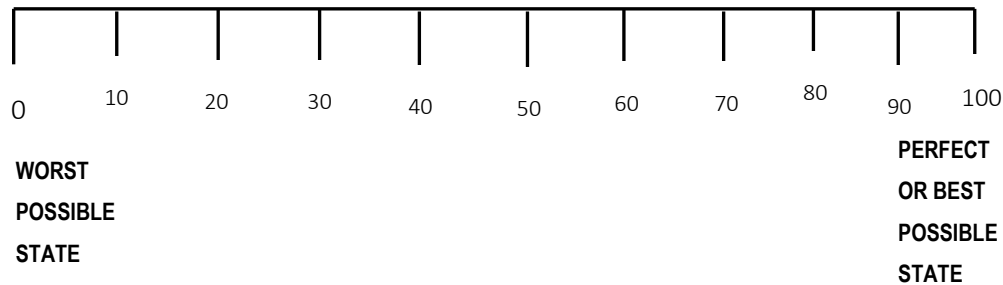


Figure 3.1: Linear analogue self-assessment scale for health-related quality of life: Participants were asked to indicate their level of quality of life by marking on the scale, zero represented the worst possible quality of life state, while 100 represented the best possible quality of life state.

3.2.3 Determination of adherence to RUTF

Adherence to RUTF was determined using two methods which were: the number of days' worth of the returned unopened sachets and objectively using the before and after comparison of serum folate levels among the ART experienced participants.

Since participants were expected to consume all the supplied sachets of RUTF (56 sachets), the number of sachets returned unopened would be divided by 2 to calculate the number of days the participant had missed taking the RUTF. Then participants were grouped in proportions according to the number of days of RUTF missed. The three groups were: No days missed, 1-3 days missed and more than 3 days missed. Adherence was also assessed objectively by comparing the baseline and week 12 serum folate levels for the malnourished ART experienced participants. Folate is one of the recommended biomarkers that can be used to assess nutrient intake (185). However, since ART interferes with folate metabolism (207), the ART experienced participants were the only group that would have the effect of ART both before and after supplementation.

3.3 RESULTS

This section contains the general results of the study including the demographic characteristics of the study population and the clinical outcomes of the nutritional supplementation among the study participants. It also describes the effect of nutritional status and nutritional supplementation on the quality of life of the study participants and the results of the qualitative study on the challenges and acceptability of Plumpy’Nut among the study population.

3.3.1 Participant numbers and loss to follow-up:

A total of 102 participants were enrolled into the study, 32 (31.4%) were moderately malnourished ART naïve (MAM), 18 (17.7%) were severely malnourished ART naïve (SAM), 27 (26.5%) were malnourished and ART experienced (ART) and 25 (24.5%) were well nourished and ART naïve (WN) (Table 3.1). 53 (52.0%) women and 49 (48.0%) men were enrolled into the study. The MAM group had 12 women and 20 men; the SAM group had 6 women and 12 men; the WN group had 15 women and 10 men, and the ART experienced group had 20 women and 7 men.

88 (86.3%) of the 102 enrolled participants completed the 12 weeks of the study. Of these 27 (30.6%) were MAM, 13 (14.8%) were SAM, 26 (29.6%) were the ART experienced participants and 22 (25.0%) were the well-nourished participants (Table 3.1). Of the 14 that were lost to follow-up, 5 (35.7%) were MAM, 5 (37.1%) were SAM, 1 (7.2%) was ART experienced and 3 (21.4%) were well-nourished. 2 participants died during the study, this represents a 2.0% mortality rate in this study. They were both in the SAM group and had been lost to follow-up. All the other participants that were lost to follow-up were ascertained to be alive but had lost interest in the study.

The immunology studies included 9 HIV negative controls. Of these, 5 were female and 4 were male. Their average age was 32 years (sd 1.5) and they were all well-nourished with an average BMI of 22.4 (IQR 20.5 – 24.5).

Table 3.1: Planned and enrolled participants at baseline and week 12 by study group

Study group	Planned	Enrolled (% of planned)	Completed 12 weeks (% of enrolled)
Overall	150	102 (68%)	88 (86.3%)
Moderately malnourished ART naive	50	32 (64%)	27 (84.4%)
Severely Malnourished	25	18 (72%)	13 (72.2%)
Malnourished ART experienced	50	27 (54%)	26 (96.3%)
Well-nourished	25	25 (100%)	22 (88.0%)

Table 3.2: Participant distribution at baseline and week 12 numbers in the randomized groups

Study group	Randomization	Planned	Enrolled (% of planned)	Completed 12 weeks (% of enrolled)
Moderately malnourished ART naive	RUTF	25	14 (56%)	13 (93%)
	No RUTF	25	18 (72%)	11 (61%)
Malnourished ART experienced	RUTF	25	16 (64%)	16 (100%)
	No RUTF	25	11 (44%)	10 (91%)

For the immunology studies, 82 participants were included in the baseline studies. The 20 missing participants are due to samples (PBMCs) of 20 participants that were lost in the liquid nitrogen tank at Trinity College Dublin. The details of the 82 participants are showed in table 3.3. Table 3.4 shows the distribution of the randomized participants at baseline and follow-up. At follow-up, the immunology studies had 61 (69%) of the 88 participants that completed the 12 weeks of the NOURISH study. Of the missing 27 participants, 10 participants declined to provide samples for immunology studies at follow-up, and samples for 17 participants were not shipped to Dublin.

These participants had not returned for their week 12 visit by the time of shipping the last batch of samples to Ireland. The ART experienced participants and well-nourished participants had one more participant at 12 weeks than at baseline. These

two were part of the participants whose baseline samples were lost in the liquid nitrogen, but had samples at week 12.

Table 3.3: Distribution of participants involved in the immunology studies:

Study group	Enrolled	Included in Immunology study at baseline (% of enrolled)	Included in immunology at 12 weeks
Overall	102	82 (79%)	61 (74%)
Moderately malnourished ART naive	32	26 (81%)	12 (46%)
Severely Malnourished	18	18 (100%)	13 (72%)
Malnourished ART experienced	27	14 (52%)	15 (107%)
Well-nourished	25	20 (80%)	21 (105%)

Table 3.4: Distribution of the randomized participants that participated in immunology studies

Study group	Randomization	Enrolled	Involved in immunology at baseline (% of enrolled)	Included in Immunology at 12 weeks (% of baseline)
MAM ART naive	RUTF	14	10 (71%)	7 (70%)
	No RUTF	18	16 (89%)	5 (31%)
MAM ART experienced	RUTF	16	9 (56%)	9 (100%)
	No RUTF	11	5 (45%)	6 (120%)

Loss to follow-up analysis

A comparison of selected baseline variables was done between the participants that completed and those that did not complete the study. This was done for the MAM, SAM and WN participants (table 3.5 – 3.7). The ART-experienced participants did not have this comparison because only one participant was lost to follow-up.

The analysis showed that for there were no statistically significant differences between the participants that completed and those that did not complete the study.

Table 3.5: Comparison of median (IQR) of selected variables between defaulters and those that completed study for the MAM participants

Variable		Defaulted N=5	Not Defaulted N=27	P-Value
Age (years)		28.1 (27.9 – 28.2)	32.3 (30.0 -40.4)	0.046
Gender	Male (%)	1 (5%)	19 (95%)	0.053
	Female (%)	4 (33.3%)	8 (66.7%)	
Plumpy Nut	Yes	1 (7.1%)	13 (92.9%)	0.355
	No	4 (22.2%)	14 (77.8%)	
Weight (Kg)		48.1 (44.7 -48.7)	50.2 (45.4 – 54.3)	0.311
BMI		18.3 (17.5 – 18.4)	17.9 (17.4 – 18.3)	0.334
CD4 (cells/ul)		222 (37-306)	184 (107 – 313)	0.856
VL (copies/ml)		34507 (11243 – 350840)	208307 (38641 – 434417)	0.311
Linear Quality of life score (%)		70 (57.5 – 75)	50 (50 – 70)	0.476
Total Energy (kcal)		2250 (1973 – 2757)	2256 (1693 -2498)	0.126
Total Protein (g)		73.4 (62.5 -73.7)	53.6 (36.4 -76.4)	0.458
Hemoglobin (g/dl)		12.1 (11.5 – 12.5)	12.7 (10.3 – 13.9)	0.640
Albumin (g/l)		43 (41-44)	37.5 (29-45)	0.374

Table 3.6: Comparison of median (IQR) of selected variables between defaulters and those that completed study for the severely malnourished

Variable		Defaulted N=5	Not Defaulted N=13	P-Value
Age (years)		33.3 (28.1 – 35.9)	32.7 (29.9 – 36.1)	0.805
Gender	Male (%)	4 (33.3%)	8 (66.7%)	0.439
	Female (%)	1 (16.7%)	5 (83.3%)	
Weight (Kg)		42.2 (41.1 – 45.8)	44.1 (40.3 – 47.6)	0.657
BMI		15.3 (15.0 – 16.0)	16.3 (15.9 -16.3)	0.180
CD4 (cells/ul)		41 (10 -153)	79 (12 – 217)	0.316
VL (copies/ml)		344355 (34952 – 1236122)	134864 (50970 – 170667)	0.460
Linear Quality of life score (%)		40 (30 -50)	50 (40-50)	0.357
Total Energy (kcal)		2374 (1538 -2595)	1424 (1034 -1742)	0.292
Total Protein (g)		51.1 (22.2 – 60.9)	33.9 (26.4 – 52.6)	0.752
Hemoglobin (g/dl)		9.2 (8.2 – 9.7)	11.1 (10.6 -13.6)	0.054
Albumin (g/l)		27 (26 -29)	34 (30 -37)	0.380

Table 3.7: Comparison of median (IQR) of selected variables between defaulters and those that completed study for the well-nourished

Variable		Defaulted N=3	Not Defaulted N=22	P-Value
Age (years)		33.0 (25.2 – 45.2)	31.1 (27.2- 35.0)	0.707
Gender	Male (%)	2 (20.0%)	8 (80.0%)	0.346
	Female (%)	1 (6.7%)	14 (93.3)	
Weight (Kg)		64.0 (59.0 -81.0)	55.0 (52.5 -64.0)	0.086
BMI		23.5 (22.2 -24.7)	22.2 (20.8 -23.3)	0.347
CD4 (cells/ul)		268 (266 – 306)	275 (169-371)	0.895
VL (copies/ml)		142457 (123569 -217431)	51984 (18568 – 138394)	0.155
Linear Quality of life score (%)		90.0 (90.0 – 90.0)	70.0 (50.0 -90.0)	0.445
Total Energy (kcal)		1747 (974 – 2519)	1897 (1506 – 2393)	0.713
Total Protein (g)		45.0 (31.7 -58.2)	50.1 (36.0 – 85.5)	0.493
Hemoglobin (g/dl)		12.6 (11.7 – 14.3)	13.3 (11.9 -15.6)	0.896
Albumin (g/l)		45.0 (45.0 -45.0)	45.5 (44.0 – 49.0)	0.208

3.3.2 Baseline characteristics of the study population

The overall mean BMI for the study population was 18.4 (sd 2.6). The mean BMI was 17.9 (sd 0.5) for MAM participants, 15.9 (sd 0.8) for SAM participants, 17.0 (sd 1.0) for the ART experienced participants, and 22.4 (sd 1.9) for the well-nourished group. The average age of the study population was 36.7 years (sd 10.7). The SAM participants had the most advanced HIV disease with 15 (83.3%) of them reporting WHO stage IV disease, and the highest C-reactive protein (CRP) levels (13.6 mg/l) indicating high inflammatory state. (Table 3.8).

Effect of nutritional status on clinical characteristics of ART naïve HIV infected adults

Table 3.9 details the clinical laboratory characteristics of the different study groups. The median viral load for the ART naïve participants was 9.0×10^4 (IQR 3.1×10^4 – 3.0×10^5) copies/ml. The MAM participants had the highest viral load of 1.8×10^5 (IQR 3.5×10^4 – 4.3×10^5) copies/ml, while the WN had the lowest viral load of 6.2×10^4

copies/ml (IQR 2.5×10^4 – 1.4×10^5) copies/ml. However, the differences in the viral loads were not statistically significant, with a Kruskal Wallis P-value of 0.415. The median CD4⁺ cell count for the study population was 191 (IQR 77 - 306) cells/ μ l of blood. The severely malnourished participants having the lowest median CD4⁺ cell count of 64 (IQR 10-133) cells/ μ l of blood; the lowest median hemoglobin level of 10.9 (IQR 8.8 – 12.8) g/dl and lowest median albumin level of 31 (IQR 27-36) g/l. The group differences for the CD4 cell count and albumin were statistically significant, P-values Of 0.002 and <0.001 respectively. The SAM group also had higher liver function tests compared to the other groups, although these were not significantly different from the other groups.

3.3.3 Effect of nutritional status on the health-related quality of life of HIV infected adults

The participants reported an average linear scale quality of life of 61.5% (sd 20.4). The SAM reported the lowest linear quality of life score of 50% (sd 17.1), the MAM participants reported 60% (sd 18.7) and the WN reported 72.0% (sd 20.4). The SAM participants also reported a mean of 1.7 (sd 6.5) days of hospital admission in the past month. The SAM participants reported more days of work lost due to illness (mean 8.7 sd 12.0) in the past one month compared to the MAM participants who reported having missed an average of 4 (sd 7.2) days and the well-nourished participants who missed 3.3 (sd 8.8) days of working (Figure 3.1).

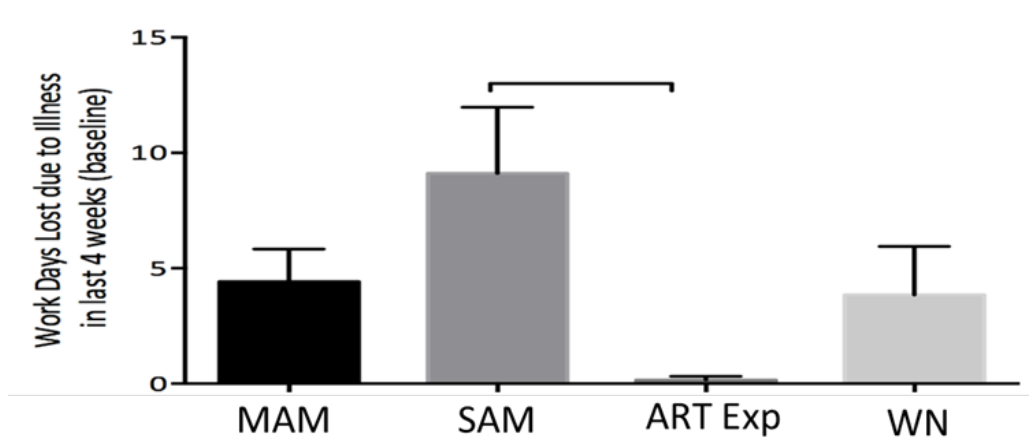


Figure 3.1: Baseline Daily Work activities lost through illness across the ART naïve groups: The moderately malnourished (MAM), severely malnourished (SAM), the ART experienced malnourished (ART) and the well-nourished (WN) HIV infected participants. Participants in each group were asked how many days of work they had missed due to illness in the past one month. Mean (sd) number of days missed were plotted for each group. The Severely malnourished (SAM) had the highest number of days missed.

Table 3.8: Baseline characteristics of the study population

Characteristic		MAM ART-naïve	SAM	WN	MAM ART-experienced	Overall
Numbers (%)		32 (31.37)	18 (17.65)	25 (24.51)	27 (26.47)	102
Mean Age/Yrs (SD)		34.7 (11.9)	32.6 (6.4)	32.0 (7.9)	46.0 (8.2)	36.7(10.7)
Gender (%)	Female	12 (37.5)	6 (33.3)	15 (60.0)	20 (60.0)	53 (52.0)
	Male	20 (62.5)	12 (66.7)	10 (40.0)	7 (40.0)	49 (48.0)
Education* (%)	None	2 (6.3)	1 (5.6)	2 (8.0)	1 (3.70)	6 (5.9)
	Primary	18 (56.3)	11 (61.1)	14 (56.0)	19 (70.4)	62 (60.8)
	Secondary	11 (34.4)	5 (27.8)	9 (36.0)	6 (22.2)	31 (30.4)
	Tertiary	1 (3.1)	1 (5.6)	0 (0.0)	0	2 (2)
WHO stage (%)	I	1 (3.13)	0 (0.0)	5 (20.0)	0	6 (5.9)
	II	6 (18.8)	1 (5.6)	15 (60.0)	3 (11.1)	25 (24.5)
	III	15 (46.9)	2 (11.1)	4 (16.0)	19 (70.4)	40 (39.2)
	IV	10 (31.2)	15 (83.3)	1 (4.0)	5 (18.5)	31 (30.4)
Mean weight/ Kg (SD)		49.5 (4.7)	43.7 (5.0)	58.5 (8.3)	44.8 (3.7)	49.5 (7.9)
Mean BMI (SD)		17.9 (0.5)	15.9 (0.8)	22.4 (1.9)	17.0 (1.0)	18.4 (2.6)
Median CD4+ T cells/ μ l of blood (IQR)		188 (101- 310)	64 (10-133)	272 (184- 360)	433 (322-661)	267 (121- 426)
Median Viral load (copies/ml), (IQR)		1.8×10^5 (3.5×10^4 – 4.3×10^5)	1.4×10^5 (4.9×10^4 – 2.2×10^5)	6.2×10^4 (2.5×10^4 – 1.4×10^5)	0 (0-0)	4.6×10^4 (1.6×10^2 – 2.2×10^5)
Serum Albumin (g/dl) (IQR)		40 (31-44)	31 (27-36)	42.5 (36.5-47)	45 (44-49)	41 (34-46)
Mean Hemoglobin (g/dl) (SD)		12.2 (2.4)	10.9 (2.5)	12.9 (2.9)	13.1 (1.6)	12.4 (2.5)
Median CRP (mg/L), (IQR)		4.28 (0.86-40.7)	13.6 (4.3-69.5)	1.52 (0.44-4.72)	0.65 (0.29-2.33)	1.53 (0.44-4.72)

Table 3.9 Effect of nutritional status on clinical laboratory characteristics of the study group

Clinical Characteristic/ Median (IQR)	Study group				P-value*
	Moderately Malnourished	Severely Malnourished	Well-nourished	Overall	
Viral load (Copies/ml)	176473 (35325- 434417)	137430 (49207- 219659)	62186 (25296- 142571)	89948 (30924 - 298447)	0.414
CD4 count (cells/ μ l)	188 (101- 310)	64 (10-133)	272 (184- 360)	199 (77 - 306)	0.002
Hemoglobin (g/dl)	12.6 (11.3 – 13.5)	10.9 (8.8 – 12.8)	13.0 (11.8 -15.0)	12.4 (10.3 – 13.9)	0.221
Aspartate Transaminases (U/L)	30 (23-39)	42 (24-75)	26.5 (19.5-30.5)	29 (22 - 42)	0.146
Alanine Transaminases (U/L)	20 15- 32	27 (12-43)	18.5 (14.5-28)	20 (14 - 32)	0.601
Alkaline Phosphatases (IU/L)	90 (73-119)	118 (72-305)	96.5 (74.5-118.5)	97 (74 - 131)	0.298
Serum Albumin (g/l)	40 (31-44)	31 (27-36)	42.5 (36.5-47)	37 (31 - 44)	<0.001
Creatinine (μ mol/l)	63 (55-71.5)	63 (53-74.5)	61 (56-66)	62 (52 -74)	0.623
Mean number work days due to illness (SD)	4.4 (9.2)	8.6 (12.0)	3.3 (8.8)	5.1 (9.2)	0.556
Mean number of days admitted (SD)	0.5 (1.0)	1.7 (6.5)	0.4 (0.2)	0.6 (3.3)	0.546
Average linear quality of life score (SD)	60% (18.70)	50% (17.1)	72 (20.4)	61.5 (20.4)	<0.01

3.3.4 Baseline characteristics of the participants in the randomization study

3.3.4.1 Baseline clinical characteristics and quality of life measures of ART naïve moderately acute malnourished (MAM) participants

This group had a total of 32 participants, of which 14 (43.7%) were randomized to receive RUTF and 18 (56.3%) were randomized to no receiving RUTF. Each randomization arm had 6 women, the RUTF group had 8 men and the No RUTF group had 12 men. The median age of the No RUTF group was 31.5 (IQR 28.9 – 40.4) years, while that of the RUTF group was 31.4 (IQR 25.9 – 34.1) years. The two groups had similar mean BMIs, but the median weight of the RUTF group was 49.1 (IQR 45.2 – 51.5) kg, and that of the No RUTF group was 50.7 (IQR 47.4 – 54.4) Kg. Clinically, both groups had the majority of participants in WHO stage

III; 8(44.44%) for the RUTF group and 7 (50.0%) for the No RUTF group. The median (IQR) viral load for the RUTF group was 2.7×10^5 (1.2×10^4 - 5.4×10^5) copies/ml, this was more than three times the median viral load for the No RUTF group. Conversely, the RUTF group had a lower CD4⁺ cell count 136 (IQR 69 – 306) cells/ μ l of blood than the CD4⁺ cells of the No RUTF group 214 (124 – 313) copies/ml. Details of the baseline comparisons for the MAM group are shown in Table 3.10.

Table 3.10 Baseline characteristics of randomization groups of the MAM study group

Clinical characteristic (Median-IQR)		RUTF	NO RUTF
Numbers (%)		14 (43.7)	18 (56.3)
Gender	Female (%)	6 (42.9)	6 (33.3)
	Male (%)	8 (57.1%)	12 (66.7)
Age (years)		32.4 (8.5)	36.5 (13.9)
BMI		17.8 (0.5)	17.9 (0.5)
Weight (Kg)		48.76 (4.19)	50.04 (5.11)
WHO stage (%)	I	0 (0.00)	1 (5.56)
	II	2 (22.22)	4 (14.29)
	III	8 (44.44)	7 (50.00)
	IV	5 (27.78)	5 (35.71)
Viral load (Copies per ml)		270119 (12423-537789)	69705 (38641 – 434417)
CD4 (cells/ μ l)		214.5 (124 – 313)	136 (69 -306)
Hemoglobin (g/dl)		12.8 (11.3 – 14.0)	12.1 (11.2 – 13.1)
Albumin (g/l)		40 (32 – 45)	39 (29 – 42)

In the quality of life, the mean score for the quality of life scale for the RUTF group was 53.1 (sd 16.0) which was 13.1 scales lower than the No RUTF group which was at 66.1% (sd 18.9). The number of days of work lost due to illness in the previous one month reported by the No RUTF group were 2.5 days more than those reported by the RUTF group, but there was no difference in number of days admitted between the two groups. (Data not shown).

3.3.4.2 Baseline clinical characteristics and quality of life measures of ART experienced malnourished participants

Table 3.11 shows comparison of the baseline clinical characteristics and quality of life measures between the participants that were randomized to receive Plumpy'Nut and nutrition counselling (RUTF) and those randomized to receive nutritional counselling only (No RUTF) among the ART experienced group. The group had 27 participants, 16 (59.3%) were randomized and 11 (40.7%) were randomized to No RUTF. Both randomization arms had more women than men; 11 (68.8%) for the RUTF and 9 (81.8%) for the NO RUTF arm. The median age for the NO RUTF arm was 41.4 (IQR 39.2 – 50.3) years compared to 46.5 (43.5 – 49.0) years for the RUTF arm; and majority of the participants in both arms had WHO stage III HIV disease at the time of initiating antiretroviral therapy (81.3% for RUTF arm and 54.5% for the NO RUTF arm). There were no major differences in most of the clinical characteristics between the participants that received and did not receive RUTF. The No RUTF arm reported a slightly higher percentage quality of life (76%) compared to the RUTF arm (70%), however, there were no differences in the other quality of life markers between the two groups.

Table 3.11 Baseline clinical characteristics and selected laboratory parameters for the randomization arms among the ART experienced participants

Clinical Characteristic (Median/IQR)		No RUTF	RUTF
Numbers (%)		11 (40.7%)	16 (59.3%)
Gender	Male (%)	2 (18.2)	5 (31.2%)
	Female (%)	9 (81.8)	11 (68.8%)
Age (years)		41.4 (39.2 – 50.3)	48.2 (43.3 – 50.7)
Weight (Kg)		44.8 (2.8)	44.9 (4.2)
BMI		17.5 (0.7)	17.3 (1.1)
WHO Stage (%)	II	3 (27.3)	0 (0.0)
	III	6 (54.5)	13 (81.3)
	IV	2 (18.2)	3 (18.7)
Median (IQR) Viral load/copies per ml		0 (0-0)	0 (0-14)
CD4 (cells/ μ l)		371 (277 – 813)	442.5 (341.5 -637.5)
Hemoglobin (g/dl)		12.6 (11.4 – 13.4)	13.3 (12.3 -15.1)
Albumin (g/l)		45 (44 – 47)	46.5 (43.5 – 49.0)

3.3.5 Effect of nutritional supplementation and ART on clinical outcomes and quality of life among HIV infected adults in Uganda.

This section describes the changes in viral load, CD4 counts, albumin, CRP and quality of life outcomes after 12 weeks of ART and nutritional supplementation in the study participants.

Figure 3.2 shows the changes in viral load, CRP, albumin in the ART-naïve participants after 12 weeks of receiving ART with or without RUTF. There were significant reductions in the viral load in all the ART-naïve groups, and a corresponding increase in CD4⁺ T cells. The MAM-ART naïve participants that received RUTF had a 64.9%% increase in their CD4 cell count compared to the 51.6%% increase in CD4 cell count among the ones that did not receive RUTF. In the SAM participants, there was significant reduction in the viral load from 1.4×10^5 copies/ml to 1.6×10^2 copies /ml (p-value <0.01); an increase in the CD4 cells from 64 cells/ μ l to 161 cells/ μ l (p-value = 0.01) and an increase in the hemoglobin level from 10.9 g/dl to 13.3 g/dl (p-value = 0.04). Tables 3.12 to 3.14

show results of the effects of nutritional supplementation on clinical outcomes in the MAM, SAM and MAM-ART participants.

Table 3.12 Effect of RUTF on moderately malnourished ART naïve HIV infected adults after 12 weeks of ART

Clinical Characteristic (Median/IQR)	No RUTF (N= 11)	RUTF (N=13)	P-Value
Median (IQR) Viral load/copies per ml	69 (22 -146)	150 (92 – 1002)	0.06
CD4 (cells/μl)	329 (299 – 359)	315 (189 – 332)	0.174
Hemoglobin (g/dl)	13.7 (13.0 – 14.9)	13.1 (11.8 – 14.5)	0.200
Albumin (g/l)	44 (39 – 47)	43 (36 – 44)	0.180
Mean linear scale self-assessed quality of life	75.4 (16.3)	79.1 (23. 4)	0.335
Mean number work days due to illness (SD)	0 (0.0)	0 (0.0)	0.531
Mean number of days admitted (SD)	0 (0 .0)	0 (0.0)	0.307

Table 3.13 Clinical and quality of life outcomes of the SAM participants after 12 weeks of ART and nutritional supplementation

Clinical Characteristic (Median/IQR)	No RUTF (N=10)	RUTF (N=16)	P-Value
Median (IQR) Viral load/copies per ml	0 (0 – 0)	0 (0 – 0)	0.166
CD4 (cells/μl)	454 (359 – 640)	465 (382 – 567)	0.824
Hemoglobin (g/dl)	12.4 (11.8 – 13.3)	13.4 (12.7 – 15.5)	0.04
Albumin (g/l)	45 (44 – 47)	46 (43 – 50)	0.780
Mean linear scale self-assessed quality of life	80 (70 – 90)	90 (80 - 90)	0.119
Mean number work days due to illness (SD)	0 (0 – 0)	0 (0 – 0)	0.832
Mean number of days admitted (SD)	0 (0 – 0)	0 (0 -0)	0.824

Table 3.14 Effect of RUTF on moderately malnourished ART experienced HIV infected adults

Clinical Characteristic (Median/IQR)	Baseline (N= 18)	Week 12 (N=13)	P- value
Median (IQR) Viral load/copies per ml	1.4*10⁵ (4.9*10⁴ – 2.2*10⁵)	1.6*10² (77 – 1435)	<0.01
CD4 (cells/μl)	64 (10 -133)	161 (83 -311)	0.01
Hemoglobin (g/dl)	10.9 (8.8 – 12.8)	13.3 (12.4 – 14.0)	0.04
Mean (SD) Percent linear scale self-assessed quality of life	50.0 (17.1)	82.5 (14.2)	0.001
Mean (SD) number work days due to illness	8.6 (12.0)	0.1 (0.3)	0.020
Mean (SD) number of days admitted	1.7 (6.5)	0.2 (0.6)	0.030

There was a general improvement in the quality of life among the study participants. The SAM participants experienced the biggest improvements in the quality of life with significant increases in the linear self-assessment quality of life score and reductions in the number of days work lost due to illness and reductions in number of days of admission. Their mean percent linear scale self-assessed quality of life improved from 50% (sd 17.1) to 82.5% (sd 14.2) (p-value <0.001) and the number of work days lost due to illness reduced from 8.6 to 0.1 (p-value 0.02). Table 3.11

Among the MAM-ART-naïve, the participants that received RUTF had a median percent change in linear scale self-assessed quality of life from 50% (20% - 70%) to 80% (70% - 90%) representing a 30% median increase compared to 7.5% increase in the no RUTF group.. Similarly, the reported linear quality of life score of the participants that received RUTF in the ART-experienced participants had increase from 70% (50% - 90%) to 90% (80% -100%) representing a 20% median increase compared to a 0% median increase in ones that did not receive RUTF.. Table 3.15

Table 3.15: Changes in the linear scale self-assessed quality of life of the participants in the two randomizations

Randomization	MAM			MAM-ART		
	No	Yes	P-value	No	Yes	P-Value
Baseline	70 % (50-80)	50 % (40-70)	0.08	80 %(70-95)	70% (50-90)	0.547
Week 12	77.5% (62.5 -90)	80% (70-90)	0.335	80% (70 -90)	90% (80 -100)	0.120
Difference	7.5%	30%		0%	20%	

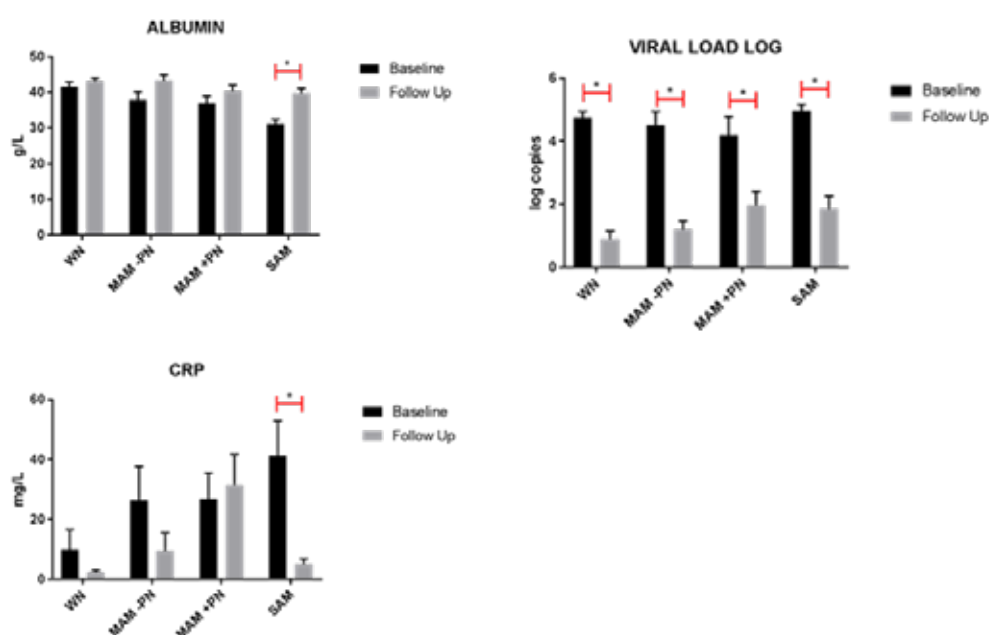


Figure 3.2: Changes in albumin, viral load and CRP in the ART naïve participants. 12 weeks of receiving ART with or without RUTF, the well-nourished (WN); severely malnourished (SAM) and the moderately malnourished who received and did not receive RUTF (MAM+PN) and (MAM-PN) experienced the changes above. The red bars indicate statistically significant changes ($P<0.05$).

3.3.6 Adherence to Nutritional supplementation

Adherence to nutritional supplementation was assessed using two methods: counting of returned empty used Plumpy'Nut sachets, and a before and after comparison of the serum folate levels among the participants that received RUTF.

Table 3.16 shows the number of days' worth of unused sachets returned by the participants in the three groups (MAM, SAM and ART-experienced). 24 (60%) of all the participants that received RUTF did not return any unused sachet indicating good adherence. Of the 14 moderately malnourished participants that received RUTF, 12 (85.7%) did not return any unused sachet, while only 53.9% of the severely malnourished and 38.5% of the ART experienced participants did the same. However, up to 77.0% of the ART experienced participants and up to 76.9% of the SAM participants returned unused sachets of 3 days or less. Overall, 82.5% of all participants returned unused sachets of 3 days or less. This level of adherence was also reported in the focus group discussions. Participants reported sharing the RUTF with family members especially children, although they also indicated that they liked it and eaten most of it (see details in results of the Focus Group Discussion).

Figure 3.3 is a graphic representation of the changes in serum folate between baseline and 12 weeks of the study. The serum folate level of the ART experienced participants increased among those the received RUTF (18.5 nmol/l to 22.1 nmol/l), but decreased among those that did not receive RUTF (14.5 nmol/l to 10.5 nmol/l). Additionally, the reduction in the serum folate level of the MAM-ART naïve participants that received RUTF were less compared to those that did not receive RUTF. In the RUTF group, the reduction was by 9.2 nmol/l compared to a reduction of 11.4 nmol/l in the No RUTF group. In the SAM participants, there was no reduction in serum folate levels. (Figure 3.3)

Table 3.16: Number of days' worth of unused RUTF sachets returned by the participants in the different study groups.

No of days' worth of unused Sachets	Moderately Malnourished N=14	Severely Malnourished N=13	Malnourished and ART-Experienced N=16	Overall N=53
None	12 (85.7%)	7 (53.9)	5 (38.5)	24 (60.0%)
1-3 days	1 (7.1%)	3 (23.0)	5 (38.5%)	9 (22.5%)
3-7 days	1 (7.1%)	1 (7.7%)	3 (23.1%)	5 (12.5%)
>7days	0 (0.0%)	2 (15.4%)	0 (0.0%)	0 (5.0%)

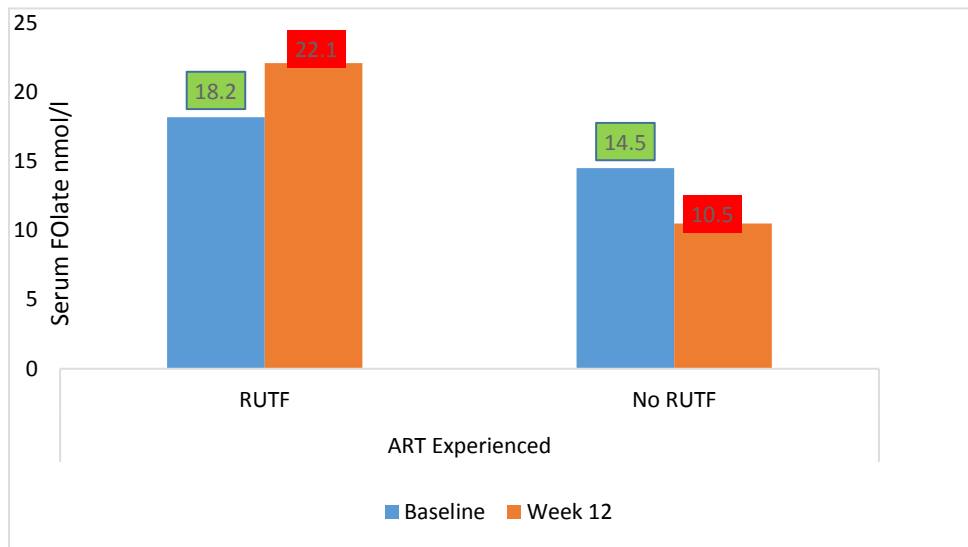


Figure 3.3: Baseline and 12-week comparison of serum folate among the ART experienced participants

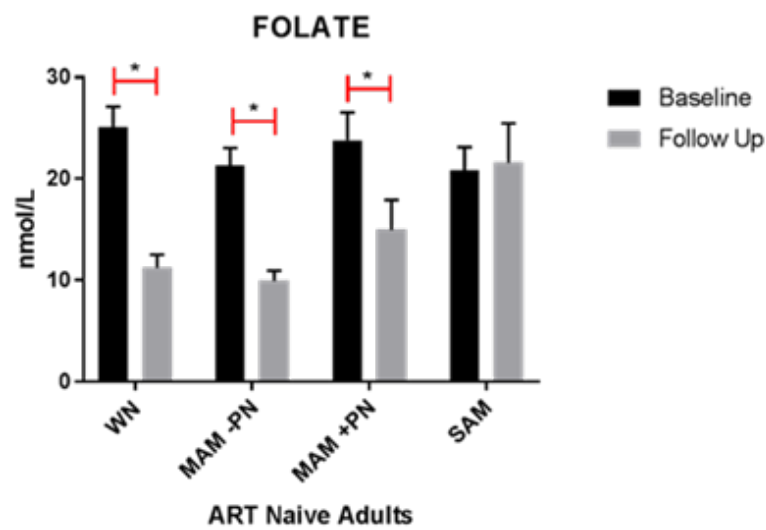


Figure 3.4: Changes in serum folate from baseline to 12 weeks among the ART-naïve participants after introduction of ART and nutritional supplementation. Red bars indicate a statistically significant difference ($P<0.05$)

3.3.7 The qualitative study (Focus Group Discussions)

This section details the findings of the focus group discussions. The key themes from the FGDs were: high acceptability of RUTF among HIV infected adults, fairly good adherence and a small number of challenges associated with using RUTF in the study population.

3.3.7.1 Acceptability, adherence, benefits and challenges associated with the use of Plumpy’Nut, a ready to use therapeutic food among malnourished HIV infected adults in Uganda

These are the results of the Focus Group Discussions that explored the acceptability, adherence, benefits and challenges associated with the use of Plumpy’Nut as a nutritional supplement among malnourished HIV infected adults.

3.3.7.1.1 Acceptability of RUTF as a nutritional supplement by malnourished HIV infected adults in Uganda.

It is important to explore the perceptions the respondents had about Plumpy’Nut because these influenced acceptability and willingness to take the supplement. The majority of respondents thought that it was paramount for an HIV positive person to feed well, i.e. eat food with all the vital nutrients like proteins and vitamins and to eat food not only in the right proportions per day, but also on time and at an affordable price. The participants implied that Plumpy’Nut possessed these qualities and this influenced their uptake of the supplement.

Others mentioned the dangers around food preparation today which they did not worry about once they started taking Plumpy’Nut, for instance excessive use of oil, which they said was not good for their health. Others said they needed to change their diet which was characterized by a few types of food eaten on a daily basis e.g. posho and matooke.

‘Being HIV positive doesn’t mean that you will hate your body; you have to eat well to look good, for example, [you have to eat] fruits, avocado, cabbage, etc.

‘I understand a balanced diet to mean whether the food I eat has all the nutrients needed by the body. For example, do I eat energy giving foods like posho, sweet potatoes, greens, vitamins, proteins?’

Others, after receiving information about the study, hoped that the Plumpy’Nut would lead to weight gain since they were emaciated at the time of recruitment into the study; and that eating Plumpy’Nut would increase their CD4 cells. Many of them later reported that they had experienced significant increases in weight and other people were telling them about it, although they did not readily report on improvement in CD4 count.

‘I decided to eat Plumpy’Nut because I was so weak with no appetite. I had less kgs (about 30). You had said that it leads to weight gain so I decided to eat it to gain weight and whenever I would come back to you, you could encourage me to continue eating and that my kgs were increasing. That is what pushed me to continue eating it. I weigh 46 kgs at the moment.’

‘It helps me look better than the way I was. For example, there is a time my mother told me that Plumpy’Nut tastes good for her but then I told her that it was given to me because I wasn’t looking good. I also feel that my body has restored energy only that I get stomach disorders.’

‘It made us look better...everyone is saying that you have put on weight, you are looking good.’

3.3.7.1.2 Adherence to a daily intake of Plumpy’Nut among malnourished HIV infected adults

Majority of respondents said that Plumpy’Nut tasted good and this encouraged them to eat it as directed by the medical personnel, hence adherence. Many said that they preferred it to their local food and that it enhanced their water intake. However, because of its pleasant taste, they inevitably shared the supplement with their children and other family members and this affected adherence. They generally described the taste as: ‘milk and soy’ taste, ‘biscuit and chocolate’ taste, and a few said that it has a sugar and salt taste. For those who shared Plumpy’Nut with the children, they prepared it together with porridge and shared with the family just like with any regular meal.

The majority of respondents said they did not mind people knowing that Plumpy’Nut was given to HIV positive people although initially some were concerned about the stigma associated with taking Plumpy’Nut.

‘The first time Plumpy’Nut was given to me I was the first to get scared but my daughter said that I had received a miracle. That there is a lady she knew who was given Plumpy’Nut and it helped her a lot.’

3.3.7.1.3 Benefits of using Plumpy’Nut among malnourished HIV infected adults

a. Physical benefits

The participants reported that their appetite improved while taking Plumpy’Nut and inevitably their weight improved and they felt a new surge of energy. A few others also reported that they did not experience any ART related side effects while taking Plumpy’Nut.

‘It increased water intake and the amount of food I eat. I used to eat a very small amount of food before taking Plumpy’Nut.’

‘It also helps in body building because I remember the first time I came here I weighed 31 kgs, but when I came back I weighed 50 kgs due to Plumpy’Nut.’

‘Plumpy’Nut has helped me gain strength, I had previously lost appetite with low fluid intake but ever since I started taking it, I can now take 3 litres [of water].’

b. Mental benefits

A few participants said that they experienced better mental health while taking Plumpy’Nut and they specifically reported experiencing improved memory.

‘I gained strength, brain rejuvenation...this is one thing I loved most because I was so forgetful. I first worked in an electronic shop but when I got sick I forgot everything. I could not remember the names of most items in the shop.’

‘My brain was nourished as soon as I started eating Plumpy’Nut. I even returned back to work because my brain was now working well. I ate Plumpy’Nut because

I didn't want to reach a stage of most people I saw in hospital, I wanted to get back on my feet and live a normal life.'

'Plumpy'Nut is of great importance; first of all, it nourishes the brain. Me personally, my brain was not working well, I was so forgetful but when I started eating Plumpy'Nut, I started recovering.

'I personally loved teaching before I got sick but when my brain stopped working well, it really hurt me a lot because I was so forgetful but after eating Plumpy'Nut it was very easy to start remembering most of the things, so I can now teach. I am a primary teacher, teaching p2 - p7. I teach English, Science and RE without forgetting anything.'

c. Economic benefits

A few participants reported that there was a decrease in expenditure especially on sugar and also felt that they needed to take Plumpy'Nut for a longer time.

'They [sachets of Plumpy'Nut] would have been three because we usually use them during our meals - one sachet at breakfast, lunch and supper.'

3.3.7.1.4 Challenges of using Plumpy'Nut as a nutritional supplement

Many participants reported that they did not experience any challenges as a result of taking Plumpy'Nut.

'Plumpy'Nut is very good food because I never experienced any problem from the time I started taking it. I had no worries and I used to eat on time. There was an increase in the kilos I weighed.'

However, a few participants reported that they had diarrhea when they had just started taking Plumpy'Nut. A few others also reported that Plumpy'Nut caused them a burning sensation in the stomach and nausea, especially when eaten alone and that it had too much sugar compared to what they were accustomed to. Some participants, in order to deal with this problem, prepared the Plumpy'Nut with porridge. Some reported experiencing bad dreams and yet others reported that they had missed taking Plumpy'Nut for about 7-30 days mainly due to

mobility. It is important to note however that for some men, it was apparent from their narratives that they had to deal with stigma attached to being given Plumpy'Nut when one is HIV positive. For instance, one man expressed that he kept the supplement in the car and not in the house.

'I am a person who usually goes on long journeys so I miscalculated. I thought that what I took was going to be enough but it was less than the days I spent there and I couldn't return home immediately. I keep them in a car, and I spent a month or more so what I carried wasn't enough yet I had left the rest at home.'

'I returned some because I was looking after my brother in Mulago Hospital. At first we thought we were returning home but he ended up being admitted and we spent almost a week in hospital yet I had packed only 2 for one day. So I returned those for the days missed. I also did not take any when I was sick because I did not like them.'

3.4 DISCUSSION

In the NOURISH study, 12 weeks of ART and nutritional supplementation with RUTF led to marginally higher proportional increases in the CD4 cell counts, compared to 12 weeks of ART only in the malnourished HIV infected adults. These results were irrespective of whether the participants were initiating ART or had been receiving ART for at least a year by the time they started the nutritional supplement. These findings are different to what was found in Malawi (135,233), where the use of RUTF and ART did not lead to higher proportional changes in CD4 or viral load. However, the Malawi studies used corn soy blend (a nutritional supplement similar to RUTF) in the control arm, and thus the comparison groups were similar with respect to the intervention in these studies. It is known that ART leads to increases in CD4 cell counts and reductions in viral loads, the marginally higher proportional increases in CD4 counts seen in the NOURISH participants that received both RUTF and ART compared to those that received ART alone may indicate that RUTF improves on HIV treatment outcomes especially among malnourished HIV infected adults. However, due to the small sample size, these data need further evaluation in larger studies.

RUTF supplementation and ART also led to improvement in the quality of life in the NOURISH study. There percent increase in the linear analogue self-assessment quality of life scale were higher among the participants that received both ART and RUTF compared to those that received ART alone. This was also found by Evans et al in 2013 (117) among South African patients that were initiating ART and were supplemented with fortified foods. Antiretroviral therapy has been found to improve quality of life of HIV infected patients (87), but the observed increases in the RUTF groups were more than those in the No RUTF groups, thus ART alone could not explain these increases., the. The SAM participants also reported reduced number of days of work lost due to illness and reduced number of days of admission. These improvements could be due to the use of either ART or RUTF or both; a randomized study would have been used to identify the effect of RUTF, but it would have been unethical to randomize severely malnourished participants not to receive nutritional supplementation. In

the randomized study, nutritional supplementation led to an increase in the quality of life of the study participants, although it was not statistically significant. This is similar to what has been reported elsewhere in Africa (135).. Similarly, participants reported improved quality of life in the Focus Group Discussions citing improved strength, ability to work and better appearance (skin and facial) as indicators of better quality of life.

Evans et al (117) found an increase in hemoglobin level among the South Africans that received food supplementation, which is similar to what was found in the ART experienced participants that received RUTF as well as the severely malnourished participants in the NOURISH study. However, this observation was not seen in the MAM ART naïve participants. This finding will need to be further described in a larger study.

There were significant increases in the serum albumin from 31 g/l at baseline to 39 g/l at 12 weeks among the SAM study participants in the NOURISH study, an observation that has not been previously reported and may need further evaluation. A study to describe the nutritional status of HIV infected adults during the first year of ART failed to demonstrate reduction in hypoalbuminemia among the study participants (178). This increase in serum albumin could also be explained by the increased intake in proteins that was observed among the participants that received RUTF (chapter 4).

The loss to follow-up in the study was 14%, which was fairly high, but less than the maximum 20% (43, 44) which has been suggested as the allowable loss to follow up in randomized clinical trials. This was slightly less than the 16% reported in a similar study in Thailand (231) and the more than 22% reported in the longitudinal study conducted in Uganda and Kenya (220). It has also been suggested that in nutritional studies examining physiological outcomes such as the NOURISH study, loss to follow-up may not impact the study negatively (257). Of the 14 lost to follow-up, 2 died and these were severely malnourished giving a mortality ratio of approximately 2% of all the severely malnourished participants. This is less than what has been reported elsewhere (220,230)but re-affirms the increased risk of mortality among severely malnourished participants within the

first three months of initiating ART (259). The NOURISH study did not aim to describe the effect of nutritional supplementation on mortality among malnourished HIV infected adults, and thus there was no further mortality analysis.

Adherence to the nutritional supplement was at 60% overall, as assessed by the returned empty sachets. This was close to what was observed in a similar study conducted in Tanzania (230), but better than what was observed in the NUSTART trial (160) and worse than the adherence in the supplementation study in Malawi (135) and Ethiopia (233). A qualitative study in Kenya also found a low adherence to Plumpy'Nut (254). The findings of these four studies suggest that there is a generally low adherence to nutritional supplementation among malnourished adults in sub Saharan Africa. However, the Focus Group Discussions revealed that the participants took their Plumpy'Nut with enthusiasm, although many reported sharing of the supplement with family members. The extent to which Plumpy'Nut was shared with family members could not be verified. These findings indicate a low level of adherence and are similar to what was found among HIV infected adults in Kenya (254).

Serum folate has been used as objective biomarker adherence to nutrient intake (260). Intake of RUTF was assessed objective using serum folate among the ART experienced participants that received RUTF. There was a statistically significant increase in serum folate (from baseline to 12 weeks) among the participants that received indicating a good level of adherence to RUTF. This result, supports the findings of good adherence (60%) as measured by returned sachets. These two findings indicate that the NOURISH participants had a good level of adherence to RUTF.

As indicated earlier, some studies reported similar or worse adherence to RUTF but found beneficial effects of RUTF, while the Malawi study (135), which had more than 80% adherence, did not show significant changes in clinical parameters due attributable to RUTF. These results contradict what would be expected in terms of how adherence affects outcomes in research. Research studies designed to examine how different doses and levels of adherence affect clinical outcomes

are needed to answer the question of the optimum dose and level of adherence to RUTF required to obtain a measurable and significant beneficial effect on clinical and other outcomes among malnourished HIV infected adults.

Although there were fears of stigma resulting from taking Plumpy’Nut among the NOURISH study participants, there was a reported high acceptability of Plumpy’Nut which was facilitated by the perceived benefit of the supplement among the study participants. High acceptability has been reported in similar study populations (255), which further indicates good adherence to the supplement.

The NOURISH study participants reported non-medical benefits from the use of Plumpy’Nut. These ranged from increased drinking of water to improved intellectual ability and economic benefits. The participants reported that since Plumpy’Nut was sweet, they did not have to buy sugar and this helped them save money. These non-medical benefits have not been previously explored and further research is needed to describe these effects.

3.4.1 Conclusion and recommendations

RUTF and ART led to marginally better improvements in the CD4 cell counts but not better reductions in viral load among malnourished HIV infected adults that were greater than the changes that were due to ART only. Additionally, RUTF and ART led to better improvements in the quality of life of the malnourished HIV infected adults. These improvements were marked among the severely malnourished participants that presented with advanced HIV disease. The focus group discussions indicated there was good acceptability of RUTF, and participants reported several benefits of RUTF including improved quality of life. Although these changes were not statistically significant, they are clinically significant given the magnitude of change observed and provide evidence for the use of RUTF as a nutritional supplement in the management of malnutrition in HIV.

However, it is important to note that all these findings were not statistically significant, and clinical significance could not be inferred from these data. This is due to the small sample size of this study which could not allow for strong

inferences to be made. . A larger randomized study, that is powered to answer the study question, in a similar population will enable adjustment for confounding, and provide more generalizable evidence of the effect of nutritional supplementation on CD4 T cell counts, viral load and quality of life in malnourished HIV infected adults.

CHAPTER FOUR

EFFECT OF NUTRITIONAL SUPPLEMENTATION ON NUTRITIONAL OUTCOMES OF HIV INFECTED ADULTS IN UGANDA

4.1 INTRODUCTION

4.1.1 Burden of HIV and malnutrition

HIV infected patients are at an increased risk of malnutrition (169). Indeed the early description of HIV as 'slim' disease (170) indicates that the disease was associated with severe weight loss. The prevalence of malnutrition among HIV infected adults in sub-Saharan Africa ranges between 20% - 40% (134,136,180,261). In Uganda, up to 25% of adults initiating antiretroviral treatment were found to be malnourished (111) with the prevalence highest in rural areas. Malnutrition in HIV infected individuals is due to both macro and micronutrient deficiencies. Hypophosphatemia, hypokalemia, a low BMI and weight loss have been cited as major complications of advanced HIV disease (161,262) and been associated with increased mortality among HIV infected adults initiating ART in many African countries(134,136,187). These studies also demonstrated that weight gain and increase in BMI were associated with reduced mortality among study participants. However, a study to assess the effect of supplementing patients with hypophosphatemia did not show an increased mortality among patients with hypophosphatemia nor a benefit from phosphate supplementation with regard to reduction in mortality (160,229)

Malnutrition in HIV is due to several causes, which may be related to opportunistic infections, HIV infection, or other factors (146,184). These may include malabsorption due to chronic diarrhea or destruction of the gut absorption mucous membrane by the HIV virus, reduced intake of nutrients due to loss of appetite and opportunistic infections in the mouth like oro-esophageal Candida or increased metabolic demands due to the increased oxidative state coupled with reduced intake. Other factors that may lead to malnutrition are

poor dietary diversity and food insecurity (184). All the above mechanisms work synergistically to lead to malnutrition in HIV.

Nutrient and dietary intake varies with regions and ethnicity (263–266). In Africa, studies have shown low protein and fat intake, but high carbohydrate intake among the general population (267,268). A reduction in nutrient intake has been reported during illness including HIV (165,269), although a study in South Africa showed no difference in nutrient intake between HIV positive and negative women (270). The effect of nutritional supplementation on nutrient intake among HIV infected adults has not been studied

4.1.2 Nutritional supplementation among malnourished HIV infected adults

WHO recommends a 10% increase in energy intake among asymptomatic HIV infected patients and 20 - 50% increase among symptomatic HIV infected patients (271). Additionally, they recommend that protein intake can be maintained at 12 - 15% of the total energy intake. Many programs in Sub-Saharan Africa have focused on nutritional support of food insecure and or malnourished HIV infected patients(111)(272–274). Most of these programs aim at improving nutritional status to improve clinical outcomes, slow progress of HIV disease and improve adherence to ART but did not intentionally set out to evaluate the effect of nutritional supplementation on the named outcomes.

Several studies have been conducted to explore the effect of nutritional supplementation on nutritional outcomes among HIV malnourished adults and found varied outcomes. A study in Malawi found that RUTF increased patients' BMI marginally more than Corn-Soy Blend (CSB) after 3 months of supplementation (19.0 Vs 18.5 $P < 0.001$)(236), although this difference did not persist after 9 months. In this study, the participants were given nutritional supplements worth 1000 kcal daily for a period of three months. This was an observational study with many possible confounding factors that may not have been accounted for. A pilot study of food supplementation to improve adherence to ART among food insecure adults in Lusaka found no differences in weight gain or CD4 count among patients receiving and not receiving food

supplementation(262). The adjusted mean weight gain among those on supplements was 5.4 kg compared to 5.1 kg at 6 months ($P = 0.68$). Another study that explored the changes in BMI and fat-free mass among wasted HIV infected adults receiving either RUTF or CSB after 14 weeks of supplementation found that patients receiving RUTF had marginal, but statistically significant, increases in BMI (2.2 vs 1.9) and fat-free mass (2.9 kg vs 2.2 kg) (135). However, 12 weeks after stopping supplementation, there were no differences in anthropometric measures between the two groups. This was a randomized study and had a big number lost to follow-up (34.4%) although it appeared non-differential; the sample size was calculated with a perceived attrition of 15% which is high; therefore, the study lacked internal validity and the findings could be due to chance. In a cohort of HIV infected adults initiating ART in rural Haiti, there was less weight loss among those receiving food supplementation in the first 6 months of the program (-0.20 vs. -0.66, $P = 0.012$) and significant weight gain at 12 months (+0.22 vs. -0.67, $P = 0.002$). This was an observational study and the method of ascertaining who needed food supplementation was not clearly defined and could have introduced bias (115). A study in south India found that macronutrient supplementation did not have significant effect on weight gain (239) and had inconclusive effects on immune recovery among HIV infected adults in India. The studies cited above showed that nutritional supplementation has a minimal effect on most nutritional outcomes including changes in weight and BMI. None of the studies explored the effect of nutritional supplementation on dietary intake, or the biomarkers on nutritional status and only one described the changes in fat-free mass of study participants. The comparison arm in the randomized study that described the differences in BMI and fat-free mass among wasted HIV infected adults in Malawi, received Corn Soy Blend, which is a nutritional supplement. Therefore, the findings of this study could have been impacted the fact that the two study groups might have been similar in terms of the intervention (both received a nutritional supplement). A randomized study to describe the impact of nutritional supplementation on immune responses, BMI and body impedance among HIV infected patients in South Africa found significant changes in weight (mean percentage change in body weight of 12.7% vs 4.9%; $p = 0.047$) and BMI

(mean percentage change in BMI of 7.8% vs 5.5%; $p = 0.007$), and BMI among the participants that received nutritional supplementation (275) compared to those who did not. The participants that received nutritional supplementation also had a higher mean percentage change in fat-free mass (16.7% vs - 3.5%, $p = 0.036$), and basal metabolic rate (5.3% vs - 0.2%, $p = 0.014$) compared to those who did not. The inclusion criteria for the study was self-reported weight loss and as observed in the baseline characteristics, the study participants were not malnourished as reflected by the median BMI of 20.4 and 19.3 for the intervention and comparison groups respectively. Additionally, nutritional supplementation in this study was for six months, which is longer than the three months that most nutritional rehabilitation programs recommend. A sample size of 45 participants was also not powered to answer the study question. A programmatic evaluation of nutritional supplementation among malnourished HIV infected adults found the average time to BMI recovery (described of achieving a BMI of at least 18.5) was four months (220). This was a programmatic evaluation and thus the result could be affected by bias and confounding.

A few studies have described the dietary intake of malnourished HIV infected adults in Africa. A reduction in nutrient intake has been reported during illness including HIV (59, 60). A study in Kenya described the nutrient intake of HIV infected adults and found the average protein intake of the participants to be 40 g/day (162), which was less than the recommended daily allowance. In addition, only one macronutrient (protein) intake was described. Mupere et al, described the nutrient intake of HIV infected adults in Kampala, Uganda (165) and found less than normal nutrient intake. This was a cross-sectional study, with no nutrient supplementation. However, two studies in South Africa showed no difference in nutrient intake between HIV positive and negative women (61,)(170).

There are few studies that have described micronutrient intake among HIV infected adults. Two studies reported adequate micronutrient intake among HIV infected adults (72, 73), while a prospective study in Kenya reported less than adequate intake of all micronutrients except for thiamine (162). The two studies that reported adequate intake of micronutrients were among men that have sex

with men in the United States of America, and this observation has not been reported elsewhere especially in sub-Saharan Africa, apart from South Africa where one study showed adequate intake of micronutrients (270). Despite the many studies, the impact of nutritional supplementation on nutritional outcomes among malnourished HIV infected adults has not conclusively been described. While most studies have focused on effects of nutritional supplementation of weight and BMI changes, there are hardly any studies that have explored the impact of nutritional supplementation on body composition; biomarkers of nutrition, and dietary intake among malnourished HIV infected adults.

The NOURISH study was therefore designed to examine the impact of nutritional supplement on nutritional outcomes (anthropometry, body mass composition, biomarkers of nutrition and dietary intake) among malnourished HIV infected adults in Uganda.

4.1.3 Study Objectives

4.1.3.1 AIM of the nutritional section

The aim of this section was to characterize the nutritional status and describe the effect of nutritional supplementation on nutritional outcomes among malnourished HIV infected adults in Uganda

4.1.3.2 Objectives

- 1- To describe the nutritional characteristics (anthropometry, nutritional biomarkers, and nutrient intake) of HIV infected adults in Uganda.
- 2- To describe the effect of nutritional supplementation on nutrition outcomes of HIV infected adults in Uganda.

4.2 METHODOLOGY

4.2.1 Study design

This was a prospective interventional study with both randomized and non-randomized aspects. Details are in chapter 2, section 2.2.

4.2.2 Nutritional Counseling

All participants received nutritional counseling and this was based on the USAID developed nutrition counseling guidelines (USAID 2013).

4.2.3 Outcomes of Interest

The outcomes of interest were:

- I) Baseline median measures of anthropometry (MUAC, BMI, and body composition), energy intake, and other nutrient intake including the macronutrients and micronutrients, and biomarkers of nutrition of the study population.
- II) Median differences in anthropometric, nutrient content and biomarkers of nutrition among the randomization arms for group A (MAM) and C (ART).
- III) Median differences between baseline and 12-week anthropometric measures, nutrient intake and biomarkers of nutrition for the severely malnourished and the well-nourished participants.

4.2.4 Measurement of outcome

Details of the measurement of each outcome are found in Chapter 2 section 2.4.

4.2.5 Data Management and analysis

Details of the data management were discussed in the general methodology. This section deals with analysis for the nutritional outcomes of the study.

Non-parametric methods were used to analyze the data since the sample size was very small and the data could not be normally distributed. The Wilcoxon rank sum test was used to test for statistical significance at a 5% level.

At baseline, the median (IQR) of anthropometric indices, nutrient intake and biomarkers of nutrition for the four groups were compared. Furthermore, the proportion of participants with less than the expected percent fat mass was calculated in anthropometry, and the proportion of participants that took adequate amounts of the macronutrients (in comparison with the recommended daily allowances) were also calculated and compared.

At follow-up, the effect of nutritional supplementation on nutritional parameters was analyzed as follows: for all participants, a before and after analysis was conducted and the medians for the baseline and week 12 of each parameter were compared. Additionally, for the moderately malnourished and the ART experienced participants, a comparison for the median of each parameter was done both at baseline and follow-up, comparing the participants that received RUTF and those that did not receive RUTF.

4.3 RESULTS

A total of 102 participants were recruited into the study, 59 were female and 43 were male. Study participants were grouped by level of malnutrition and history of using antiretroviral drugs for treatment of HIV. Of the 102, 32 (31.4%) were moderately malnourished and ART naïve (MAM), 18 (17.7%) were severely malnourished and ART naïve (SAM), 27 (26.5%) were malnourished and had been on ART for treatment of HIV for at least one year (ART), and 25 (24.5%) were well nourished and ART naïve (WN). The average age of the study population was 36.7 years (sd 10.7), with the ART experienced group being the oldest. The SAM participants had the most advanced disease with 83.3% of them having HIV stage IV disease. Detailed results of the demographic characteristics of the study population are found in results section of Chapter 3. Baseline characteristics of the study population by level of nutrition are shown in Table 3 .1 (Chapter 3).

4.3.1 Objective I: Nutritional Characteristics of HIV infected adults in Uganda

This section describes the nutritional characteristics of the study population and is presented by the different methods of assessing these characteristics i.e. anthropometry, biomarkers of nutrition and nutrient intake.

4.3.1.1 Anthropometry

Table 4.1 shows the median (IQR) of the baseline anthropometric characteristics of the study population stratified by study group.

The median body mass index for the study participants was 17.9 (IQR 16.4 - 18.9). The median weight of the study population was 48.2 kg (IQR 43.9 – 53.4); the well-nourished (WN) participants were the heaviest with median weight of 56.4 kg (IQR 52.5 – 64.0).

The MAM participants had the biggest median fat-free mass 45.9 kg (IQR 39.4 – 50.2) while the ART experienced participants had the least fat-free mass 37.5 kg (IQR 35.6 – 40.4). All the three (MAM, SAM and ART) malnourished groups had

a median MUAC that was less than the normal range (22 – 25cm)(279) although the median MUAC for the entire study population was 23.0cm (IQR 21.2 – 24.5) table 4.1. Tables 4.2 shows the gender differences in body mass composition. The women in all groups had more fat (total fat mass and higher percentage fat mass) compared to the men. This difference was observed between the participants that were randomized to RUTF and No RUTF in the MAM and MAM-ART groups as well.

Table 4.1: Baseline anthropometry by study group

Median (IQR) Nutritional Characteristic	Overall (n=78)	Moderately Malnourished ART naïve (n=26)	Severely Malnourished ART Naïve (n=18)	Malnourished on Chronic HIV treatment (n=14)	Well Nourished ART naïve (n=20)
Weight/ Kgs	48.2 (43.9 – 53.4)	50.2 (47.2 – 54.3)	43.5 (40.3 – 47.6)	44.4 (42.5 – 47.0)	56.4 (52.5 – 64.0)
Body Mass Index	17.9 (16.4 – 18.9)	18.1 (17.4 – 18.3)	16.3 (15.9 – 16.4)	17.5 (16.8 – 18.10)	22.0 (20.8 – 22.9)
Fat Mass (Kgs)	6.1 (3.6 – 9.9)	4.9 (1.6 – 7.5)	3.5 (2.0 – 4.8)	6.3 (5.6 – 8.4)	11.8 (10.3 – 14.8)
% Fat mass	13.6 (7.2 – 19.4)	8.8 (3.2 – 24.4)	6.9 (4.2 – 10.7)	14.6 (12.8 – 19.3)	21.8 (17.1 – 26.9)
Fat Free Mass (Kgs)	40.9 (36.7 – 47.4)	45.9 (39.4 – 50.2)	39.8 (36.1 – 44.4)	37.5 (35.6 – 40.4)	42.1 (40.6 – 48.2)
MUAC (cm)	23.0 (21.2 – 24.5)	21.8 (21.0 – 24.0)	21.0 (19.0 – 22.0)	22.0 (21.0 – 23.0)	25.6 (24.5 – 27.0)
BMR (Kcal)	1210 (1124 – 1366)	1323 (1194 – 1438)	1171 (1111 – 1279)	1104 (1064 – 1193)	1322 (1211- 1394)

Table 4.2: Baseline gender differences of body mass composition by study group

Variable	Gender	MAM	P-value	SAM	P-value	ART	P-value	WN	P-value
% Fat	Female	15.0 (12.5 -20.3)	<0.01	10.7 (10.6 -13.6)	0.04	17.0 (4.0 -20.3)	<0.01	26.5 (22.5-29.6)	<0.01
	Male	3.3 (2.5 -9.0)		4.1 (3.4 – 6.2)		11.8 (2.6 -12.8)		13.8 (6.6-18.4)	
Fat Mass (Kg)	Female	7.9 (5.9 – 9.9)	<0.01	4.8 (4.3-5.7)	0.06	7.9 (6.0 -9.3)	<0.01	14.3 (11.1–17.3)	0.01
	Male	1.8 (1.3 – 4.8)		1.9 (1.5 -3.0)		5.0 (1.3 -6.2)		8.3 (3.9-12.0)	
Fat Free Mass (Kg)	Female	37.1 (35.1-39.7)	<0.01	36.1 (36.1-39.8)	0.10	36.2 (34.4-38.9)	<0.01	40.7 (37.2-41.7)	<0.01
	Male	48.1 (46.0 – 53.0)		43.4 (40.7-46.8)		42.8 (37.5-48.5)		54.1 (47.8-58.2)	

4.3.1.2 Nutrient intake

4.3.1.2.1 Macronutrients

The median energy intake for the study population was 1871.7 kcal (IQR 1422.2 - 2421.8). The MAM participants had the highest protein (60.0 g); fat 47.7g; carbohydrate (382.2g) and energy (2253.5 kcal) intake while the SAM participants had the lowest energy (1538.3 kcals); protein (33.9g and carbohydrate (244.5g) intake. (Table 4.3).. The ART participants had the highest percent calorie intake of carbohydrates (79.1%) but lowest fat (12.6%) intake.. 74% of all study participants had more than the recommended daily intake of carbohydrates, 29% had adequate protein and 58.6% had adequate fat intake at baseline.

4.3.1.2.2 Micronutrients

Overall, there was inadequate intake for all but two of the 9 assessed micronutrients by all the study groups. The two micronutrients that were taken in adequate amounts were: Iron 10.2 mg/day (RDA: 8 - 18 mg/day) and Vitamin B6 1.4 µg/day (RDA 1.3 – 1.7 µg/day). The SAM participants had inadequate intake for all the micronutrients and had the least intake for most of the micronutrients while the MAM had the highest intake.

Table 4.3 Baseline nutrient intake by study group

Median (IQR) Nutritional Characteristic	Overall	Moderately Malnourished ART naïve	Severely Malnourished ART Naïve	Malnourished on Chronic HIV treatment	Well Nourished ART naïve
Energy (Kcal)	1871.7 (1422.2 – 2421.8)	2253.5 (1734.9- 2576.5)	1538.3 (1088.3– 2374.4)	1888.6 (1428.2– 2100)	1897.4 (1416.3–2419.3)
Fat intake (g)	38.6 (17.6 – 53.4)	47.7 (25.4 – 61.7)	43.6 (28.1 – 53.1)	27.2 (13.5 – 47.6)	33.6 (14.3 -56.8)
% Fat Calorie	17.7 (9.7 – 26.0)	19.5 (11.2- 26.5)	23.1 (10.7 – 29.4)	12.6 (8.5 – 21.0)	18.5 (9.2 -25.9)
Protein intake (g)	50.8 (34.6 -69.6)	60.0 (38.4 -76.7)	33.9 (24.7 – 54.8)	46.4 (34.3 -59.5)	50.1 (35.1 -84.9)
% Protein calorie	10.6 (8.6 -12.5)	11.3 (8.6 – 13.4)	8.6 (6.4 – 11.4)	10.1 (9.2 – 11.7)	11.2 (10.3 – 13.0)
Carb*intake(g)	335.3 (250.0– 452.0)	382.3 (293.0 – 473.4)	244.5 (182.4– 414.9)	324.9 (271.7 – 415.9)	356.4 (210.0 -466.3)
% Carb* calorie	75.4 (64.9 -82.7)	74.4 (62.2 – 82.7)	71.2 (65.4 – 86.0)	79.1 (72.3 -84.5)	70.9 (62.0 – 80.6)
Thiamine (mg)	0.8 (0.6 -1.1)	0.9 (0.7 – 1.2)	0.6 (0.5 – 1.2)	0.7 (0.6 -1.0)	0.7 (0.5 – 1.0)
Riboflavin (mg)	0.9 (0.6 – 1.4)	1.1 (0.6 – 1.7)	0.6 (0.3 – 1.4)	0.8 (0.5 – 1.1)	1.1 (0.6 -1.3)
Niacin (mg)	11.4 (7.1 -16.4)	12.9 (9.2 – 16.2)	7.8 (5.1 – 16.8)	11.5 (7.4 – 14.7)	8.7 (5.9 -17.8)
Vitamin B6 (mg)	1.4 (0.7 – 2.5)	1.8 (1.1 – 2.5)	1.0 (0.7 - 2.5)	1.2 (0.7 – 2.3)	0.9 (0.7 – 2.5)
Vitamin B12 (µg)	0.9 (0.0 – 2.3)	1.7 (0.3 – 3.1)	0.8 (0.0 – 1.5)	1.2 (0.0 – 2.7)	0.9 (0.4 – 1.0)
Vitamin C (mg)	56.9 (36.8 – 106.7)	60.9 (50.2 – 115.9)	64.6 (34.1 – 126.10)	49.0 (33.8 – 99.9)	48.2 (34.8 – 109.5)
Total Folate (µg)	252.9 (146.3– 384.1)	292. 4 (190.6– 404.3)	139.5 (113.8– 356.8)	260.0 (152.9 – 346.5)	221.5 (137.7 – 400.5)
Iron (mg)	10.2 (6.7 -13.8)	11.4 (8.7 – 15.0)	7.6 (4.0 – 12.9)	10.5 (8.0 – 12.4)	9.7 (6.0 – 13.5)
Zinc (masseg)	6.0 (4.2 -8.0)	7.1 (5.4 – 9.2)	4.2 (2.6 - 6.6)	5.4 (3.9 – 6.6)	5.8 (4.5 – 8.1)
Fibre (g)	25.4 (14.2 – 34.9)	26.8 (15.6 -37.8)	15.5 (9.1 – 30.2)	29.2 (19.9 – 34.1)	20.8 (10.9 – 34.5)

4.3.1.2.3 Biomarkers of nutrition status

The median levels for all the assessed biomarkers of nutrition were within normal ranges for all the study groups except for the high-density lipoproteins (HDL) which was 1.0 mmol/l (IQR 0.7 – 1.4) (reference range >1.6 mmol/l). Only the ART participants had their HDL level within the normal range 1.6 mmol/l (1.2 – 2.0). The SAM participants, whose serum levels of the different biomarkers was lowest, had their HDL levels as low as 0.7 mmol/l (IQR 0.4 – 0.8) (Table 4.4). Figure 4.4 is a comparison of the baseline lipid profiles of the four study groups.

The majority of participants had low levels in HDL, total cholesterol, sodium, calcium and fasting blood sugar, with the SAM participants having the levels for these biomarkers except fasting blood glucose. Folate, low density lipoproteins (LDL), triglycerides, vitamin B12, magnesium and potassium were the biomarkers found to be within normal ranges for most of the participants. As expected, the WN participants had the highest proportion of participants with serum levels of these biomarkers that were within the normal ranges.

Table 4.4: Baseline biomarkers of nutrition by study group

Median (IQR) Nutritional Characteristic	Normal range	Overall	Moderately Malnourished ART naïve	Severely Malnourished ART Naïve	Malnourished on Chronic HIV treatment	Well Nourished ART naïve
Folate (nmol/l)	10.4 – 42.4	19.9 (15.0 – 6.10)	21.2 (159 – 26.9)	22.0 (11.5 – 28.2)	16.0 (12.3 – 20.6)	24.5 (16.9 – 30.9)
Phosphates (mmol/l)	1.1 – 1.5	1.2 (1.0 – 1.3)	1.2 (1.0 – 1.3)	1.2 (1.1 -1.4)	1.1 (0.9 – 1.2)	1.2 (1.1 -1.3)
Vitamin B12 (pmol/l)	141 - 489	302.6 (209.0 -431.9)	317.3 (205.9 – 501.0)	366.7 (250.3 – 508.9)	276.2 (180.4 – 358.7)	300.5 (218.9 – 428.2)
Triglycerides (mmol/l)	0.1 – 2.2	1.2 (0.9 – 1.8)	1.1 (1.0 – 1.6)	1.7 (1.1 2.3)	1.2 (0.9 – 1.6)	1.2 (0.8 -1.7)
HDL (mmol/l)	>1.6	1.0 (0.7 -1.4)	0.9 (0.6 – 1.0)	0.7 (0.4 – 0.8)	1.6 (1.2 – 2.0)	1.0 (0.7 – 1.2)
LDL (mmol/l)	<3.0	2.2 (1.6 – 2.7)	2.4 (1.7 – 2.6)	1.6 (0.9 – 2.5)	2.4 (1.8 – 3.0)	2.0 (1.7 – 2.7)
Total Cholesterol (mmol/l)	3.6 – 5.2	4.0 (3.3 – 4.6)	3.6 (3.4 – 4.3)	3.0 (2.6 -3.7)	4.4 (4.1 – 5.3)	3.7 (3.1 – 4.3)
Sodium (mmol/l)	136 -145	134.5 (132.0 – 37.0)	134.0 (131.0 – 135.5)	131.0 (131.0 – 135.0)	136.0 (133.0 – 139.0)	136.0 (133.0 -138.0)
Potassium (mmol/l)	3.5 – 5.5	4.5 (4.1 – 5.3)	4.9 (4.3 – 5.5)	4.5 (3.8 – 5.2)	4.3 (4.1 – 4.6)	4.4 (4.0 – 5.0)
Calcium (mmol/l)	2.2 – 2.5	2.2 (2.1 – 2.3)	2.2 (2.1 – 2.3)	2.1 (2.0 – 2.2)	2.2 (2.1 – 2.3)	2.2 (2.2 -2.4)
Magnesium mmol/l)	0.7 – 1.5	0.8 (0.7 – 0.9)	0.8 (0.7 – 0.8)	0.7 (0.7 – 0.8)	0.8 (0.8 -0.9)	0.8 (0.7 – 0.8)
Fasting Blood Sugar (mmol/l)	5.6 -6.9	4.6 (4.2 – 5.0)	4.4 (4.2 – 4.8)	4.5 (4.0 – 5.6)	4.6 (4.3 -5.1)	4.7 (4.3 -5.0)
C-Reactive Protein (mg/l)	0 - 4	2.1 (0.5 – 4.2)	4.4 (0.9 – 40.7)	13.3 (4.3 – 69.5)	0.7 (0.3 – 2.3)	1.5 (0.4 – 14.2)

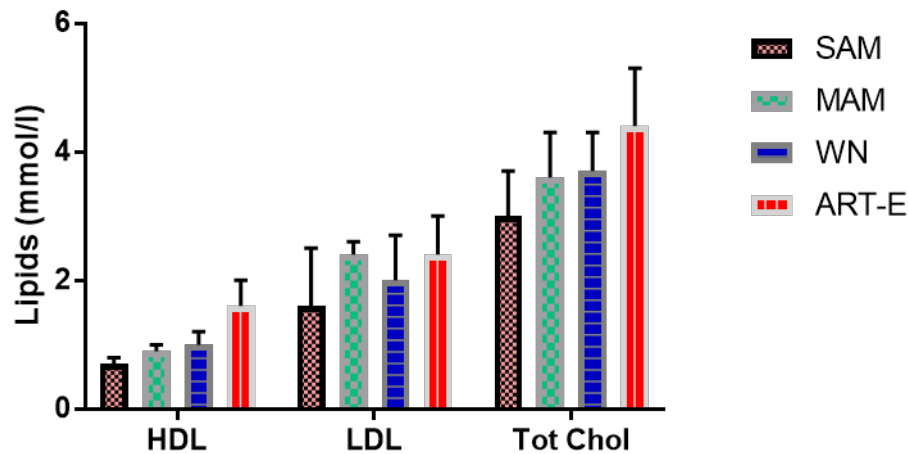


Figure 4.4: Baseline comparison of the lipid profiles of the four study groups.
The SAM participants had the lowest levels of all the lipid profiles.

4.3.2 Objective II: Effect of Nutritional Supplementation on Nutritional outcomes among HIV infected adults in Uganda

This section describes the second objective of the nutrition section of the study, which was to describe the effect of nutritional supplementation on nutritional outcomes among HIV infected adults in Uganda. The results are presented according to the type of study design that was used. First, the results of the randomized trials (one for that involved the ART-naïve MAM participants, and the other which involved the ART-experienced malnourished participants) are presented. These are followed by the results of the non-randomized interventional study involving the severely malnourished and the well-nourished participants.

The randomized studies

4.3.2.1 Effect of nutritional supplementation on nutritional outcomes among MAM-ART naïve

This section describes the results of the randomized study among the ART-naïve MAM participants. The baseline characteristics of the randomization arms are first presented, followed by the effects of a nutritional supplementation on the nutritional outcomes in this study group.

4.3.2.1.1 Comparison of baseline nutritional characteristics between the randomization arms:

This section compares the nutritional characteristics of randomization groups of the moderately malnourished ART naïve at baseline.

Table 4.5 shows that the participants that were randomized to receive RUTF had lower medians for most of the nutritional characteristics. There were no major differences were observed in anthropometry and nutritional intake. As expected, the women in both randomization arms had more fat.

For nutritional intake, the notable difference between the RUTF and no RUTF group were; lower energy intake among the RUTF arm (2052.0 kcal Vs 2434.9 kcal); reduced protein intake (54.5g Vs 64.4 g); reduced fat intake (33.3 g Vs 60.4 g) and reduced percent fat calorie intake (14.2% Vs 26.1%). All these differences were not statistically significant except for total fat intake ($P = 0.005$) and percent fat calorie ($P = 0.007$). Participants from both study arms had a carbohydrate-based diet. All participants had at least 45 g of carbohydrate, which is the minimum recommended daily allowance. Over 80% of participants in both groups had inadequate intake of fat, and approximately 60% had inadequate protein intake, at baseline. There were no major differences in the micronutrient intake except for iron and folate. The participants that received RUTF had adequate intake for 4 of the 8 assessed micronutrients, while those that did not receive had adequate intake for 3 of the 8 micronutrients. The biomarkers of nutritional status for both groups were within the normal physiological ranges except for HDL which was less than normal in both groups, and CRP which was raised for the group that received RUTF.

Table 4.5: Baseline comparison of nutritional parameters of the ART-naïve MAM participants by randomization arm.

Median (IQR)		No RUTF	RUTF	P-value
Weight (Kg)		50.7 (47.7 - 54.7)	49.1 (45.3 - 51.5)	0.403
MUAC (cm)		23.0 (22.5 - 24.0)	22.9 (21.8 - 24.0)	0.620
BMI		18.3 (17.5 - 18.3)	17.9 (17.5 - 18.2)	0.580
Fat Mass (Kg)	General	4.9 (2.8 - 7.5))	2.5 (1.3 - 8.2)	0.525
	Female	7.0 (5.8 - 8.9)	9.2 (6.0 - 14.3)	0.337
	Male	3.6 (1.8 - 4.9)	1.3 (1.2 - 1.4)	0.023
% Fat Mass	General	9.2 (4.8 - 14.8)	3.9 (2.5 - 13.2)	0.204
	Female	15.7 (12.9 - 20.3)	15.9 (8.6 - 20.9)	0.749
	Male	7.2 (3.3 - 9.2)	2.6 (2.3 - 3.0)	0.026
Fat Free Mass (Kgs)	General	46.0 (42.3 - 51.4)	44.9 (38.4 - 49.5)	0.592
	Female	36.7 (35 - 42.3)	37.2 (35.6 - 39.4)	0.849
	Male	48.1 (46.0 - 53.1)	48.2 (46.4 - 51.7)	0.772
Potassium		5.0 (4.6 - 6.4)	4.7 (4.1 - 5.3)	0.196
Phosphates (mmol/l)		1.2 (1.0 - 1.3)	1.2 (1.1 - 1.3)	0.903
Low Density Lipoproteins (mmol/l)		2.5 (1.7 - 3.4)	2.3 (1.9 - 2.6)	0.460
High Density Lipoproteins (mmol/l)		0.9 (0.8 - 1.1)	0.9 (0.4 - 1.0)	0.662
Triglycerides (mmol/l)		1.2 (1.0 - 1.7)	1.0 (0.7 - 1.4)	0.119
Total Cholesterol mmol/l)		4.1 (3.5 - 5.10)	3.6 (3.2 - 3.9)	0.274
Serum Folate (nmol/l)		21.2 (15.0 - 25.4)	20.5 (16.5 - 31.9)	0.658
Fasting Blood Sugar (mmol/l)		4.3 (4.0 - 4.5)	4.8 (4.2 - 4.9)	0.204
Sodium (mmol/l)		134.0 (130.0 - 135.0)	134.0 (132.0 - 136.0)	0.315
Calcium (mmol/l)		2.3 (2.2 - 2.3)	2.2 (2.1 - 2.3)	0.159
Magnesium (mmol/l)		0.8 (0.8 - 0.8)	0.8 (0.7 - 0.8)	0.318
Vitamin B 12 (pmol/l)		252.7 (204.1 - 468.7)	394.7 (298.5 - 543.2)	0.121
Total Energy Intake (Kcal)		2434.9 (1805.1 - 3078.2)	2052.0 (1329.2 - 2425)	0.088
Protein Intake (g)		64.4 (45.7 - 77.9)	53.5 (35.4 - 74.9)	0.280
% Protein Calorie		10.4 (8.4 - 13.5)	11.4 (8.6 - 13.4)	0.678
Fat intake (g)		60.4 (43.7 - 96.0)	33.1 (17.7 - 50.2)	0.004
% Fat Calorie		26.1 (17.1 - 30.8)	14.2 (8.6 - 19.7)	<0.01
Carbohydrate Intake (g)		364.3 (303.3 - 508.4)	396.2 (232.6 - 467.8)	0.678
% Carbohydrate calorie		64.4 (56.2 - 76.3)	78.7 (73.1 - 83.9)	0.022
Thiamine (mg)		1.0 (0.6 - 1.3)	0.8 (0.8 - 1.2)	0.708
Riboflavin (mg)		1.2 (0.7 - 1.9)	1.0 (0.6 - 1.6)	0.454
Niacin (mg)		13.4 (8.6 - 20.3)	12.7 (11.0 - 13.7)	0.771
Vitamin B6 (mg)		1.7 (1.2 - 2.6)	2.0 (1.0 - 2.5)	0.901
Vitamin C (mg)		61.3 (34.0 - 109.3)	74.3 (55.7 - 132.5)	0.171
Zinc (mg)		8.4 (6.4 - 10.6)	6.2 (3.2 - 7.2)	0.031
Iron (mg)		12.8 (9.1 - 17.0)	10.4 (6.6 - 14.0)	0.244
Folate Intake (µg)		336.0 (201.3 - 414.5)	277.7 (179.7 - 365.4)	0.561

4.3.2.1.2 12-week outcome of nutritional supplementation among moderately malnourished HIV infected adults initiating ART

Table 4.6 is a comparison of the medians of the nutritional outcomes at week 12 of between the randomization arms. , There were no statistically significant differences between the RUTF and NO RUTF groups in the anthropometry and biomarkers of nutrition after 12 weeks. There were no statistically significant differences in the body mass composition of women or men between the two randomization arms. Regarding nutrient intake, the median fat intake for the RUTF group was higher than the NO RUTF group by 59.6 g (not significant). In contrast, the percentage carbohydrate calorie for the RUTF group was less than that of the NO RUTF group by 22.3% ($P=0.027$). This was mainly due to the reduction in total carbohydrate intake by the RUTF group. There were significant differences for all the assessed micronutrients between the two groups after 12 weeks of follow-up. There were increases in all micronutrients for the RUTF group to the recommended daily allowance ranges. There were no differences in the biomarkers of nutrition except for serum folate level, with the RUTF group having a higher level of folate than the NO RUTF group, $P<0.001$ at 12 weeks.

A comparison of the absolute and proportional changes between baseline and 12 weeks for all the parameters between the randomization arms of the MAM-ART naïve is shown in table 4.7. This analysis showed that the RUTF participants had higher absolute and proportional increases between baseline and 12 weeks in most of the nutritional parameters compared to the No RUTF participants. The increases in total fat mass were significant higher for men (P -value 0.053) but not the women. Additionally the RUTF group in general and men specifically had significantly higher increases in percent fat mass (P -values 0.052 for both increases). The weight gain in both arms and gender were mainly due to increases in fat mass and not fat free mass. In the NO RUTF arm, the absolute change in weight 2.9 (IQR 0.9 – 5.9) representing a 6.0% increase in weight, while in the RUTF arm, the absolute change in weight was 4.0 (IQR 1.3-7.9) representing 8.2% increase. However, there was only a 2.5% increase in the fat free mass in the NO RUTF arm, and a 4.4% reduction in the RUTF arm. On the contrary, there was a

55.4% increase in the fat mass in the NO RUTF ARM and 205.6% increase in the RUTF arm. In the gender specific analysis, there was minimal change among in the fat free mass of both gender regardless of the randomization arm. The women in the NO RUTF arm had a 4.1 increase in fat free mass, while the men had a 0.2% reduction. In the RUTF arm, the women had a 4.4% increase, while the men had a 1.7% increase in fat free mass. The changes were significant increases in fat mass. In the NO RUTF ARM, the women had a fat mass increase of 27.7% and the men had an increase of 60.4%, while in the RUTF arm the increases were 79.5% and 327.3% respectively.

The absolute and proportional increases were also higher for most of the biomarkers of nutrition were also higher in the RUTF participants. Of specific interest were the changes in serum folate levels of the RUTF arm compared to the No RUTF arm. Much as both arms had a reduction in the serum folate levels, the reduction in the RUTF was less (34.8%) compared to the 58.1% reduction in the NO RUTF arm ($P<0.01$). Additionally, there was a reduction in the serum triglycerides in the RUTF arm (16% reduction) compared to the 8.6% increase in the No RUTF arm, although this difference was not statistically significant ($P>0.05$).

The increases in nutrient intake between the two randomization arms mirrored the differences in the week 12 medians of the nutrient intake. Although the difference in the medians of protein intake was borderline at week 12 ($P=0.09$), the increases in protein intake were significantly higher among the RUTF arm ($P<0.01$). The RUTF participants had a more than 100% reduction in carbohydrate intake compared to the 6% reduction in the No RUTF participants (P -value 0.02). This was not seen in the end line comparison of the medians of total carbohydrate intake. Although the percent carbohydrate intake was significantly reduced among the RUTF group ($P<0.01$) Table 4.6. There micronutrient increases mirrored between baseline and week 12, mirrored the observed differences at week 12 between the RUTF and No RUTF arms.

Table 4.6: Week 12 comparison of the nutritional parameters of MAM initiating ART

Median (IQR)		No RUTF	RUTF	P-Value
Weight (Kg)		55.3 (50.8 -57.8)	52.4 (50.0 -54.4)	0.355
MUAC (cm)		23.5 (23.0 -24.0)	24.0 (22.5 - 26.0)	0.565
BMI		19.0 (18.7 -20.0)	19.3 (18.3 -20.0)	0.624
Fat Mass (Kg)	General	7.5 (5.7 - 8.5)	6.4 (4.7 – 11.1)	0.878
	Female	8.6 (8.3- 10.7)	9.7 (7.2-14.6)	0.827
	Male	6.0 (4.2-8.2)	6.0 (4.3 -6.5)	0.858
% Fat Mass	General	13.0 (10.1 - 15.7)	12.7 (8.7 - 20.7)	0.902
	Female	20 (17.1-22.9)	19.1 (15.3-24.6)	0.965
	Male	11.7 (7.3-14.2)	10.7 (8.0 – 12.7)	0.775
Fat Free Mass (Kgs)	General	49.7 (43.7 - 53.0)	45.8 (41.8 - 49.3)	0.498
	Female	36.2 (33.2- 41.8)	40.6 (39.8-43.6)	0.288
	Male	51.4 (45.6-53.2)	49.1 (45.8 – 49.7)	0.759
Potassium		4.5 (4.3 – 5.0)	4.3 (4.0 -4.7)	0.279
Phosphates (mmol/l)		1.3 (1.0 - 1.4)	1.2 (1.1 - 1.2)	0.793
Low Density Lipoproteins (mmol/l)		2.9 (1.6 - 3.5)	2.8 (2.3 - 3.4)	0.839
High Density Lipoproteins (mmol/l)		1.5 (1.1 - 1.9)	1.3 (1.0 - 1.5)	0.401
Triglycerides (mmol/l)		1.5 (0.93 - 2.0)	1.1 (0.8 - 1.5)	0.212
Total Cholesterol mmol/l)		4.6 (3.3 - 5.2)	4.1 (4.1 - 4.9)	0.601
Serum Folate (nmol/l)		9.8 (6.4 - 10.7)	11.3 (9.2 – 16.3)	0.137
Fasting Blood Sugar (mmol/l)		5.2 (4.8 - 5.3)	4.9 (4.3 - 5.3)	0.399
Sodium (mmol/l)		136.0 (134.0 – 138.0)	136.0 (135.0 – 139.0)	0.579
Calcium (mmol/l)		2.3 (2.2 – 2.4)	2.3 (2.2 – 2.3)	0.817
Magnesium (mmol/l)		0.8 (0.7 - 0.8)	0.8 (0.7 – 0.8)	0.433
Vitamin B 12 (pmol/l)		301.3 (217.4 – 436.6)	354.2 (277.7 – 800.6)	0.291
Total Energy Intake (Kcal)		2081.0 (1693.3 - 2218.9)	3285.8 (2596.4 -3373.5)	0.059
Protein Intake (g)		55.6 (36.4 - 75.4)	84.0 (58.8 -116.4)	0.099
% Protein Calorie		11.4 (9.9 - 13.6)	10.1 (9.1 - 14.2)	0.976
Fat intake (g)		38.8 (22.4 - 54.6)	130.8 (80.7 - 148.5)	0.008
% Fat Calorie		18.6 (11.9 -23.3)	32.4 (28.0 - 39.7)	0.009
Carbohydrate Intake (g)		367.6 (308.9 - 393.3)	352.4 (289.1 - 401 .7)	0.937
% Carbohydrate calorie		70.9 (66.7 - 78.7)	48.6 (39.2 - 61.7)	0.008
Thiamine (mg)		1.1 (0.8 - 1.2)	1.8 (1.0 - 2.0)	0.034
Riboflavin (mg)		1.1 (0.7 - 1.3)	3.9 (1.7 - 4.6)	0.004
Niacin (mg)		12.3 (9.7 - 15.1)	19.3 (12.6 - 28.9)	0.068
Vitamin B6 (mg)		1.7 (1.2 – 2.5)	2.8 (1.9 – 3.0)	0.099
Vitamin C (mg)		48.4 (27.8 – 88.9)	134.0 (118.1 – 186.0)	0.005
Zinc (mg)		7.5 (4.8 – 8.4)	27.4 (7.1 – 32.6)	0.016
Iron (mg)		13.9 (11.9 - 16.3)	30.5 (12.2 - 34.9)	0.059
Folate Intake (µg)		338.6 (285.7 - 406.3)	629.5 (397.1 - 683.0)	0.014

Table 4.7: Absolute and percentage change between baseline and week 12 in nutritional outcomes of the MAM initiating ART.

Median (IQR)		No RUTF	% Change	RUTF	% Change	P-Value
Weight (Kg)		2.9 (0.9-5.9)	6.0	4.0 (1.3-7.9)	8.2	0.497
MUAC (cm)		0.5 (0.3-1.0)	1.1	1.4 (0.3-2.6)	2.3	0.275
BMI		0.9 (0.3 -2.3)	5.2	1.5 (0.5 - 2.3)	8.4	0.511
Fat Mass (Kg)	General	3.6 (1.2 -4.9)	55.4	7.6 (4.0 - 9.7)	205.6	0.066
	Female	1.8 (1.8 – 2.9)	27.7	4.9 (-1.0 - 8.7)	79.5	0.479
	Male	2.4 (0.6 – 3.2)	60.4	4.7 (3.0 – 5.1)	327.3	0.053
% Fat Mass	General	2.3 (0.7 – 2.9)	43.5	4.7 (2.3 - 5.1)	143.0	0.052
	Female	3.0 (2.6-4.2)	17.6	6.9 (-1.4 - 11)	41.2	0.479
	Male	4.0 (0.5- 5.6)	62.5	7.6 (5.3 - 8.9)	304	0.052
Fat Free Mass (Kgs)	General	1.1 (-0.3 -2.0)	2.5	-2.0 (-1.1 - 3.0)	-4.4	0.723
	Female	1.3 (1.2 – 3.6)	4.1	1.7 (0.1- 6.4)	4.4	0.565
	Male	-0.1 (-2.1 – 1.9)	-0.2	-0.8 (-2.1 – 2.9)	-1.7	0.319
Potassium		-0.5 (-2.4 - -0.2)	-10.9	-0.3 (-1.0 – 0.4)	-5.7	0.437
Phosphates (mmol/l)		0.1 (-0.1 – 0.1)	6.5	0.0 (-0.1 – 0.8)	0.0	0.619
Low Density Lipoproteins (mmol/l)		0.3 (- 0.2 – 1.0)	10.3	0.6 (0.1 – 0.8)	34.1	0.301
High Density Lipoproteins (mmol/l)		0.6 (0.4-1.0)	66.7	0.35 (0.23-0.68)	36.1	0.343
Triglycerides (mmol/l)		0.11 (-0.2 – 0.6)	8.6	-1.0 (-0.6 – 0.4)	-16	0.383
Total Cholesterol mmol/l)		0.8 (0.3- 1.2)	21.0	0.8 (0.4 – 1.0)	20.6	0.877
Serum Folate (nmol/l)		-11.9 (-15.1 - -11.2)	-58.1	-6.0 (-7.3 - -4.4)	-34.8	<0.01
Sodium (mmol/l)		5.0 (3 – 7)	3.8	3.0 (0 – 4)	2.2	0.071
Calcium (mmol/l)		0.05 (-0.1 – 0.1)	2.5	0.07 (0.03 -0.13)	3.2	0.374
Magnesium (mmol/l)		0.4 (-0.4 – 0.5)	4.2	0.03 (-0.03 – 0.09)	4.1	0.661
Vitamin B 12 (pmol/l)		22.9 (12.5 – 80.1)	9.7	64.6 (10.5 – 324.5)	12.4	0.537
Total Energy Intake (Kcal)		-315 (-102 - 218)	-14.1	1415 (399 – 1577)	72.1	<0.01
Protein Intake (g)		-2.0 (-16.2 – 20.1)	-6.0	30.4 (-13.4 – 76.9)	75.9	0.09
Fat intake (g)		-10.6 (-32 – 16)	-24.1	80.3 (47 – 130)	181.6	<0.001
Carbohydrate Intake (g)		-30.7 (-133.0 – 43.6)	-9.9	-8.2 (-84.0 – 153.3)	-4.2	0.356
Thiamine (mg)		-0.05 (-0.52 – 0.29)	-6.1	1.0 (-0.03 – 1.60)	100.5	0.022
Riboflavin (mg)		-0.33 (-0.54 – 0.16)	-29.2	2.75 (0.3 – 3.4)	276.8	0.002
Niacin (mg)		-0.8 (-4.7 – 4.8)	-7.0	7.4 (-1.0 – 17.0)	49.0	0.068
Vitamin B6 (mg)		-0.2 (- 1.0 - 0.5)	-16.8	0.7 (-0.5 – 1.8)	33.3	0.052
Vitamin C (mg)		-22.4 (- 48.7 – 14.5)	-41.5	35.7 (-17.0 – 92.8)	43.3	0.052
Zinc (mg)		-1.3 (-2.3 – 1.3)	-13.2	23.5 (1.7 – 25.9)	388.2	<0.01
Iron (mg)		2.2 (0.5 – 5.12)	13.7	19.9 (2.1 – 22.9)	133.9	0.019
Folate Intake (µg)		41 (-50.6 – 105.6)	9.5	332 (91.1 – 546.2)	76.6	0.008

4.3.2.2 Effect of nutritional supplementation on nutritional outcomes among ART experienced malnourished HIV infected adults

This section describes the results of the randomized study among the ART experienced participants. The baseline characteristics of the randomization arms are first presented, followed by the effects of a nutritional supplementation on the nutritional outcomes in this study group.

4.3.2.2.1 Comparison of baseline nutritional characteristics between randomizations arms

Table 4.8 shows that most of the baseline nutritional characteristics of the RUTF and No RUTF groups were similar.

In anthropometry, all indices were comparable in both groups of participants. The median weight of the no RUTF group was 45.0 kg (IQR 42.5 – 47.0) compared to 43.5 kg (IQR 42.7 – 47.0) of the RUTF group. As expected the women had more body fat than the men in both randomization arms, but there were no statistically significant differences in the body fat of either men or women between the randomization arms

Participants in both groups had adequate intake of carbohydrates. There was a low intake of proteins and fat by all the study participants. 72.7% and 87.5% of the participants in the NO RUTF and RUTF groups respectively had less than adequate intake of proteins, and 81.8% and 75.0% of the NO RUTF and RUTF participants had less than adequate intake of fat. Participants in both groups had less than the recommended daily intake for all the assessed micronutrients at baseline.

Much as all biomarkers of nutrition were within normal ranges for both groups, the phosphate level for the RUTF, 1.2 mmol/l (1.1 – 1.3) was significantly higher than the NO RUTF, 1.0 (0.9 – 1.1) ($P = 0.023$), and this was true for fasting blood sugar with a median of 4.7 mmol/l (4.5 – 5.4) for the RUTF group compared to 4.3 mmol/l (4.1 – 4.5), $P = 0.048$.

Table 4.8: Baseline comparison of nutritional parameters for the malnourished ART experienced participants.

Median (IQR)		No RUTF N=11	RUTF N=16	P-value
Weight (Kg)		45.0 (42.5-47.0)	43.9 (42.7-47.0)	0.751
MUAC (cm)		22.0 (21.0-23.0)	22.0 (21.0-23.5)	0.595
BMI (Kg/M ²)		17.5 (16.8-18.2)	17.4 (16.7-18.1)	0.937
Fat Mass(Kg)	General	6.3 (6.0-8.4)	6.5 (5.5-8.6)	0.979
	Female	7.2 (6.1-8.9)	7.9 (5.7 – 9.3)	0.836
	Male	4.4 (3.8-5.0)	5.4 (1.3-6.2)	0.699
% Fat Mass	General	14.7 (13.0 -17.2)	14.5 (12.4-19.8)	0.752
	Female	15.9 (14.4-18.4)	17.5(14.3-21.3)	0.482
	Male	10.0 (8.1-11.8)	12.0 (2.6-12.8)	0.695
Fat Free Mass (Kg)	General	38.2 (36.2-40.1)	37.2 (34.3-40.8)	0.493
	Female	37.7 (36.2-39.5)	40.6 (37.5 – 43.7)	0.063
	Male	40.6 (37.5-43.6)	42.8 (40.9 -48.5)	0.789
Phosphate (mmol/l)		1.0 (0.9-1.1)	1.2 (1.1-1.3)	0.023
High Density Lipoproteins (mmol/l)		1.7 (1.3-1.9)	1.5 (1.0-2.1)	0.461
Low Density Lipoproteins (mmol/l)		2.7 (2.2-3.1)	2.3 (1.7-3.0)	0.257
Triglycerides (mmol/l)		1.2 (0.9-1.4)	1.3 (1.2-1.8)	0.514
Total cholesterol (mmo/l)		4.7 (4.0 – 5.4)	4.3 (4.1 – 5.4)	0.771
Fasting blood sugar (mmol/l)		4.3 (4.1-5.0)	4.7 (4.5-5.4)	0.042
Folate (nmol/l)		14.5 (10.9-16.4)	18.2 (14.6-18.2)	0.126
Sodium (mmol/l)		136.0 (133.0 – 138.0)	136.5 (133.0 -139.5)	0.751
Magnesium (mmol/l)		0.8 (0.8 – 0.9)	0.8 (0.8 - 0.9)	0.916
Potassium (mmol/l)		4.3 (4.1 – 5.0)	4.3 (4.1 -4.6)	0.812
Vitamin B12 (pmol/l)		178.7 (152.9 – 358.5)	289.2 (229.1 – 359.3)	0.065
Calcium (mmol/l)		2.2 (2.1 – 2.3)	2.1 (2.1 – 2.2)	0.493
Protein Intake (g)		48.9(43.5-62.0)	42.2(31.3-58.2)	0.268
% Protein		10.4 (8.4 – 13.5)	11.4 (8.6 – 13.4)	0.039
Fat intake (g)		25.0(11.1-46.8)	28.0(15.1-48.0)	0.598
% Fat Calorie		12.2 (7.7 - 19.6)	12.6 (9.7 -23.2)	0.493
Carbohydrate Intake (g)		388.9 (294.5 - 416.0)	309.4 (271.1 -411.3)	0.598
% Carbohydrate calorie		79.2 (72.5 - 81.8)	77.6 (72.2 -85.0)	0.833
Total energy (Kcal)		1947.7 (1672.9 - 2100.0)	1681.2 (1384.9 -2080.0)	0.833
Thiamine (mg)		0.7(0.6-1.0)	0.8(0.7-1.1)	0.102
Riboflavin (mg)		0.8(0.5-0.9)	0.8(0.6-1.3)	0.916
Niacin (mg)		11.6(8.8-14.7)	9.6(7.3-14.5)	0.958
Vitamin B6 (mg)		1.1 (0.7 -2.1)	1.4 (0.8 -2.4)	0.792
Vitamin C (mg)		51.2 (33.2 – 99.9)	44.5 (35.4 -98.9)	0.958
Iron (mg)		10.5(8.3-12.4)	10.4(7.7-12.5)	0.874
Folate Intake (µg)		219.4(146.9-379.1)	213.4(136.3-262.2)	0.562
Zinc (mg)		5.8 (5.1 – 6.6)	4.8 (3.7 – 7.1)	0.399

4.3.2.2.2 12 Week outcome of nutritional supplementation among ART experienced malnourished HIV infected adults

Although there were no significant differences in the anthropometry and body composition between the RUTF and NO RUTF arms in this group (table 4.9) after 12 weeks, the absolute and percentage changes between baseline and 12 weeks in these parameters were significantly higher among the RUTF group than the NO RUTF group. (Table 4.10). Specifically, the changes in weight were significantly higher among the RUTF group (absolute change of 0.3 kg among the No RUTF group compared to 6.1 kg increase in the RUTF group, $P<0.01$). The increase in weight, meant a significant increase in BMI among the RUTF compared to the no RUTF. The RUTF had a 1 unit increase in BMI compared to the 0.1 unit increase in the No RUTF arm ($P=0.02$).

There was hardly any weight gain in in the NO RUTF arm with change in median weight between baseline and 12 weeks being 0.3 kg representing a 6% increase. In the RUTF arm, the median weight gain was 2.5 kg representing a 7.4% increase. Similar to the ART naïve participants, these increases in weight, though minimal, were mainly due to mainly due to increases in fat mass and not fat free mass. In both arms, there was very little change in fat free mass. There was a 1.7% reduction in the fat free mass of the NO RUTF, and only a 1.5% increase in the RUTF group. On the contrary, the increases in fat mass were 10% in the NO RUTF group and 26.6 % in the RUTF group. In the gender specific analysis, there was minimal change among in the fat free mass of both gender regardless of the randomization arm. The women in the NO RUTF arm had a 1.7% decrease in fat free mass, while the men had a 1.1% increase. In the RUTF arm, the women had a 0.9% increase, while the men had a 2.3% increase in fat free mass. The changes were significant increases in fat mass. In the NO RUTF ARM, the women had a fat mass increase of 10% and the men had an increase of 6.7%, while in the RUTF arm the increases were 26.2% and 41.8% respectively.

Similarly, the biomarkers of nutrition had no significant difference at the end of the study except for serum folate ($P < 0.001$). The absolute and proportional changes in the biomarkers of nutrition were much higher among the RUTF arm.

While the median nutrient intake of all the macronutrients were not significantly different at the end of the study, the absolute and proportional changes in the RUTF participants were significantly higher than those of the NO RUTF participants, especially in total energy intake ($P < 0.01$) and weight change ($P < 0.01$). There was a 43.7% increase in total energy intake and a 37.9% increase in the protein intake of the RUTF participants compared to a 5% reduction in energy intake and almost 45% reduction in protein intake in the No RUTF participants. There was a more than 100% increase in the fat intake in the RUTF participants compared to the 22% in the NO RUTF participants, although this had no statistical difference.

At week 12, the RUTF participants had higher intake of riboflavin, Niacin, Iron and Zinc. These observations were confirmed by similarly higher absolute and proportional changes in the same micronutrients except for riboflavin. The changes in folate intake were also higher for the RUTF participants.

Table 4.9 Nutritional characteristics and outcomes following 12 weeks of supplementation with RUTF among the ART experienced participants.

Median (IQR)		No RUTF	RUTF	P-Value
Weight (Kg)		45.7 (43.6 - 47.2)	45.7 (44.2 - 51.0)	0.477
MUAC (cm)		22.4 (22.0 - 23.0)	23.4 (22.0 - 24.3)	0.221
BMI (Kg/M ²)		17.4 (16.7 - 18.3)	18.4 (17.5 - 19.0)	0.624
Fat Mass(Kg)	General	6.6 (5.7 - 10.2)	9.4 (7.2 - 10.0)	0.444
	Female	8.6 (6.6 - 10.3)	9.5 (7.8 - 10.6)	0.439
	Male	6.0 (4.2 - 8.2)	6.0 (4.3 - 6.5)	0.113
% Fat Mass	General	14.6 (13.8 - 21.1)	18.7 (16.4 - 21.6)	0.396
	Female	18.2 (14.6 - 21.6)	21.2 (17.5 - 22.8)	0.224
	Male	9.9 (8.4 - 11.3)	13.1 (8.5 - 16.3)	0.414
Fat Free Mass (Kg)	General	38.5 (37.1 - 39)	36.7 (35 - 41.8)	0.571
	Female	38.3 (35.6 - 38.6)	35.3 (34.7 - 37.0)	0.436
	Male	40.8 (39.9 - 1.8)	43.1 (42.7 - 48.8)	0.245
Phosphate (mmol/l)		1.0 (0.9 - 1.1)	1.2 (1.1 - 1.4)	0.01
High Density Lipoproteins (mmol/l)		1.8 (1.6 - 1.9)	1.6 (1.2 - 2.1)	0.222
Low Density Lipoproteins (mmol/l)		2.9 (2.4 - 3.4)	2.5 (1.9 - 3.8)	0.506
Triglycerides (mmol/l)		0.7 (0.6 - 1.0)	1.2 (0.7 - 1.8)	0.06
Total cholesterol (mmo/l)		4.5 (4.3 - 5.6)	4.8 (4.1 - 5.6)	0.697
Fasting blood sugar (mmol/l)		4.4 (4.2 - 4.8)	4.9 (4.2 - 6.7)	0.182
Folate (nmol/l)		10.5 (9.0 - 13.8)	22.1 (16.9 - 28.5)	<0.001
Sodium (mmol/l)		136 (135 - 136)	137 (136 - 140)	0.068
Magnesium (mmol/l)		0.8 (0.8 - 0.9)	0.9 (0.8 - 0.9)	0.486
Potassium (mmol/l)		4.9 (4.3 - 5.4)	4.2 (4.0 - 4.5)	0.075
Vitamin B12 (pmol/l)		254.9 (229.0 - 549.1)	441.4 (302.2 - 750.7)	0.046
Calcium (mmol/l)		2.2 (2.1 - 2.3)	2.3 (2.2 - 2.4)	0.487
Protein Intake (g)		32.1 (24.4 - 51.7)	63.7 (34.1 - 92.7)	0.078
% Protein		8.5 (7.5 - 10.8)	9.5 (7.3 - 11.5)	0.673
Fat intake (g)		17.6 (14.3 - 43.6)	76.0 (34.4 - 119.3)	0.396
% Fat Calorie		14.6 (13.8 - 21.1)	18.7 (16.7 - 21.6)	0.171
Carbohydrate Intake (g)		367.6 (308.9 - 393.3)	352.4 (289.1 - 402.7)	0.931
% Carbohydrate calorie		79.8 (69.4 - 85.2)	57.5 (47.6 - 72.9)	0.027
Total energy (Kcal)		2081.0 (1693.3 - 2218.9)	3285.8 (2596.4 - 3373.5)	0.059
Thiamine (mg)		0.8 (0.5 - 0.9)	1.6 (0.7 - 2.1)	0.071
Riboflavin (mg)		0.7 (0.4 - 1.5)	2.9 (0.9 - 3.8)	0.02
Niacin (mg)		7.7 (6.5 - 11.4)	18.8 (9.7 - 24.7)	0.039
Vitamin B6 (mg)		1.0 (0.6 - 1.9)	2.2 (1.5 - 2.8)	0.065
Vitamin C (mg)		87.5 (7.5 - 10.8)	150.5 (77.3 - 198.1)	0.493
Iron (mg)		7.6 (6.6 - 10.9)	24.5 (8.2 - 33.4)	0.031
Folate Intake (µg)		238.0 (218.5 - 293.0)	510.8 (210.7 - 801.6)	0.065
Zinc (mg)		4.3 (2.9 - 6.7)	22.9 (5.0 - 32.1)	0.023

Table 4. 10: Absolute and percentage change in anthropometry and body mass composition of the ART experienced participants after 12 weeks

Median (IQR)		No RUTF	% Change	RUTF	% Change	P-Value
Weight (Kg)		0.3 (0.5 – 0.7)	6.1	2.5 (1.3 -3.3)	7.4	<0.01
MUAC (cm)		0.8 (0 -1)	0.6	1.1 (0.6 – 1.8)	5.6	0.171
BMI		0.1(0.4 – 0.6)	0.6	1 (0.6 -1.4)	4.7	0.02
Fat Mass (Kg)	General	0.6 (0.6 -1.5)	10	1.9 (0.7 – 3.1)	28.2	0.08
	Female	0.6 (0.6 -1.8)	10	1.7 (0.9 – 3.6)	26.6	0.146
	Male	0.1 (1.3 – 3.6)	6.7	2.8 (0.4 – 2.9)	41.8	0.245
% Fat Mass	General	1.6 (0.5-3.2)	8.2	2.9 (0.9 – 5.5)	19.0	0.192
	Female	1.6 (0.5 -4.4)	8.2	2.8 (1.4 -4.7)	17.1	0.222
	Male	1 (3.2 -3.4)	5.3	4.3 (0.3 - 4.8)	35.8	0.241
Fat Free Mass (Kgs)	General	0.6 (1.8 - 0.3)	1.7	0.7 (0.4 – 1.5)	1.5	0.182
	Female	0.6 (2 – 0.3)	1.7	0.3 (1.1 – 1.60)	0.9	0.237
	Male	0.3 (1.8 – 2.4)	1.1	1.1 (0.3 – 1.4)	2.3	0.974
Potassium		0.2 (0.4 – 1.1)	4.5	0.1 (0.6 – 0.4)	2.5	0.487
Phosphates (mmol/l)		0.03 (0.12 – 0.15)	3.1	0.4 (0.1 – 0.2)	4.4	0.589
Low Density Lipoproteins (mmol/l)		0.24 (0.1 – 0.5)	5.5	0.24 (0.1 – 0.8)	14.2	0.846
High Density Lipoproteins (mmol/l)		0.1 (0.1 – 0.3)	5.4	0.2 (0.0 – 0.4)	12.0	0.579
Triglycerides (mmol/l)		0.4 (0.1 - 0.2)	28.7	0.3 (0.6 – 0.1)	23.4	0.907
Total Cholesterol (mmol/l)		0.5 (0.2 – 0.2)	1.0	0.0 (0.5 – 0.5)	0	0.845
Serum Folate (nmol/l)		2.6 (6.0 - 0.74)	17.3	3.4 (1.9 – 18.3)	13.9	0.032
Sodium (mmol/l)		1.0 (1.0 – 4)	0.8	3 (1- 5)	2.2	0.559
Calcium (mmol/l)		0.03 (0.01 – 0.1)	1.1	0.13(0.1- 0.2)	5.9	0.076
Magnesium (mmol/l)		0.01 (0.07 -0.1)	0.6	0.03 (0.05 – 0.06)	3.6	0.541
Vitamin B 12 (pmol/l)		106 (76.5- 157.1)	61.1	224.3 (5.0 – 459.8)	63.3	0.771
Total Energy Intake (Kcal)		75.6 (650 – 358)	5.2	692.1(284 – 1131)	43.7	<0.01
Protein Intake (g)		19.7 (31.8 – 4.4)	44.9	15.9 (6.7 – 41.0)	37.9	<0.01
Fat intake (g)		8.8 (3.1 – 25.7)	21.9	27.9 (4.4 – 86.3)	107.5	0.171
Carbohydrate Intake (g)		36.4 (124.0 – 10.8)	11.7	10 (128 – 136)	2.9	0.527
Thiamine (mg)		0.1 (0.26 – 0.3)	16.0	0.5(0.2 – 1.0)	45.1	0.246
Riboflavin (mg)		0.04 (0.3 – 0.4)	5.1	1.2 (0.04 – 3.4)	93.3	0.094
Niacin (mg)		2.1 (5.2 – 2.4)	23.5	5.4 (2.0 – 9.3)	51.9	0.040
Vitamin B6 (mg)		0.0 (1.0 – 0.6)	2.0	0.7 (0.0 – 1.2)	50.0	0.171
Vitamin C (mg)		53.4 (9.0 – 116.0)	71.5	77.8 (7.9- 143.6)	111.7	0.521
Zinc (mg)		1.4 (2.1 - 0.24)	30.0	10 (0.1 – 27.3)	128.2	<0.01
Iron (mg)		3.1 (5.0 – 1.4)	35.0	7.9 (0.3- 22.2)	46.3	<0.01
Folate Intake (µg)		2.4 (95.1 – 99.4)	6.3	246.3 (21.0 – 494.2)	121.0	0.04

The non-randomized studies

4.3.2.3 Effect of nutritional supplementation on nutritional outcomes among the severely malnourished HIV initiating ART in Uganda

The results of the effect of nutritional supplementation on nutritional outcomes among the SAM participants are presented here.

Table 4.11 shows the nutritional outcomes among severely malnourished (SAM) adults after 12 weeks of nutritional supplementation and ART.

4.3.2.3.1 Anthropometry

After 12 weeks of nutritional supplementation with RUTF and ART (Efavirenz, Tenofovir and Lamivudine), there were statistically significant increases in the medians of MUAC of 2.0 cm (P-value 0.02); BMI of 1.2 kg/m² (P-value 0.001); total fat mass of 2.3kgs (P-value 0.007) and percent fat mass of 4.7% (P-value 0.009).

Additionally, there was a 5.2 kg median weight gain from 43.3 kg to 48.5 kg (P-value 0.001). These increases represent an 11.9% increase in weight; an 8.7% increase in MUAC; a 7.5% increase in BMI; a 92% increase in fat mass and an 87% increase in percentage fat mass, however, there was no changes in fat-free mass.

4.3.2.3.2 Nutrient intake

There were statistically significant increases in the median total energy intake by 593 kcal (P = 0.034); median fat intake by 28.3 g (P = 0.034). Median protein intake increased by 30.3g, but this had marginal statistical significance (P = 0.059).

Much as there was an increase in the total carbohydrate intake from 244.5 g to 308.5 g, there was a drop in the percent carbohydrate calorie from 71% to 49%.

Anthropometry:

There was a general increase in all anthropometric indices except fat free mass.

Nutrient Intake:

Increase in all intake of all nutrients except for percent carbohydrate calorie intake.

Biomarkers of Nutrition:

Minor but statistically significant increase in HDL; LDL and total cholesterol

Changes in nutritional characteristics among severely malnourished HIV infected adults after 12 weeks of RUTF and ART

After 12 weeks of ART and supplementation with RUTF, there were statistically significant increases in most of the micronutrients such as: Thiamine ($P = 0.007$); niacin ($P = 0.007$); iron ($P = 0.009$) and folate ($P = 0.02$); zinc ($P = 0.012$); riboflavin ($P = 0.023$) and vitamin B6 ($P = 0.05$). Only 2 (Vitamin C and Vitamin B12) did not have significant increases at the end of 12 weeks.

4.3.2.3.3 Biomarkers of nutritional status

After 12 weeks of supplementation and ART, the severely malnourished participants had modest but statistically significant increases in median LDL of 0.5 mmol/l ($P = 0.033$), median HDL of 0.5 mmol/l ($P = 0.002$). The median total cholesterol increased from 3.1 mmol/l to 4.3 mmol/l ($P = 0.002$) which is within the physiological range.

All the biomarkers that were below the physiological range for this group of participants were restored to the normal ranges after 12 weeks of supplementation. There was a reduction in C-reactive protein from 13.3 mg/l to 3.3 mg/l ($P = 0.05$) and an increase in the serum sodium from the below physiological level of 131 mmol/l to 137 mmol/l ($P = 0.008$). There were no significant changes for the trace elements assessed. There was a reduction in serum folate levels which was not statistically significant.

Table 4.11: Baseline and 12-week median nutritional characteristics of the severely malnourished participants

Median (IQR)	SAM		
	Baseline (N=18)	Week 12 (N=13)	P-Value
Weight (Kg)	43.3 (40.3 - 47.6)	48.5 (45.6 - 52.0)	0.001
MUAC (cm)	21.0 (19.0 - 22.0)	23.0 (22.5 - 24.3)	0.02
BMI	16.1 (15.3 - 16.3)	17.3 (17.0 - 18.5)	0.001
Fat Mass (Kg)	2.5 (1.5 - 4.8)	4.8 (4.4 - 6.9)	0.007
% Fat Mass	5.4 (3.8 - 10.7)	10.1 (8.6 - 14.9)	0.009
Fat Free Mass (Kgs)	41.7 (36.1 - 45.9)	42.0 (37.3 - 49.2)	0.26
Potassium (mmol/l)	4.5 (3.8 - 5.2)	4.3 (4.1 - 4.7)	0.144
Phosphates (mmol/l)	1.2 (1.1 - 1.4)	1.3 (1.1 - 1.3)	0.612
Low Density Lipoproteins (mmol/l)	1.8 (0.9 - 2.0)	2.3 (1.7 - 2.8)	0.033
High Density Lipoproteins (mmol/l)	0.7 (0.4 - 0.8)	1.2 (1.0 - 1.9)	0.002
Triglycerides (mmol/l)	1.5 (1.1 - 2.3)	1.4 (0.9 - 1.8)	0.861
Total Cholesterol mmol/l)	3.1 (2.6 - 3.7)	4.3 (4.0 - 4.6)	0.002
Serum Folate (nmol/l)	22.0 (11.5 - 28.0)	18.5 (14.1 - 28.8)	0.138
Fasting Blood Sugar (mmol/l)	4.5 (4.0 - 5.6)	4.7 (3.9 - 5.2)	0.665
C-Reactive Protein (mg/l)	13.3 (4.3 - 69.5)	3.3 (0.8 - 7.1)	0.050
Vitamin B12 (pmol/l)	366.7 (250.3 - 508.9)	451.9 (302.2 - 524.9)	0.893
Sodium (mmol/l)	131.0 (130.0 - 135.0)	137.0 (135.0 - 137.0)	0.008
Magnesium (mmol/l)	0.7 (0.7 - 0.8)	0.8 (0.8 - 1.0)	0.075
Calcium (mmol/l)	2.1 (2.0 - 2.2)	2.2 (2.2 - 2.3)	0.035
Total Energy Intake (Kcal)	1538.3 (1088.3 - 2574.4)	2131.6 (1947.9 - 2451.6)	0.034
Protein Intake (g)	33.9 (24.8 - 54.8)	64.2 (51.5 - 78.5)	0.059
% Protein Calorie	8.6 (6.4 - 11.4)	10.9 (9.8 - 13.1)	0.136
Fat intake (g)	43.6 (28.1 - 53.1)	71.9 (48.3 - 92.3)	0.034
% Fat Calorie	23.1 (10.7 - 29.4)	29.8(20.4 - 33.2)	0.432
Carbohydrate Intake (g)	244.5 (182.4 - 414.9)	308.5 (253.5 - 354.0)	0.388
% Carbohydrate calorie	71.2 (65.4 - 85.9)	49.4 (45.5 - 69.2)	0.1
Thiamine (mg)	0.6 (0.5 - 1.2)	1.3 (1.0 - 1.7)	0.07
Riboflavin (mg)	0.6 (0.3 - 1.4)	3.2 (1.5 - 3.4)	0.023
Niacin (mg)	7.8 (5.1 - 16.9)	13.5 (11.0 - 17.7)	0.07
Vitamin B6 (mg)	1.0 (0.7 - 2.5)	1.5 (1.2 - 1.9)	0.050
Vitamin B12 (µg)	0.8 (0.0 - 1.5)	0.4 (0.0 - 2.0)	0.723
Vitamin C (mg)	64.6 (34.1 - 126.1)	93.5 (57.2 - 126.7)	0.999
Iron (mg)	7.7 (4.0 - 12.9)	24.6 (11.8 - 26.9)	0.009
Zinc (mg)	4.2 (2.5 - 6.6)	26.0 (6.7 - 28.1)	0.012
Folate Intake (µg)	139.5 (113.8 - 356.8)	517.8 (246.3 - 593.6)	0.02

4.3.2.4 Nutritional outcomes of well-nourished HIV infected adults after 12 weeks of antiretroviral therapy

This section described the results of 12 weeks of ART alone on the nutritional outcomes among the well-nourished participants.

The statistically significant changes among the well-nourished participants were in the anthropometry and biomarkers of nutrition status. In anthropometry, there were marginal but statistically significant increases in median weight by 1.3 kg ($P = 0.014$) and total fat mass by 0.5 kg ($P = 0.033$). There was a 1.9 kg increase in the fat-free mass, but this was not statistically significant.

In the biomarkers of nutrition status, there was a marked reduction in the serum folate by 14.6 nmol/l ($P < 0.001$). LDL and HDL had minor but statistically significant changes after 12 weeks of ART. These were 0.5 mmol/l ($P = 0.013$) for LDL and 0.1 mmol/l ($P = 0.023$) for HDL. There were no significant changes between the baseline and 12-week nutrient intake in this study group (Table 4.12).

Table 4.12: Baseline and 12-week median nutritional characteristics of the well-nourished participants

Median (IQR)	Well-Nourished		
	Baseline (N= 27)	Week 12 (N= 22)	P-Value
Weight (Kg)	56.4 (52.6 - 64.7)	57.7 (52.9 - 66.3)	0.014
MUAC (cm)	25.8 (24.5 - 27.0)	26.0 (25.0 - 28.0)	0.086
BMI	22.2 (20.9- 23.6)	22.8 (21.0 - 23.3)	0.199
Fat Mass (Kg)	12.0 (10.3 - 15.1)	12.5 (9.2 - 17.8)	0.033
% Fat Mass	22.2 (17.1 - 27.3)	23.1 (14.7 - 29.8)	0.1
Fat Free Mass (Kgs)	42.1 (39.4 - 48.2)	44.0 (39.5 - 53.8)	0.13
Potassium (mmol/l)	4.4 (4.0 - 5.0)	4.2 (4.0 - 4.6)	0.099
Phosphates (mmol/l)	1.2 (1.1-1.3)	1.1 (1.0 - 1.2)	0.205
LDL (mmol/l)	2.0 (1.6 - 2.60)	2.5 (1.8 - 3.9)	0.013
HDL (mmol/l)	1.0 (0.7 - 1.2)	1.1 (1.0 -1.4)	0.023
Triglycerides (mmol/l)	1.2 (0.8 - 1.8)	1.3 (0.9 - 2.1)	0.836
Total Cholesterol mmol/l)	3.7 (3.1 - 4.4)	4.1 (3.6 - 5.0)	0.113
Serum Folate (nmol/l)	24.5 (16.9 - 30.9)	9.9 (7.3 - 14.1)	<0.001
Fasting Blood Sugar (mmol/l)	4.7 (4.3 - 5.0)	5.4 (4.8 - 5.6)	0.198
C-Reactive Protein (mg/l)	1.5 (0.4 - 4.7)	1.2 (0.6 - 3.8)	0.145
Vitamin B12 (pmol/l)	300.5 (218.9 - 428.2)	313.2 (205.0 - 431.6)	0.557
Sodium (mmol/l)	136.0 (133.0 - 138.0)	137.0 (136.0 - 138.0)	0.086
Magnesium (mmol/l)	0.8 (0.7 - 0.8)	0.8 (0.8 - 0.8)	0.852
Calcium (mmol/l)	2.2 (2.2 - 2.4)	2.2 (2.2 - 2.3)	0.239
Total Energy Intake (Kcal)	1897.0 (1416.3 - 2419.0)	1622.8 (1234.2 - 2592.4)	0.478
Protein Intake (g)	50.1 (35.1 -84.9)	51.4 (31.2 - 76.2)	0.37
% Protein Calorie	11.2 (10.3 - 13.0)	13.0 (8.4 - 17.1)	0.97
Fat intake (g)	33.6 (14.3 -56.8)	40.8 (20.3 - 56.4)	0.627
% Fat Calorie	18.5 (9.2 -25.9)	22.5 (14.6 - 31.3)	0.135
Carbohydrate Intake (g)	356.4 (250.0 - 466.3)	247.9 (165.0 - 473.5)	0.313
% Carbohydrate calorie	71.0 (62.0 - 80.6)	62.9 (58.1 - 76.7)	0.411
Thiamine (mg)	0.7 (0.5 - 1.0)	0.8 (0.6 - 1.1)	0.88
Riboflavin (mg)	1.1 (0.6 - 1.1)	1.0 (0.6 -1.7)	0.765
Niacin (mg)	8.7 (5.9 - 17.8)	11.9 (6.5 - 15.7)	0.941
Vitamin B6 (mg)	0.9 (0.7 - 2.5)	1.2 (0.8 - 2.0)	0.999
Vitamin B12 (µg)	0.9 (0.4 - 1.9)	1.3 (0.4 - 3.1)	0.563
Vitamin C (mg)	48.2 (34.9 - 109.5)	46.7 (28.4 - 94.6)	0.654
Iron (mg)	9.9 (6.0 - 13.5)	9.8 (7.0 - 13.8)	0.35
Zinc (mg)	5.7 (4.5 - 8.1)	7.0 (4.6 - 8.3)	0.681
Folate Intake (µg)	221.5 (137.7 - 400.5)	277.5 (194.0 - 407.8)	0.627

4.4 DISCUSSION

At baseline, the NOURISH study found that all the anthropometric indices (BMI, weight, MUAC, total fat mass and percent fat mass) were reduced among the malnourished participants with the severely malnourished participants had the lowest measurements for these parameters. This is similar to what has been described previously in literature (124,157). The findings in the severely malnourished have not been described before among HIV patients since most studies did not categorize participants by level of malnutrition. Most studies have described the differences in anthropometric measurements between the malnourished and well-nourished HIV infected adults, but have not explored any differences in anthropometry by severity of malnutrition (134,239,262). The very low anthropometric indices among the malnourished could be as a result of a combination of factors including a reduced appetite coupled with lack of food due to inability to work because of the advanced HIV disease(170,280) and increased catabolism (185,281). The body composition findings in the NOURISH study were lower than those reported in similar studies in India (234,239), although the Indian participants had higher BMIs than the participants in the NOURISH study. However, it should bioelectric impedance analysis as a method of body composition analysis has been reported to overestimate body fat among lean or underweight people compared to dual-energy X-ray scanning. (282). This implies that the NOURISH study participants and the Indian studies might have had much less body fat percentage than what is reported.

In the NOURISH study, women had more fat than men, and this was not surprising but has not been reported before. Most studies in HIV have focused on regional fat redistribution and body fat abnormalities in HIV as opposed to the global differences between men and women (283,284). One study described the preferential loss of fat among men compared to women as an effect of HIV (285) or compartmental distribution of fat (286).

Despite the very low anthropometric measurements in the severely participants at baseline, 12 weeks of treatment with ART and RUTF led to significant increases in all the anthropometric measures except for fat-free mass among the severely malnourished participants the received RUTF. These changes are not surprising since similar findings have been previously reported in Kenya (255), but the Kenyan study used a more energy dense locally made therapeutic food compared to Plumpy'Nut. Previous studies have demonstrated minor increases in BMI and weight(135,220,229), while others have shown increases in BMI, weight, MUAC and fat-free mass (117,239).

At the end of 12 weeks, there were increases in anthropometry and body composition in both supplemented and un-supplemented participants in the NOURISH study in the randomization studies. The absolute and percent changes were in the RUTF arm were higher than the NO RUTF arm.

These findings are consistent with what has been reported in other studies. (117,135,229,234,239) Swaminathan found that nutritional supplementation increased weight by 0.9 kg and BMI by 0.4. In our study, we found that in the ART experienced group, RUTF participants had a 2.5 kg in weight and a 1.1 increase in BMI, while in the MAM-ART naïve, and the RUTF participants experienced a 4.0 kg increase in weight and 1.5 increase in BMI. Evans and other (117) showed a 12.7% increase in weight and 7.8 % increase in BMI in their study, while the NOURISH study found an 8.2% increase in weight and 8.4% increase in BMI among the ART naïve participants, and a 7.4% increase in weight and 4.7% increase in BMI in the ART experienced participants. These differences in effect might be due to the fact that the participants in the Swaminathan and Evans study received 400 kilocalories per day for 6 months, while the NOURISH participants received 1000 kilocalories per day for 3 months . It should also be noted that the baseline BMIs for the Swaminathan's and Evans' study participants were above 20, while in the NOURISH study, the baseline BMI were below 18.5.

Increase in fat free mass a common finding in other supplementation studies (114,117,135,233,239), however this was not the case in the NOURISH study. Some of the studies that reported increase in fat free mass had longer

supplementation periods, while others included participants with BMIs >20 or the study reported increased physical exercise. The longer supplementation period and increased physical exercise could explain the increase in fat free mass which was not observed in NOURISH. Ndekha and others (135) reported minor but statistically significant increases between the participants that received RUTF and corn soy blend, a nutritional supplement almost similar to RUTF in nutrient composition. These observations imply that the observed increases in weight and BMI in the NOURISH study were due to increases in fat mass and not lean mass.

In the MAM ART naïve participants, the men had higher absolute and percentage increase in fat mass and percentage fat mass. The RUTF men had a 327% increase in percent fat and 304% increase in fat mass compared to a 79.5% increase in percent fat and 41.2% increase in fat mass among the women that received RUTF. Among the NO RUTF, then men had 60% increase in fat mass and a 62.5% increase in percent fat mass compared to the 27.7% and 17.6% increases respectively among women. This is a novel finding that has not been reported before and further studies are needed to evaluate these changes.

Like many studies, NOURISH demonstrated better changes in anthropometry and body composition in the participants that received RUTF, but did not demonstrate statistically significant differences in these changes.

At baseline, the median total calorie intake in this study was 1871.7 kcal (IQR 1422.2 - 2421.8). This is lower than the daily recommended intake of 2200 -2500 kcal per day (287), but slightly above the average daily energy intake of Ugandans of 1700 kcal (111) and what has been reported among HIV infected patients in Botswana (288) and India (239) although another study reported higher energy intake (234). Like most of the diets in sub-Saharan Africa (265)(266) the predominant macronutrient taken was carbohydrate, with fat and protein intake very low. These findings are similar to what Mupere found in his study about nutrient intake among patients with tuberculosis(165), and what has been reported from large national surveys in sub-Saharan Africa (267)(268). Additionally, there was inadequate intake of folate, vitamin B12, vitamin C, zinc, thiamin and niacin, but adequate intake of vitamin A, iron, riboflavin and vitamin

B6. This is in reference to the recommended daily allowances for these micronutrients (289). The available literature indicates adequate intake of micronutrients by HIV infected people (290) (291), but these were studies conducted in the United States of America which has a completely different dietary patterns from Uganda. The few micronutrients that were taken in adequate amounts might be due to food fortification of the local foods with these micronutrients since there is increased fortification of foods with these micronutrients (111).

Like in all supplementation studies (234,239), all malnourished participants that received RUTF had an increased nutrient intake after 12 weeks of nutritional supplements in the NOURISH study. However, there was a reduction in carbohydrate intake and this might be due to the fact that the RUTF used is known to cause stomach filling. Unfortunately, there are no studies that have reported changes in carbohydrate intake following nutritional supplementation, therefore the NOURISH study provides a beginning source of data for such studies.

Although the level for most of biomarkers for all the malnourished participants were within normal ranges at baseline, they were mostly on the lower end of the normal range and a big proportion of participants in all the study groups had less than normal ranges of these biomarkers including HDL, total cholesterol, calcium and fasting blood sugar. Low levels of HDL, LDL, but high triglycerides have been reported in HIV infected patients regardless of nutrition status (292–294). These alterations in lipids has been attributed to the infection of T cells by HIV which leads to production of cytokines that affect lipid metabolism(295). The low sodium levels found in the severely malnourished participants was an incidental finding since sodium is regulated by homeostasis. Other studies have reported low levels of several biomarkers in HIV infected patients including vitamin B12, folate and phosphate and potassium levels (183)(296); low serum phosphate levels(161)(230)(160) . However, in the NOURISH study, we found normal levels of Vitamin B 12 and folate, similar to one study which reported normal levels of these biomarkers (297), but the study population were Caucasian. Large population studies are needed to verify this funding. The findings together

reinforce the observation that advanced HIV infection and malnutrition are associated with reduced serum levels of biomarkers of nutrition (292).

After 12 weeks of nutritional supplementation, there were changes in the serum lipids with normalization of HDL, increases in LDL and total cholesterol, but reductions in triglycerides. The changes were more marked in the RUTF participants especially in the ART naïve patients; in the ART experienced participants, there were no changes in total cholesterol, but rather a high drop in the triglycerides and moderate increases in HDL and LDL. Swaminathan reported a reduction in triglycerides in both supplemented and none supplemented participants (239), but did not report on HDL, LDL and total cholesterol. Two studies showed that ART restores the pre-infection levels of LDL and total cholesterol, but not HDL (292,298). With the exemption of the changes in HDL, it is not clear whether the observed changes in the NOURISH study were due to nutritional supplementation or introduction of ART to the participants. Longitudinal studies involving ART naïve participants would be of use to explore the effect of nutritional supplements alone, unfortunately with the policy change of treating every identified HIV infected patient, this is no longer be possible.

The NOURISH study also demonstrated an increase in the serum folate levels in the RUTF participants and a reduction in the No RUTF participants. ART has been shown to interact with folate metabolism, which is reversed with supplementation (299). Therefore the reduction in serum folate among the participants that did not receive RUTF was due to the effects of ART on folate metabolism. These effects are cancelled by the RUTF which had very high concentration of folate.

4.4.1 Conclusion

Supplementation with RUTF is beneficial to malnourished HIV infected adults in Uganda. Supplementation with RUTF led to higher absolute and proportional changes in the anthropometry, biomarkers of nutrition and nutrient intake of malnourished HIV infected adults in Uganda. Although most of the differences were not statistically significant, this was not unique to NOURISH as most of the nutritional interventional studies did not demonstrate statistical significance and

was partly due to the small sample size. However, the magnitude of the changes in the RUTF supplemented participants were large enough to indicate a beneficial effect of RUTF supplementation in malnourished HIV infected adults.

The small sample size, and did not allow for multivariate analysis as non-parametric methods were used in analysis, however the findings provide a basis for a larger study. A larger study that allows for multivariate analysis including adjusting for confounding may provide stronger evidence on the effect of nutritional supplementation on nutritional outcomes among malnourished HIV infected adults.

Chapter five will explore the effects of nutritional status and nutritional supplementation on immune responses among HIV infected adults.

CHAPTER FIVE

EFFECT OF NUTRITIONAL STATUS AND NUTRITIONAL SUPPLEMENTATION ON IMMUNE RESPONSES AMONG HIV INFECTED ADULTS IN UGANDA

5.1 INTRODUCTION

5.1.1 HIV and the Immune system

The human immunodeficiency virus (HIV) is a member of the retrovirus family and the genus of lentiviruses (7, 8), and consists of two types; HIV1 and HIV2 (8). Over 40 million people worldwide are living with HIV infection and nearly 25 million of these are in sub Saharan Africa (1, 221).

The HIV virus specifically infects variety of immune cells through specific target cell receptors; in macrophages and CD4 T cells, infection occurs through an interaction between viral surface glycoprotein (gp 120) and CD4 and the chemokine receptors CCR5 or CXCR4 (20). The HIV virus uses seven steps to infect and replicate in these cells (Figure 5.1). Initial infection is usually followed by a period of latency during which the virus undergoes dormancy for a varying period of time in different individuals and remains undetected with no expression of symptoms of infection (29, 222–224). If left untreated, HIV gradually takes a toll on the immune system affecting and weakening it (67) and this leads to increased susceptibility to other infections such as tuberculosis and meningococcal infections (69,70).

5.1.1.1 Innate Immune responses to HIV Infection

Major focus has been on the T cell response to HIV infection, but there are other cells in the cellular immunity, especially the cells of innate immunity that may be involved in the immune response to HIV.

5.1.1.1.1 Natural Killer cells

Natural killer (NK) cells are lymphocytes of the innate immune system; they represent 10% of the cells in the total peripheral blood mononuclear cell (MNC) population of circulating human lymphocytes. NK cells are also present as resident

cells in the vaginal, uterine and gut mucosa; forming a rapid first line of defense against incoming pathogens (304). NK cells are widely known for their roles in tumor immunity but have also demonstrated effector cell functions in other pathogenic infections. NK cells act rapidly and do not require prior stimulation to perform their effector functions (305). Morphologically, NK cells can be described as large, granular, bone marrow-derived lymphocytes and phenotypically, they express CD56 and CD16 on the cell membrane (306). NK cells can either be labelled “cytotoxic” (CD56^{dim} NK cells), predominantly producing perforin and granzyme B; or “immune-regulatory” (CD56^{bright} NK cells), secreting IFN- γ , TNF- α , IL-10, IL-13 and GM-CSF (307).

NK cells activities depend on the presence of activating or inhibitory signals. They express cell-surface receptors for self HLA class I, ligation of which transduces inhibitory signals to the NK cell. However, HLA class I expression is frequently downregulated on virus-infected and tumor cells, allowing them to escape recognition by cytotoxic T cells, but this leaves them visible to NK cells. Mature NK cells, in the absence of their self-HLA ligand, become sensitive to the presence of activating ligands (induced or altered self) expressed by virally infected or tumor transformed target cells. NK cells activating receptors include CD16 (Fc γ IIIa) and natural toxicity receptors (NCR) while inhibitory receptors consist of Killer-cell immunoglobulin-like receptors (KIRs) and CD94/NKG2 heterodimers. These activating and inhibitory receptors will usually act antagonistically depending on the expressed immune signals.

During acute HIV-1 infection, there is increase of NK cells associated with the expansion of CD56^{dim} NK cells and early depletion of the CD56^{bright} NK cells (308), but with time the CD56^{dim} are also depleted leading to loss of function of the NK cells (308). The decrease in cytotoxic NK cells is a result of the reduced expression of activating receptors (aKIRs and NCRs) (308), an increase in expression of inhibitory NK cell receptors and reduced secretion of cytokines (IFN- γ , TNF- α and GM-CSF) (309). High and chronic viremia dampens NK cell activity by impairing their antiviral functioning, ultimately contributing to the disease progression (310). Anti-retroviral therapy is associated with restoration

towards normal NK cell subset distribution, suggesting that the impact of HIV-I on NK cell distribution can be reversible [43].

Although NK cells have been shown to restrict viral replication by producing CC chemokines (264, 265), their numbers reduce with HIV disease progress (313–315). Additionally, their function is reduced by increased viral load (312), just as their ability to produce IFN- γ is reduced with progression of disease and increased viremia (269, 270). This means that, their role in the immune response is mainly during early infection when there is low viremia, but is not restored by ART (318).

5.1.1.1.2 $\gamma\delta$ T cells

$\gamma\delta$ T cells are comprised of three major subsets V δ 1, V δ 2 and V δ 3 T cells, with V δ 2 T cells being the most predominant subset in human peripheral blood comprising 1-8% of peripheral T cells in healthy adults (319). $\gamma\delta$ T cells differ from $\alpha\beta$ T cells in that they recognize non-peptide antigens in an MHC-unrestricted manner; they can be activated without the need for prior antigen priming; and they respond by killing target cells, releasing cytokines and activating other cells.

Changes in the peripheral distribution of $\gamma\delta$ T cells has been reported, with an expansion in the V δ 1 cells in HIV infection (320–323), and a reduction in the V δ 2 cells (274, 276, 277). The $\gamma\delta$ cells have a cytotoxic activity against HIV infected cells and limit infection of CCR5 expressing CD4+ T cells by production of CCR5-binding chemokines (278, 279). $\gamma\delta$ T cells produce perforins and cytokines IFN- γ and TNF- α (280, 281).

a. V δ 1 T

V δ 1 TCRs are diverse and can recognize the stress-inducible proteins MICA and MICB, which are expressed by some tumor and virus-infected cells (329) glycolipid antigens presented by CD1c (330) and CD1d (284, 285) and the algal protein phycoerythrin (333). In addition to the TCR, V δ 1 T cells can be activated via ligation of other stimulatory receptors, including NKG2C, NKG2D, NKp30, toll-like receptors and the β -glucan receptor, dectin 1 (334–337). Upon activation, V δ 1 T cells proliferate, release cytokines such as IFN- γ , TNF- α and IL-17,

chemokines such as CCL3, CCL4 and CCL5, and they can kill CD4⁺ T cells in vitro (338). V δ 1 T cell numbers are significantly increased and sustained in patients with HIV irrespective of ART. This increase has been attributed to microbial translocation in a study in SIV infected Rhesus Macaques (339). V δ 1 T cells can lyse HIV-infected as well as uninfected CD4⁺T lymphocytes and other cells, resulting in immunopathology and AIDS in HIV-1–infected individuals (340). HIV infection affects the cytolytic activity of $\gamma\delta$ T cells (341), proliferation and production of Th1 cytokines (342,343).

b. V δ 2 T cells

V δ 2 T cells represent the largest $\gamma\delta$ T cell population in blood and their TCRs display specificity for pyrophosphate antigens produced by some bacteria and protists, such as 4-hydroxy-3-methyl-but-2-enyl pyrophosphate, an intermediate of the non-mevalonate pathway of isoprenoid synthesis (344). V δ 2 T cells have been reported to quickly expand during microbial infections both locally and systemically (345).

Activated V δ 2 T cells produce significant amounts of pro-inflammatory cytokines such as IFN- γ TNF- α , IL-17 and IL-21 important effectors of viral clearance. V δ 2 T cells also promote the maturation of dendritic cells into professional APCs and the maturation of B cells into antibody secreting plasma cells (346). The interplay between V δ 2 T cells and DCs also further increases V δ 2 T cell production of IFN- γ (347). During an active HIV-1 and HIV-2 infection, V δ 2⁺ T cells are significantly diminished, leading to increased susceptibility to opportunistic infections and progress of HIV disease (323,348).

c. V δ 3 T cells

$\gamma\delta$ T cells that express the V δ 3 T cell receptor constitute a minute population of V δ T cells compared to the V δ 1 and V δ 2 T cell populations (349). The antigens recognized by the V δ 3 TCR remain to be identified, but V δ 3 T cells that recognize HLA-A2 (350) and CD1d (350) have been reported. V δ 3 T cells are expanded in patients with cytomegalovirus infection (303, 304). V δ 3 T cells have been demonstrated to release T helper 1, T helper 2 and T helper 17 cytokines coupled

with a role in facilitating dendritic cell maturation (353). This ability to recognize different antigens, unique functionality, anatomical localization and cytokine productivity further expound on the importance and roles of non- $\alpha\beta$ T cells, such as the $\gamma\delta$ T cells, in the immune system.

5.1.1.1.3 Invariant Natural Killer T cells (iNK T cells)

iNK T cells are a heterogeneous group of T cells that recognize glycolipid antigens presented by the MHC class I-like molecule CD1d. Two classes of iNK T cells are found in humans and mice. Type 1 or invariant NKT (iNKT) cells express a TCR composed of an invariant TCR α -chain ($V\alpha 24$ -J $\alpha 18$ in human and $V\alpha 14$ -J $\alpha 18$ in mice) which pairs with a limited number of β -chains, whereas type 2 NKT cells express a diverse array of TCRs that recognize CD1d. Type 1 and type 2 NKT cells also express a number of NK cell stimulatory receptors, such as NK1.1 in mice and NKG2C and NKG2D in humans. The semi-invariant TCR on iNKT cells recognizes a number of self and microbial glycosphingolipids, however, most of the understanding of NKT cells comes from studies of murine and human iNKT cells stimulated with the xenogeneic glycolipid, α -galactosylceramide (α -GC). Upon activation with α -GC in vitro, iNKT cells kill target cells and secrete a diverse range of growth factors and cytokines. iNKT cells are notorious for their ability to produce Th1 (IFN- γ and TNF- α), Th2 (IL-4, IL-5 and IL-13), Th9 (IL-9), Th17 (IL-17A and IL-22) and regulatory T cell (IL-10) cytokines, sometimes simultaneously [154, 155]. Cytokines released by iNKT cells contribute to the activation of T cells, NK cells, macrophages and suppression of functions of neutrophils and MDSC. iNKT cells can also promote the maturation of dendritic cells into APCs and the maturation of B cells into antibody-producing plasma cells. iNKT cells comprise subsets that present as $CD4^+$, $CD4^+CD8^-$ or $CD8^+$ and are characterized by high expression of CXCR3 and CXCR4 coupled with an effector/memory phenotype (354). A study by Van der Vliet and others (355) on iNKT cells and HIV infections demonstrated that iNKT cells are greatly depleted during infection from first year of seroconversion up to five years into the infection suggesting their active role in HIV disease progression and pathogenesis.

5.1.1.1.4 CD56⁺ T cells

A subset of human T cells that expresses CD56 and a variable number of NK cell stimulatory and inhibitory receptors on their cell surfaces are sometimes termed as natural T cells. CD56 expression by T cells confers on them the ability to be activated by TCR-independent stimuli, including cytokines and NK cell ligands, in addition to specific antigen recognized by the TCR. CD56⁺ T cells express memory phenotypes and respond more rapidly than CD56⁻ T cells to stimulation. Compared to CD56⁻ T cells, CD56⁺ T cells display enhanced cytotoxic activity against a broader range of target cell types and more rapid and potent Th1 and Th2 cytokine production. CD56⁺ T cells generally account for less than 5% of peripheral T cells but they accumulate in the human liver and intestine, representing up to 50% of hepatic T cells. CD56⁺ T cells have also been well characterized by their role in tumor cell lysis following activation by IL-2. Almehmadi and Flanagan have also demonstrated the role of these cells in immunity against CMV (356). The cells produce cytokines such as IFN- γ (357). There is scanty information or studies directly linking CD56⁺ T cells and HIV infection, however a study in China demonstrated that HIV-I infected children infected through mother to child transmission had a dramatic increase in the CD56⁺ T cell population, this was attributed to the immune mechanisms aimed to limit the transmission of maternal HIV hence curbing the spread of virus or slowing disease progression (358)

5.1.2 Rationale for immunology studies

Despite the fact that many studies have described the effects of malnutrition on immunity and the cellular immune responses to HIV, few studies have described the combined effect of HIV and malnutrition on the cellular immune responses, as well as the effect of nutritional supplementation on these responses. Additionally, most of the studies describing the effect of malnutrition on the immune system among HIV infected patients have been in children.

5.1.2.1 Aim

The aim of the immunology section was to describe the effects of HIV infection, malnutrition and nutritional supplementation on circulating phenotypes and functions of innate T cells among adults in Uganda.

5.1.2.2 Objectives

- I. To describe the effect of HIV on the frequencies and numbers of innate T cell phenotypes in HIV infected adults in Uganda.
- II. To investigate the effects of nutritional status on the frequencies and numbers of innate T cell phenotypes and functions (cytotoxicity and cytokine production) in HIV infected adults in Uganda.

5.2 METHODOLOGY

This was a longitudinal study with interventional and observational aspects. Details of the study design are described in Chapter Two.

The study enrolled HIV infected adults in the following categories: Well-nourished ART naïve (WN); moderately malnourished ART naïve (MAM); severely malnourished ART naïve (SAM), and ART- experienced malnourished (ART). The listed categories were assigned according to the participant's BMI at the time of enrolment. The well-nourished participants had a BMI greater than 18.5; the moderately malnourished participants had a BMI between 18.5 and 16.5, while the severely malnourished participants had a BMI less than 16.5.

All severely malnourished participants received Plumpy'Nut, a ready to use therapeutic food (RUTF) used to treat malnutrition among malnourished HIV infected persons. The moderately malnourished participants, and the ART experienced participants were randomized to either receive the RUTF or not, while the well-nourished participants did not receive any nutritional supplementation. The ART naïve participants were all initiated on HIV treatment following the Ministry of Health HIV treatment guidelines in Uganda. All participants were followed up for 12 weeks.

The study also included HIV negative controls. These were known to be HIV negative, and did not receive any intervention. They provided blood samples for PBCM isolation at one time point, and their immune parameters were also used to compare with the HIV infected persons only at baseline. The demographic characteristics of these HIV negative controls are found in chapter three.

5.2.1 Outcomes of Interest

The study had a number of outcomes of interest, but this chapter is concerned with the immunological outcomes of interest. These included:

- I. The median differences in the lymphocyte subset frequencies and numbers among the study groups at baseline.

- II. The median differences in the cytotoxicity and cytokine production in the different study groups at baseline.

5.2.2 Materials

Details of the materials used in the immunology experiments are found in appendix four.

5.2.3 Laboratory procedures

5.2.3.1 Preparation of peripheral blood mononuclear cells (PBMC)

The PBMCs were isolated in a biosafety level three (BSL3) laboratory at the Medical Research Council immunology laboratories in Uganda and all the other immunology experiments were done in a biosafety level two laboratory at the Trinity Translational Medicine Institute (TTMI) at St. James's Hospital in Trinity College Dublin. All laboratory processes involved in this laboratory are in a highly supervised and monitored environment. These measures are taken to ensure safety and sterility in the laboratory.

5.2.3.1.1 Isolation

Venous blood was collected into an Acid Citrate Dextrose (ACD) bottle and PBMCs isolation was done within 4 hours of collecting the blood by density gradient method as detailed below; the collected venous blood was diluted 1:1 with phosphate buffered saline (PBS) containing 1% foetal calf serum (FCS: heat-inactivated for 30 min at 56°C) and carefully layered onto Lymphoprep in a ratio of 2:1. It was then centrifuged for 25 min at 400 x g (1,410 rpm in an Eppendorf 5810 centrifuge) with the brake off (minimum deceleration). The components of blood were separated according to their density during the centrifugation as shown in the figure 5.6. The plasma (top) layer was removed and discarded, and the buffy coat (cloudy layer that sits on top of the Lymphoprep) plus the Lymphoprep layer (in order to obtain all the buffy coat) was collected into a clean tube using a sterile Pastuer pipette. Care was taken to ensure that none of the red cell pellet was taken. The buffy coat layer (which contained the PBMCs) was

washed twice by topping it up with PBS + 1% FCS, mix and centrifuged for 8 min at $450 \times g$ (1,500 rpm in an Eppendorf 5810 centrifuge) with the brake on. The supernatant was discarded and the pellet resuspended in a small amount of complete RPMI medium (RPMI 1640 containing 25 mM HEPES, 2 mM L-glutamine, 50 $\mu\text{g}/\text{ml}$ streptomycin, 50 U/ml penicillin and 10% heat-inactivated FCS).

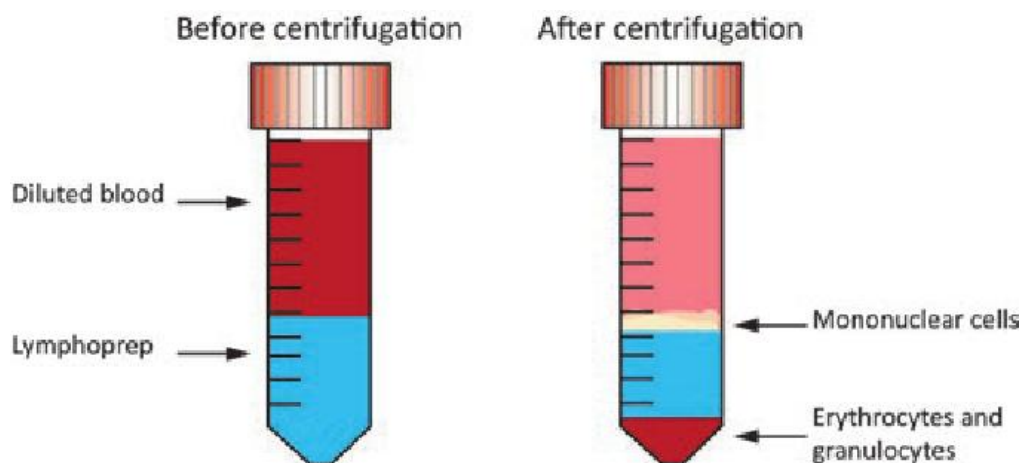


Figure 5.6: Separation of blood components by density gradient

Centrifugation: After careful layering of the diluted blood onto lymphoprep, the blood was centrifuged and the different components will separate according to their density gradient. (www.researchgate.net).

5.2.3.1.2 Counting of cells

The cells were then counted by adding 10 μl cell suspension to 190 μl ethidium bromide/acridine orange (EB/AO) and counted on a Neubauer haemocytometer slide. A working solution of EB/AO was made up by diluting a 100 X solution (15 mg AO, 50 mg EB, 1 mL 95% ethanol and 49 mL dH_2O) 1 in 100 in PBS). Under ultraviolet light, ethidium bromide highlights dead cells orange, while acridine orange stains live cells green. The original number of cells (millions) per ml was calculated as the number of cells counted in two groups of 16 small squares divided by 10. The 16 small squares contained a volume of 0.1 mm^3 of cell suspension.

5.2.3.1.3 Storage of PBMCs

Fresh freezing mixture (90% FCS + 10% dimethyl sulphoxide) was made up each time by adding 0.5 ml dimethyl sulphoxide (DMSO) to 4.5 ml FCS and allowing it to cool for about 10 minutes. The resuspended PBMC mixture was centrifuged for 7 min at 450 x g, and the supernatant discarded. The cell pellet was resuspended in 2 ml of freezing mixture and immediately transferred cells to 2 previously-labelled cryovials (PID, date, and number of cells) and frozen at -80°C overnight in a strata cooler. The cells were transferred to liquid nitrogen after 24 hours.

5.2.3.1.4 Shipping of PBMCs to Trinity College Dublin

The frozen PBMCs were then transferred to Trinity College in Dublin, where the immunological experiments were done. The cells were transported by air in liquid nitrogen, and upon arrival in Dublin, they were immediately placed in the liquid nitrogen freezers at TTMI.

5.2.3.1.5 Recovery of PBMC from liquid nitrogen

On the day the experiments were to be done, the PBMCs were removed from the liquid nitrogen and thawed rapidly without allowing them to heat up beyond room temperature and then diluted rapidly in culture medium. This was by continuously shaking the vial containing the PBMCs under a hot tap or a water bath. This helped to transfer the heat throughout the vial. The vial was removed from heat the before it completely thawed (i.e. leave a lump of ice floating in the liquid to ensure that the liquid doesn't heat up too much, which could kill the cells). The thawed cell suspension was then transferred to a universal or Falcon tube using a sterile pastette and complete RPMI medium was added dropwise, while shaking the tube to mix continuously. The tubes were then centrifuged for 10 minutes at 450 x g, supernatant discarded and resuspend pellets in ~2 ml complete RPMI medium. The cells were then counted as described above.

5.2.3.2 Phenotypic analysis of PBMC

Cells were added to labelled flow cytometer tubes (100,000 cells per flow cytometry tube) and pelleted by centrifuging at 450g for 7 minutes. The pelleted

cells were then resuspended in 50 µl Fixable Viability Dye efluor 506 (FVD), which was diluted 1/1000 in PBS, and incubated at room temperature (r/t) for 20 minutes in the dark.

Each participant had 3 experimental tubes, and 5 single-stain control tubes for setting compensations, as well as fluorescent minus one (FMOs) tubes. Table 5.6 details the labelling of the flow cytometer tubes.

Table 5.6: Flow cytometry tubes that were used for each PBMC sample that were enumerated.

A, Control samples – 1 drop of One Comp Beads was used

Tube	FITC	PE	APC	PECy7	PB
1	CD8α	-	-	-	-
2	-	CD3	-	-	-
3	-	-	CD8α	-	-
4	-	-	-	CD4	-
5	-	-	-	-	CD3

B. Test samples – done for each sample

Tube	FITC	PE	APC	PECy7	PB
6	-	-	-	-	-
7	Vδ1	iNKT	CD8α	CD4	CD3
8	Vδ2	CD56	Vδ3	CD19	CD3

Fluorescence-minus-one (FMO) control stains were done, for antibodies that stain few cells, i.e. iNKT, Vδ1, Vδ2, Vδ3 and these were used as negative controls. In an FMO control, all the flouorochromes that contained the panel of antibody stains are included in the tube, except the one being measured. The FMO controls ensure that any spread of the flouorochromes into the channel of interest are distinctly identified.

After incubation with the FVA dye, cells were centrifuged for 7 minutes at 450xg and supernatant discarded. A cocktail of antibodies that had been pre-prepared was added to each tube according to the table above (6, 7 and 8). The cells were then incubated for 15 minutes at room temperature in the dark (covered in tin foil) and washed by adding ~3 ml PBA buffer, centrifuged for 7 minutes at 450 x g, and supernatant discarded. The cells were then resuspended cells in ~0.4 ml 1% paraformaldehyde, covered in tin foil and left in the fridge until the time to acquire on the flow cytometry.

In NOURISH, the flow cytometer used was the BD FACSCanto II from BD Biosciences. Table 5.7 below shows how each cell was identified on flow cytometry and Figures 5.7-5.9 below are a representation of the gating strategy that was used to identify the different immunophenotypes for the NOURISH study.

Table 5.7: The phenotypes that were enumerated in the NOURISH study

Cell	Phenotype
T- Lymphocytes	CD3 ⁺
B- Lymphocytes	CD19 ⁺ CD3 ⁻
NK cells	CD56 ⁺ CD3 ⁻
CD4 ⁺	CD4 ⁺ CD8 ⁻
CD8 ⁺	CD8 ⁺ CD4 ⁻
Double Negatives	CD4 ⁻ CD8 ⁻
Double Positives	CD4 ⁺ CD8 ⁺
NT cells	CD56 ⁺ CD3 ⁺
iNKT	CD3 ⁺ Vα-24Jα18
Vδ1	CD3 ⁺ Vδ1 ⁺
Vδ2	CD3 ⁺ Vδ2 ⁺
Vδ3	CD3 ⁺ Vδ3 ⁺

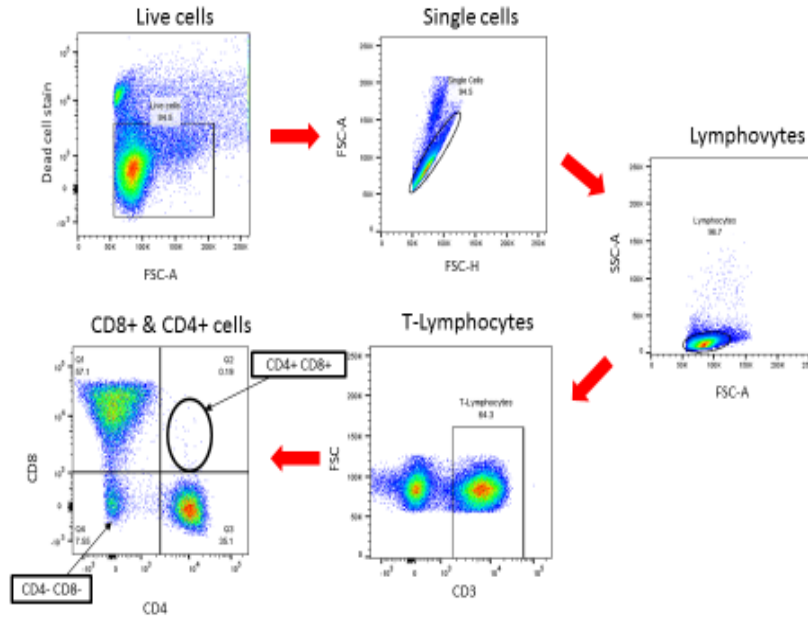


Figure 5.7: Gating strategy for the NOURISH project. Live cells were identified as those that did not stain with the dead cell stain (Top left), then a doublet analysis retained only single cells, (top middle). Lymphocytes were identified by their physical characteristics of side scatter-area against the forward scatter-area dot plots (extreme right), and T-lymphocytes were identified as those that were positive for the CD3⁺ stain (bottom middle). CD4⁺ and CD8⁺ T cells were then identified within gated T cells, by plotting of the stains for each of the cells against each other (bottom left). CD4⁺CD8⁺ and CD4⁻CD8⁻ cells were also identified as shown in the figure.

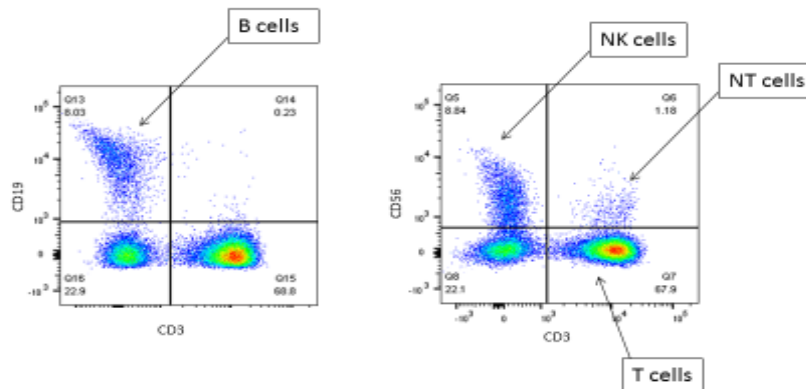


Figure 5.8: Flow cytometric enumeration of cells: B-cells were identified as cells that stained CD19⁺ CD3⁻ (left). NK cells stained positive for CD56⁺ CD3⁻, while NT cells stained CD56⁺ CD3⁺ (right)

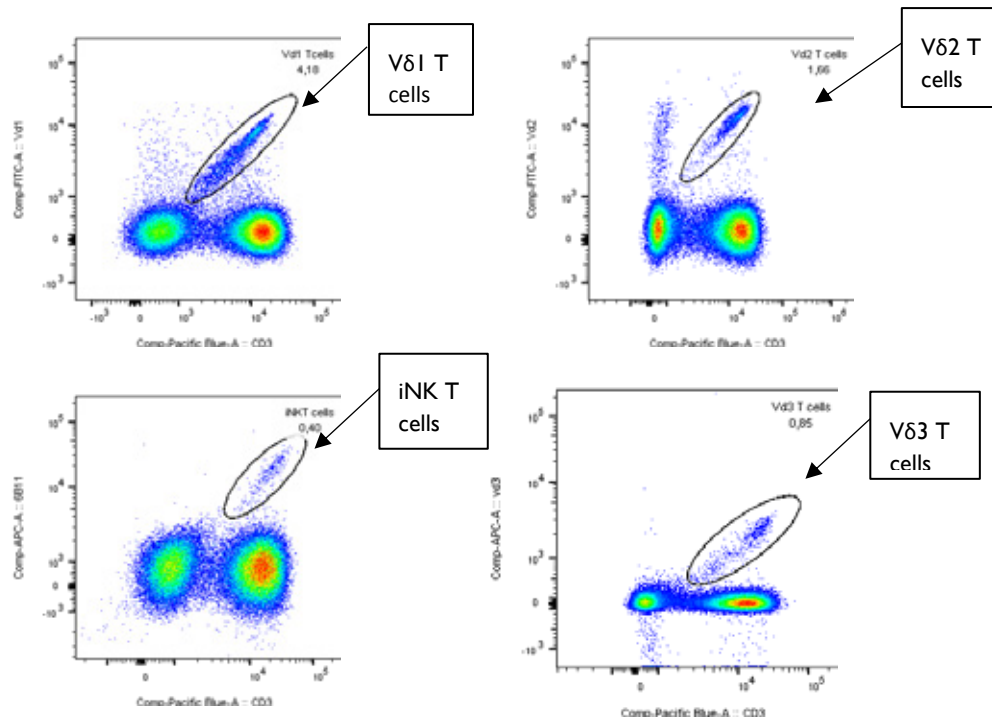


Figure 5.9: Flow cytometric enumeration of Vδ1, Vδ2, Vδ3 and iNK

T cells: All cells were identified as those that stained positive for that particular stain and CD3, since all are T-cells.

5.2.3.3 Measurement of cytolytic degranulation by T cells, NK cells and Vδ1 T cells

Frozen PBMCs were thawed as described above and the final cell pellet was resuspended in 2 ml complete RPMI medium (cRPMI) and cells were counted. 4 wells of a 96-well round bottom plates were labelled as below and each filled with $0.1-0.25 \times 10^6$ cells. One well was labelled 'Medium'; the second well was labelled 'T-cell activator'; the third well was labelled 'PMA/I (phorbol myristate acetate and ionomycin); and the fourth was labelled 'Unstained'. 50 ng/ml PMA (10 μ l of 1/1000 dilution of stock PMA) and 1 μ g/ml ionomycin (0.2 μ l of a 1/10 dilution of ionomycin) were added to the well-marked PMA/I. 5 μ l/ml 'ImmunoCult™ Human

CD3/CD28/CD2 T Cell Activator' (Stem Cell Technologies) were added to the well-marked 'T cell activator'. At time 0, 2 μ l of anti-CD107a-FITC antibody were

to the four wells, and after and after 1 hour, 10 µl monensin were added to all wells.

The cells were then incubated for 4-6 hours in a CO₂ incubator. After the incubation, cells were transferred to appropriately labelled flow cytometry tubes and 2 ml of PBS was added to each tube. The cells were pelleted by centrifugation and then re-suspended in 100 µl of Dead Cell Stain and incubated for 15 minutes in the dark at room temperature. After 15 minutes, the cells were washed and resuspended in 50 µl antibody cocktail by pipetting gently up and down 3 times and transferring them to labelled FACS tubes. The antibody cocktail used for staining the cells is shown in table 5.8. The cells were then vortexed and incubated in dark (covered with tin foil) for 15 minutes at room temperature. The cells were then washed with 3 ml of PBA buffer as described above, and supernatant discarded. They were then resuspended in 0.5 ml of 1% paraformaldehyde (PFA) and kept at 4°C until the time of flow cytometry when they were analyzed to determine the percentage of T cells, NK cells and Vδ1T cells that express CD107a

Table 5.8: Antibodies used to stain cells for cytolytic activity

Antibody stain	µl/tube	µl/ 3tubes
CD56-PE	5	15
CD3-PB	1	3
Vδ1-APC	2	6
CD4-PE/Cy7	1	3
CD8 PerCP	1	3
PBA buffer	40	120

5.2.3.4 Analysis of cytokine production by T cells, NK cells, Vδ1 and Vδ3 T

The steps for stimulating cells for cytokine release were similar to the ones for staining cells for cytolytic activity (section on staining for cytolytic activity). However, for cytokine release, 10 µl of monensin were added to all wells. After

the cells had been stained with the antibodies (table 5.9 has the list of antibodies used), they were fixed with 0.5% of PFA and incubated at room temperature in the dark for 10 minutes.

Table 5.9: Antibodies used to stain cells for cytokine release

Antibody stain	µl/tube	µl/ 3 tubes
CD3-PB	1	3
Vδ1-APC	2	6
CD4-PE/Cy7	1	3
CD8 PerCP	1	3
PBA buffer	45	135

The tubes with the cells were topped up with 2 ml of PBA buffer, centrifuged and supernatant discarded. The cells were then permeabilised by adding 1 ml of freshly made 0.2% saponin to the cells and incubating in dark for 10 minutes at room temperature. 50 µl of cytokine antibody cocktail were then added to the tubes, and later vortexed and incubated for 20-30 minutes in the dark at room temperature. The cytokine antibody cocktail that was used is detailed in table 5.10.

Table 5.10: Cytokine antibodies used in the intracellular staining for cytokine release

Antibody stain	µl/tube	µl/ 3tube
IFN-γ-FITC	2	6
IL-4-PE	8	24
IL-17-APC/Cy5.5	2	6
0.2% saponin	38	114

Cells were washed with PBA buffer as described above, and resuspended in ~0.5 ml 1% PFA and then incubated for 10 minutes in the dark at room temperature. The cells were pelleted by centrifugation and re-suspended in 0.3 ml PBA buffer, then stored in dark overnight in the 4°C fridge and later analyzed by flow

cytometry to determine the % of T cells, NK cells, V δ 1 and V δ 3 T cells that produce IFN- γ , IL-4 and IL-17.

5.2.4 Principles of Flow cytometry

Flow cytometry is a method that analyses or characterizes small particles like cells in liquid suspension. The size of particles analyzed by flow cytometry ranges between 0.2 to 50 μ m. Flow cytometry analyzes the relative size, internal complexity or granularity and fluorescence characteristics of the particles which must be stained with fluorochrome –conjugated antibodies.

A flow cytometer is made up of three subsystems, the fluidics, optics and electronic subsystems. The fluidics subsystem has the tubing apparatus which transports a sample of liquid suspension, the 'sheath fluid' circulating through the flow cytometer. This fluid carries the cell suspension through a narrow channel to the 'interrogation point' where there are laser beams.

The optics subsystem contains a set of lasers, filters, mirrors and photomultipliers. A laser beam from the lasers is used to interrogate or illuminate the cells. This light is scattered by the cell and causes an emission of the fluorescence.

In the electronic subsystem, a series of sensors detect the emitted or scattered light from the optics system, and collect, digitize and convert it into data that can be used to make deductions about the size and granularity of the cells evaluated. This light is detected by sensors either placed directly in front of the laser source (forward scatter channel) or at 90° angle (side scatter channel and the fluorescence channels). The forward scatter gives the relative size of the cell, while the side scatter gives the granularity or complexity of the cell. The higher the side scatter, the higher the granularity, and the higher the forward scatter, the bigger the cell. The fluorescence channels have mirrors and band pass filters that enable collection of fluorescence at a given wavelength. The photomultiplier tubes then amplify the detected signal and send it for digitization and interpretation.

The fluidics and optics are located in the flow cytometer, while the electronics are external devices comprising of a computer and appropriate software. The collected data from the optics system is converted into digital data which is graphically displayed on the computer screen and allows the operator to analyze the data.

5.2.5 Data management and statistical analysis

5.2.5.1 Data management

Data files from the flow cytometer were then transferred to Flowjo software for further analysis. Percentages of each of the phenotypes were obtained from Flowjo software and absolute counts were then calculated from the results of the full blood count for each participant. The absolute counts of the T cells, B cells and NK cells were calculated by multiplying the percentage of respective cell by the total lymphocyte count that was obtained from the full blood count. The absolute counts of the T cell subsets and the innate T cells were calculated by multiplying their percentages with the absolute count of the T cells that had been calculated from the total lymphocyte count.

The data was then transferred to excel spreadsheets for cleaning, and after the cleaning, data was transferred to prism analysis software for statistical analysis.

Frequencies of the different immunophenotypes were represented as means, and comparisons were made between the different study groups as described in the section on measurement of outcome above, using Mann-Whitney non-parametric methods. All means that had a 0.05 P-value were considered to be statistically significant.

5.2.5.2 Statistical analysis

All statistical analyses were done using Prism Graph pad version 5.0. Comparisons between two groups was done using the Mann-Whitney test. Results with a p-value less than 0.05 were considered significant.

For phenotypic analysis, comparison was made between the medians of the absolute counts and frequencies between the different groups under study. For

examples, the medians of the SAM were compared to the medians of the MAM participants. In the randomizations, comparison was done between the medians of the RUTF and the no RUTF groups.

In the functional studies, median proportions of the different cells producing the cytokines or CD104a were compared both at baseline and 12 weeks.

5.3 RESULTS

5.3.1 Study Participants

This section contains the results of the baseline analysis for the immunological studies.

82 participants were included in the baseline studies. The 20 missing participants are due to samples (PBMCs) of 20 participants that were lost in the liquid nitrogen tank at Trinity College Dublin. The details of the 82 participants are showed in table 5.11 Table 5.12 shows the distribution of the randomized participants at baseline and follow-up. At follow-up, the immunology studies had 61 (69%) of the 88 participants that completed the 12 weeks of the NOURISH study. Of the missing 27 participants, 10 participants declined to provide samples for immunology studies at follow-up, and samples for 17 participants were not shipped to Dublin.

These participants had not returned for their week 12 visit by the time of shipping the last batch of samples to Ireland. The ART experienced participants and well-nourished participants had one more participant at 12 weeks than at baseline. These two were part of the participants whose baseline samples were lost in the liquid nitrogen, but had samples at week 12.

Table 5.11: Distribution of participants involved in the immunology studies:

Study group	Enrolled	Included in Immunology study at baseline (% of enrolled)	Included in immunology at 12 weeks
Overall	102	82 (79%)	61 (74%)
Moderately malnourished ART naive	32	26 (81%)	12 (46%)
Severely Malnourished	18	18 (100%)	13 (72%)
Malnourished ART experienced	27	14 (52%)	15 (107%)
Well-nourished	25	20 (80%)	21 (105%)

Table 5.12: Distribution of the randomized participants that participated in immunology studies

Study group	Randomization	Enrolled	Immunology at baseline (% of enrolled)	Included in Immunology at 12 weeks (% of baseline)
Moderately malnourished ART naïve	RUTF	14	10 (71%)	7 (70%)
	No RUTF	18	16 (89%)	5 (31%)
Malnourished ART experienced	RUTF	16	9 (56%)	9 (100%)
	No RUTF	11	5 (45%)	6 (120%)

The immunology studies included 9 HIV negative controls. Of these, 5 were female and 4 were male. Their average age was 32 years (sd 1.5) and they were all well-nourished with an average BMI of 22.4 (IQR 20.5 – 24.5).

5.3.2 Impact of HIV infection and nutritional status on circulating lymphocytes among ART-naïve HIV infected adults

Peripheral blood mononuclear cells (PBMCs) were isolated from 61 ART naïve HIV infected adults (HIV +ve) and 9 HIV negative controls (HIV –ve). Of the ART naïve participants, 40 were malnourished (HIV +ve Mal) and 21 were well nourished (HIV +ve WN). Cells were stained with monoclonal antibodies (mAb) specific for CD3, CD4, CD8, CD19, CD56, and the V δ 1, V δ 2, V δ 3 and V α 24J α 18 TCRs and analysed by flow cytometry.

5.3.2.1 Impact of HIV infection and nutritional status on frequencies of circulating B cells, NK cells and T cells in ART-naïve HIV-infected adults

The ART naïve HIV infected adults (HIV +ve) had lower circulating frequencies of B cells than the HIV negative controls (HIV –ve). However, these differences were not statistically significant except in the malnourished HIV infected adults (HIV +ve mal) (**Figure 5.01**). Similarly, the HIV +ve participants had lower circulating frequencies of NK cells than the HIV –ve controls but this difference was not statistically significant. Nutritional status of the HIV +ve participants did not lead to statistically significant differences (**Figure 5.02**). There were no differences in the circulating frequencies of the T cells of the HIV +ve and HIV –ve participants. (**Figures 5.03**).

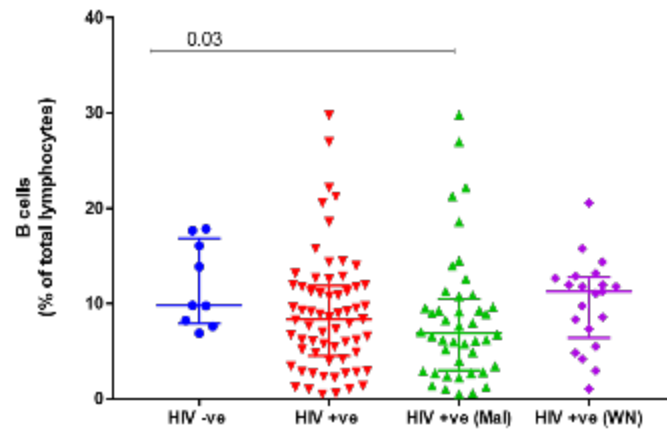


Figure 5.01 Effect of HIV infection and nutritional status on circulating frequencies of B cells : PBMCs were isolated from HIV negative adults (HIV -ve), and ART naïve HIV infected adults (HIV +ve), some of whom were malnourished (HIV +ve Mal) and some were well-nourished (HIV +ve WN). Cells were stained with monoclonal antibodies specific for CD3 and CD19 analysed by flow cytometry. B cells were defined as CD3⁻ CD19⁺ lymphocytes. Scatter plot show frequencies of B cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test. The numbers in the graph show the significant P-value.

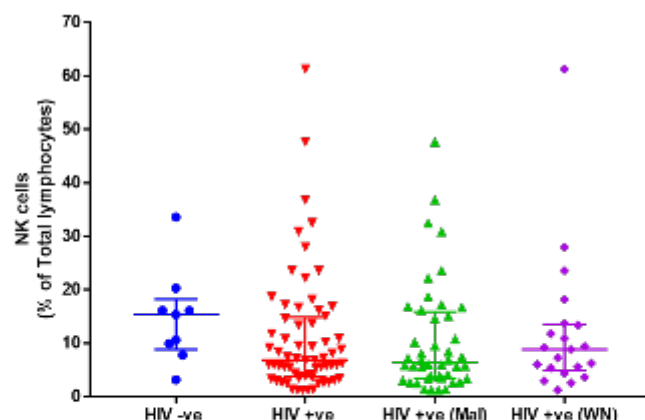


Figure 5.02 Effect of HIV infection and nutritional status on circulating frequencies of NK cells : PBMCs were isolated from HIV negative adults (HIV -ve), and ART naïve HIV infected adults (HIV +ve), some of whom were malnourished (HIV +ve Mal) and some were well-nourished (HIV +ve WN). Cells were stained with monoclonal antibodies specific for CD3 and CD56 analysed by flow cytometry. NK cells were defined as CD3⁻ CD56⁺ lymphocytes. Scatter plot show frequencies of NK cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test.

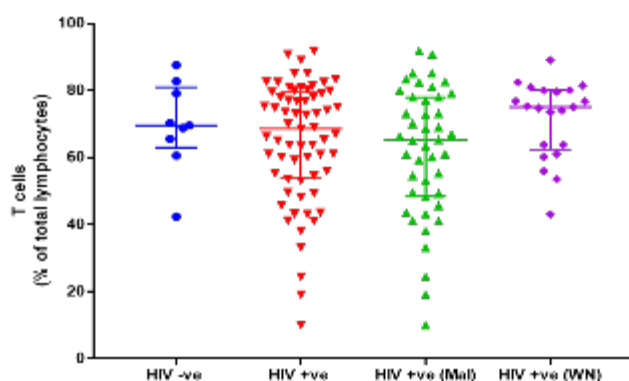


Figure 5.03 Effect of HIV infection and nutritional status on circulating frequencies of T cells: PBMCs were isolated from HIV negative adults (HIV -ve), and ART naïve HIV infected adults (HIV +ve), some of whom were malnourished (HIV +ve Mal) and some were well-nourished (HIV +ve WN). Cells were stained with monoclonal antibodies specific for CD3 analysed by flow cytometry. T cells were defined as CD3⁺ lymphocytes. Scatter plot show frequencies of T cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test.

5.3.2.2 Impact of HIV infection and nutritional status on frequencies of circulating T cell subsets in ART-naïve HIV-infected adults

The CD4⁺ T cells frequencies were significantly lower among the HIV +ve participants compared to the HIV –ve controls. This was irrespective of the nutritional status of the HIV +ve participants (**Figure 5.04**). Conversely, the CD8⁺ T cells frequencies of the HIV +ve participants were significantly higher. This effect was also observed among the malnourished and well-nourished HIV +ve participants (**Figure 5.05**). The HIV +ve participants had higher frequencies of double negative T cells and lower frequencies of double positive T cells, but these differences were not statistically significant (**Figures 5.06 -5.07**)

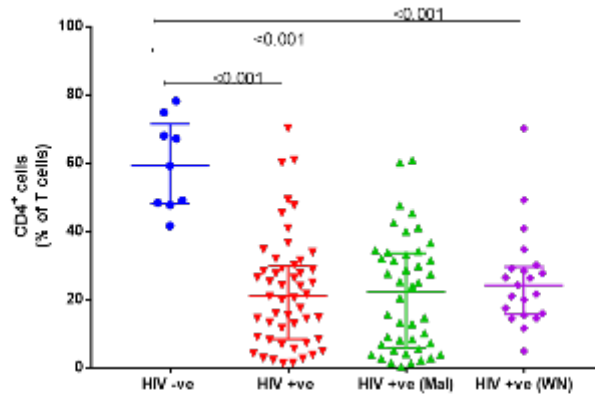


Figure 5.04 Effect of HIV infection and nutritional status on circulating frequencies of CD4⁺T cell : PBMCs were isolated from HIV negative adults (HIV –ve), and ART naïve HIV infected adults (HIV +ve), some of whom were malnourished (HIV +ve Mal) and some were well-nourished (HIV +ve WN). Cells were stained with monoclonal antibodies specific for CD3, CD4 and CD8 analysed by flow cytometry. B cells were defined as CD3⁺ CD4⁺ CD8[–] lymphocytes. Scatter plot show frequencies of CD4⁺T cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test. The numbers in the graph show the significant P-value

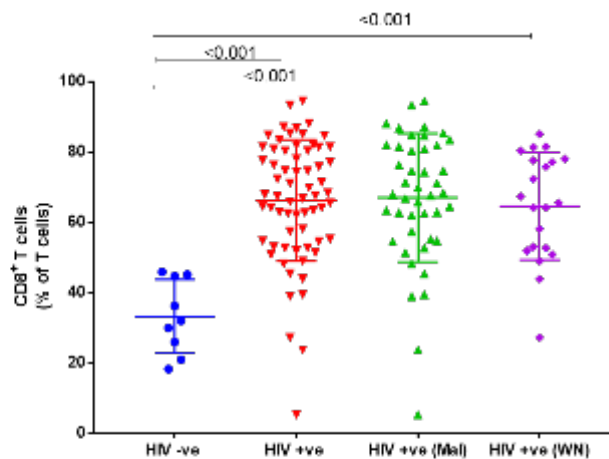


Figure 5.05 Effect of HIV infection and nutritional status on circulating frequencies of CD8⁺T cell : PBMCs were isolated from HIV negative adults (HIV –ve), and ART naïve HIV infected adults (HIV +ve), some of whom were malnourished (HIV +ve Mal) and some were well-nourished (HIV +ve WN). Cells were stained with monoclonal antibodies specific for CD3, CD4 and CD8 analysed by flow cytometry. B cells were defined as CD3⁺ CD4[–] CD8⁺ lymphocytes. Scatter plot show frequencies of CD8⁺T cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test. The numbers in the graph show the significant P-value

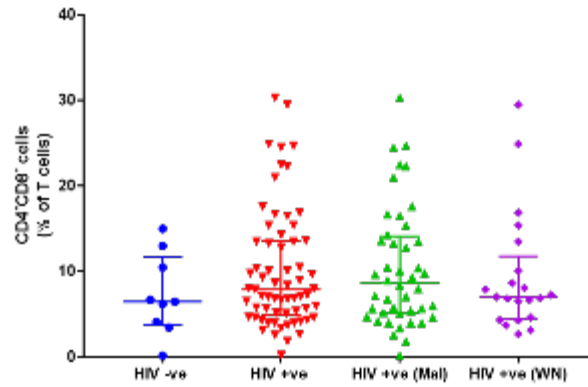


Figure 5.06 Effect of HIV infection and nutritional status on circulating frequencies of CD4⁺ CD8⁺ T cell : PBMCs were isolated from HIV negative adults (HIV -ve), and ART naïve HIV infected adults (HIV +ve), some of whom were malnourished (HIV +ve Mal) and some were well-nourished (HIV +ve WN). Cells were stained with monoclonal antibodies specific for CD3, CD4 and CD8 analysed by flow cytometry. Scatter plot show frequencies of CD4⁺ CD8⁺ T cells present. Error bars show medians and interquartile ranges. Data were compared using the MannWhitney statistical test.

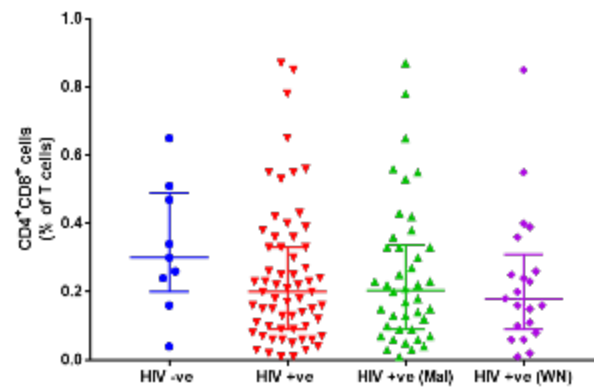


Figure 5.07 Effect of HIV infection and nutritional status on circulating frequencies of CD4⁺ CD8⁺ T cell : PBMCs were isolated from HIV negative adults (HIV -ve), and ART naïve HIV infected adults (HIV +ve), some of whom were malnourished (HIV +ve Mal) and some were well-nourished (HIV +ve WN). Cells were stained with monoclonal antibodies specific for CD3, CD4 and CD8 analysed by flow cytometry. Scatter plot show frequencies of CD4⁺ CD8⁺ T cells present. Error bars show medians and interquartile ranges. Data were compared using the MannWhitney statistical test.

5.3.2.3 Impact of HIV infection and nutritional status on frequencies of circulating T cell subsets in ART-naïve HIV-infected adults

The HIV +ve participants had higher frequencies of circulating Vδ1 T cells than the HIV –ve participants but statistical difference was only found with the malnourished HIV +ve participants (**Figure 5.08**). The frequencies of the circulating Vδ2 T cells were lower among the HIV +ve participants, but this difference was statistically significant with the malnourished HIV +ve participants (**Figure 5.09**). There was no difference in the frequencies of Vδ3 T cells between the HIV +ve participants and the HIV-ve controls (**Figure 5.10**). Additionally, the HIV +ve participants had lower frequencies of iNK T cells, although this difference was not statistically significant with the HIV +ve well-nourished participants. Finally, the circulating frequencies of CD56⁺ T cells were significantly lower among the HIV +ve participants regardless of their nutritional status. (**Figures 5.11 -5.12**).

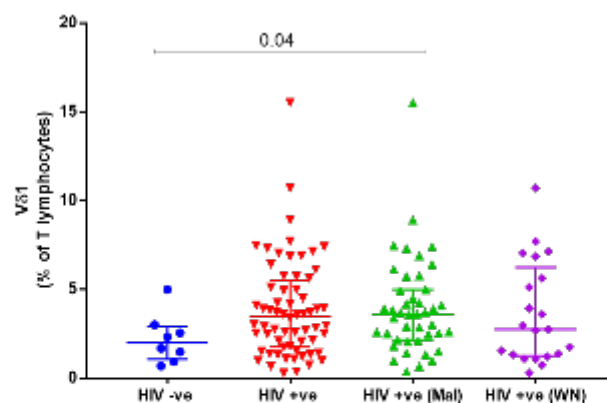


Figure 5.08 Effect of HIV infection and nutritional status on circulating frequencies of Vδ1 cells: PBMCs were isolated from HIV negative adults (HIV –ve), and ART naïve HIV infected adults (HIV +ve), some of whom were malnourished (HIV +ve Mal) and some were well-nourished (HIV +ve WN). Cells were stained with monoclonal antibodies specific for CD3 and Vδ1 analysed by flow cytometry. B cells were defined as CD3⁺Vδ1⁺ lymphocytes. Scatter plot show frequencies of Vδ1 cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test. The numbers in the graph show the significant P-value

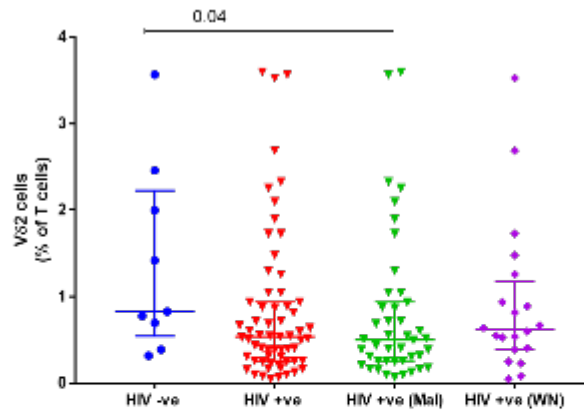


Figure 5.09 Effect of HIV infection and nutritional status on circulating frequencies of Vδ2 cells: PBMCs were isolated from HIV negative adults (HIV -ve), and ART naïve HIV infected adults (HIV +ve), some of whom were malnourished (HIV +ve Mal) and some were well-nourished (HIV +ve WN). Cells were stained with monoclonal antibodies specific for CD3 and Vδ2 analysed by flow cytometry. B cells were defined as CD3⁺Vδ2⁺ lymphocytes. Scatter plot show frequencies of Vδ2 cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test. The numbers in the graph show the significant P-value.

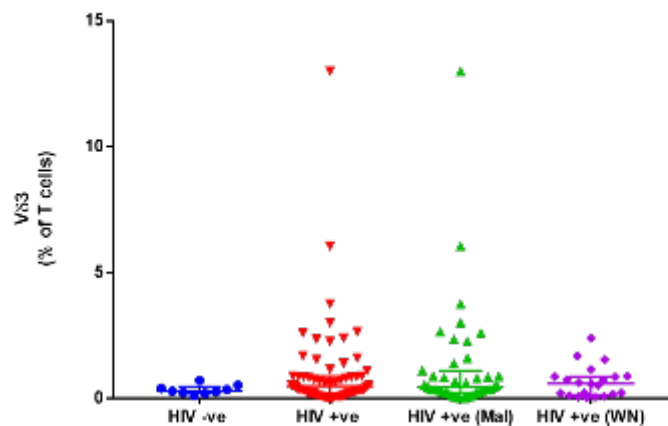


Figure 5.10 Effect of HIV infection and nutritional status on circulating frequencies of Vδ3 cells: PBMCs were isolated from HIV negative adults (HIV -ve), and ART naïve HIV infected adults (HIV +ve), some of whom were malnourished (HIV +ve Mal) and some were well-nourished (HIV +ve WN). Cells were stained with monoclonal antibodies specific for CD3 and Vδ2 analysed by flow cytometry. B cells were defined as CD3⁺Vδ3⁺ lymphocytes. Scatter plot show frequencies of Vδ3 cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test.

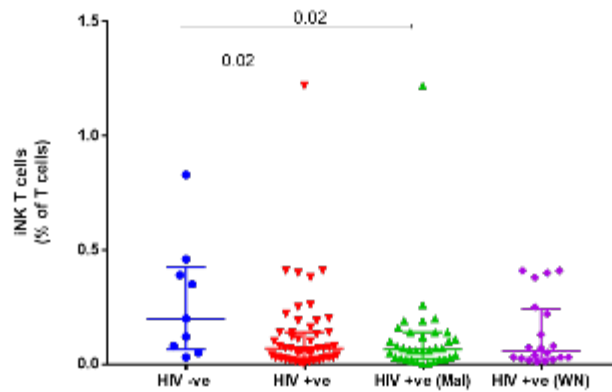


Figure 5.11 Effect of HIV infection and nutritional status on circulating frequencies of iNKT cells: PBMCs were isolated from HIV negative adults (HIV -ve), and ART naïve HIV infected adults (HIV +ve), some of whom were malnourished (HIV +ve Mal) and some were well-nourished (HIV +ve WN). Cells were stained with monoclonal antibodies specific for CD3 and Va24 α 18 analysed by flow cytometry. B cells were defined as CD3⁺Va24 α 18⁺ lymphocytes. Scatter plot show frequencies of iNKT T cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test. The numbers in the graph show the significant P-value

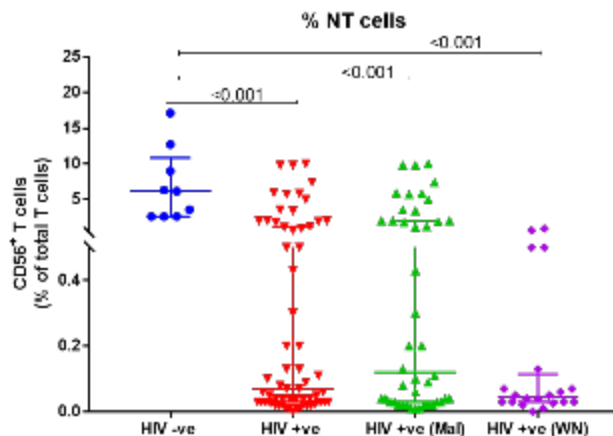


Figure 5.12 Effect of HIV infection and nutritional status on circulating frequencies of NT cells: PBMCs were isolated from HIV negative adults (HIV -ve), and ART naïve HIV infected adults (HIV +ve), some of whom were malnourished (HIV +ve Mal) and some were well-nourished (HIV +ve WN). Cells were stained with monoclonal antibodies specific for CD3 and CD56 analysed by flow cytometry. NT cells were defined as CD3⁺ CD56⁺ lymphocytes. Scatter plot show frequencies of NT cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test. The numbers in the graph show the significant P-value.

Summary:

Compared to the HIV negatives, the V δ 1 T cells were expanded among, while the V δ 2 T cells were reduced in the HIV infected participants. The changes in these two cell lines were similar to the changes in the CD8⁺ T cells and CD4⁺ T cells respectively. All the other cells were reduced among the HIV infected participants compared to the HIV uninfected.

Malnutrition accentuated the effects of HIV on the different cells that were studied.

5.3.3 Impact of nutritional status on circulating lymphocyte frequencies and numbers in ART-naïve HIV-infected adults

Peripheral blood mononuclear cells (PBMCs) were isolated from 61 ART naïve and 14 ART-experienced participants. Of the ART naïve participants, 26 were moderately-acutely malnourished (MAM), 14 were severely acutely malnourished (SAM) and 21 were well-nourished (WN). All the ART-experienced participants were malnourished. Cells were stained with monoclonal antibodies (mAb) specific for CD3, CD4, CD8, CD19, CD56, and the V δ 1, V δ 2, V δ 3 and V α 24J α 18 TCRs and analysed by flow cytometry.

5.3.3.2 Impact of nutritional status on frequencies and numbers of circulating T cells, B cells and NK cells in ART-naïve HIV-infected adults

The frequencies of T cells were found to be similar among the three ART-naïve groups (MAM, SAM and WN), however, the absolute counts of the T cells in SAM patients were significantly lower than the absolute counts in WN participants ($p < 0.05$) (**Figure 5.13**). There were no significant differences in the absolute counts of the T-cells between the MAM and SAM or between the MAM and WN groups. Just like the T-cells, there were no statistically significant differences in the frequencies of the B-cells among the three groups, but the absolute counts of the B-cells in the MAM and SAM patients were significantly lower than the absolute counts in the well-nourished participants ($P < 0.05$) (**Figure 5.14**). There were no significant differences in the frequencies or absolute counts of NK cells in the three groups. (**Figure 5.15**).

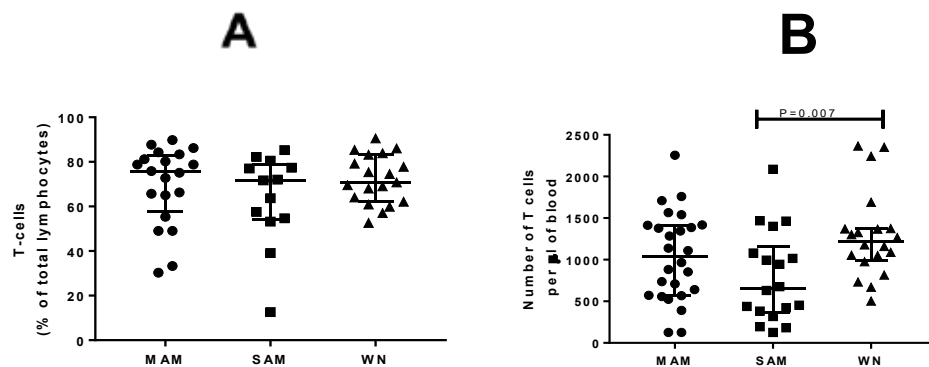


Figure 5.13 Circulating T cell frequencies and numbers in HIV-infected ART-naïve adults of different nutrition status. Cells were stained with monoclonal antibodies specific for CD3 and analysed by flow cytometry. T cells were defined as CD3⁺ lymphocytes. Absolute numbers were calculated from full blood counts taken at the time of blood collection. Scatter plot show frequencies (A) and absolute numbers (B) of T cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test. Numbers in the plots show P values.

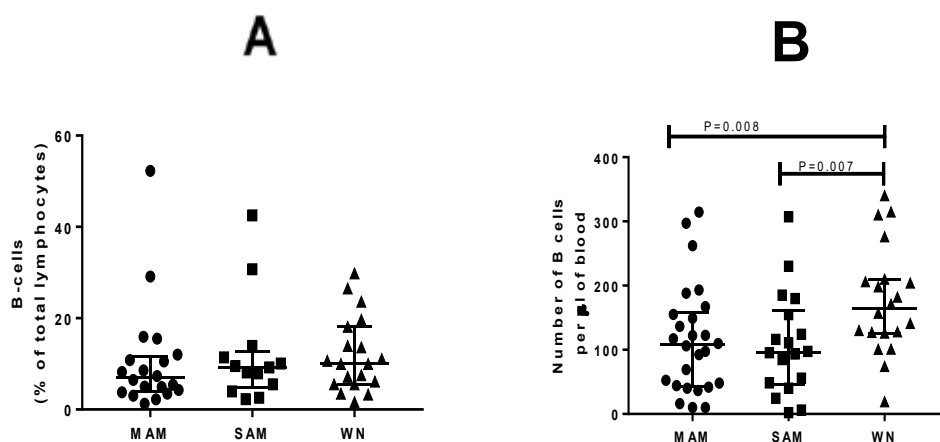


Figure 5.14 Circulating B cell frequencies and numbers in HIV-infected ART-naïve adults of different nutrition status. Cells were stained with monoclonal antibodies specific for CD3 and CD19, and analysed by flow cytometry. B cells were defined as CD3⁺ CD19⁺ lymphocytes. Absolute numbers were calculated from full blood counts taken at the time of blood collection. Scatter plot show frequencies (A) and absolute numbers (B) of B cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test. Numbers in the plots show P values.

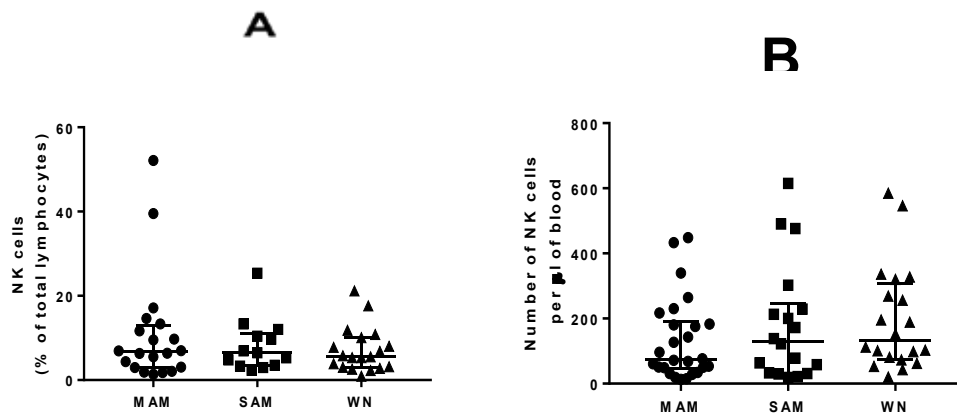


Figure 5.15 *Circulating NK cell frequencies and numbers in HIV-infected ART-naïve adults of different nutrition status.* Cells were stained with monoclonal antibodies specific for CD3 and CD56, and analysed by flow cytometry. NK cells were defined as CD3⁺ CD56⁺ lymphocytes. Absolute numbers were calculated from full blood counts taken at the time of blood

5.3.3.2 Impact of nutritional status on frequencies and numbers of circulating T cell subsets in ART-naïve HIV-infected adults

This section describes the differences in the distribution of the T cell subsets among HIV infected patients of different nutritional status. The severely malnourished participants had the lowest CD4⁺ T cell frequencies. The differences of frequencies between the severely malnourished and the other groups were significantly different ($P < 0.05$). Similarly, the severely malnourished had the lowest CD4⁺ T cell absolute counts compared to the other participants, and the differences of the absolute counts between the severely malnourished and the other two groups were significantly different (**Figure 5.16**).

The CD8⁺ T cell frequencies of the severely and moderately malnourished participants were higher than the CD8⁺ T cell frequencies of the well-nourished participants. The difference between the severely malnourished and well-nourished participants being statistically significant, $P < 0.05$. On the contrary, the well-nourished participants had higher CD8⁺ absolute counts than the MAM and SAM (**Figure 5.17**); with the difference between the well-nourished and severely malnourished being statistically significant.

The frequencies and absolute counts of the CD4⁺ CD8⁺ T cells for all the ART naïve participants were found to be similar (**Figure 5.18**) and this was also true for the CD4⁺ CD8⁺ T cells (**Figure 5.19**).

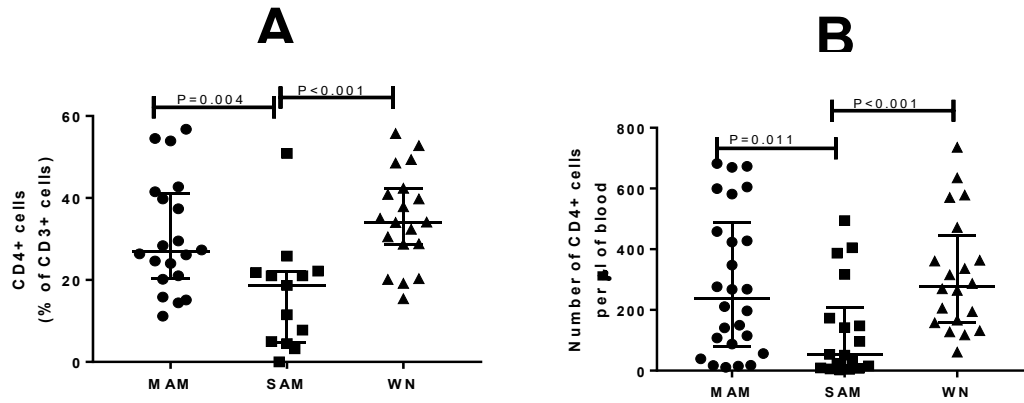


Figure 5.16 Circulating CD4⁺ T cell frequencies and numbers in HIV-infected ART-naïve adults of different nutrition status. Cells were stained with monoclonal antibodies specific for CD3, CD8 and CD4, and analysed by flow cytometry. CD4⁺ T cells were defined as CD3⁺ CD4⁺ CD8⁺ lymphocytes. Absolute numbers were calculated from full blood counts taken at the time of blood collection. Scatter plot show frequencies (**A**) and absolute numbers (**B**) of CD4⁺ T cells present. Error bars show medians and

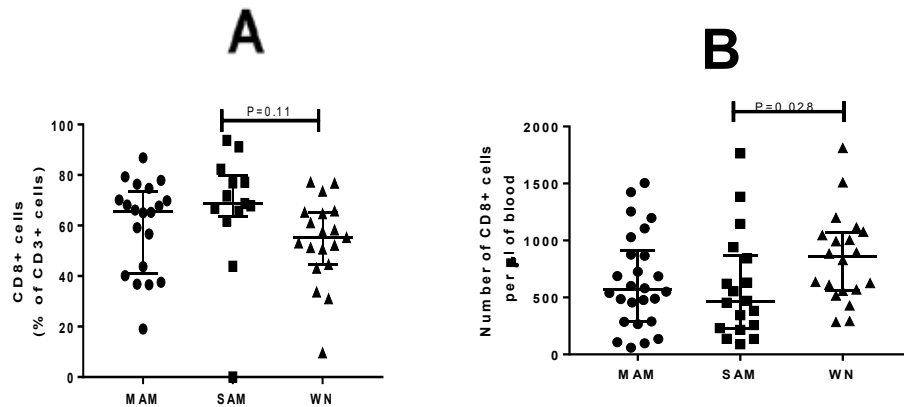


Figure 5.17 Circulating CD8⁺ T cell frequencies and numbers in HIV-infected ART-naïve adults of different nutrition status. Cells were stained with monoclonal antibodies specific for CD3, CD4 and CD8, and analysed by flow cytometry. CD8⁺ T cells were defined as CD3⁺ CD4⁻ CD8⁺ lymphocytes. Absolute numbers were calculated from full blood counts taken at the time of blood collection. Scatter plot show frequencies (A) and absolute numbers (B) of CD8⁺ T cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test. Numbers in the plots show P values.

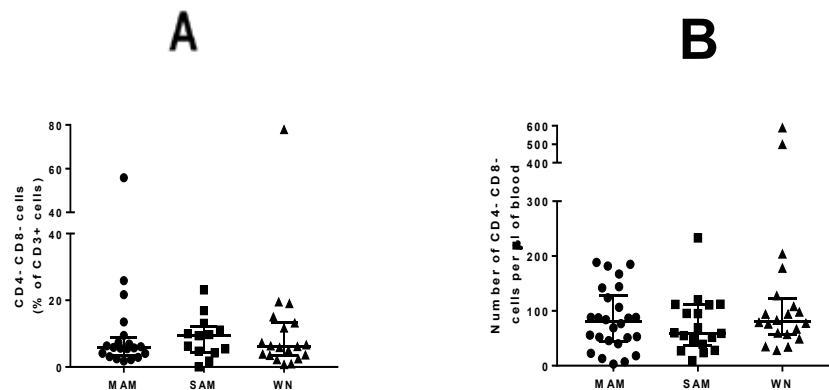


Figure 5.18 Circulating CD4⁻ CD8⁻ T cell frequencies and numbers in HIV-infected ART-naïve adults of different nutrition status. Cells were stained with monoclonal antibodies specific for CD3, CD4, and CD8, and analysed by flow cytometry. CD4⁻ CD8⁻ T cells were defined as CD3⁺ CD4⁻ CD8⁻ lymphocytes. Absolute numbers were calculated from full blood counts taken at the time of blood collection. Scatter plot show frequencies (A) and absolute numbers (B) of CD4⁻ CD8⁻ T cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test.

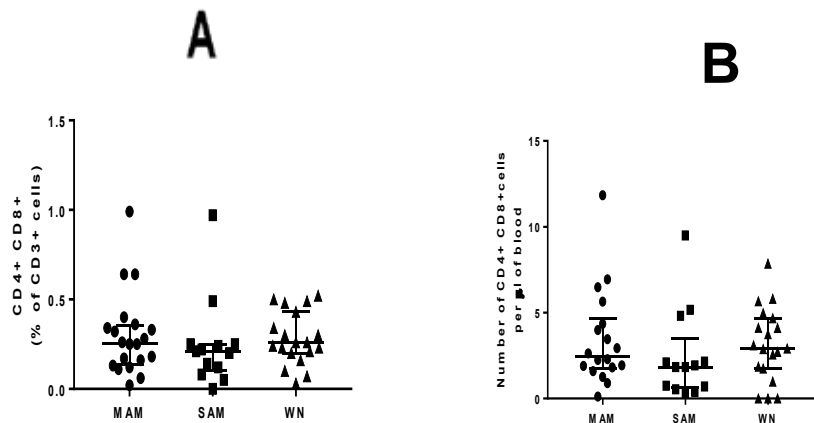


Figure 5.19 Circulating $CD4^+ CD8^+$ T cell frequencies and numbers in HIV-infected ART-naïve adults of different nutrition status. Cells were stained with monoclonal antibodies specific for CD3, CD8 and CD4, and analysed by flow cytometry. $CD4^+ CD8^+$ T cells were defined as $CD3^+ CD4^+ CD8^+$ lymphocytes. Absolute numbers were calculated from full blood counts taken at the time of blood collection. Scatter plot show frequencies (A) and absolute numbers (B) of $CD4^+ CD8^+$ T cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test.

5.3.3.3 Impact of nutritional status on frequencies and numbers of circulating innate T cell populations in ART-naïve HIV-infected adults

Among the ART naïve HIV infected participants, the distribution of the innate T cells was as follows: the moderately malnourished participants had significantly higher frequencies of $V\delta 1$ T cells than the well-nourished participants ($P < 0.05$), but there were no significant differences in the absolute counts of the $V\delta 1$ T cells among the three groups (**Figure 5.20**). The frequencies of the $V\delta 2$ T cells in the three groups were similar. However, the absolute counts of $V\delta 2$ T cells in the moderately malnourished and well-nourished participants were higher than in the severely malnourished participants ($P < 0.05$) (**Figure 5.21**).

There were no significant differences between the frequencies and absolute counts of V δ 3 T cells for the three groups (**Figure 5.22**). This was also observed among the iNKT cells (**figure 5.23**) and CD56⁺ T cells (**Figure 5.24**).

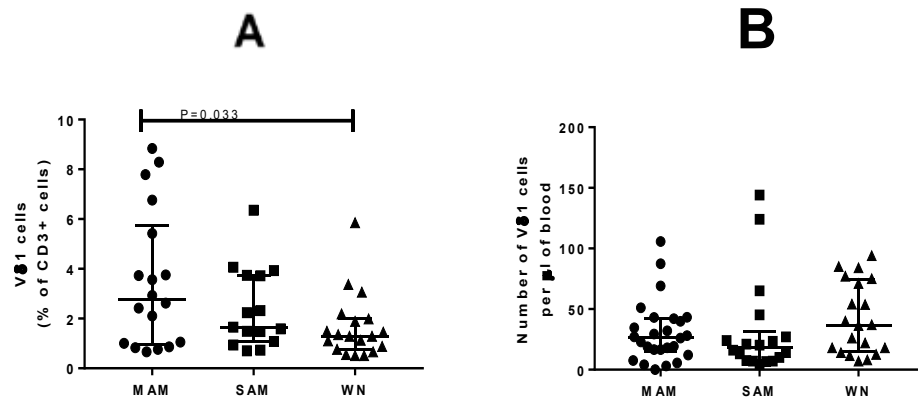


Figure 5.20 Circulating V δ 1 T cell frequencies and numbers in HIV-infected ART-naïve adults of different nutrition status. Cells were stained with monoclonal antibodies specific for CD3 and V δ 1 and analysed by flow cytometry. V δ 1 T cells were defined as CD3⁺ V δ 1⁺ lymphocytes. Absolute numbers were calculated from full blood counts taken at the time of blood collection. Scatter plot show frequencies (A) and absolute numbers (B) of V δ 1 cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test. Numbers in the plots show P values.

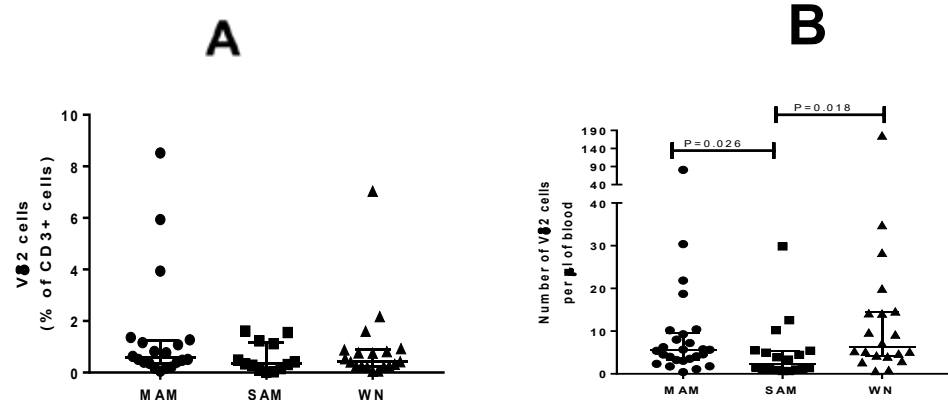


Figure 5.21 Circulating V δ 2 T cell frequencies and numbers in HIV-infected ART-naïve adults of different nutrition status. Cells were stained with monoclonal antibodies specific for CD3 and V δ 2 and analysed by flow cytometry. V δ 2 T cells were defined as CD3⁺ V δ 2⁺ lymphocytes. Absolute numbers were calculated from full blood counts taken at the time of blood collection. Scatter plot show frequencies (A) and absolute numbers (B) of V δ 2 cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test. Numbers in the plots show P values.

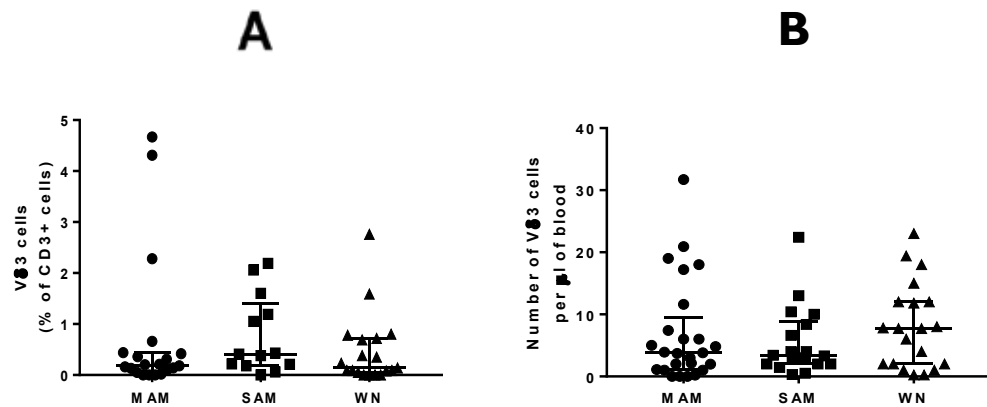


Figure 5.22 Circulating Vδ3 T cell frequencies and numbers in HIV-infected ART-naïve adults of different nutrition status. Cells were stained with monoclonal antibodies specific for CD3 and Vδ3 and analysed by flow cytometry. Vδ3 T cells were defined as CD3⁺ Vδ3⁺ lymphocytes. Absolute numbers were calculated from full blood counts taken at the time of blood collection. Scatter plot show frequencies (A) and absolute numbers (B) of CD4⁺ T cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test.

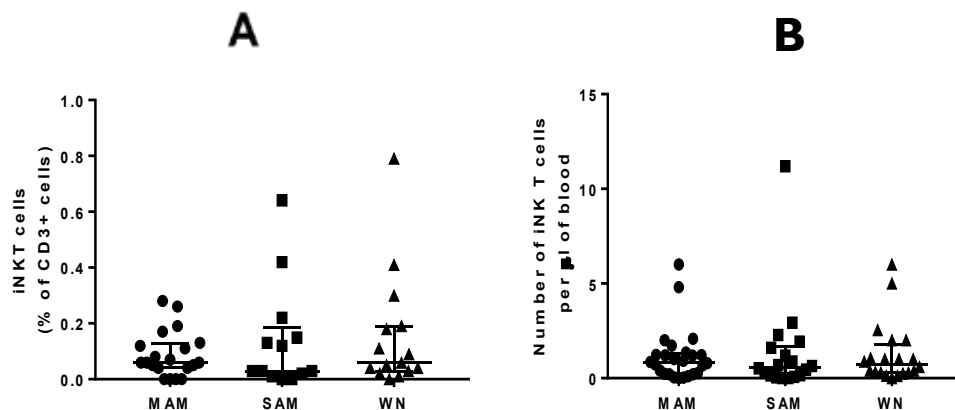


Figure 5.23 Circulating iNKT cell frequencies and numbers in HIV-infected ART-naïve adults of different nutrition status. Cells were stained with monoclonal antibodies specific for CD3 and Va24Jα18 and analysed by flow cytometry. iNKT cells were defined as CD3⁺ Va24Jα18⁺ lymphocytes. Absolute numbers were calculated from full blood counts taken at the time of blood collection. Scatter plot show frequencies (A) and absolute numbers (B) of iNKT cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test.

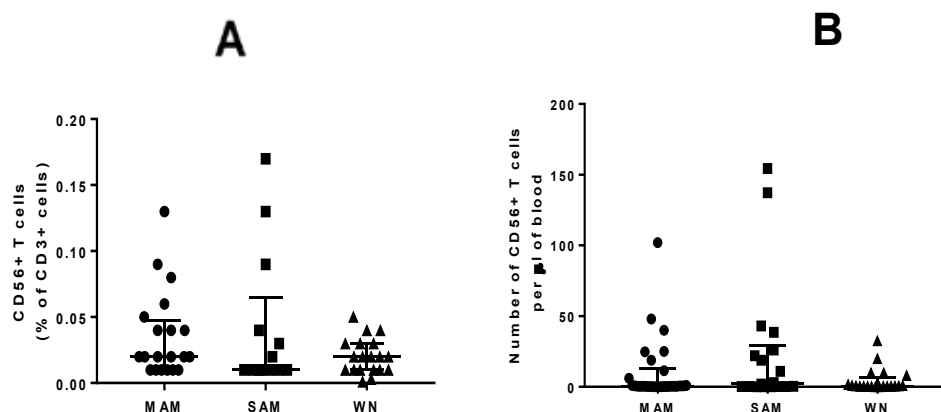


Figure 5.24 Circulating CD56⁺ T cell frequencies and numbers in HIV-infected ART-naïve adults of different nutrition status. Cells were stained with monoclonal antibodies specific for CD3 and CD56 and analysed by flow cytometry. CD56⁺ T cells were defined as CD3⁺ CD56⁺ lymphocytes. Absolute numbers were calculated from full blood counts taken at the time of blood collection. Scatter plot show frequencies (A) and absolute numbers (B) of CD56⁺ T cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test.

Summary:

Changes in the immunophenotypes of the malnourished participants compared to the well-nourished were similar to the changes in the immunophenotypes of the HIV infected participants when compared to the HIV negative controls.

Participants in the malnourished groups (MAM and SAM) had lower absolute T cell and B cell counts, and lower Vδ2 T cell expression compared to the well-nourished participants. The Vδ1 T cells were more expressed among the malnourished participants.

5.3.4 Effect of nutritional status on cytolytic degranulation and cytokine release in HIV infected adults

5.3.4.1 Effect of nutritional status on cytolytic degranulation by CD4⁺ T cells, CD8⁺ T cells, NK cells and Vδ1 T cells from ART-naïve with HIV infection after stimulation ex vivo

PBMC were isolated from 30 ART-naïve adult patients with HIV infection, of whom 10 were well nourished (WN), 9 were moderately acutely malnourished (MAM) and 11 were severely acutely malnourished (SAM). Cells were stimulated

for 4 hours with medium alone, PMA and ionomycin, or monoclonal antibodies (mAb) specific for CD3, CD28 and CD2 in the presence of a fluorochrome-conjugated mAb specific for CD107a. Cells were subsequently stained with fluorochrome-conjugated mAbs specific for CD3, CD4, CD8 and CD56, and analysed by flow cytometry.

The CD4⁺ T cells did not exhibit much degranulation, and there were no differences in the degranulation by the CD4⁺ T cells among the different participant groups (SAM, MAM and WN) regardless of the mode of stimulation. The degranulation by the CD8⁺ T cells of the well-nourished participants and the severely malnourished participants were significantly different. These differences were seen the cells incubated with anti-CD3/CD28/CD2 mAb ($P = 0.01$) and in PMA and ionomycin ($P = 0.04$). There were no significant differences in degranulation from NK cells among the three groups. The MAM participants showed higher levels of degranulation by V δ 1⁺ T cells than the SAM participants in the cells incubated with anti-CD3/CD28/CD2 mAb ($P = 0.03$) (**figure 5.25 – 5.28**)

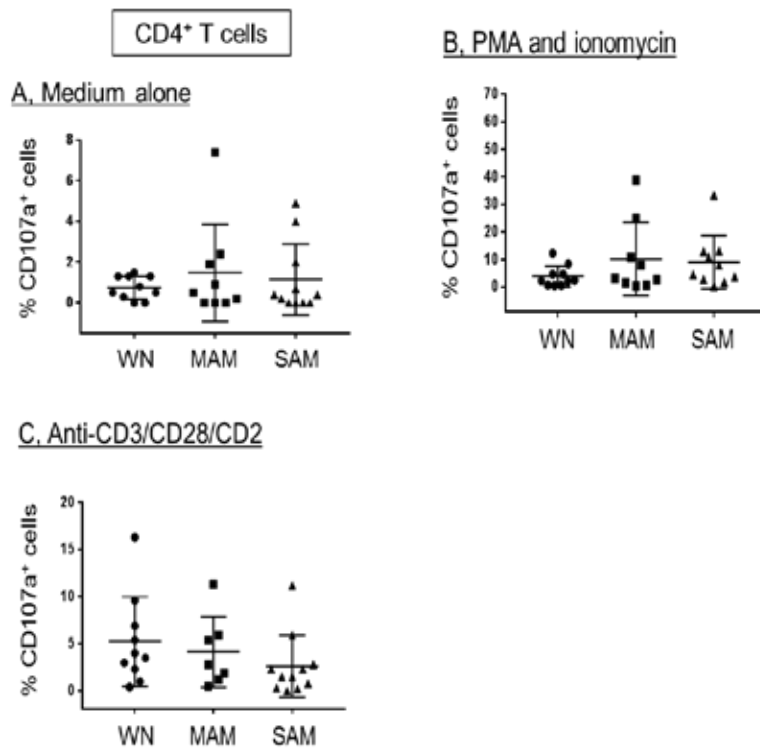


Figure 5.25 Effect of nutritional status on cytolytic degranulation by CD4⁺ T cells from ART-naïve adults with HIV-infection after stimulation ex vivo. PBMC were isolated from 30 ART-naïve adult patients with HIV infection, of whom 10 were well-nourished (WN), 9 were moderately acutely malnourished (MAM) and 11 were severely acutely malnourished (SAM). Cells were stimulated for 4 hours with medium alone (**A**), PMA and ionomycin (**B**), or monoclonal antibodies (mAb) specific for CD3, CD28 and CD2 (**C**) in the presence of a fluorochrome-conjugated mAb specific for CD107a. Cells were subsequently stained with fluorochrome-conjugated mAbs specific for CD3, CD4, and analysed by flow cytometry. Scatter plot show frequencies of CD4⁺ T cells that expressed cell-surface CD107a. Data were compared using the Mann Whitney U statistical test.

CD8⁺ T cells

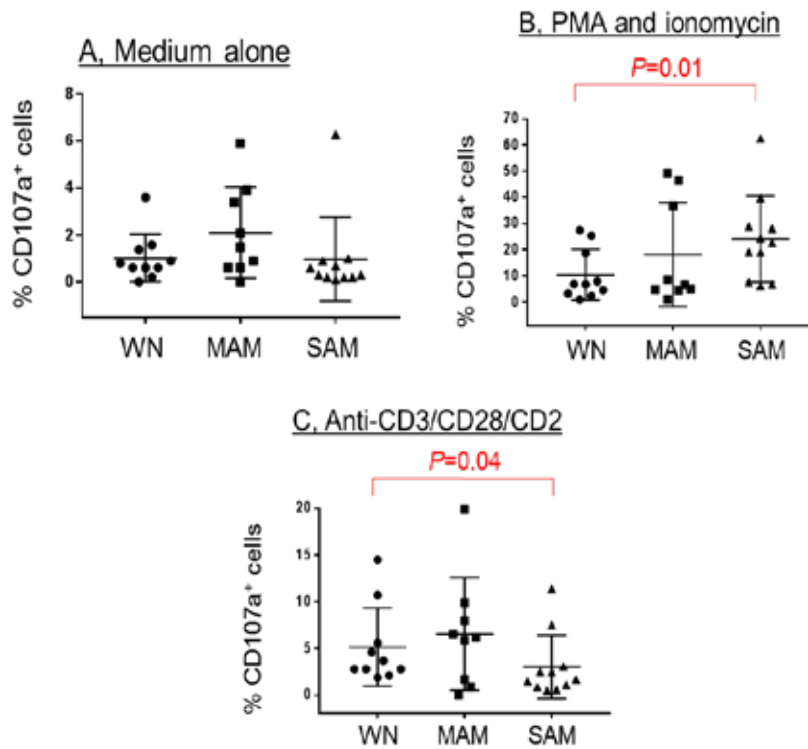


Figure 5.26 Effect of nutritional status on cytolytic degranulation by CD8⁺ T cells from ART-naïve adults with HIV-infection after stimulation ex vivo. PBMC were isolated from 30 ART-naïve adult patients with HIV infection, of whom 10 were well-nourished (WN), 9 were moderately acutely malnourished (MAM) and 11 were severely acutely malnourished (SAM). Cells were stimulated for 4 hours with medium alone (**A**), PMA and ionomycin (**B**), or monoclonal antibodies (mAb) specific for CD3, CD28 and CD2 (**C**) in the presence of a fluorochrome-conjugated mAb specific for CD107a. Cells were subsequently stained with fluorochrome-conjugated mAbs specific for CD3, CD8, and analysed by flow cytometry. Scatter plot show frequencies of CD8⁺ T cells that expressed cell-surface CD107a. Data were compared using the Mann Whitney U statistical test. A significant difference is indicated in red print.

NK cells

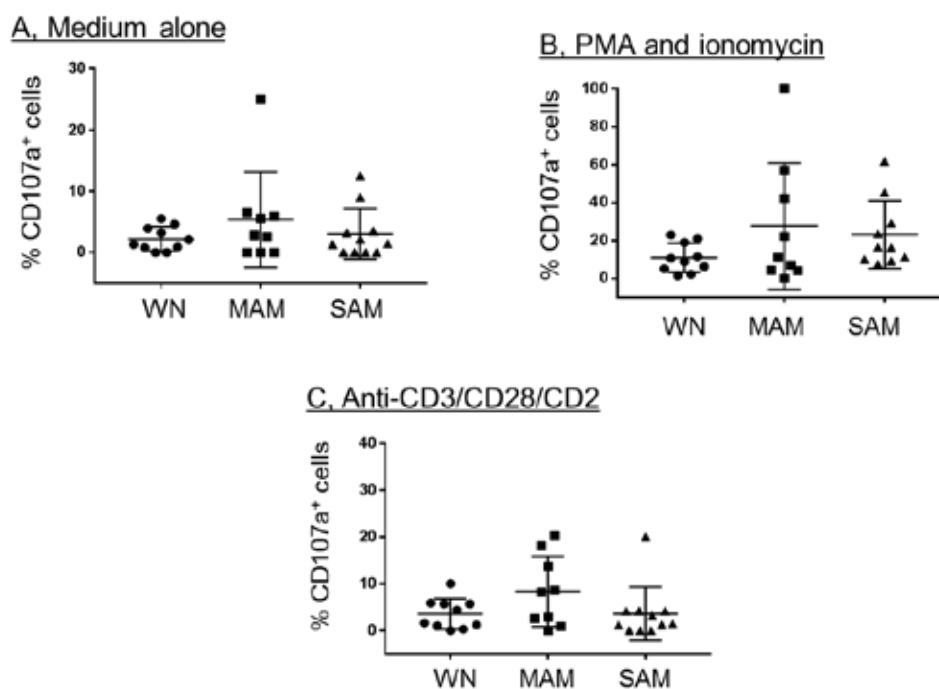


Figure 5.27 Effect of nutritional status on cytolytic degranulation by NK cells from ART-naïve adults with HIV-infection after stimulation ex vivo. PBMC were isolated from 30 ART-naïve adult patients with HIV infection, of whom 10 were well-nourished (WN), 9 were moderately acutely malnourished (MAM) and 11 were severely acutely malnourished (SAM). Cells were stimulated for 4 hours with medium alone (**A**), PMA and ionomycin (**B**), or monoclonal antibodies (mAb) specific for CD3, CD28 and CD2 (**C**) in the presence of a fluorochrome-conjugated mAb specific for CD107a. Cells were subsequently stained with fluorochrome-conjugated mAbs specific for CD3, CD56 and analysed by flow cytometry. Scatter plot show frequencies of NK cells that expressed cell-surface CD107a. Data were compared using the Mann Whitney U statistical test

V δ 1 T cells

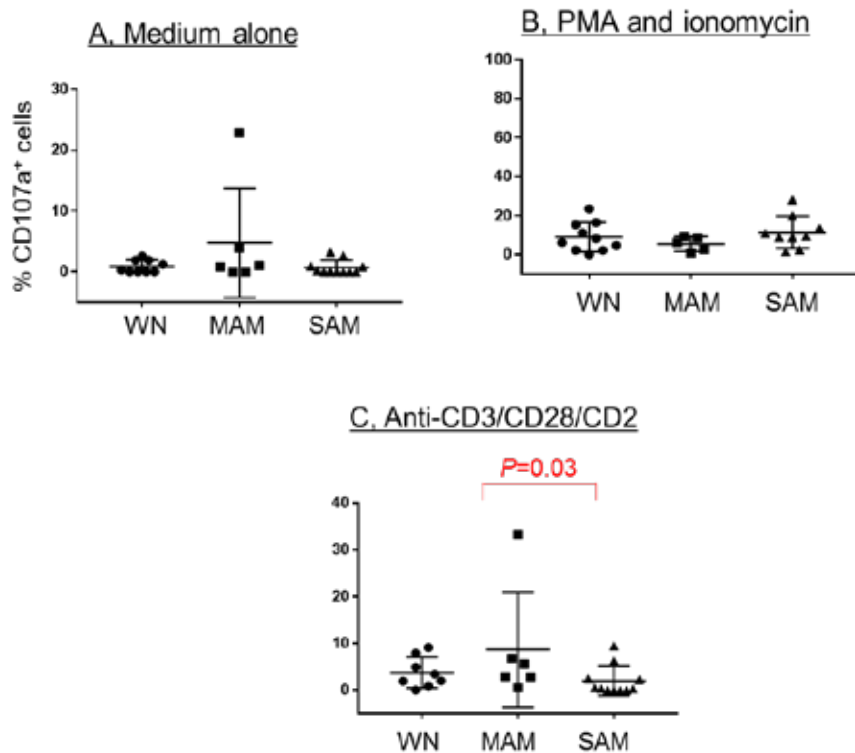


Figure 5.28 Effect of nutritional status on cytolytic degranulation by V δ 1⁺ T cells from ART-naïve adults with HIV-infection after stimulation ex vivo. PBMC were isolated from 30 ART-naïve adult patients with HIV infection, of whom 10 were well-nourished (WN), 9 were moderately acutely malnourished (MAM) and 11 were severely acutely malnourished (SAM). Cells were stimulated for 4 hours with medium alone (**A**), PMA and ionomycin (**B**), or monoclonal antibodies (mAb) specific for CD3, CD28 and CD2 (**C**) in the presence of a fluorochrome-conjugated mAb specific for CD107a. Cells were subsequently stained with fluorochrome-conjugated mAbs specific for CD3 and V δ 1 and analysed by flow cytometry. Scatter plot show frequencies of V δ 1⁺ T cells that expressed cell-surface CD107a. Data were compared using the Mann Whitney U statistical test. A significant difference is indicated in red print.

Summary

No clear association between level of malnutrition and CD107a degranulation among ART naïve HIV infected adults.

5.3.4.2 Effect of nutritional status on cytokine production by CD4⁺ T cells, CD8⁺ T cells and Vδ1 T cells from ART-naïve adults with HIV-infection after stimulation ex vivo

5.3.6.2.1 Effect of nutritional status on IFN-γ production by CD4⁺ T cells, CD8⁺ T cells and Vδ1 T cells from ART-naïve adults with HIV-infection after stimulation ex vivo.

PBMC were isolated from 30 ART-naïve adult patients with HIV infection, of whom 10 were well nourished (WN), 9 were moderately acutely malnourished (MAM) and 11 were severely acutely malnourished (SAM). Cells were stimulated for 4 hours with medium alone (A), PMA and ionomycin (B), or monoclonal antibodies (mAb) specific for CD3, CD28 and CD2 (C) and subsequently stained with fluorochrome-conjugated mAbs specific for cell-surface CD3, CD4, CD8 and Vδ1 and intracellular IFN-γ and analysed by flow cytometry. Data were compared using the Mann Whitney U statistical test.

The proportion of CD4⁺ T cells of the WN participants that produced IFN-γ were greater than those of the MAM and SAM participants. For the cells incubated in PMA and ionomycin, these differences were significantly higher than those of the MAM participants ($P = 0.04$). For cells incubated with anti-CD3/CD28/CD2 mAb, they were significantly higher than those of both the MAM ($P=0.006$) and SAM participants ($P = 0.017$).

The proportions of CD8⁺ T cells from the WN participants that produced IFN-γ were greater than those of the MAM and SAM participants. These differences were statistically significant between the WN and MAM participants ($P < 0.05$), and more pronounced in the PMA and ionomycin stimulations. Just like for the CD8⁺ T cells, the proportion of Vδ1 T cells of the WN participants that produced were higher than those of the MAM and SAM participants. However, these differences were not statistically significant (**figure 5.29 -5.31**)

CD4⁺ T cells

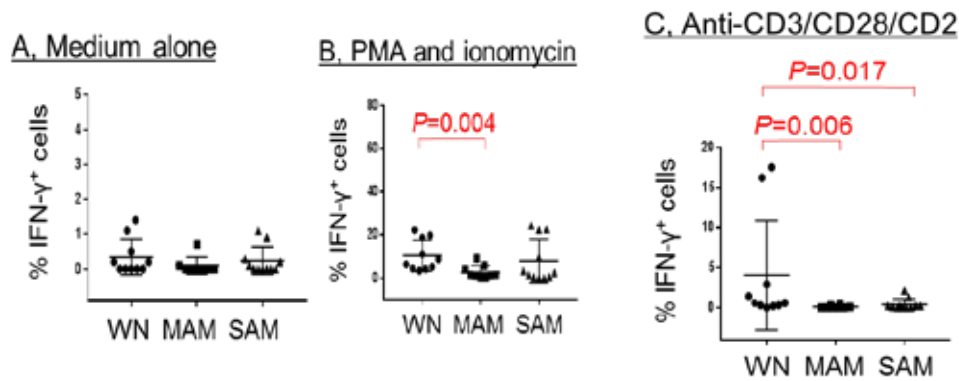


Figure 5.29 Effect of nutritional status on IFN- γ production by CD4⁺ T cells, from ART-naïve adults with HIV-infection after stimulation *ex vivo*. PBMC were isolated from 30 ART-naïve adult patients with HIV infection, of whom 10 were well-nourished (WN), 9 were moderately acutely malnourished (MAM) and 11 were severely acutely malnourished (SAM). Cells were stimulated for 4 hours with medium alone (**A**), PMA and ionomycin (**B**), or monoclonal antibodies (mAb) specific for CD3, CD28 and CD2 (**C**) and subsequently stained with fluorochrome-conjugated mAbs specific for cell-surface CD3, CD4, and intracellular IFN- γ and analysed by flow cytometry. Scatter plot show frequencies of CD4⁺ T cells that expressed IFN- γ . Data were compared using the Mann Whitney U statistical test. Significant differences are indicated in red print.

CD8⁺ T cells

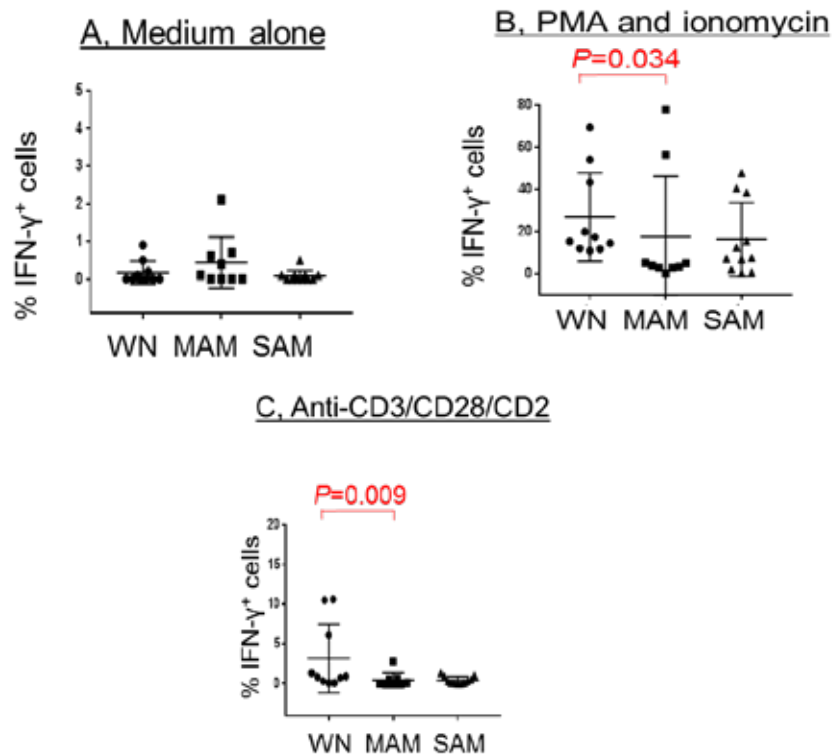


Figure 5.30 Effect of nutritional status on IFN- γ production by CD8⁺ T cells from ART-naïve adults with HIV-infection after stimulation ex vivo. PBMC were isolated from 30 ART-naïve adult patients with HIV infection, of whom 10 were well-nourished (WN), 9 were moderately acutely malnourished (MAM) and 11 were severely acutely malnourished (SAM). Cells were stimulated for 4 hours with medium alone (A), PMA and ionomycin (B), or monoclonal antibodies (mAb) specific for CD3, CD28 and CD2 (C) and subsequently stained with fluorochrome-conjugated mAbs specific for cell-surface CD3, CD8 and intracellular IFN- γ and analysed by flow cytometry. Scatter plot show frequencies of CD8⁺ T cells that expressed IFN- γ . Data were compared using the Mann Whitney U statistical test. Significant differences are indicated in red print.

V δ 1 T cells

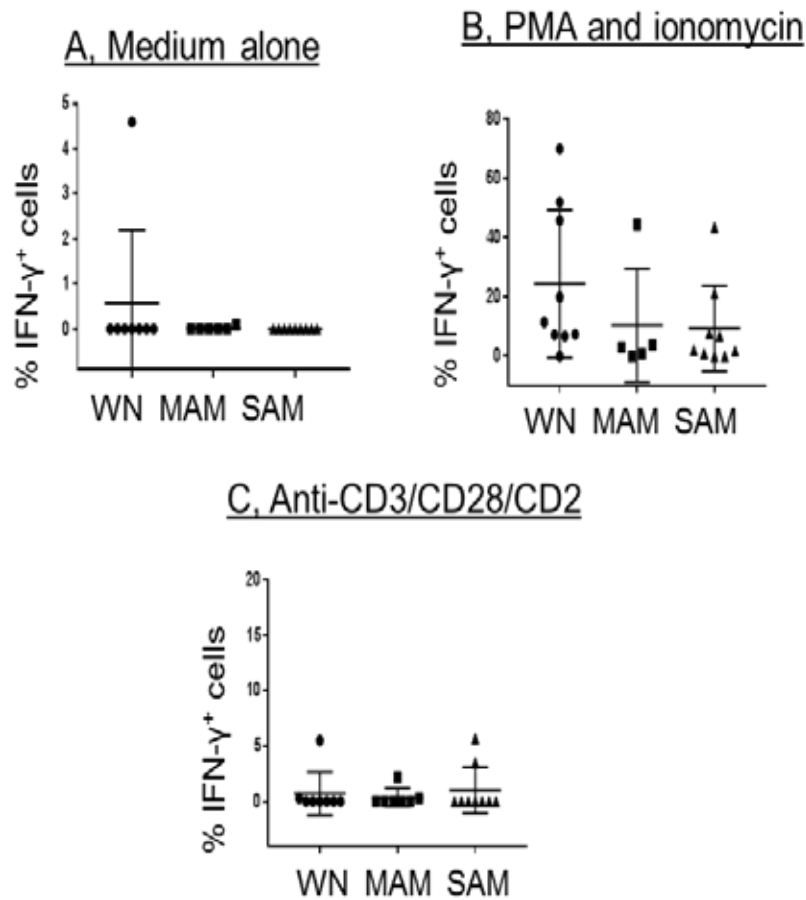


Figure 5.3I Effect of nutritional status on IFN- γ production by V δ 1 T cells from ART-naïve adults with HIV-infection after stimulation *ex vivo*. PBMC were isolated from 30 ART-naïve adult patients with HIV infection, of whom 10 were well-nourished (WN), 9 were moderately acutely malnourished (MAM) and 11 were severely acutely malnourished (SAM). Cells were stimulated for 4 hours with medium alone (**A**), PMA and ionomycin (**B**), or monoclonal antibodies (mAb) specific for CD3, CD28 and CD2 (**C**) and subsequently stained with fluorochrome-conjugated mAbs specific for cell-surface CD3, V δ 1 T cells and intracellular IFN- γ and analysed by flow cytometry. Scatter plot show frequencies of V δ 1 T cells that expressed IFN- γ . Data were compared using the Mann Whitney U statistical test.

5.3.6.2.2 Effect of nutritional status on IL-4 production by CD4⁺ T cells, CD8⁺ T cells and Vδ1 T cells from ART-naïve adults with HIV-infection after stimulation ex vivo

PBMC were isolated from 30 ART-naïve adult patients with HIV infection, of whom 10 were well-nourished (WN), 9 were moderately acutely malnourished (MAM) and 11 were severely acutely malnourished (SAM). Cells were stimulated for 4 hours with medium alone, PMA and ionomycin or monoclonal antibodies (mAb) specific for CD3, CD28 and CD2 and subsequently stained with fluorochrome-conjugated mAbs specific for cell-surface CD3, CD4, CD8 and Vδ1 and intracellular IL-4 and analysed by flow cytometry. Scatter plot show frequencies of CD4⁺ T cells, CD8⁺ T cells and Vδ1 T cells that expressed IL-4. Data were compared using the Mann Whitney U statistical test.

The proportion of CD4⁺ T cells that produced IL-4 was very small for all the three groups and there were no significant differences in the proportion of CD4⁺ T cells that produced IL-4 among the three study groups. There was a high proportion of CD8⁺ T cells that were incubated in medium only produced IL-4 among the MAM and SAM participants. Additionally, the SAM participants had significantly higher proportion of CD8⁺ T cells producing IL-4 compared to the WN participants ($P = 0.045$). The proportion of Vδ1 T cells that produced IL-4 was very small for all the three groups with no significant differences. **(Figure 5.32 – 5.34)**

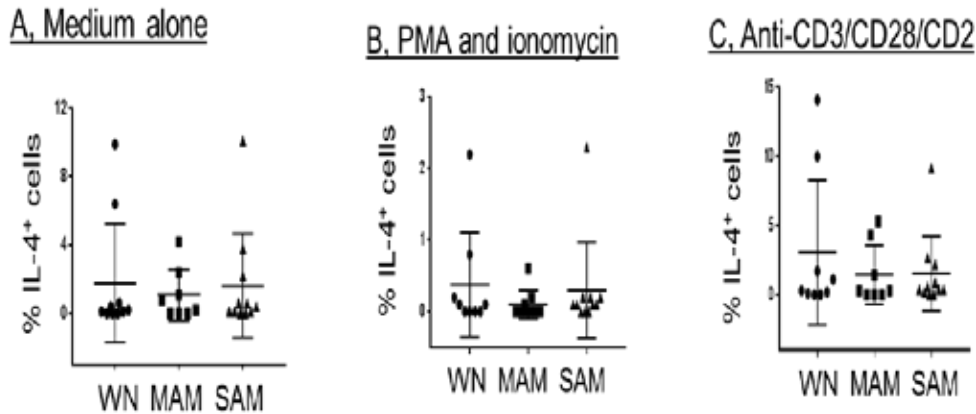


Figure 5.32 Effect of nutritional status on IL-4 production by CD4⁺ T cells from ART-naïve adults with HIV-infection after stimulation ex vivo. PBMC were isolated from 30 ART-naïve adult patients with HIV infection, of whom 10 were well-nourished (WN), 9 were moderately acutely malnourished (MAM) and 11 were severely acutely malnourished (SAM). Cells were stimulated for 4 hours with medium alone (A), PMA and ionomycin (B), or monoclonal antibodies (mAb) specific for CD3, CD28 and CD2 (C) and subsequently stained with fluorochrome-conjugated mAbs specific for cell-surface CD3, CD4 and intracellular IL-4 and analysed by flow cytometry. Scatter plot show frequencies of CD4⁺ T cells left panels), CD8⁺ T cells (centre panels) and Vδ1 T cells (right panels) that expressed IL-4. Data were compared using the Mann Whitney U statistical test.

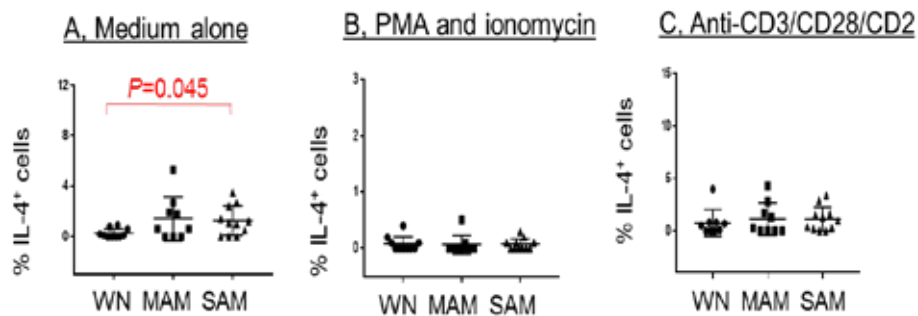


Figure 5.33 Effect of nutritional status on IL-4 production by CD8⁺ T cells from ART-naïve adults with HIV-infection after stimulation ex vivo. PBMC were isolated from 30 ART-naïve adult patients with HIV infection, of whom 10 were well-nourished (WN), 9 were moderately acutely malnourished (MAM) and 11 were severely acutely malnourished (SAM). Cells were stimulated for 4 hours with medium alone (A), PMA and ionomycin (B), or monoclonal antibodies (mAb) specific for CD3, CD28 and CD2 (C) and subsequently stained with fluorochrome-conjugated mAbs specific for cell-surface CD3, CD8 and intracellular IL-4 and analysed by flow cytometry. Scatter plot show frequencies of CD8⁺ T cells that expressed IL-4. Data were compared using the Mann Whitney U statistical test. A significant difference is indicated in red.

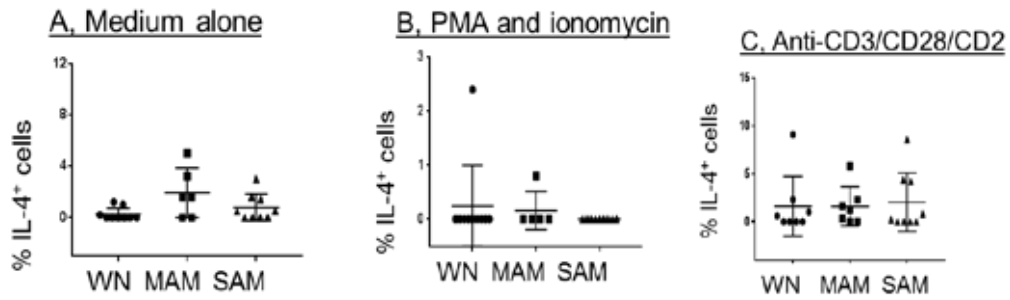


Figure 5.34 Effect of nutritional status on IL-4 production by Vδ1 T cell from ART-naïve adults with HIV-infection after stimulation ex vivo. PBMC were isolated from 30 ART-naïve adult patients with HIV infection, of whom 10 were well-nourished (WN), 9 were moderately acutely malnourished (MAM) and 11 were severely acutely malnourished (SAM). Cells were stimulated for 4 hours with medium alone (A), PMA and ionomycin (B), or monoclonal antibodies (mAb) specific for CD3, CD28 and CD2 (C) and subsequently stained with fluorochrome-conjugated mAbs specific for cell-surface CD3, Vδ1 T cells and intracellular IL-4 and analysed by flow cytometry. Scatter plot show frequencies of Vδ1 T cells (right panels) that expressed IL-4. Data were compared using the Mann Whitney U statistical test.

5.3.6.2.3 Effect of nutritional status on IL-17 production by CD4⁺ T cells, CD8⁺ T cells and Vδ1 T cells from ART-naïve adults with HIV-infection after stimulation ex vivo

PBMC were isolated from 30 ART-naïve adult patients with HIV infection, of whom 10 were well-nourished (WN), 9 were moderately acutely malnourished (MAM) and 11 were severely acutely malnourished (SAM). Cells were stimulated for 4 hours with medium alone, PMA and ionomycin, or monoclonal antibodies (mAb) specific for CD3, CD28 and CD2 and subsequently stained with fluorochrome-conjugated mAbs specific for cell-surface CD3, CD4, CD8 and Vδ1 and intracellular IL-17 and analysed by flow cytometry. Scatter plot show frequencies of CD4⁺ T cells CD8⁺ T cells and Vδ1 T cells that expressed IL-17. Data were compared using the Mann Whitney U statistical test.

There was hardly any production of IL-17 by the CD4⁺ and CD8⁺ T cells for all the three study groups. The Vδ1 T cells, had a similar pattern of IL-17 production like the CD4⁺ T cells and CD8⁺ T cells. However, there was a significant difference between the proportion of Vδ1 T cells producing IL-17 among the well-nourished and moderately malnourished participants (P=0.05) (**Figure 5.35-5.37**).

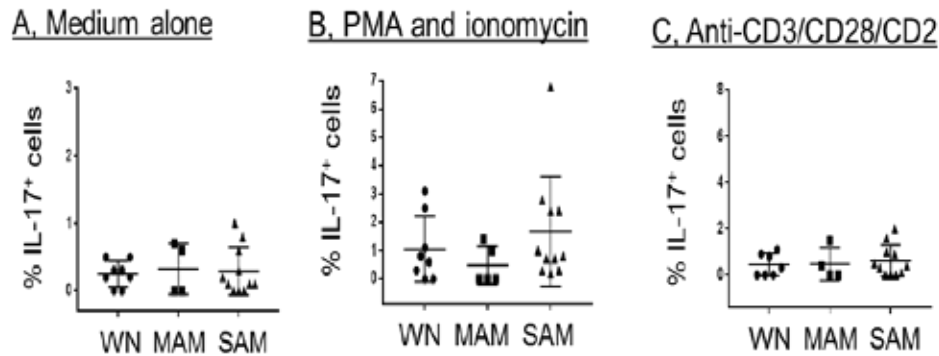


Figure 5.35 Effect of nutritional status on IL-17 production by CD4⁺ T cells from ART-naïve adults with HIV-infection after stimulation ex vivo. PBMC were isolated from 30 ART-naïve adult patients with HIV infection, of whom 10 were well-nourished (WN), 9 were moderately acutely malnourished (MAM) and 11 were severely acutely malnourished (SAM). Cells were stimulated for 4 hours with medium alone (A), PMA and ionomycin (B), or monoclonal antibodies (mAb) specific for CD3, CD28 and CD2 (C) and subsequently stained with fluorochrome-conjugated mAbs specific for cell-surface CD3, CD4 and intracellular IL-17 and analysed by flow cytometry. Scatter plot show frequencies of CD4⁺ T cells that expressed IL-17. Data were compared using the Mann Whitney U statistical test.

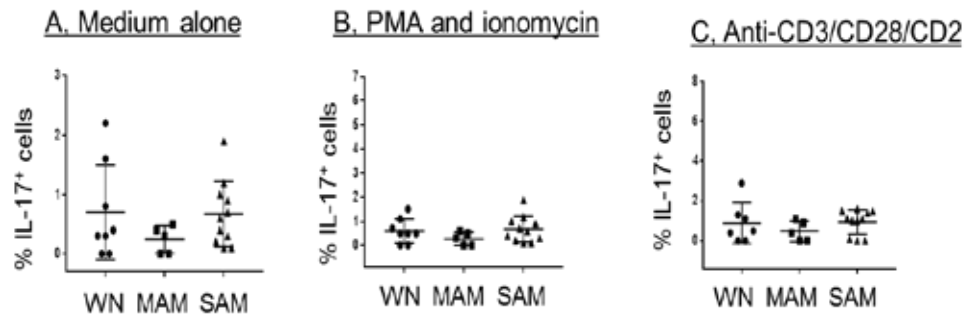


Figure 5.36 Effect of nutritional status on IL-17 production by CD8⁺ T cells from ART-naïve adults with HIV-infection after stimulation ex vivo. PBMC were isolated from 30 ART-naïve adult patients with HIV infection, of whom 10 were well-nourished (WN), 9 were moderately acutely malnourished (MAM) and 11 were severely acutely malnourished (SAM). Cells were stimulated for 4 hours with medium alone (A), PMA and ionomycin (B), or monoclonal antibodies (mAb) specific for CD3, CD28 and CD2 (C) and subsequently stained with fluorochrome-conjugated mAbs specific for cell-surface CD3, CD8 and intracellular IL-17 and analysed by flow cytometry. Scatter plot show frequencies of CD8⁺ T cells that expressed IL-17. Data were compared using the Mann Whitney U statistical test.

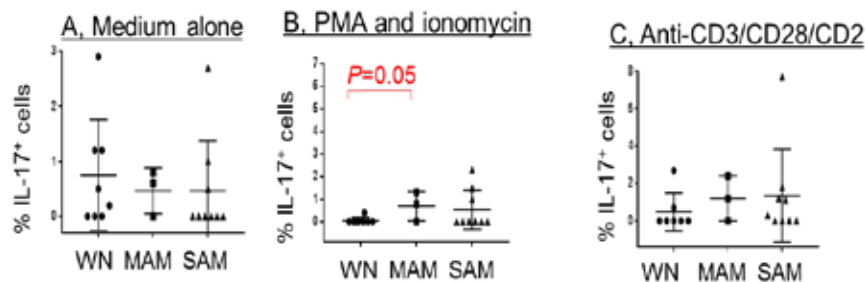


Figure 5.37 Effect of nutritional status on IL-17 production by Vδ1T cells from ART-naïve adults with HIV-infection after stimulation ex vivo. PBMC were isolated from 30 ART-naïve adult patients with HIV infection, of whom 10 were well-nourished (WN), 9 were moderately acutely malnourished (MAM) and 11 were severely acutely malnourished (SAM). Cells were stimulated for 4 hours with medium alone (A), PMA and ionomycin (B), or monoclonal antibodies (mAb) specific for CD3, CD28 and CD2 (C) and subsequently stained with fluorochrome-conjugated mAbs specific for cell-surface CD3, CD8 and intracellular IL-17 and analysed by flow cytometry. Scatter plot show frequencies of Vδ1T cells that expressed IL-17. Data were compared using the Mann Whitney U statistical test. A significant difference is indicated in red.

SUMMARY

Malnourished participants produced less IFN- γ than the well-nourished participants. Differences more pronounced in CD4⁺ T cells, CD8⁺ T cells. No major differences in the production of IL-4 and IL-17 in the three ART-naïve groups

5.4 DISCUSSION

The discussion of chapter five will be combined with the discussion of chapter six.

6.0 CHAPTER SIX:

**EFFECTS OF ART AND NUTRITIONAL
SUPPLEMENTATION ON IMMUNOLOGICAL
OUTCOMES**

6.1 INTRODUCTION

This chapter contains the results of the effects of ART and the effects of nutritional supplementation on immunological outcomes of the NOURISH study.

Details of the background to this chapter are contained in chapter 5.

6.1.1 OBJECTIVES:

- I. To describe the effects of ART on the frequencies, numbers, phenotypes and functions (cytotoxicity and cytokine production) among HIV infected adults in Uganda.
- II. To describe the effect of nutritional supplementation on the frequencies, numbers, phenotypes and functions (cytotoxicity and cytokine production) among HIV infected adults in Uganda.

6.2 METHODOLOGY:

The methods of this chapter have been detailed in chapter 5.

The immunological outcomes of the ART-experienced participants were compared to the outcomes of the ART-naïve participants at baseline. All the ART-naïve participants regardless of nutritional status were included in this analysis.

For the effect of nutritional supplementation on immunophenotypes and functions (cytotoxicity and cytokine production), only participants in the randomization groups were included in the analysis. All participants that were lost to follow-up were not included in the analysis, and those that did not have results at baseline (lost samples) or those whose samples had not been shipped from Uganda were not included in this analysis. Details of these participants are in table 5.2

6.2.1 Outcomes of interest

- III. The median differences in the lymphocyte subsets between the HIV negative controls and ART-naïve HIV infected adults.
- IV. The median differences in lymphocyte subset frequencies and absolute counts between the randomization arms of participants in the MAM and ART groups at 12 weeks.
- V. The median differences in cytokine production and cytotoxicity of selected cells between the randomization arms of the MAM and ART groups at 12 weeks.

6.3 RESULTS

6.3.1 Effect of ART on circulating lymphocyte frequencies and numbers in malnourished HIV infected patients

This section describes the effect of ART on the numbers and frequencies of circulating lymphocytes.

6.3.1.1 Effect of ART on circulating T cell, B cell and NK frequencies and numbers in malnourished HIV infected patients

PBMC were isolated from 57 malnourished patients with HIV infection, of whom 43 were ART naïve (Naïve) and 14 had been on ART (ART) for at least one year.

The ART-naïve participants had significantly lower frequencies and absolute counts of T cells compared to the ART-experienced participants, $P < 0.05$ (**Figure 6.1**). However, there were no significant differences in the frequencies or absolute counts of B cells (**Figure 6.2**) and NK cells in these study participants (**Figure 6.3**).

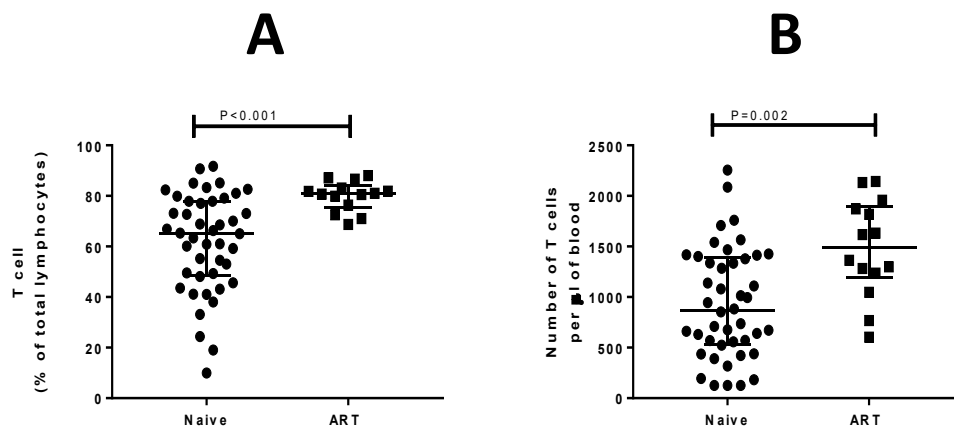


Figure 6.1 Effect of ART on circulating T cell frequencies and numbers in malnourished HIV-infected adults. Cells were stained with monoclonal antibodies specific for CD3 and, analysed by flow cytometry. T cells were defined as CD3⁺ lymphocytes. Absolute numbers were calculated from full blood counts taken at the time of blood collection. Scatter plot show frequencies (**A**) and absolute numbers (**B**) of T cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney

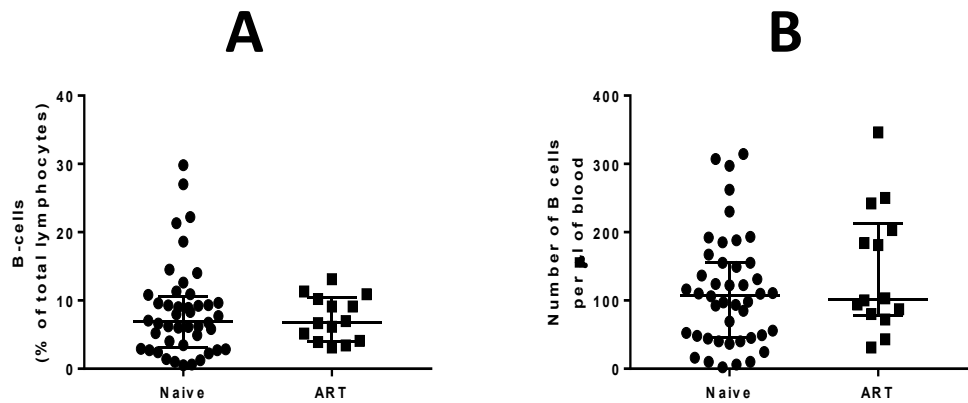


Figure 6.2 Effect of ART on circulating B cell frequencies and numbers in malnourished HIV-infected adults. Cells were stained with monoclonal antibodies specific for CD3 and CD19, and analysed by flow cytometry. B cells were defined as CD3⁺ CD19⁺ lymphocytes. Absolute numbers were calculated from full blood counts taken at the time of blood collection. Scatter plot show frequencies (A) and absolute numbers (B) of B cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test.

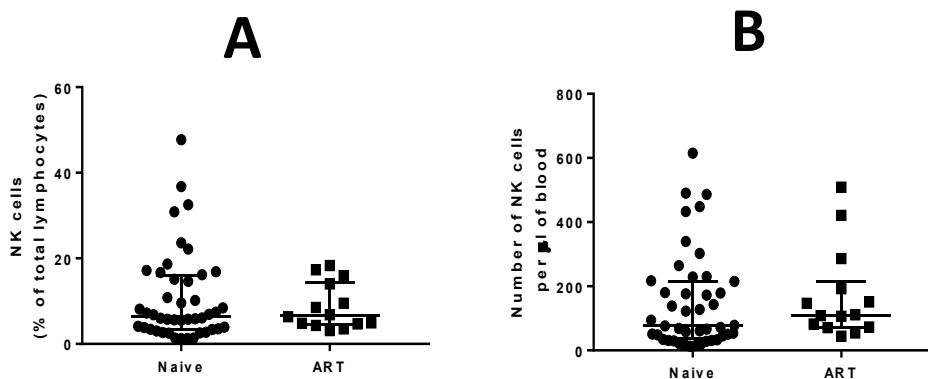


Figure 6.3 Effect of ART on circulating NK cell frequencies and numbers in malnourished HIV-infected adults. Cells were stained with monoclonal antibodies specific for CD3 and CD56 and analysed by flow cytometry. NK cells were defined as CD3⁺ CD56⁺ lymphocytes. Absolute numbers were calculated from full blood counts taken at the time of blood collection. Scatter plot show frequencies (A) and absolute numbers (B) of NK cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test.

6.3.1.2 Effect of ART on frequencies and numbers of circulating T cell subsets in malnourished HIV-infected adults

The effect of ART on the circulating T cell subsets was analyzed by comparing distribution of the T cell subsets among the ART experienced participants with

those of the ART naïve participants. As expected, the ART-experienced participants had significantly higher frequencies and absolute counts of the CD4⁺ T cell subset, $p < 0.05$ (**Figure 6.4**), but significantly lower frequencies of the CD8⁺ T cell subset. However, there were no significant differences in the CD8⁺ T cell absolute counts (**Figure 6.5**). There were no significant differences in the frequencies and absolute counts of the CD4⁺ CD8⁺ cells in the two participant groups (**Figure 6.6**). The frequencies of the CD4⁺ CD8⁺ cells were also not significantly different (**Figure 6.7**), but the absolute counts were higher in the ART-experienced subjects ($p < 0.05$).

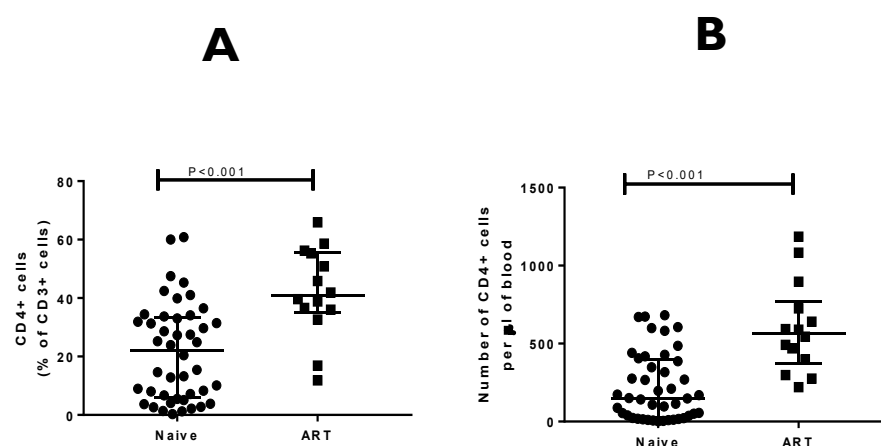


Figure 6.4 Effect of ART on circulating CD4⁺ T cell frequencies and numbers in malnourished HIV-infected adults. Cells were stained with monoclonal antibodies specific for CD3, CD4 and CD8, and analysed by flow cytometry. CD4⁺ T cells were defined as CD3⁺ CD4⁺ CD8⁺ lymphocytes. Absolute numbers were calculated from full blood counts taken at the time of blood collection. Scatter plot show frequencies (**A**) and absolute numbers (**B**) of CD4⁺ cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test. Numbers in the plots

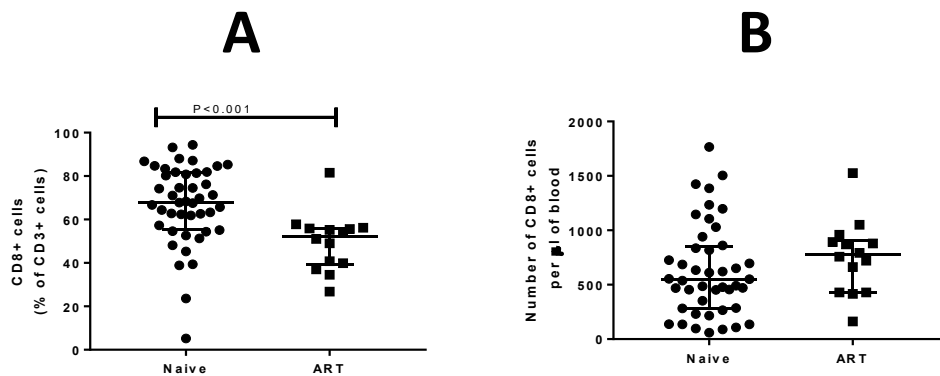


Figure 6.5 Effect of ART on circulating CD8⁺ T cell frequencies and numbers in malnourished HIV-infected adults. Cells were stained with monoclonal antibodies specific for CD3, CD4 and CD8, and analysed by flow cytometry. CD8⁺ T cells were defined as CD3⁺ CD4⁻ CD8⁺ lymphocytes. Absolute numbers were calculated from full blood counts taken at the time of blood collection. Scatter plot show frequencies (A) and absolute numbers (B) of CD8⁺ cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test. Numbers in the plots show *P* values.

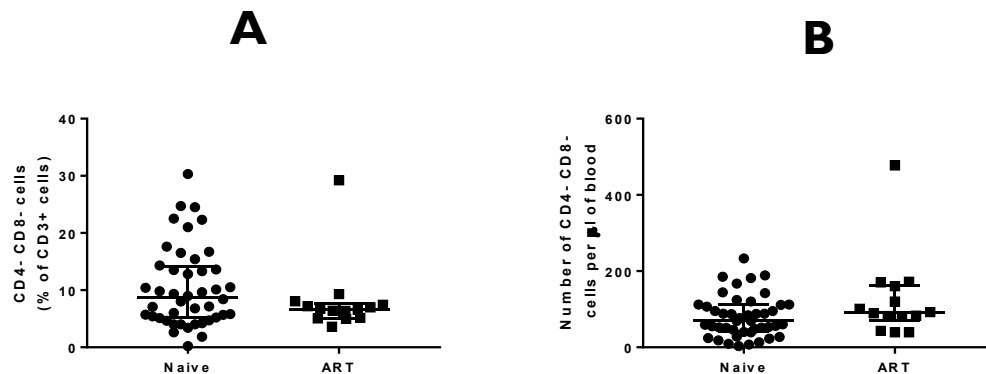


Figure 6.6 Effect of ART on circulating CD4⁻ CD8⁻ T cell frequencies and numbers in malnourished HIV-infected adults. Cells were stained with monoclonal antibodies specific for CD3, CD4 and CD8, and analysed by flow cytometry. CD4⁻ CD8⁻ T cells were defined as CD3⁺ CD4⁻ CD8⁻ lymphocytes. Absolute numbers were calculated from full blood counts taken at the time of blood collection. Scatter plot show frequencies (A) and absolute numbers (B) of CD4⁻ CD8⁻ cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test.

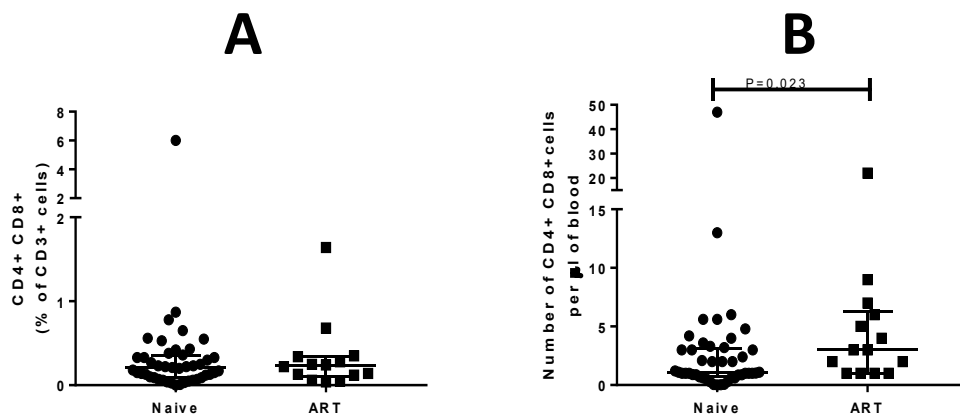


Figure 6.7 Effect of ART on circulating CD4⁺ CD8⁺ T cell frequencies and numbers in malnourished HIV-infected adults. Cells were stained with monoclonal antibodies specific for CD3, CD4 and CD8, and analysed by flow cytometry. CD4⁺ CD8⁺ T cells were defined as CD3⁺ CD4⁺ CD8⁺ lymphocytes. Absolute numbers were calculated from full blood counts taken at the time of blood collection. Scatter plot show frequencies (A) and absolute numbers (B) of CD4⁺ CD8⁺ cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test. Numbers in the plots show P value.

6.3.1.3 Effect of ART on frequencies and numbers of circulating innate T cell populations in malnourished HIV-infected adults

The effect of ART on the innate T cells among malnourished HIV infected adults is described as below. The ART-naïve participants had significantly higher Vδ1 T-cell frequencies than the ART-experienced participants ($p < 0.05$). Although the absolute counts of these cells were also higher among the naïve participants, this difference was not statistically significant (**Figure 6.8**).

The frequencies and absolute counts of Vδ2 T cells in the two groups were not significantly different (**Figure 6.9**). Although the ART naïve participants had slightly higher frequencies of Vδ3 T cells, neither the frequencies nor absolute numbers of Vδ3 T cells were significantly different than those in the ART-experienced subjects (**Figure 6.10**). Similarly, the ART-naïve participants had slightly higher frequencies, but not absolute numbers, of iNK T cells compared to the ART-experienced participants (**Figure 6.11**).

Finally, the frequencies, but not absolute numbers, of the CD56⁺ T cells in the naïve participants were significantly higher than those in the ART-experienced participants (**Figure 6.12**).

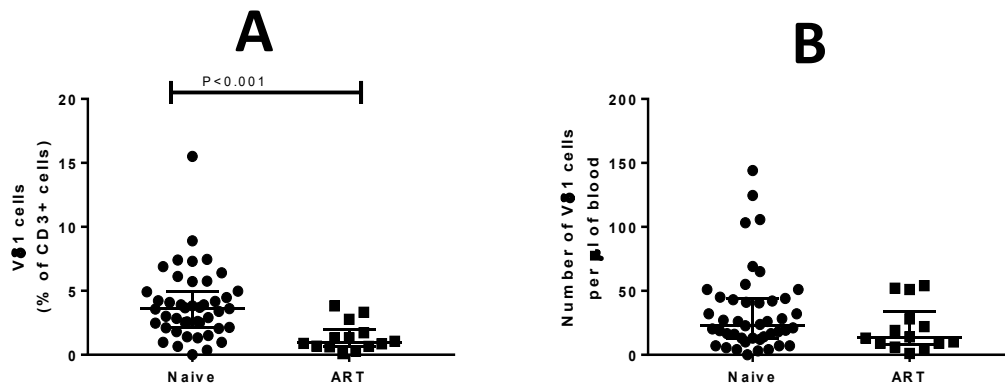


Figure 6.8 Effect of ART on circulating Vδ1 T cell frequencies and numbers in malnourished HIV-infected adults. Cells were stained with monoclonal antibodies specific for CD3 and Vδ1 and analysed by flow cytometry. Vδ1 T cells were defined as CD3⁺ Vδ1⁺ lymphocytes. Absolute numbers were calculated from full blood counts taken at the time of blood collection. Scatter plot show frequencies (**A**) and absolute numbers (**B**) of Vδ1 cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test. Numbers in the plots show P values.

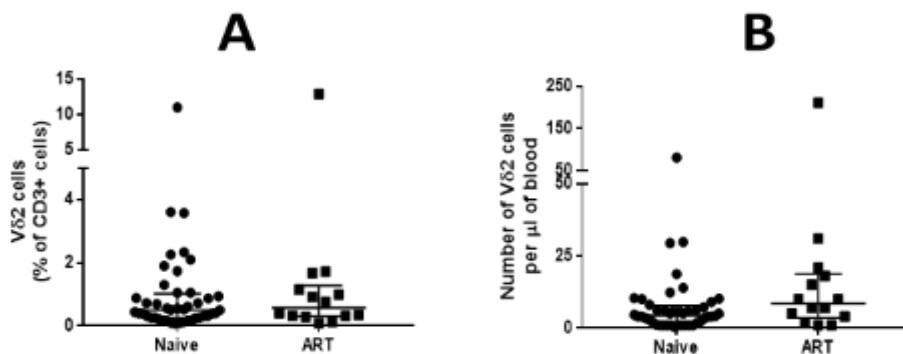


Figure 6.9 Effect of ART on circulating Vδ2 T cell frequencies and numbers in malnourished HIV-infected adults. Cells were stained with monoclonal antibodies specific for CD3 and Vδ2 and analysed by flow cytometry. Vδ2 T cells were defined as CD3⁺ Vδ2⁺ lymphocytes. Absolute numbers were calculated from full blood counts taken at the time of blood collection. Scatter plot show frequencies (**A**) and absolute numbers (**B**) of Vδ2 cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test. Numbers in the plots show P values.

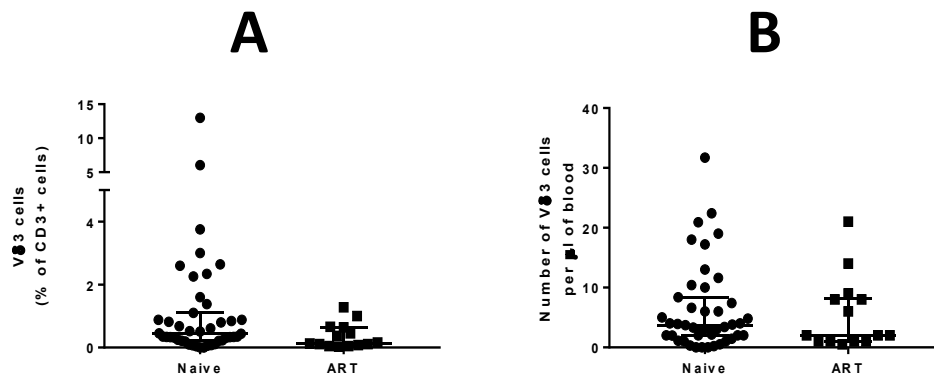


Figure 6.10 Effect of ART on circulating Vδ3 T cell frequencies and numbers in malnourished HIV-infected adults. Cells were stained with monoclonal antibodies specific for CD3 and Vδ3, and analysed by flow cytometry. Vδ3 T cells were defined as CD3⁺ Vδ3⁺ lymphocytes. Absolute numbers were calculated from full blood counts taken at the time of blood collection. Scatter plot show frequencies (A) and absolute numbers (B) of Vδ3 cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test.

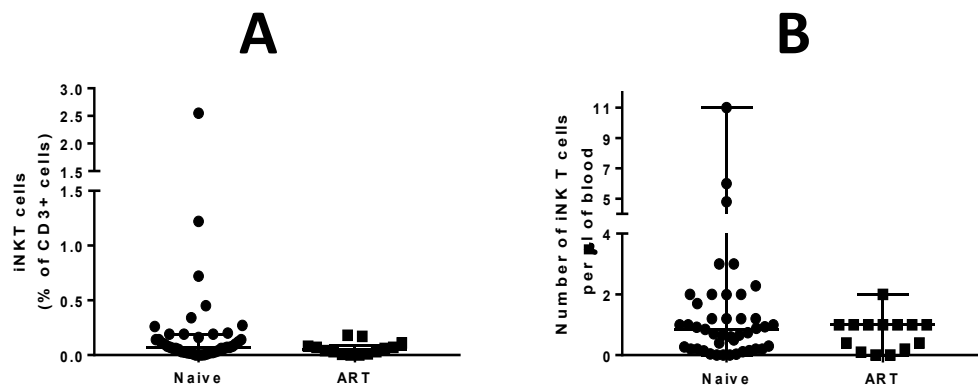


Figure 6.11 Effect of ART on circulating iNKT cell frequencies and numbers in malnourished HIV-infected adults. Cells were stained with monoclonal antibodies specific for CD3 and Va24Jα18 and analysed by flow cytometry. iNKT cells were defined as CD3⁺ Va24Jα18⁺ lymphocytes. Absolute numbers were calculated from full blood counts taken at the time of blood collection. Scatter plot show frequencies (A) and absolute numbers (B) of iNKT cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test.

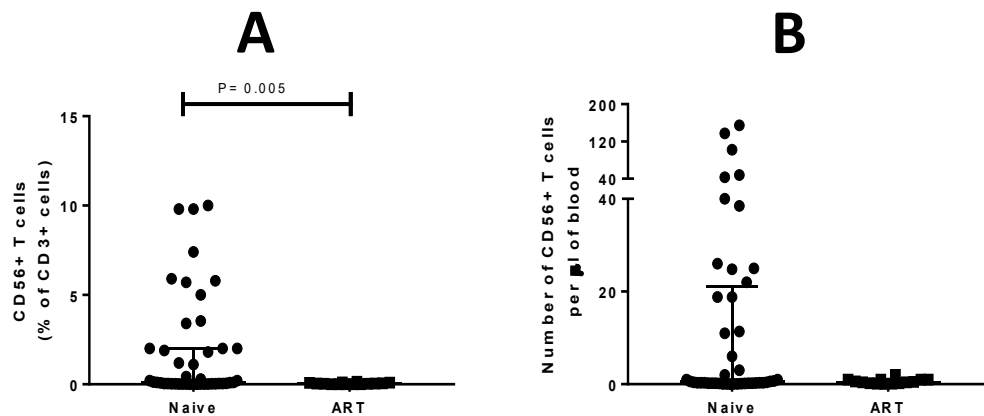


Figure 6.12 Effect of ART on circulating CD56⁺ T cell frequencies and numbers in malnourished HIV-infected adults. Cells were stained with monoclonal antibodies specific for CD3 and CD56, and analysed by flow cytometry. CD56⁺ T cells were defined as CD3⁺ CD56⁺ lymphocytes. Absolute numbers were calculated from full blood counts taken at the time of blood collection. Scatter plot show frequencies (**A**) and absolute numbers (**B**) of CD56⁺ T cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test. Numbers in the plots show P values.

SUMMARY

ART reverses the effect of HIV on V δ I T-cell, CD4⁺ T cell and CD8⁺ T cell among malnourished HIV infected patients.

6.3.2 Effect of ART on cytolytic degranulation and cytokine release in HIV infected adults

6.3.2.1 Effect of ART on cytolytic degranulation by CD4⁺ T cells, CD8⁺ T cells, NK cells and Vδ1 T cells from moderately or severely malnourished adults with HIV-infection after stimulation ex vivo.

PBMC were isolated from 28 moderately or severely malnourished adult patients with HIV infection, of whom 20 had never received ART (naïve) and 8 have been on ART for at least 12 months. Cells were stimulated for 4 hours with medium alone, PMA and ionomycin, or monoclonal antibodies (mAb) specific for CD3, CD28 and CD2 in the presence of a fluorochrome-conjugated mAb specific for CD107a. Cells were subsequently stained with fluorochrome-conjugated mAbs specific for CD3, CD4, CD8, CD56 and Vδ1, and analysed by flow cytometry. Scatter plot show frequencies of CD4⁺ T cells, CD8⁺ T cells, NK cells and Vδ1 T cells that expressed cell-surface CD107a. Data were compared using the Mann Whitney U statistical test.

No significant differences were found in the level of degranulation from the four (4) cell types between the ART naïve and ART experienced participants. **(Figures 6.13 and 6.14)**

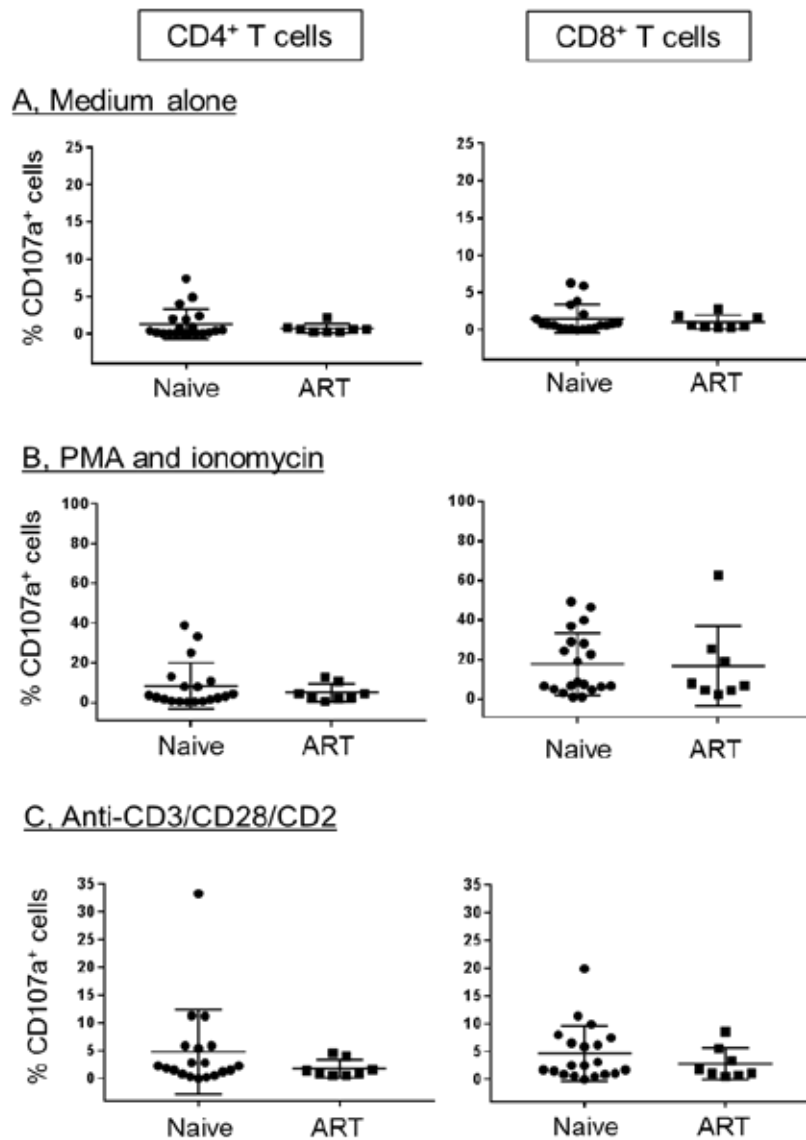


Figure 6.13 Effect of antiretroviral therapy (ART) on cytolytic degranulation by CD4⁺ T cells and CD8⁺ T cells from malnourished adults with HIV-infection after stimulation *ex vivo*. Scatter plot show frequencies of CD4⁺ T cells (left panels) and CD8⁺ T cells (right panels) that expressed cell-surface CD107a. Data were compared using the Mann Whitney U statistical test. No significant differences were found.

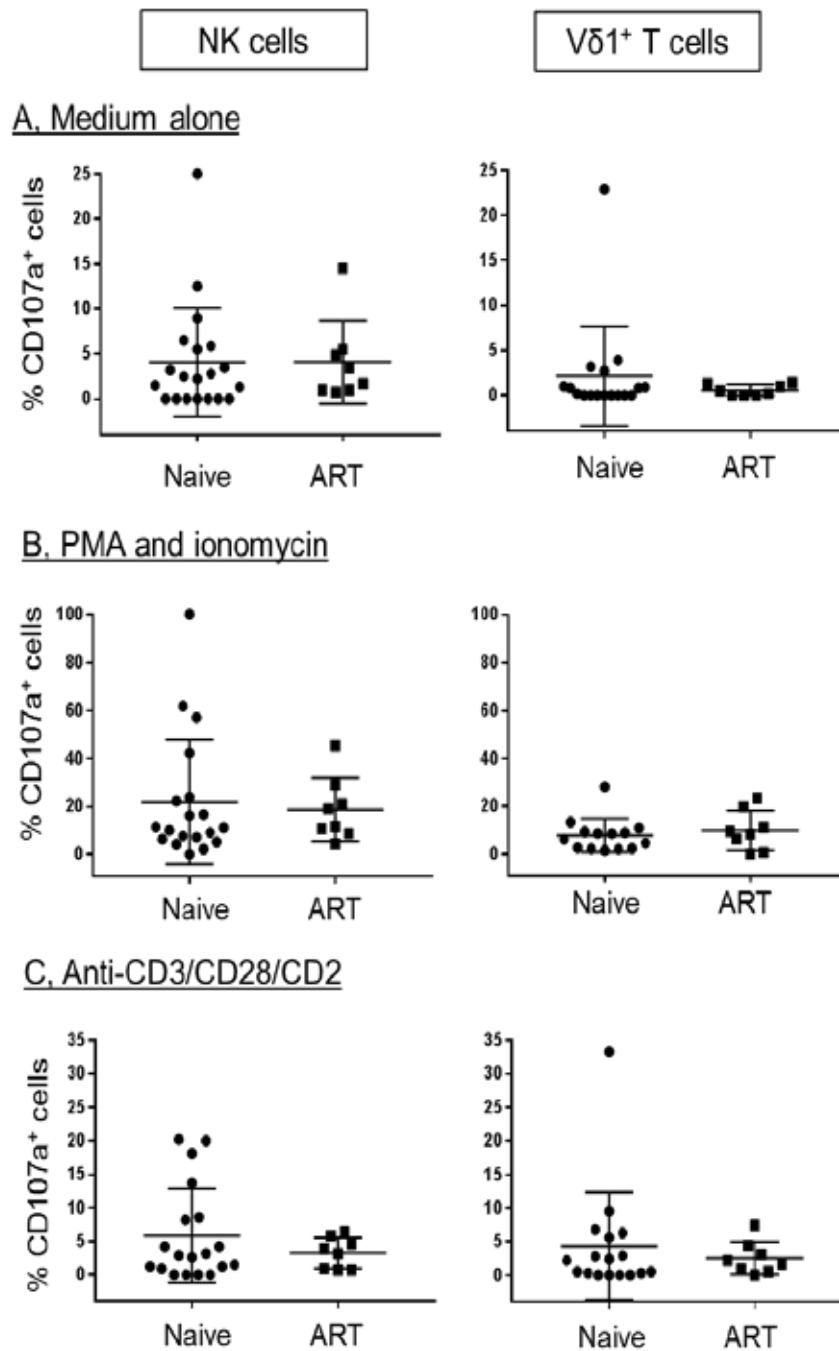


Figure 6.14 Effect of antiretroviral therapy (ART) on cytolytic degranulation by NK cells and Vδ1 T cells from malnourished adults with HIV-infection after stimulation *ex vivo*. Scatter plot show frequencies of NK cells (left panels) and Vδ1 T cells (right panels) that expressed cell-surface CD107a. Data were compared using the Mann Whitney U statistical test. No significant differences were found.

6.3.2.2 Effect of antiretroviral therapy (ART) on IFN- γ , IL-4 and IL-17 production by CD4⁺ T cells, CD8⁺ T cells and V δ 1 T cells from malnourished adults with HIV-infection after stimulation ex vivo

PBMC were isolated from 28 moderately or severely malnourished adult patients with HIV infection, of whom 20 had never received ART (naïve) and 8 have been on ART for at least 12 months. Cells were stimulated for 4 hours with medium alone (**A**), PMA and ionomycin (**B**), or monoclonal antibodies (mAb) specific for CD3, CD28 and CD2 (**C**) and subsequently stained with fluorochrome-conjugated mAbs specific for cell-surface CD3, CD4, CD8 and V δ 1 and intracellular IFN- γ , IL-4 and IL-17, and analysed by flow cytometry.

Production of IFN- γ by the cells incubated with anti-CD3/CD28/CD2 mAb, the CD4⁺ T cells of the ART participants was significantly higher than that of the ART naïve participants. There were no significant differences in the production of IFN- γ by CD8⁺ T cells and V δ 1 T cells (**Figure 6.15**). There were no differences in the production of IL-4 and IL-17 between the ART experienced and ART naïve participants from all the three cell types. (**Figure 6.16 - 6.17**)

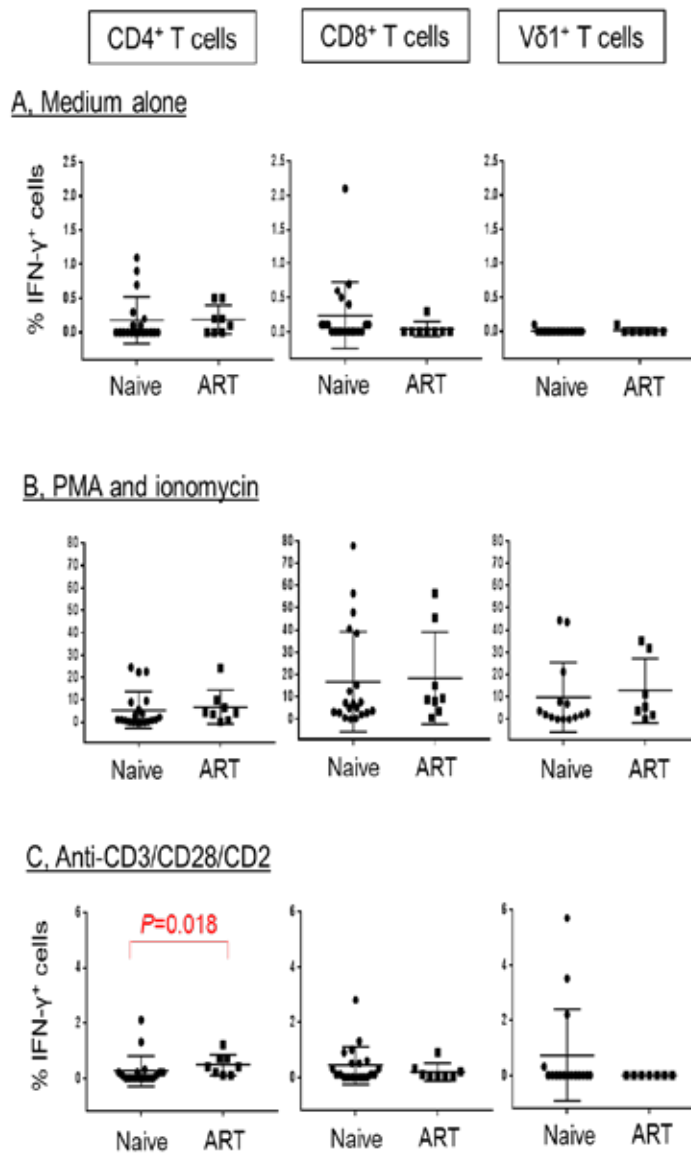


Figure 6.15 Effect of antiretroviral therapy (ART) on IFN- γ production by CD4⁺ T cells, CD8⁺ T cells and V δ 1⁺ T cells from malnourished adults with HIV-infection after stimulation ex vivo. Scatter plot show frequencies of CD4⁺ T cells (left panels), CD8⁺ T cells (centre panels) and V δ 1⁺ T cells (right panels) that expressed IFN- γ . Data were

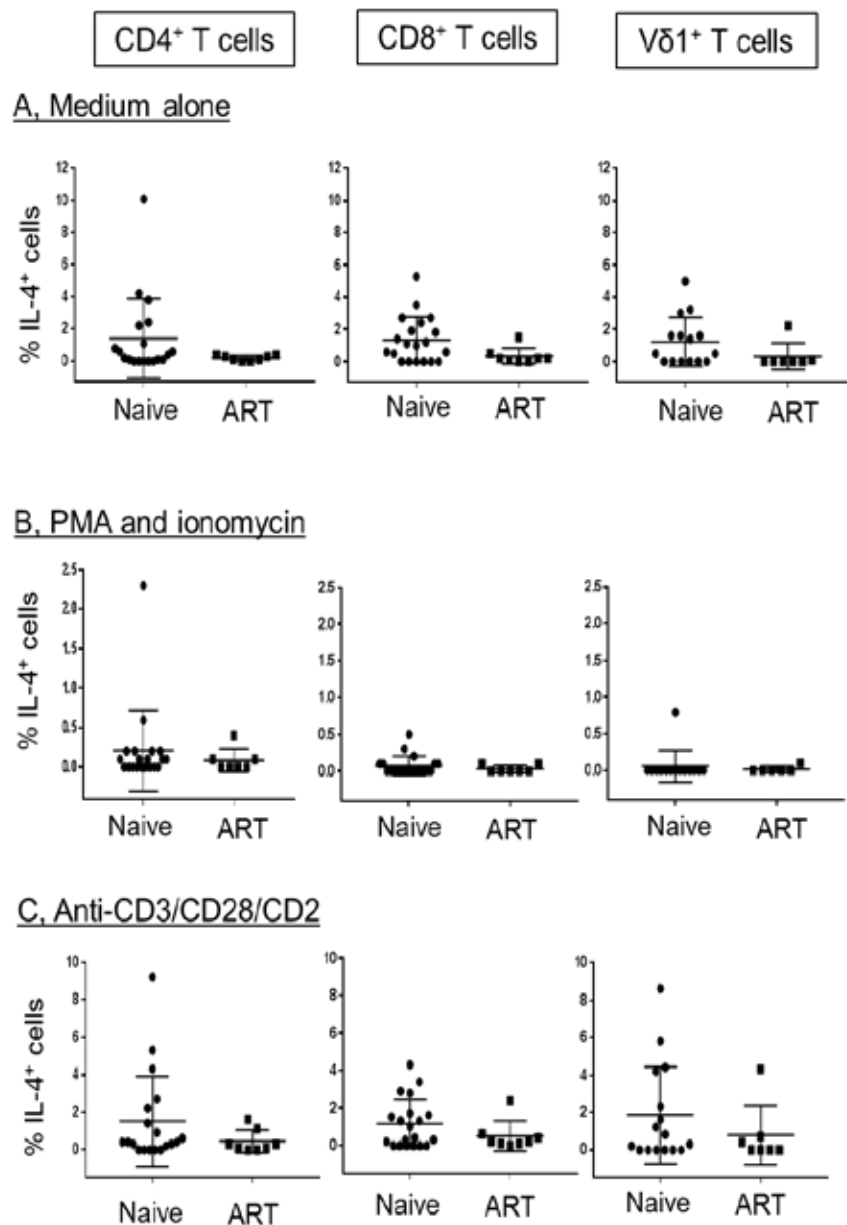


Figure 6.16 Effect of antiretroviral therapy (ART) on IL-4 production by CD4⁺ T cells, CD8⁺ T cells and Vδ1 T cells from malnourished adults with HIV-infection after stimulation ex vivo

Scatter plot show frequencies of CD4⁺ T cells (left panels), CD8⁺ T cells (center panels) and Vδ1 T cells (right panels) that expressed IL-4. Data were compared using the Mann

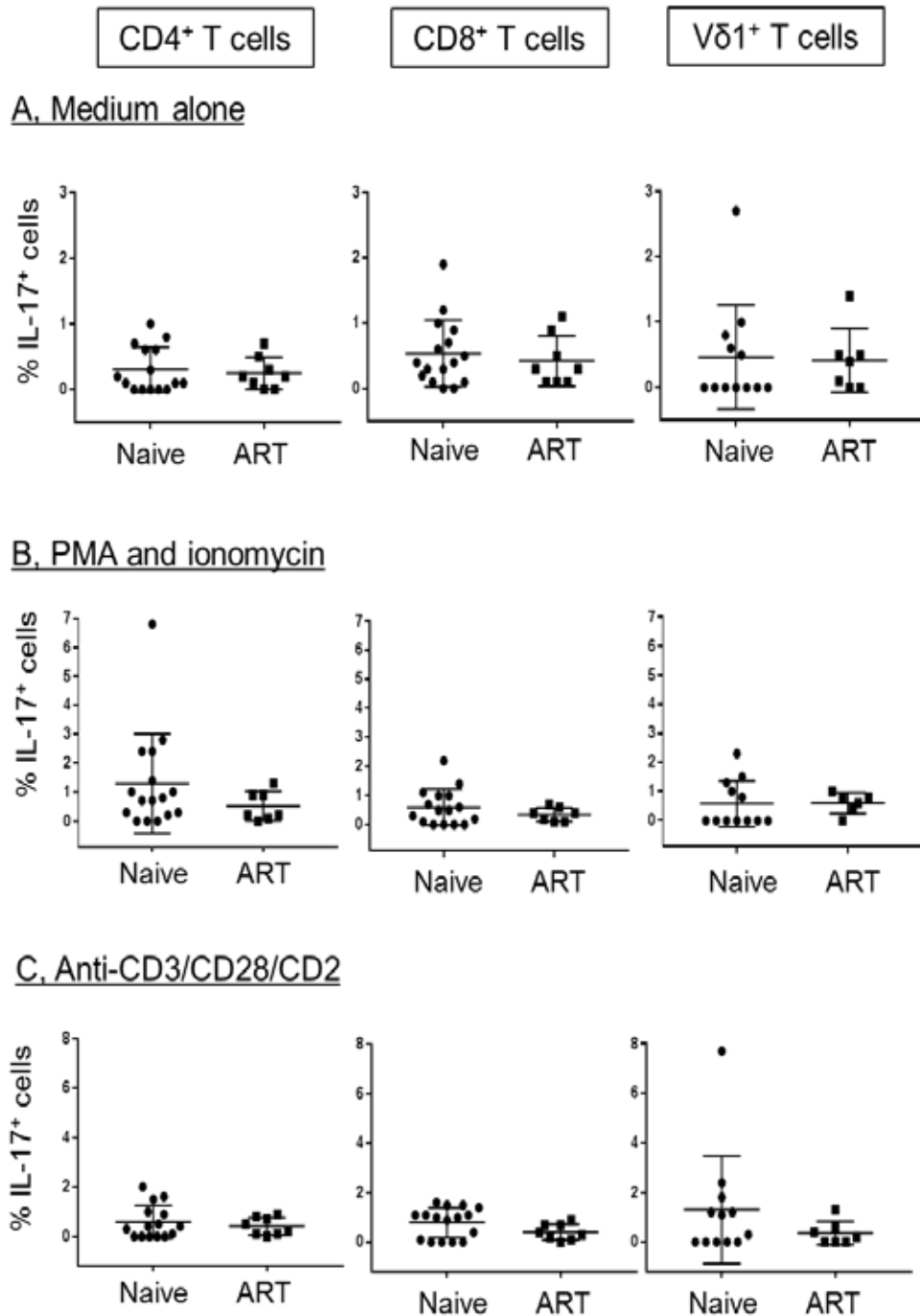


Figure 6.17 Effect of antiretroviral therapy (ART) on IL-17 production by CD4⁺ T cells, CD8⁺ T cells and Vδ1 T cells from malnourished adults with HIV-infection after stimulation *ex vivo* Scatter plot show frequencies of CD4⁺ T cells (left panels), CD8⁺ T cells (centre panels) and Vδ1 T cells (right panels) that expressed IL-17. Data were compared using the Mann Whitney U statistical test. No significant differences were found.

6.3.3 Effect of 12 weeks supplementation with RUTF on circulating lymphocyte frequencies and numbers in moderately malnourished HIV infected adults initiating ART

Moderately malnourished adults initiating ART were randomized to receive nutritional counselling and a daily supply of RUTF (Plumpy'Nut^R) or nutritional counselling only. After 12 weeks, PBMC were isolated from 18 of the patients, of whom 6 had received RUTF and 12 received nutritional counselling only. PBMC were stained with mAbs specific for CD3, CD4, CD8, CD19, CD56, and the V δ 1, V δ 2, V δ 3 and V α 24J α 18 TCRs and analysed by flow cytometry.

6.3.3.2 Effect of RUTF supplementation on circulating T cell, B cell and NK cell frequencies and numbers in moderately malnourished HIV infected adults initiating ART

The frequencies and absolute counts of the circulating T cells, B cells and NK cells were similar between the participants that received nutritional counselling only and those that received both nutritional counselling and RUTF (**Figures 6.18-6.20**)

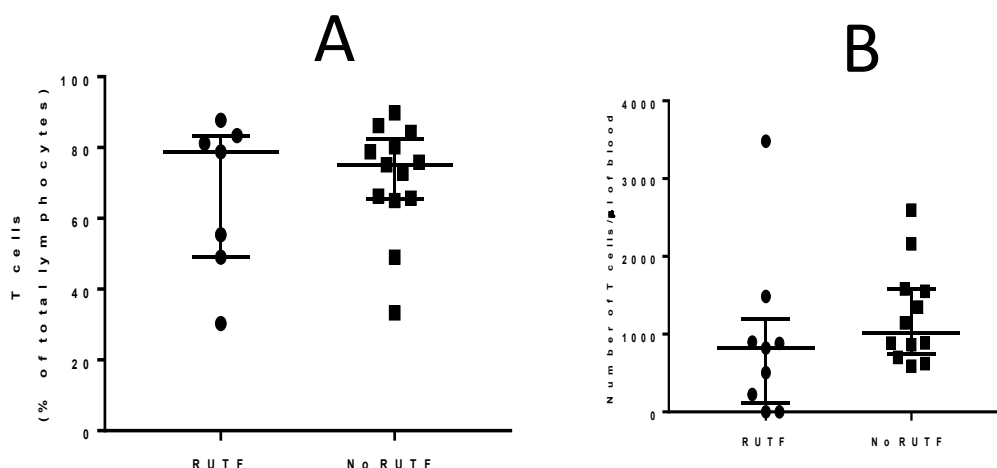


Figure 6.18 Effect of 12-week supplementation with a ready-to-use therapeutic food (RUTF) given to moderately-malnourished adults with HIV-infection initiating ART on circulating T cell frequencies and numbers. Cells were stained with monoclonal

antibodies specific for CD3 and analysed by flow cytometry. T cells were defined as CD3⁺ lymphocytes. Absolute numbers were calculated from full blood counts taken at the time of blood collection. Scatter plot show frequencies (A) and absolute numbers (B) of T cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test

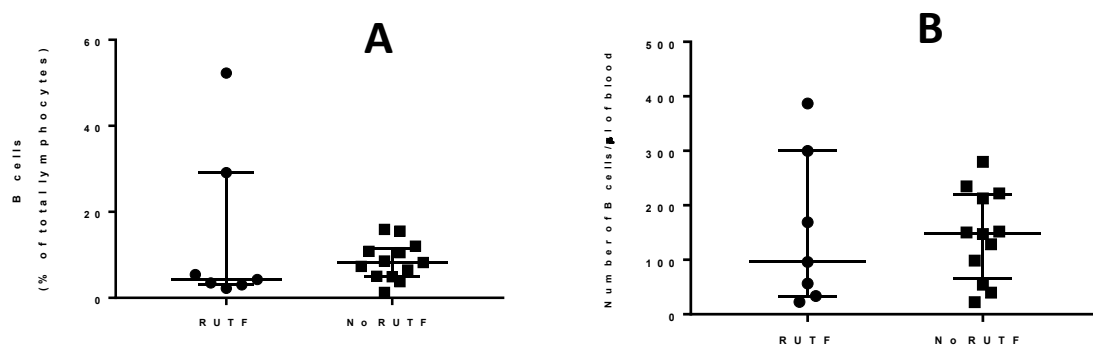


Figure 6.19 Effect of 12-week supplementation with a ready-to-use therapeutic food (RUTF) given to moderately-malnourished adults with HIV-infection initiating ART on circulating B cell frequencies and numbers. Cells were stained with monoclonal antibodies specific for CD3 and CD19 and analysed by flow cytometry. B cells were defined as CD3⁻ CD19⁺ lymphocytes. Absolute numbers were calculated from full blood counts taken at the time of blood collection. Scatter plot show frequencies (A) and absolute numbers (B) of B cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test.

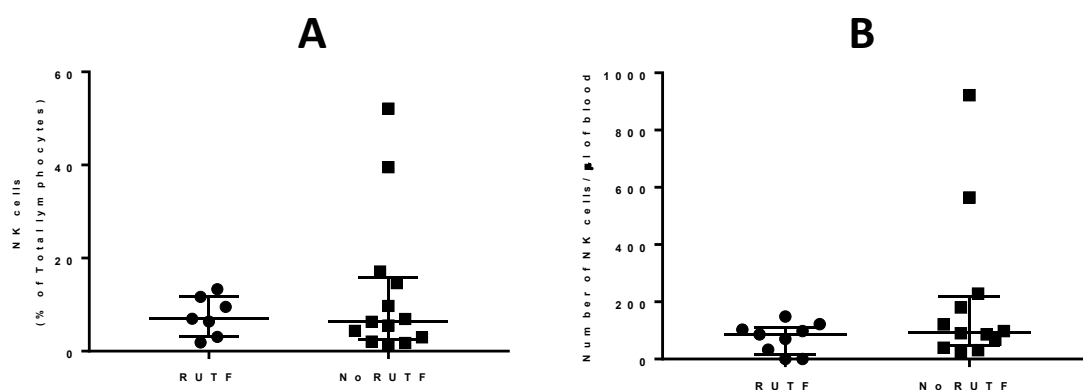


Figure 6.20 Effect of 12-week supplementation with a ready-to-use therapeutic food (RUTF) given to moderately-malnourished adults with HIV-infection initiating ART on circulating NK cell frequencies and numbers. Cells were stained with monoclonal antibodies specific for CD3 and CD56 and analysed by flow cytometry. NK cells were defined as CD3⁻ CD56⁺ lymphocytes. Absolute numbers were calculated from full blood counts taken at the time of blood collection. Scatter plot show frequencies (A) and absolute numbers (B) of NK cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test.

6.3.3.3 Effect of RUTF supplementation on circulating T cell subset frequencies and numbers in moderately malnourished HIV infected adults initiating ART

There were no significant differences in all the frequencies and absolute counts of the T-cell subsets between the participants in the randomization groups of the moderately malnourished HIV infected patients initiating ART (**Figures 6.21 to 6.24**).

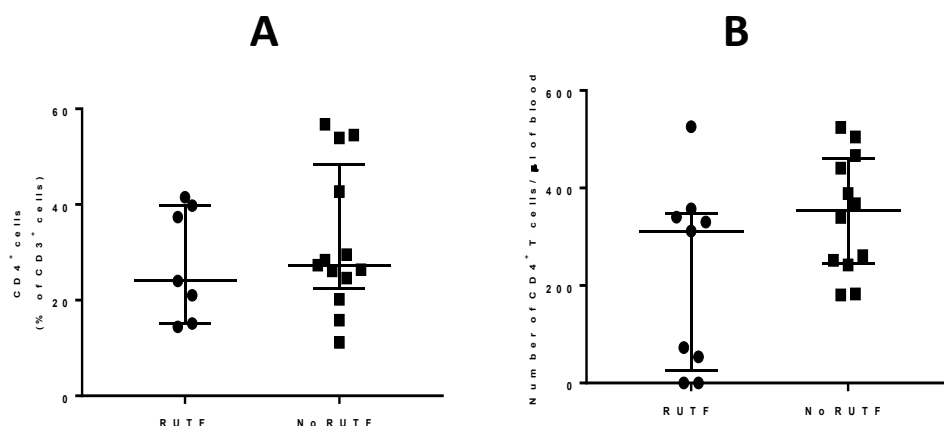


Figure 6.21 Effect of 12-week supplementation with a ready-to-use therapeutic food (RUTF) given to moderately-malnourished adults with HIV-infection initiating ART on circulating CD4⁺ T cell frequencies and numbers. Cells were stained with monoclonal antibodies specific for CD3, CD4 and CD8 and analysed by flow cytometry. CD4⁺ T cell were defined as CD3⁺ CD4⁺ CD8⁻. Absolute numbers were calculated from full blood counts taken at the time of blood collection. Scatter plot show frequencies (**A**) and absolute numbers (**B**) of CD4⁺ T cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test

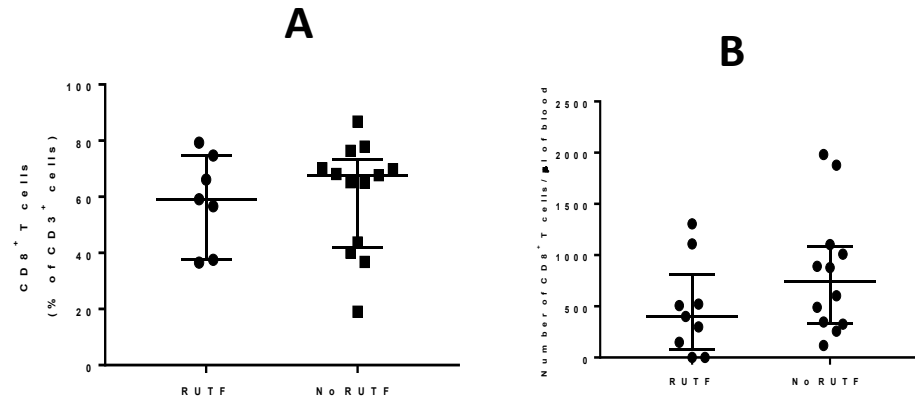


Figure 6.22 Effect of 12-week supplementation with a ready-to-use therapeutic food (RUTF) given to moderately-malnourished adults with HIV-infection initiating ART on circulating CD8⁺ T cell frequencies and numbers. Cells were stained with monoclonal antibodies specific for CD3, CD4 and CD8 and analysed by flow cytometry. CD8⁺ T cell were defined as CD3⁺ CD4⁻ CD8⁺. Absolute numbers were calculated from full blood counts taken at the time of blood collection. Scatter plot show frequencies (A) and absolute numbers (B) of CD8⁺ T cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test

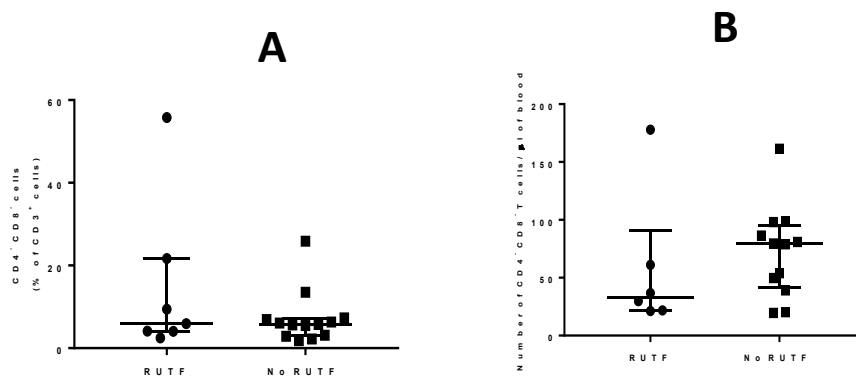


Figure 6.23 Effect of 12-week supplementation with a ready-to-use therapeutic food (RUTF) given to moderately-malnourished adults with HIV-infection initiating ART on circulating CD4⁻ CD8⁻ T cell frequencies and numbers. Cells were stained with monoclonal antibodies specific for CD3, CD4 and CD8 and analysed by flow cytometry. CD4⁻ CD8⁻ T cells were defined as CD3⁺ CD4⁻ CD8⁻. Absolute numbers were calculated from full blood counts taken at the time of blood collection. Scatter plot show frequencies (A) and absolute numbers (B) of CD4⁻ CD8⁻ T cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test

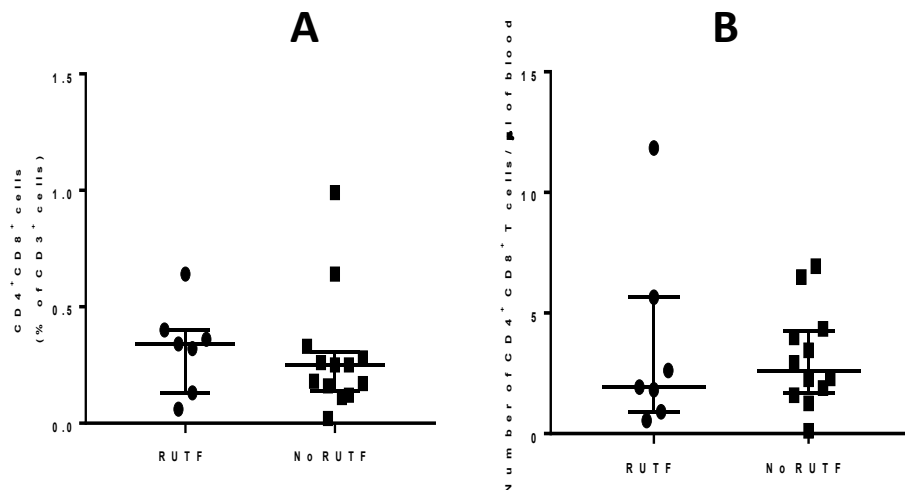


Figure 6.24 Effect of 12-week supplementation with a ready-to-use therapeutic food (RUTF) given to moderately-malnourished adults with HIV-infection initiating ART on circulating CD4⁺CD8⁺ T cell frequencies and numbers. Cells were stained with monoclonal antibodies specific for CD3, CD4 and CD8 and analysed by flow cytometry. CD4⁺CD8⁺ T cells were defined as CD3⁺CD4⁺CD8⁺. Absolute numbers were calculated from full blood counts taken at the time of blood collection. Scatter plot show frequencies (A) and absolute numbers (B) of CD4⁺CD8⁺ T cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test

6.3.3.4 Effect of RUTF supplementation on circulating innate T cell frequencies and numbers in moderately malnourished HIV infected adults initiating ART

As observed among the T cell subsets, there were no significant differences in the frequencies and absolute counts of all the innate cell populations studied in the two comparison groups, after 12 weeks of receiving either nutritional counselling only, or RUTF and nutritional counselling (**Figures 6.25 – 6.29**)

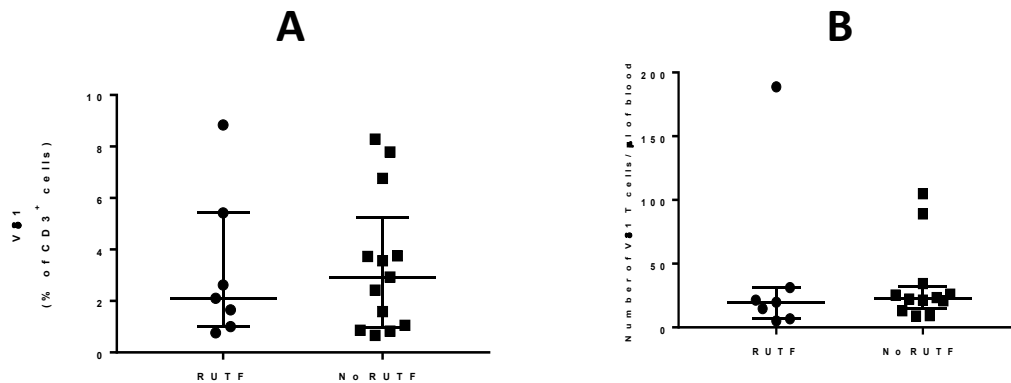


Figure 6.25 Effect of 12-week supplementation with a ready-to-use therapeutic food (RUTF) given to moderately-malnourished adults with HIV-infection initiating ART on circulating Vδ1 cell frequencies and numbers. Cells were stained with monoclonal antibodies specific for CD3 and Vδ1 and analysed by flow cytometry. Vδ1 cells were defined as CD3⁺ Vδ1⁺ lymphocytes. Absolute numbers were calculated from full blood counts taken at the time of blood collection. Scatter plot show frequencies (A) and absolute numbers (B) of Vδ1 cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test.

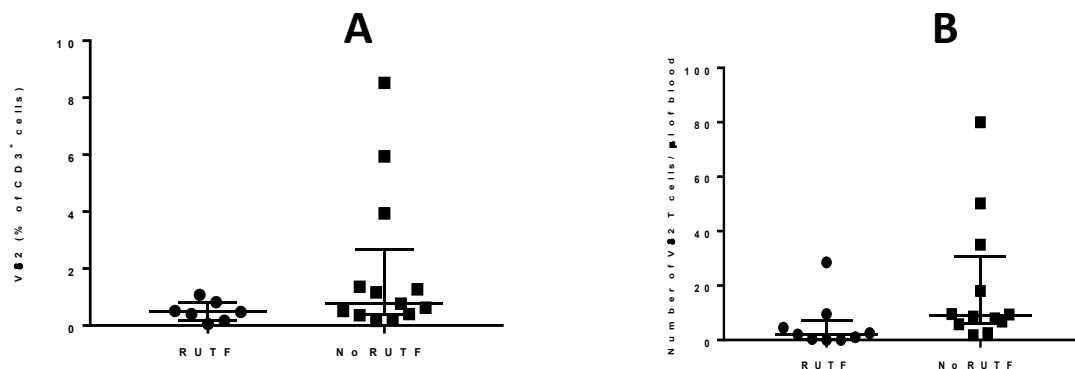


Figure 6.26 Effect of 12-week supplementation with a ready-to-use therapeutic food (RUTF) given to moderately-malnourished adults with HIV-infection initiating ART on circulating Vδ2 cell frequencies and numbers. Cells were stained with monoclonal antibodies specific for CD3 and Vδ2 and analysed by flow cytometry. Vδ2 cells were defined as CD3⁺ Vδ2⁺ lymphocytes. Absolute numbers were calculated from full blood counts taken at the time of blood collection. Scatter plot show frequencies (A) and absolute numbers (B) of Vδ2 cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test.

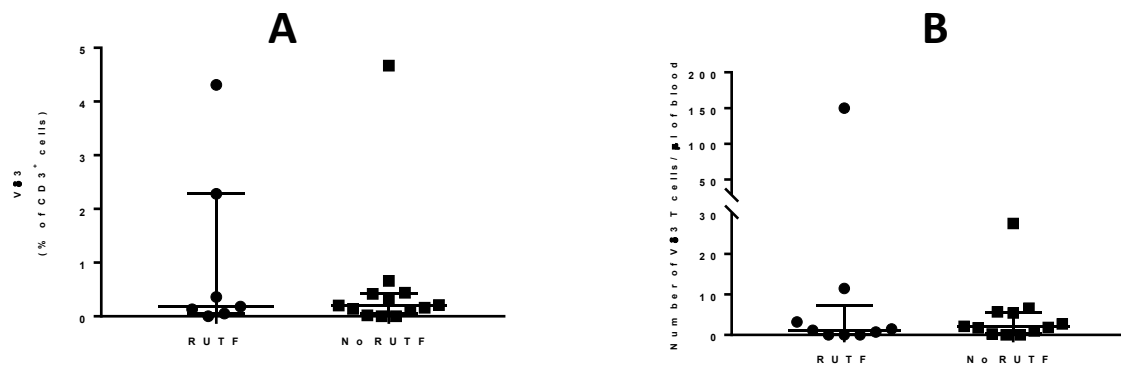


Figure 6.27 Effect of 12-week supplementation with a ready-to-use therapeutic food (RUTF) given to moderately-malnourished adults with HIV-infection initiating ART on circulating Vδ3 cell frequencies and numbers. Cells were stained with monoclonal antibodies specific for CD3 and Vδ3 and analysed by flow cytometry. Vδ3 cells were defined as CD3⁺ Vδ3⁺ lymphocytes. Absolute numbers were calculated from full blood counts taken at the time of blood collection. Scatter plot show frequencies (A) and absolute numbers (B) Vδ3 of cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test.

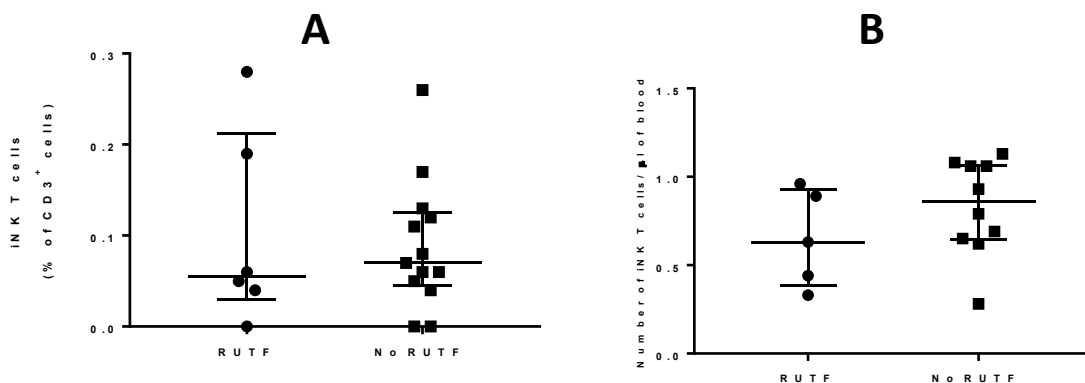


Figure 6.28 Effect of 12-week supplementation with a ready-to-use therapeutic food (RUTF) given to moderately-malnourished adults with HIV-infection initiating ART on circulating iNK T cell frequencies and numbers. Cells were stained with monoclonal antibodies specific for CD3 and Vα24Jα18 and analysed by flow cytometry. iNK T cells were defined as CD3⁺ Vα24Jα18⁺ lymphocytes. Absolute numbers were calculated from full blood counts taken at the time of blood collection. Scatter plot show frequencies (A) and absolute numbers (B) of iNK T cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test.

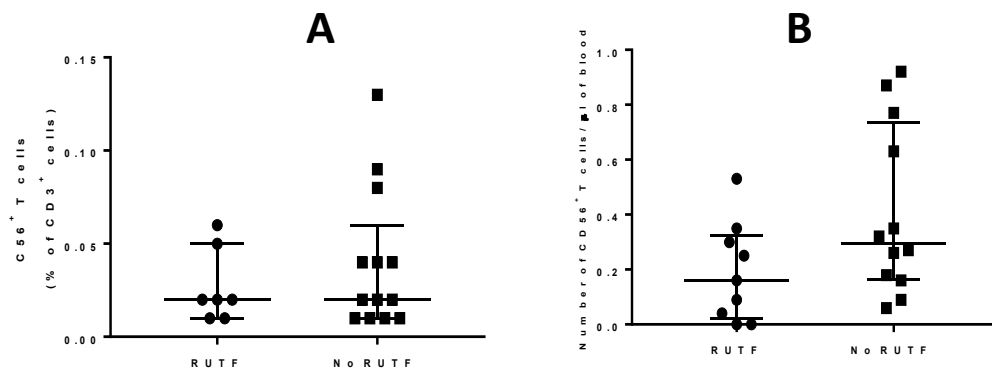


Figure 6.29 Effect of 12-week supplementation with a ready-to-use therapeutic food (RUTF) given to moderately-malnourished adults with HIV-infection initiating ART on circulating

CD56⁺ T cell frequencies and numbers. Cells were stained with monoclonal antibodies specific for CD3 and CD56 and analysed by flow cytometry. CD56⁺ T cell cells were defined as CD3⁺ CD56⁺ lymphocytes. Absolute numbers were calculated from full blood counts taken at the time of blood collection.

Scatter plot show frequencies (A) and absolute numbers (B) of CD56⁺ T cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test.

6.3.4 Effect of 12-week supplementation with RUTF on circulating lymphocyte frequencies and numbers in ART-experienced malnourished HIV infected adults

Malnourished HIV infected adults that had been on ART for at least 12 months were randomized to receive nutritional counselling and a daily supply of RUTF (Plumpy'Nut^R) or nutritional counselling only. After 12 weeks, PBMC were isolated from 18 of the patients, of whom 6 had received RUTF and 12 received nutritional counselling only. Immunophenotypes were analyzed using flow cytometry.

6.3.4.1 Effect of RUTF supplementation on circulating T cell, B cell and NK cell frequencies and numbers in ART-experienced malnourished HIV infected adults

12-week supplementation with RUTF did not lead to significant changes in the frequencies and absolute numbers of the circulating T cells, B cells and NK cells in ART-experienced patients with HIV (Figures 6.30-6.32).

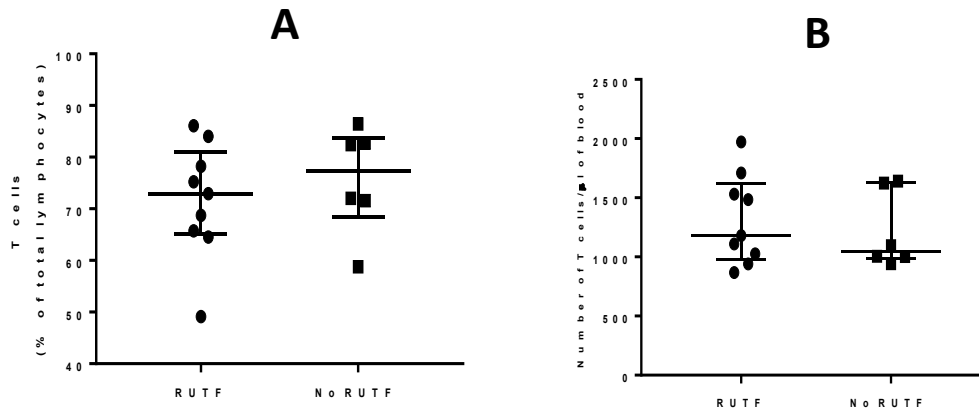


Figure 6.30 Effect of 12-week supplementation with a ready-to-use therapeutic food (RUTF) given to malnourished to ART experienced HIV-infection adults on circulating T cell frequencies and numbers. Cells were stained with monoclonal antibodies specific for CD3 and analysed by flow cytometry. T cells were defined as CD3⁺ lymphocytes. Absolute numbers were calculated from full blood counts taken at the time of blood collection. Scatter plot show frequencies (A) and absolute numbers (B) of T cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test

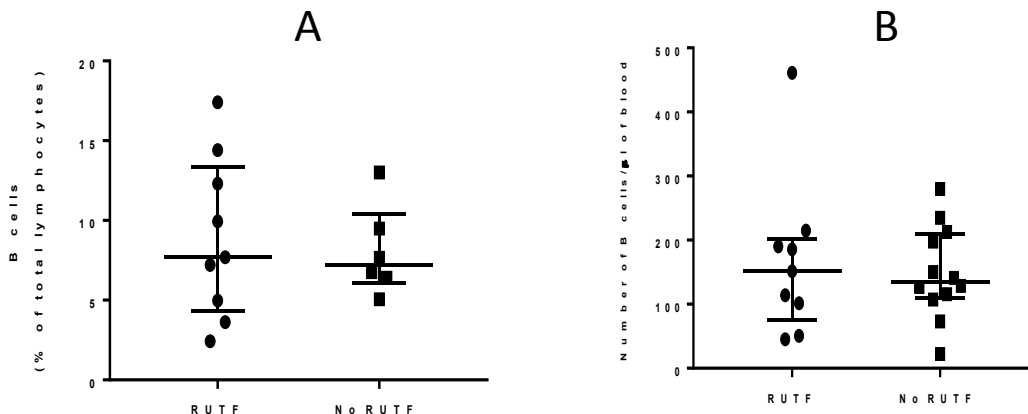


Figure 6.31 Effect of 12-week supplementation with a ready-to-use therapeutic food (RUTF) given to malnourished to ART experienced HIV-infection adults on circulating B cell frequencies and numbers. Cells were stained with monoclonal antibodies specific for CD3 and CD19 analysed by flow cytometry. B cells were defined as CD3⁻CD19⁺ lymphocytes. Absolute numbers were calculated from full blood counts taken at the time of blood collection. Scatter plot show frequencies (A) and absolute numbers (B) of B cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test

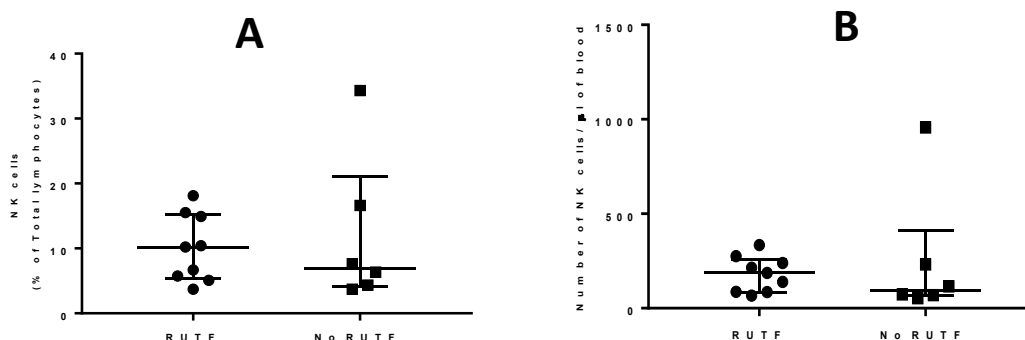


Figure 6.32 Effect of 12-week supplementation with a ready-to-use therapeutic food (RUTF) given to malnourished to ART experienced HIV-infection adults on circulating NK cell frequencies and numbers. Cells were stained with monoclonal antibodies specific for CD3 and CD56 analysed by flow cytometry. NK cells were defined as CD3⁺ CD56⁺ lymphocytes. Absolute numbers were calculated from full blood counts taken at the time of blood collection. Scatter plot show frequencies (A) and absolute numbers (B) of NK cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test

6.3.4.2 Effect of RUTF supplementation on circulating T cell subtype frequencies and numbers in ART-experienced malnourished HIV infected adults

12-week supplementation with RUTF did not have any significant effect on the frequencies and absolute counts of the circulating T cell subsets defined by the presence of CD4 and CD8 (Figures 6.33 – 6.36).

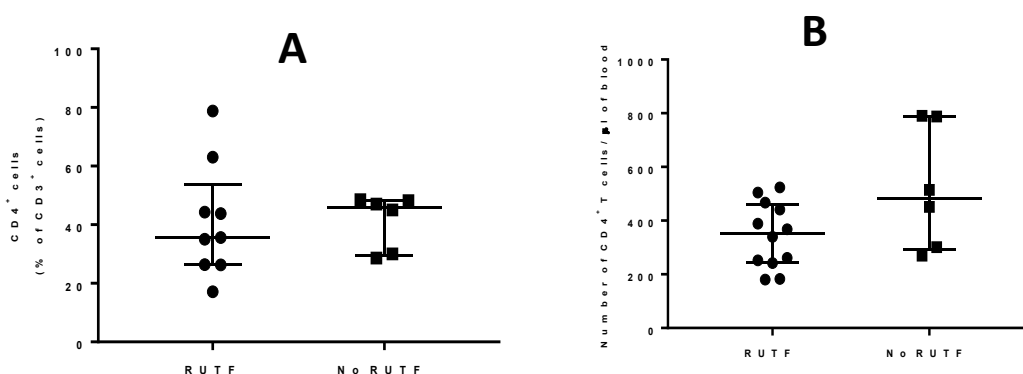


Figure 6.33 Effect of 12-week supplementation with a ready-to-use therapeutic food (RUTF) given to malnourished to ART experienced HIV-infection adults on circulating CD4⁺ T cell frequencies and numbers. Cells were stained with monoclonal antibodies specific for CD3, CD4 and CD8 analysed by flow cytometry. CD4⁺ T cells were defined as CD3⁺ CD4⁺ CD8⁺ lymphocytes. Absolute numbers were calculated from full blood counts taken at the time of blood collection. Scatter plot show frequencies (A) and absolute numbers (B) of CD4⁺ T cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test

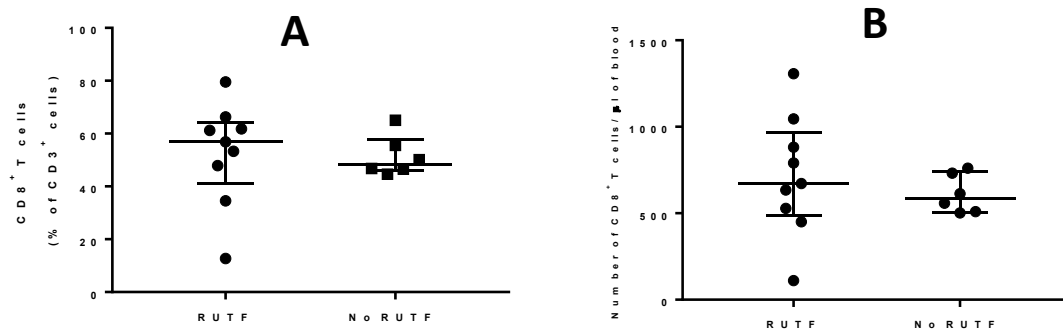


Figure 6.34 Effect of 12-week supplementation with a ready-to-use therapeutic food (RUTF) given to malnourished to ART experienced HIV-infection adults on circulating CD8⁺ T cell frequencies and numbers. Cells were stained with monoclonal antibodies specific for CD3, CD4 and CD8 analysed by flow cytometry. CD4⁺ T cells were defined as CD3⁺ CD4⁺ CD8⁺ lymphocytes. Absolute numbers were calculated from full blood counts taken at the time of blood collection. Scatter plot show frequencies (A) and absolute numbers (B) of CD8⁺ T cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test

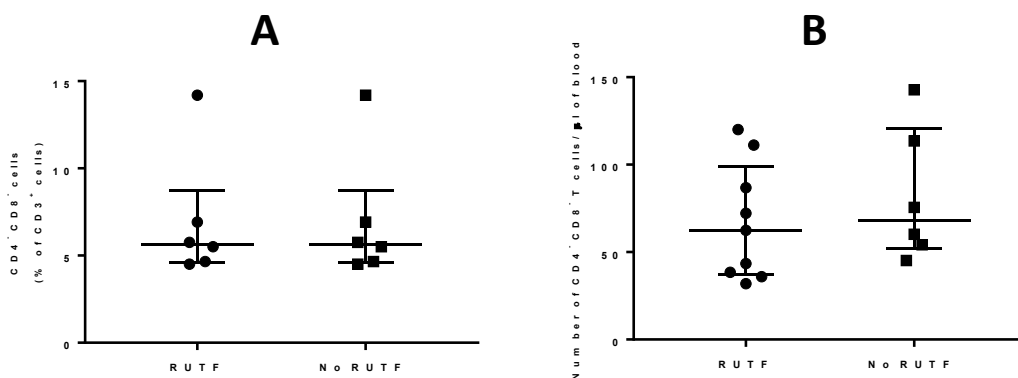


Figure 6.35 Effect of 12-week supplementation with a ready-to-use therapeutic food (RUTF) given to malnourished to ART experienced HIV-infection adults on circulating CD4⁺ CD8⁻ T cell frequencies and numbers. Cells were stained with monoclonal antibodies specific for CD3, CD4 and CD8 analysed by flow cytometry. CD4⁺ CD8⁻ T cells were defined as CD3⁺ CD4⁺ CD8⁻ lymphocytes. Absolute numbers were calculated from full blood counts taken at the time of blood collection. Scatter plot show frequencies (A) and absolute numbers (B) of CD4⁺ CD8⁻ T cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test

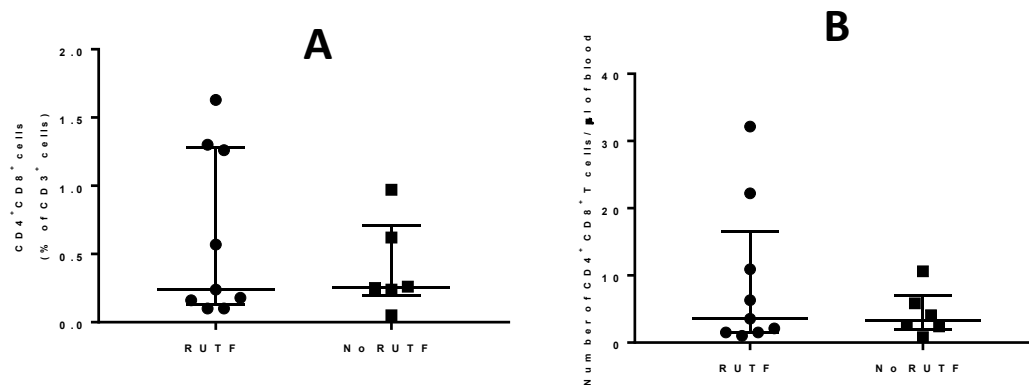


Figure 6.36 Effect of 12-week supplementation with a ready-to-use therapeutic food (RUTF) given to malnourished to ART experienced HIV-infection adults on circulating CD4⁺ CD8⁺ T cell frequencies and numbers. Cells were stained with monoclonal antibodies specific for CD3, CD4 and CD8 analysed by flow cytometry. CD4⁺ CD8⁺ T cells were defined as CD3⁺ CD4⁺ CD8⁺ lymphocytes. Absolute numbers were calculated from full blood counts taken at the time of blood collection. Scatter plot show frequencies (A) and absolute numbers (B) of CD4⁺ CD8⁺ T cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test

6.3.4.3 Effect of RUTF supplementation on circulating innate T cell frequencies and numbers in ART-experienced malnourished HIV infected adults

12-week supplementation with RUTF led to decreases in the frequencies and absolute counts of V δ 1 cells (**figure 6.37**), however, there were no changes in the frequencies and absolute counts of V δ 2 and V δ 3 (**figures 6.38 and 6.39**). Conversely, there was a reduction in the CD56⁺ T cells which was not statistically significant (**figure 6.40**).

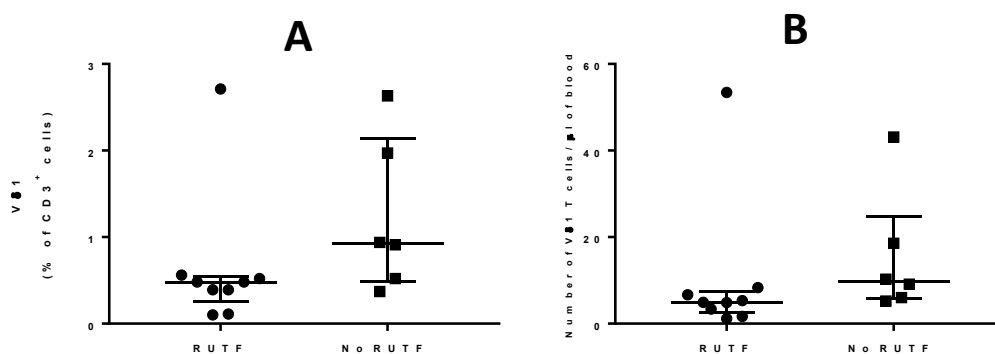


Figure 6.37 Effect of 12-week supplementation with a ready-to-use therapeutic food (RUTF) given to malnourished to ART experienced HIV-infection adults on circulating Vδ1 T cell frequencies and numbers. Cells were stained with monoclonal antibodies specific for CD3 and Vδ1 and analysed by flow cytometry. Vδ1 T cells were defined as CD3+ Vδ1+ lymphocytes. Absolute numbers were calculated from full blood counts taken at the time of blood collection. Scatter plot show frequencies (A) and absolute numbers (B) of Vδ1 T cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test

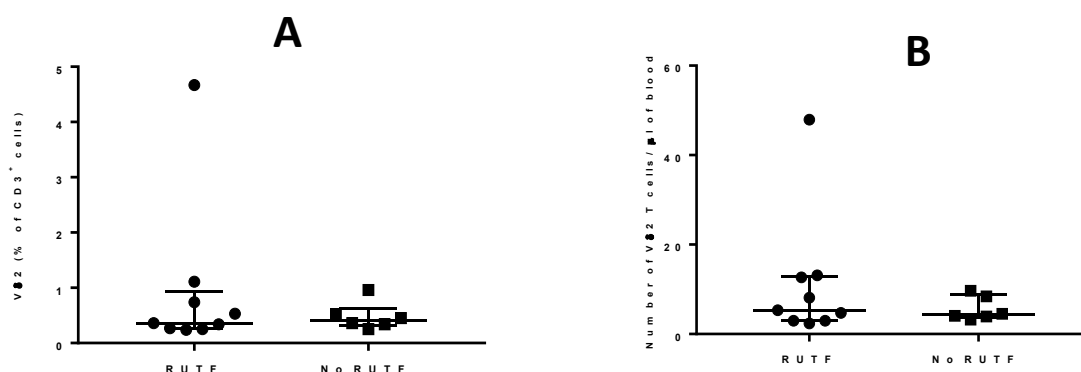


Figure 6.38 Effect of 12-week supplementation with a ready-to-use therapeutic food (RUTF) given to malnourished to ART experienced HIV-infection adults on circulating Vδ2 T cell frequencies and numbers. Cells were stained with monoclonal antibodies specific for CD3 and Vδ2 and analysed by flow cytometry. Vδ2 T cells were defined as CD3+ Vδ2+ lymphocytes. Absolute numbers were calculated from full blood counts taken at the time of blood collection. Scatter plot show frequencies (A) and absolute numbers (B) of Vδ2 T cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test

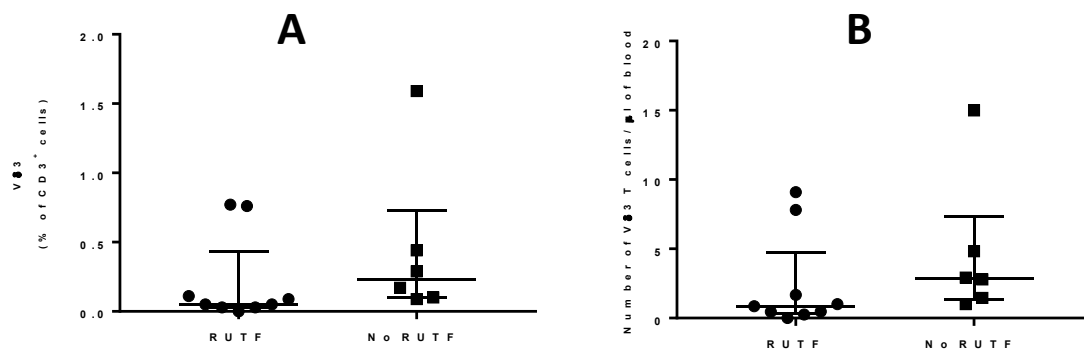


Figure 6.39 Effect of 12-week supplementation with a ready-to-use therapeutic food (RUTF) given to malnourished to ART experienced HIV-infection adults on circulating Vδ3 T cell frequencies and numbers. Cells were stained with monoclonal antibodies specific for CD3 and Vδ3 and analysed by flow cytometry. Vδ3 T cells were defined as CD3⁺ Vδ3⁺ lymphocytes. Absolute numbers were calculated from full blood counts taken at the time of blood collection. Scatter plot show frequencies (A) and absolute numbers (B) of Vδ3 T cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test

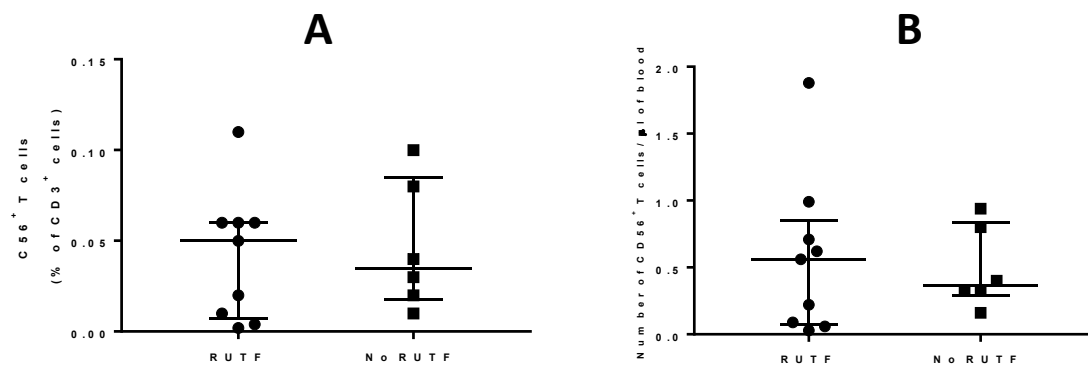


Figure 6.40 Effect of 12-week supplementation with a ready-to-use therapeutic food (RUTF) given to malnourished to ART experienced HIV-infection adults on circulating CD56⁺ T cell frequencies and numbers. Cells were stained with monoclonal antibodies specific for CD3 and CD56 analysed by flow cytometry. CD56⁺ T cells were defined as CD3⁺ CD56⁺ lymphocytes. Absolute numbers were calculated from full blood counts taken at the time of blood collection. Scatter plot show frequencies (A) and absolute numbers (B) of CD56⁺ T cells present. Error bars show medians and interquartile ranges. Data were compared using the Mann Whitney statistical test

Summary:

There were no significant differences in the immunophenotypes (except for V δ I T cells) between the participants that received and did not receive RUTF. The supplemented participants had lesser V δ I T cells but this was not statistically significant.

6.4 Effect of a 12-week programme of nutritional supplementation at the time of ART initiation on cytolytic degranulation by CD4⁺ T cells, CD8⁺ T cells, NK cells and V δ I T cells from malnourished adults with HIV-infection

6.4.1 Effect of 12-week supplementation with RUTF and ART given to malnourished adults with HIV-infection on cytolytic degranulation by CD4⁺ T cells and CD8⁺ T cells.

Moderately malnourished adults initiating ART were randomised to receive nutritional counselling and a daily supply of RUTF (Plumpy'Nut) or nutritional counselling only. After 12 weeks, PBMC were isolated from 9 of the patients, of whom 6 had received RUTF and 3 received nutritional counselling only. Cells were stimulated for 4 hours with medium alone, PMA and ionomycin, or monoclonal antibodies (mAb) specific for CD3, CD28 and CD2 in the presence of a fluorochrome-conjugated mAb specific for CD107a. Cells were subsequently stained with fluorochrome-conjugated mAbs specific for CD3, CD4, CD8, CD56 and V δ I, and analysed by flow cytometry.

All the four cell types of the participants that received RUTF showed higher levels of degranulation compared to those who did not receive RUTF. However, the only statistically significant difference was in the CD4⁺ T cells incubated with anti-CD3/CD28/CD2 mAb. **(Figure 6.41- 6.42)**

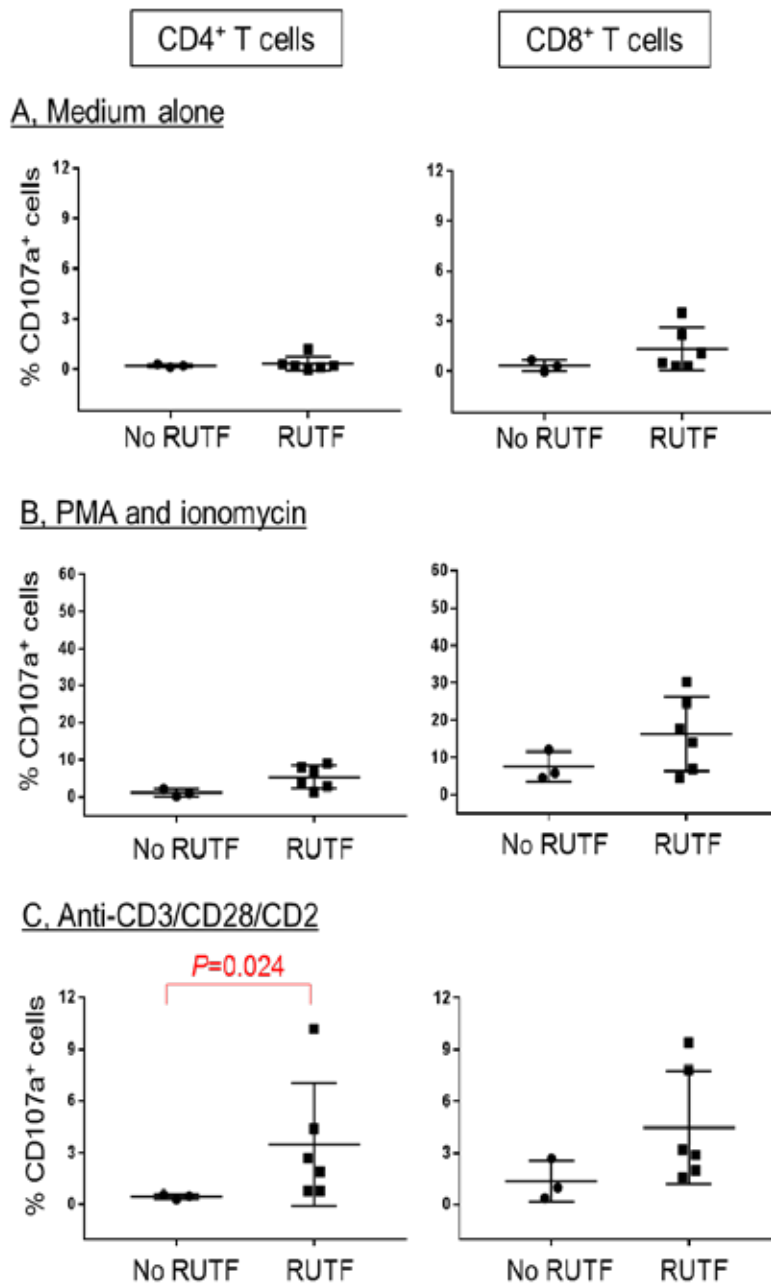


Figure 6.41 Effect of 12-week supplementation with ready-to-use therapeutic food antiretroviral therapy (RUTF) given to malnourished adults with HIV-infection on cytolytic degranulation by CD4⁺ T cells and CD8⁺ T cells: Scatter plot show frequencies of CD4⁺ T cells (left panels) and CD8⁺ T cells (right panels) that expressed cell-surface CD107a. Data were compared using the Mann Whitney U statistical test. A

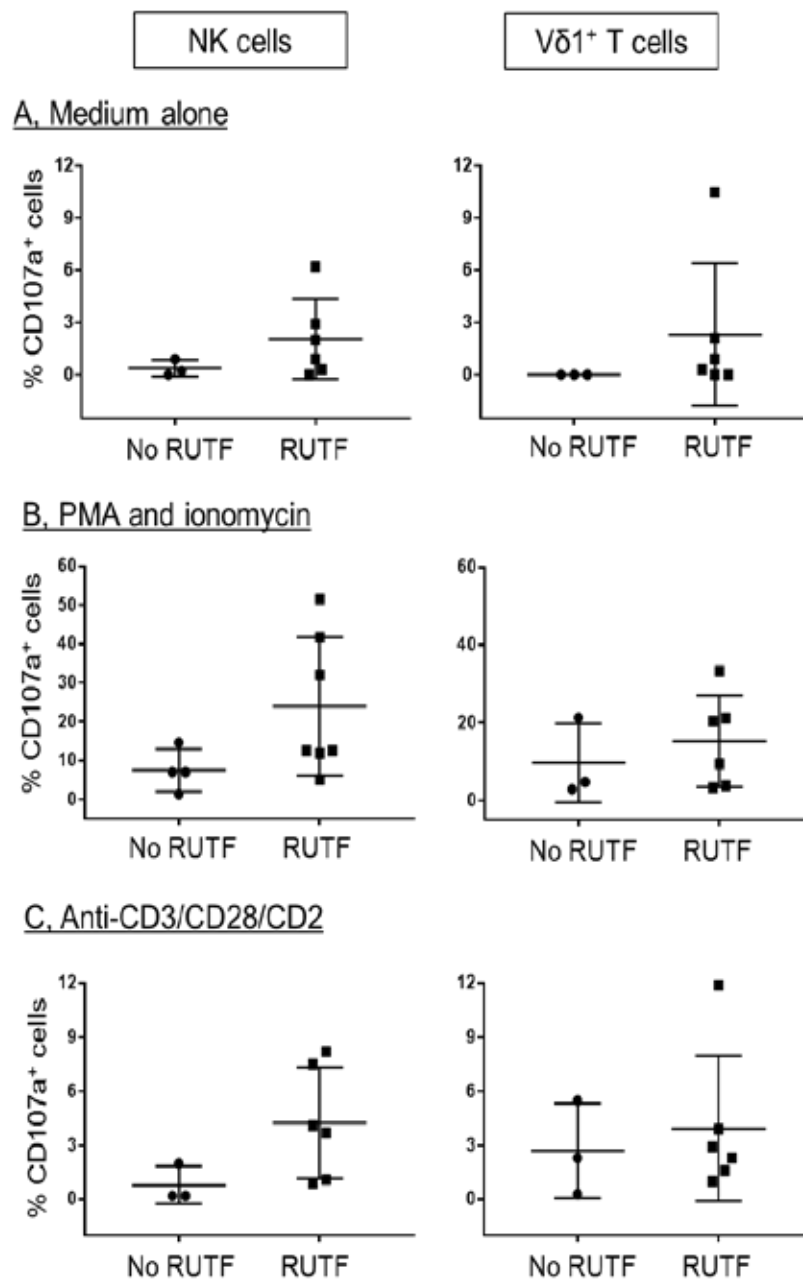
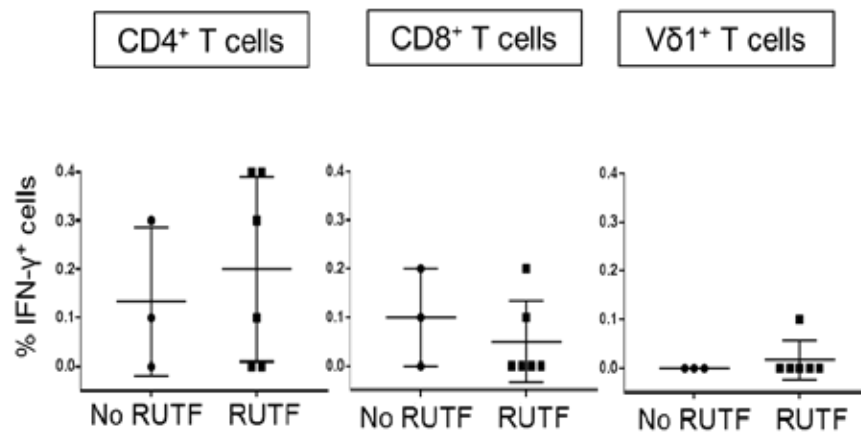


Figure 6.42. Effect of 12-week supplementation with ready-to-use therapeutic food antiretroviral therapy (RUTF) given to malnourished adults with HIV-infection on cytolytic degranulation by NK cells and Vδ1 T cells: Scatter plot show frequencies of NK cells (left panels) and Vδ1⁺ T cells (right panels) that expressed cell-surface CD107a. Data were

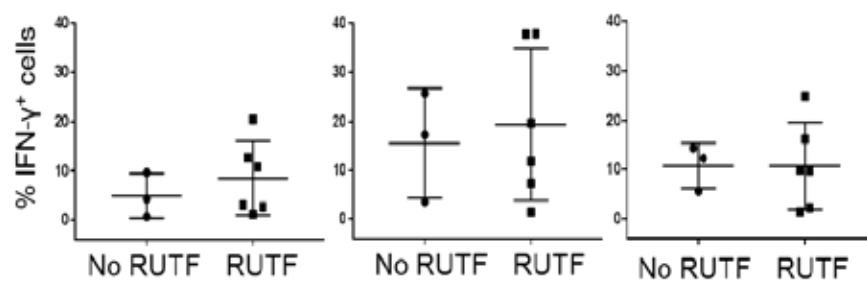
6.4.1.1 Effect of 12-week supplementation with ready-to-use therapeutic food (RUTF) and antiretroviral therapy given to malnourished adults with HIV-infection on IFN- γ , IL-4 and IL-17 production by CD4⁺ T cells, CD8⁺ T cells and V δ 1 T cells.

Moderately malnourished adults initiating ART were randomised to receive nutritional counselling and a daily supply of RUTF (Plumpy'Nut) or nutritional counselling only. After 12 weeks, PBMC were isolated from 9 of the patients, of whom 6 had received RUTF and 3 received nutritional counselling only. Cells were stimulated for 4 hours with medium alone, PMA and ionomycin, or mAb specific for CD3, CD28 and CD2 and subsequently stained with fluorochrome-conjugated mAbs specific for cell-surface CD3, CD4, CD8 and V δ 1 and intracellular IFN- γ , IL-14 and IL-17 and analysed by flow cytometry.

There were no significant differences in the production of all the three cytokines. **(Figures 6.43-6.45)**



B. PMA and ionomycin



C. Anti-CD3/CD28/CD2

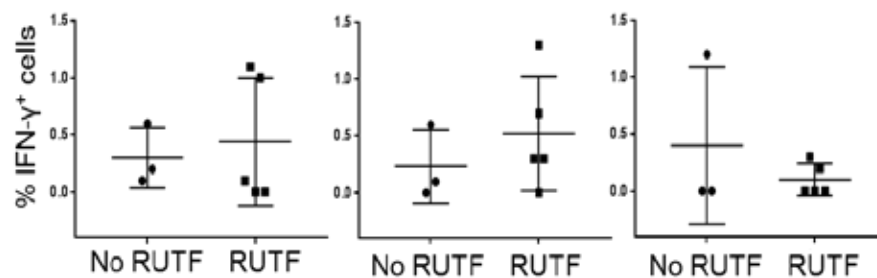


Figure 6.43 Effect of 12-week supplementation with ready-to-use therapeutic food antiretroviral therapy (RUTF) given to malnourished adults with HIV-infection on IFN- γ production by CD4⁺ T cells, CD8⁺ T cells and V δ 1 T cells:

Scatter plot show frequencies of CD4⁺ T cells (left panels), CD8⁺ T cells (centre panels) and V δ 1 T cells (right panels) that expressed IFN- γ . Data were compared using the Mann Whitney U statistical test. No significant differences were found

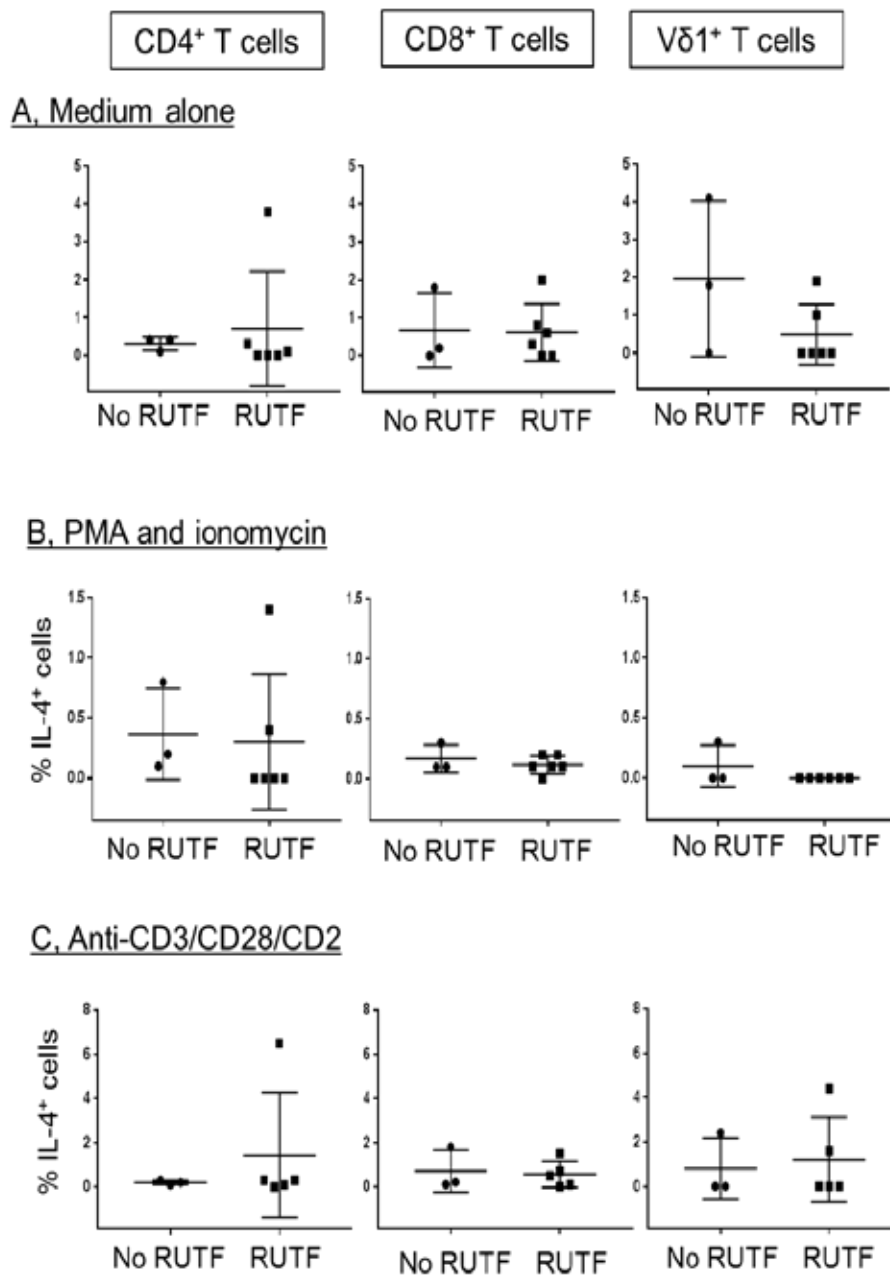


Figure 6.44 Effect of 12-week supplementation with ready-to-use therapeutic food antiretroviral therapy (RUTF) given to malnourished adults with HIV-infection on IL-4 production by CD4⁺ T cells, CD8⁺ T cells and Vδ1 T cells. Scatter plot show frequencies of CD4⁺ T cells (left panels), CD8⁺ T cells (centre panels) and Vδ1 T cells (right panels) that expressed IL-4. Data were compared using the Mann Whitney U statistical test. No significant differences were found

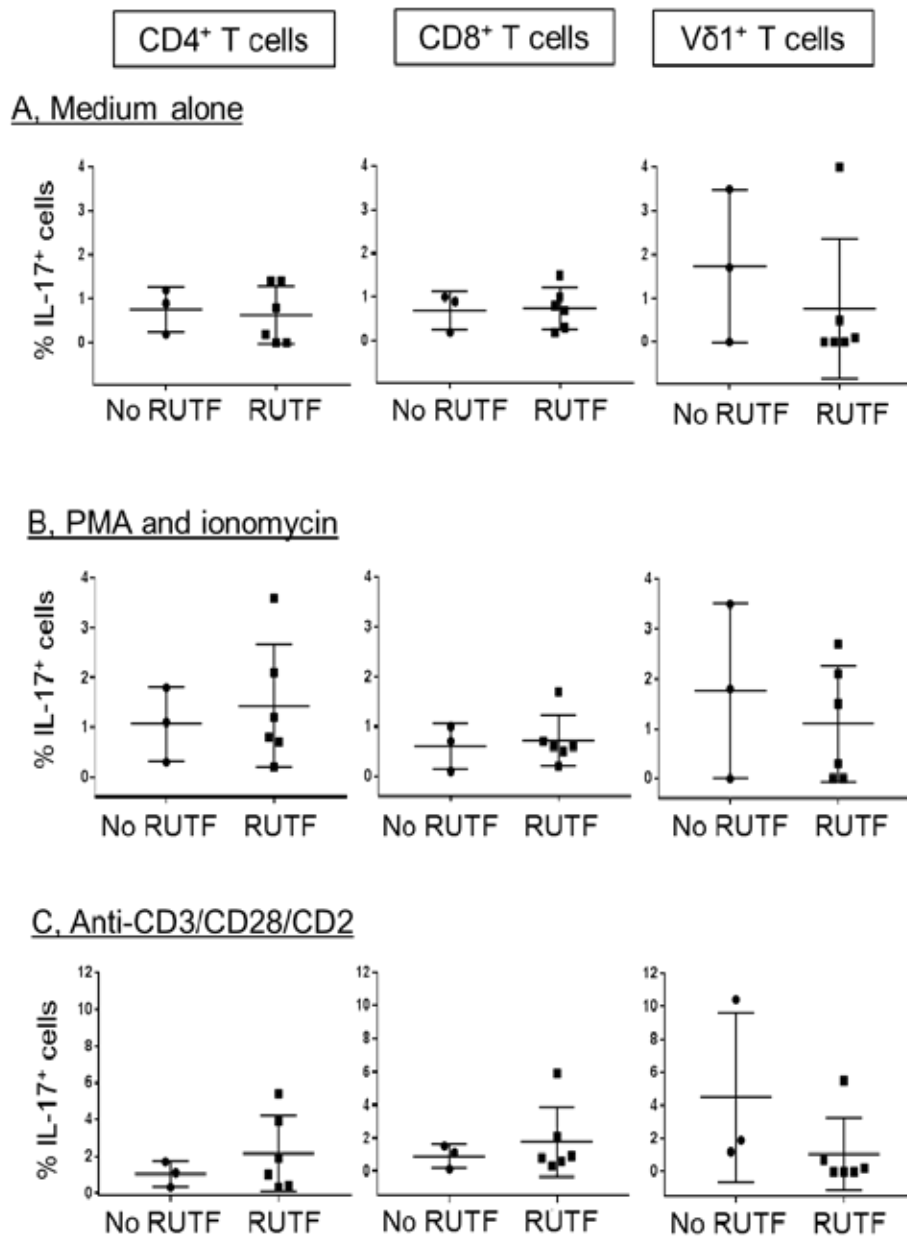


Figure 6.45 Effect of 12-week supplementation with ready-to-use therapeutic food antiretroviral therapy (RUTF) given to malnourished adults with HIV-infection on IL-17 production by CD4⁺ T cells, CD8⁺ T cells and Vδ1 T cells. Scatter plot show frequencies of CD4⁺ T cells (left panels), CD8⁺ T cells (centre panels) and Vδ1 T cells (right panels) that expressed IL-17. Data were compared using the Mann Whitney U statistical test. No significant differences were found.

6.5 COMBINED DISCUSSION FOR CHAPTER FIVE AND SIX

The burden of HIV continues to be high in Sub Saharan Africa, and malnutrition is one of the major complications of advanced HIV disease. Each of these conditions has a suppressive effect on the immune system and they present a double suppressive effect in individuals that have both. Although many studies have described individual effects of malnutrition and of HIV infection on immunity, few studies have described the combined effect of HIV and malnutrition. Furthermore, fewer studies have explored the effect of nutritional supplementation on immunity, and those carried out have mainly been in children.

The aim of the immunology section of the NOURISH study was to investigate the effects of HIV infection, malnutrition and nutritional supplementation on circulating lymphocyte phenotypes and functions among adults in Uganda. We specifically studied innate T cells because they play key roles in shaping immune responses, but they have not been studied in malnutrition, and the few studies on these cells in HIV have not been in African populations. An understanding of the effects of malnutrition and HIV on immunity, and how nutritional supplementation affects these changes will provide immunological evidence to inform the policy of treating malnutrition in HIV using RUTF.

In the NOURISH study, there was a reduction in CD4⁺ T cells numbers and a corresponding increase in the CD8⁺T cells among the ART-naïve HIV infected participants compared to the HIV negative control participants. This was expected since HIV is known to preferentially kill CD4⁺ T cells (26, 27, 229, 233), These changes were more pronounced in the malnourished participants with the CD4⁺ T cell counts significantly lower in SAM, compared to MAM and WN HIV infected participants. This effect of malnutrition has not been described before and it suggests either that malnutrition predisposes HIV-infected individuals to more rapid progress of HIV disease, or that malnutrition has independent effects on CD4⁺ T cell numbers. The production of IFN- γ by both CD4⁺ and CD8⁺ T cells in response to stimulation *ex vivo* was also impaired in malnourished HIV infected participants, compared to the WN participants, whereas IL-4 production was not affected. Malnutrition did not affect cytolytic degranulation by CD4⁺ T

cells, however, we found that CD8⁺ T cells from SAM participants degranulated more frequently when stimulated with PMA and ionomycin, but less frequently when stimulated with antibodies that crosslink CD2, CD3 and CD28. Further studies with larger sample sizes are needed to resolve this contradictory finding. The numerical reduction and impaired IFN- γ production by circulating T cells implies that ART-naïve HIV infected patients with malnutrition are at an increased risk of progression of HIV disease and its related complications such as increased opportunistic infections and worse health outcomes. Other studies have described faster progress of disease, and increased mortality and morbidity among malnourished HIV infected patients (38, 58, 96, 336).

The numbers of CD4⁻ CD8⁻, and CD4⁺ CD8⁺ T cells have not been described before in patients infected with HIV. CD4⁺ CD8⁺ exhibited similar patterns to those of CD4⁺ T cells, being numerically reduced, whereas CD4⁻ CD8⁻ T cells had patterns similar to CD8⁺ T cell patterns being proportionally increased. Their role in HIV immunity has not been well described and needs further exploration.

The numbers and frequencies of the total T cells was higher among the participants who were ART experienced compared to the ART naïve participants. This difference was mainly due to the higher CD4⁺ T cells among ART experienced participants. This is expected given that HIV preferentially kills CD4⁺ T cells, but treatment with ART leads to recovery of the CD4⁺ T cells through inhibition of viral replication (89,90,360,361). Although the CD8⁺ T cells frequencies in the ART experienced participants were lower than the ART naïve participants, there was no difference in the numbers of the CD8⁺ T cells between the two patient populations. (361). Other studies have described this persistence of CD8⁺ T cells in HIV infected patients on effective ART in the setting of good CD4⁺ T cell recovery (362–364), and found that such patients had heightened CD8⁺ T cell activation.

In the NOURISH study, no differences were seen between the numbers and frequencies of CD4⁻ CD8⁻ T cells of the ART-experienced participants and ART-naïve participants at baseline. However, the numbers of CD4⁺ CD8⁺ T cells were higher in the ART experienced participants compared to the ART naïve

participants. Further research is needed to describe the distribution and function of these cells in ART experienced HIV infected adults. The observed differences in the circulating numbers and frequencies of T cell subsets between the ART-experienced and ART-naïve participants were similar to the observed changes in the well-nourished participants between baseline and after 12 weeks of receiving ART (data not shown).

The production of cytokines (IFN- γ , IL-4 and IL-17) and cytotoxic degranulation by CD4⁺ and CD8⁺ T cells following *ex-vivo* stimulation in the ART-experienced participants was not different from that of the ART-naïve participants. This implies that despite the changes in circulating numbers of these cells, their functionality is not restored by ART. Data suggest that the loss of CD8⁺ T cell functionality including cytotoxic degranulation and production of IL-17 in chronic HIV infection which is not restored by ART (365,366). However, ART has been shown to restore the cytokine production of CD4⁺ T cells (66,367), and therefore the observed lack of difference in functionality of the CD4⁺ T cells between the ART-experienced and ART naïve participants needs further exploration since it has not been reported before.

In the ART-naïve MAM participants, RUTF supplementation did not lead to major improvements in the distributions of T cell subsets. No differences in T cell subsets were observed between the RUTF-supplemented participants and those who did not receive RUTF. Furthermore, no differences were observed in the ART-experienced group, which would detect changes that could be attributed to RUTF alone. RUTF supplementation did not lead to improvements in cytolytic degranulation and IFN- γ , IL-4 or IL-17 production. This might explain why studies have found no significant effect of RUTF on clinical outcomes such as mortality, morbidity, CD4⁺ cell counts and viral load that are directly related to immune responses in HIV infected patients (135).

Circulating B cells were present in similar numbers and frequencies in HIV-infected, and HIV- negative adults, but were reduced among the severely malnourished HIV infected participants. Reductions in circulating B cells in HIV has been described before (318, 322), but the B cell changes in malnourished HIV

patients has not been described. Because the reduction in B cells among HIV patients is associated with B cell dysfunction (55–59), it is expected that among malnourished HIV infected adults, these effects are amplified leading to worse patient outcomes. ART has been shown to reverse the effects of HIV on B cells (60), but this was not seen in the NOURISH study. RUTF did not have an effect on the effects of malnutrition on circulating B cells.

There were reductions in the numbers and frequencies of NK cells among the HIV infected adults (regardless of nutritional status) compared to HIV- negative donors in the NOURISH study, but these reductions were not statistically significant. NK cells from well-nourished and malnourished participants also displayed similar cytotoxicity and cytokine production. Reductions in the NK cells in HIV patients have been previously reported by others, and is more profound in advanced HIV disease (265, 266, 310). Since the NK cells provide innate immunity, their depletion and reduction in function leads to impairment of their antiviral activity leading to not only progress of HIV disease (268, 310, 337), but also increased susceptibility to other infections and cancer .

The increases in circulating NK cells due to ART were slightly higher among participants that received RUTF and there was better cytotoxic activity of the NK cells among the participants that received RUTF. The improved effects of RUTF on NK cells has not been previously described and should not only lead to better viral control but also better immunoregulatory abilities among the participants that received RUTF.

There was an increase in the V δ 1 T cells and corresponding reduction in V δ 2 T cells among the ART-naïve HIV infected participants, compared to the HIV-negative donors in the NOURISH study. These differences were significant between the ART naïve malnourished HIV infected adults and the HIV negative donors. Similar changes were also observed between the malnourished participants and the well-nourished participants. Previous studies have described similar changes in HIV patients (276, 292), but not in malnourished HIV infected patients. The increase in V δ 1 T cells in the ART-naïve malnourished HIV infected adults was not followed by an increase in the cytotoxic degranulation and

production of IFN- γ , IL-4 or IL-17 by V δ 1 T cells. Changes in the functionality of V δ 1 T cells in ART naïve HIV infected adults has not been described before. It is not clear how these changes affect immune responses in HIV infected patients, but since they are involved in the production of cytokines such as IFN- γ and IL-17 and the V δ 1 T cells also have cytolytic activity (294, 295), it can be assumed that the observed changes in V δ 1 T cells in HIV disease leads to acceleration of HIV disease progress. Indeed V δ 1 T cells have been implicated in HIV disease progress mainly due to their cytolytic activity against HIV uninfected CD4⁺ T cells (295, 296). An acceleration of HIV disease due to a reduction in V δ 2 T cells has been reported and is thought to be due to increased susceptibility of opportunistic infections (348).

ART led to a reduction in the circulating V δ 1 T cells and but no increases in V δ 2 T cells in the all the HIV infected adults in the NOURISH study. RUTF did not lead to further changes in both the V δ 1 T cells and V δ 2 T cells, in the ART experienced, and ART-naïve participants. Furthermore, ART and RUTF did not have an effect on the cytotoxic degranulation and cytokine production by V δ 1 T cells. The effects ART and RUTF on the numbers and frequencies of V δ 1 T cells and V δ 2 T cells, as well as on the functionality of V δ 1 T cells have not been described before. In one study, ART led to a reversal of the distributions of V δ 1 T cells and V δ 2 T cells, and restored the functionality of the $\gamma\delta$ T cells (342). However, these data reported production of IL-2 by V δ 2 T cells, and response to opportunistic infections, which our study did not examine.

In the NOURISH study, there were no differences in the numbers and frequencies in V δ 3 T cells of the ART-naïve HIV infected participants and the HIV negative participants. Malnutrition, ART or supplementation with RUTF did not affect the numbers and frequencies of V δ 3 T cells of the HIV infected participants. The frequencies of invariant NK T cells (iNK T cells) were reduced in the ART-naïve HIV infected participants compared to the HIV negative donors in the NOURISH study, however, nutritional status did not affect the distribution of these cells in the ART-naïve HIV infected adults. Just like the V δ 3 T cells, RUTF and ART did

not affect the distribution of the iNK T cells in the HIV infected participants. Similarly, the CD56⁺ T cells were reduced in the ART-naïve HIV infected participants in the NOURISH study. Nutritional status, ART or supplementation with RUTF did not lead to changes in the distribution of CD56⁺ T cells in the NOURISH study. The distribution and functionality of circulating Vδ3 T cells, iNK T cells and CD56⁺ T cells in HIV infected adults has not been studied, therefore more research is needed.

Conclusion and Recommendations

Malnutrition accentuated the effects of HIV on CD4⁺ T cells, CD8⁺ T cells, Vδ1 T cells and Vδ2 T cells numbers and functionality among ART-naïve HIV infected adults. Malnutrition did not have significant effects on NK cells, B cells, Vδ3 T cells, and the CD56⁺ T cells in the malnourished ART-naïve participants initiating ART. Additionally, malnutrition did not lead to major changes in the circulating lymphocytes among the ART experienced participants.

While ART led to a reduction in Vδ1 T cells and restoration and reduction of CD4⁺ and CD8⁺ T cells respectively, it did not affect the Vδ2 T cells, nor the functionality of all these cells in the malnourished HIV infected participants in the NOURISH study. Nutritional supplementation with RUTF led to subtle improvements on the effects of ART on the numbers and functionality CD4⁺ T cells, CD8⁺ T cells, Vδ1 T cells and Vδ2 T cells numbers. Therefore a nutritional supplementation with RUTF has a subtle benefit among malnourished HIV infected adults initiating ART.

However, supplementation with RUTF did not have an effect on the circulating lymphocytes and their functionality in the ART experienced participants meaning that RUTF may not benefit the malnourished ART-experienced participants in terms of immune responses.

As noted in chapter four, the sample size of the study was small and a larger study is needed to provide stronger evidence on the effect of nutritional status and nutritional supplementation on the studied immune parameters among HIV

infected adults. Further research to describe the functionality of the innate T cells and B cells (production of antibodies) in malnourished HIV infected adults will provide a comprehensive understanding of the changes in the immune responses in this population.

CHAPTER SEVEN

7.1 GENERAL DISCUSSION

HIV remains a global health burden with over 37 million people currently living with HIV/AIDS and more than 70% of these live in Sub-Saharan Africa (83,369). Malnutrition is a major complication of HIV infection (172–174). Malnutrition in HIV is treated using nutritional supplementation such as RUTF. Although several studies have described the effect of nutritional supplementation on different outcomes among HIV infected adults (135,219,237), the varied results from these studies provide reason for further evaluation of these effects. Furthermore, there is hardly a study that has described the effect of nutritional status on these outcomes among HIV infected adults.

The NOURISH study set out to describe the effects of nutritional status and nutritional supplementation on clinical, nutritional and immunological outcomes among HIV infected adults in Africa. The study used a mixed methods approach incorporating recruitment of newly diagnosed HIV patients initiating ART and stratified according to nutritional status as severely malnourished, moderately malnourished or well nourished. The moderately malnourished group were then randomized to either receive RUTF in the form of Plumpy Nut or not for a 12 week treatment period. In addition a second group of malnourished ART experienced patients were recruited and randomized to either receive RUTF or not for 12 weeks. Patients underwent a baseline and 12 week comprehensive assessment of nutrition, physical examination, anthropometry as well as routine laboratory investigations including nutritional biomarkers such as albumin folate, sodium, markers of inflammation such as CRP, lymphocyte subsets and viral load. Peripheral blood mononuclear cells were also reserved for immunophenotyping.

In describing our cohorts; at baseline, we found that malnourished participants had more advanced HIV disease and worse clinical parameters than the well-nourished participants. Up to 83% of the SAM participants had HIV stage IV disease, and more than 45% of the MAM participants had HIV stage III disease and they all had a raised CRP level. This advanced disease was confirmed by the very high viral load and CD4 count below 100 cells/ μ l in these participants. These data

are in agreement what has been previously described about malnourished HIV infected patients (180)(56,70). Malnutrition and advanced HIV disease are like Siamese twins and operate in a vicious cycle where malnutrition leads to HIV disease progression which in turn leads to increased malnutrition through several mechanisms (180,372). This subsequently leads to a reduced health related quality of life, just as was the case in this study. This was expected since lower quality of life scores have been associated with low BMI and poor nutritional status among HIV infected adults (261). These findings, while not surprising are of importance to public health programs where the emphasis has focused on early identification and treatment of HIV infected persons to prevent complications such as malnutrition, and improve quality of life and other health outcomes (100,101).

The malnourished participants had lower anthropometric measures such as MUAC, total fat mass and expected percentage of fat mass; had less than normal levels in other the biomarkers associated with nutrition such as albumin and sodium although the low sodium finding was likely to be an incidental finding. They also had a lower nutrient intake compared to the well-nourished patients.

It is notable that with respect to lipid values although within acceptable limits of standard measurement SAM patients had lower total cholesterol, as well as LDL and HDL cholesterol at baseline than either MAM or VN participants. The main improvement for SAM was a rise in HDL and a reduction in CRP although the latter did not fully return to normal limits. Together, these results suggest that in addition to nutritional deficits, SAM patients may be experiencing a degree of ongoing immunosuppression or low grade inflammation along with catabolism. Such a phenomenon is referenced in a paper (373). However their patients were in ICU and had severe sepsis or burns and did not improve with regular supplements.

The malnourished participants were also found to have low counts of circulating phenotypes of the lymphocytes except for increases in $\gamma\delta$ T cells and CD8⁺ T cells. They also exhibited low cytokine production and degranulation patterns. The similarity in the patterns of these outcomes indicates a similar effect of malnutrition on the different parameters among malnourished HIV infected adults.

However, because there is paucity of literature describing the effects of nutritional status on these parameters, the NOURISH study results must be interpreted with caution. More research is needed to explore this phenomenon before generalizable conclusions can be made.

The observed changes in circulating lymphocytes numbers, and functionality seen in the malnourished HIV infected adults indicates a reduced capacity of the immune system to respond to disease. This predisposes the participants to opportunistic infections, and an acceleration in progress of HIV disease, which in turn leads to further damage to the immune system and nutritional status of the patients. This vicious cycle of disease progress and malnutrition has been previously described (374) and calls for the need of interventions to address both the progress of HIV disease (treatment with ART) and malnutrition with nutritional supplementation.

The ART-experienced malnourished HIV infected participants highlight another mechanism for malnutrition in HIV disease. These participants had controlled their viral load, and had high CD4 cells counts, and thus '*their*' malnutrition could not be associated with advanced HIV disease. They either were malnourished right from the time of initiating ART and never recovered from it, or malnutrition was possibly due to food insecurity, a phenomenon that has been described previously.(54,215).

The NOURISH study demonstrated significant beneficial effects of nutritional supplementation with RUTF on clinical; nutritional and immunological outcomes among malnourished HIV infected adults.

In the clinical outcomes, RUTF led to higher gains in CD4 cell counts and improved haemoglobin among the malnourished participants. The study also demonstrated better improvements in quality of life following 12 weeks of nutritional supplementation.

In the nutritional outcomes, the study found that RUTF led to significantly higher increases in anthropometry, body fat and nutrient intake, which findings are similar to other studies (135,234,239,275). One of the key findings in the changes

in anthropometry is the higher increases in total body fat and percent fat mass in men compared to women regardless of supplementation status. This novel finding was more marked in ART naïve malnourished participants. Similarly, we found that the increases in weight and BMI were due to increases in fat as opposed to what other studies found where the increases in anthropometry were driven by increases in both fat and lean mass (234,239,275). In the biomarkers of nutrition, supplementation with RUTF to normalization of serum lipids with increases in HDL and LDL, and reductions in triglycerides. These changes in the lipid profile together with reductions in CRP lower the risk of cardiovascular disease. It is known that low HDL and high triglycerides, together with an elevated CRP increase cardiovascular disease risk in individuals due to the persistent inflammation and catabolism in the setting of immunosuppression (373). In addition to the changes in the lipid profiles, RUTF led to increases in other biomarkers of nutrition. Of note is the serum folate levels, which other studies have showed to be reduced in HIV (183)(299). This is another novel finding which has not been reported in nutritional supplementation studies. All participants would have been expected to have a reduction in serum folate levels due to the interactions between ART and folate metabolism, however, this was not so among the RUTF recipients since because RUTF increased the RUTF intake which had an effect on the serum RUTF levels. This finding is important especially to women in the reproductive age group. RUTF would be of benefit to such women that are HIV infected and are on ART since it would help prevent reduction in folate in these mothers.

After 12 weeks, ART and nutritional supplementation with RUTF led to significant improvements in the CD4 cell count; reductions in viral load and quality of life in the

RUTF led to a subtle improvement in the effects of ART on numbers and frequencies of the circulating CD4⁺ T cells, reductions in CD8⁺ T cells and Vδ1 T cells in the malnourished HIV infected adults that were initiating ART. It however did not have an effect on the changes in these cells observed in the ART-experienced malnourished HIV infected adults. The changes in CD4⁺, CD8⁺ T

cells and Vδ1 T cells have been well described in other studies (339), but the effect of RUTF has not.

Therefore, ART-naïve malnourished HIV infected adults initiating ART get immunological benefit from nutritional supplementation, and this may explain the reported reduction in mortality and morbidity in this group of patients (161,230,376). The benefit is more pronounced among the severely malnourished participants.

These benefits in the clinical, nutritional and immunological parameters, together with the findings of the focus group discussions with regard to the benefits of receiving the RUTF provide evidence for the use of RUTF in the nutritional rehabilitation of malnourished HIV infected adults.

The NOURISH is one of the few randomized studies that did not use another nutritional supplement as a comparator. Most nutritional interventional studies use an inferior nutritional supplement as a comparator due to the ethical challenges involved in randomized nutritional studies (117,135,239).

Although many of the observed changes did not have statistically significant findings, this was not unique to the NOURISH study, as many of the interventional studies cited reported changes that were deemed clinically significant but were not statistically significant (117,234,239). The biggest reason for lack of statistical significance in the NOURISH study and these studies was the small sample size. In the NOURISH study, the challenge was to find malnourished ART-naïve HIV infected patient. This is discussed in detail in the section on challenges and limitations.

The other possible reason for lack of statistical significance was the placebo effect (377). It is possible the NOURISH study had a placebo effect and this was especially seen among the ART-experienced participants. The changes observed in the NO RUTF participants among the ART-experienced participants were not expected. While in the ART naïve participants, changes in the NO RUTF group could be attributed to introduction of ART, and the potential reversing of the effects of HIV by the ART; in the ART-experienced participants, such changes are not expected since the effects of ART on any changes would have happened at

least a year prior to joining the NOURISH study. Therefore the changes in the clinical and nutritional outcomes observed in the NO RUTF ART-experienced participants could have been due to 'placebo effect' and could have masked the statistical significance were it existed. The possible explanation for this placebo effect, was the NO RUTF participants did receive other forms of locally made nutritional supplements similar to RUTF. This was not verified during the study as it had not been anticipated.

The NOURISH study had a 14% loss to follow-up, this was much less than what has been reported in other studies which ranged from 17%- more than 40% (117,230,239)

As in other studies, patients found the RUTF to be convenient and palatable (135,229,230,254), with adherence to RUTF in the NOURISH study satisfactory. However, there was reported sharing of RUTF with family members and this further underscores the importance of careful consideration of amount of nutrition supplement provided to participants in nutritional supplementation studies. One study provided more than was required by the participant, to allow for the possibility of participants sharing with family members (117), however this is not a sustainable strategy at policy level. This phenomenon may be responsible for the less than optimal nutritional recovery observed in nutritional rehabilitation programs. There is a need to for research into approaches of improving adherence to nutritional supplements in malnourished HIV infected adults which might lead to more improved outcomes of nutritional rehabilitation programs. Secondly, a longer duration of supplementation might be required in order for the nutritional supplement to have a measurable effect on the different outcomes especially in the moderately malnourished the HIV infected adults.

No other study have detailed the effect of nutritional status and nutritional supplementation on immunological outcomes among HIV infected adults initiating ART, therefore the results of this study provide preliminary data into this area.

7.2 CONCLUSION AND RECOMMENDATION

The NOURISH study sought to examine the intersection between HIV and malnutrition. It demonstrated that malnutrition lowers the clinical, nutritional and

immunological parameters in HIV infected adults in Uganda, and that RUFT leads higher absolute and proportional changes in clinical, quality of life, nutritional and immunological outcomes compared to ART only among malnourished participants.

Data from the NOURISH study are a valuable addition to the general body of knowledge in the field of nutritional interventional studies in HIV infection. However, the outcomes of the study may be improved in a study that improves adherence to the RUTF, and or increases the duration of the supplementation to at least 6 months.

7.3 LIMITATIONS AND CHALLENGES OF THE STUDY:

As already discussed, one of the challenges faced by the study was the small sample size. This affected the internal validity of the study affecting the ability of the study to demonstrate a difference where it really exists. The small sample size due to the change in policy by the government of Uganda from initiating ART at a CD4 of 350 cells/ μ l to a CD4 of 500 cells/ μ l. This meant that almost all ART-naïve participants would be recruited before they ever became malnourished since it's in advanced HIV ($CD4 < 350$ cells/ μ l) that malnutrition is common. This was coupled with the limited time available for data collection. The immunological studies had fewer participants than the clinical and nutritional studies because a number of vials of baseline PBMCs were lost in liquid nitrogen at Trinity College Dublin, and follow up samples for 20 participants were not shipped to Dublin, because their week 12 visit happened after the last batch had been shipped.

The small sample size also made it difficult to conduct a multivariate analysis, and thus adjust for any effects of confounding. However this was not possible since non-parametric methods were used and the data are only descriptive. This was not unique to the NOURISH study, as most of the nutritional interventional studies had small sample sizes and did not carry out multivariate analysis (114,234,239).

The 24-hour dietary recall method was liable to a recall bias thus nutrient intake data may not be the true reflection of the actual dietary intake. However, all

methods of collecting dietary or nutrient intake data are based on participant recall, and thus liable recall bias. The 24-hour dietary recall method is less liable to recall bias than the other methods, since the information needed is within the past 24 hours compared to others which collect weekly, monthly or annual information. Additionally, counting the returned empty and unopened sachets and self-reported missing of RUTF to assess adherence was also liable to social disability bias with participants not telling the truth. Participants might have emptied the sachets or shared the RUTF with other family members and return empty sachets indicating good adherence, yet it would not have been the case. However, analysis of the changes in serum folate, which is a biomarker used to objectively assess nutrient intake, were also used to assess adherence to RUTF. This reduced the effect of the social disability bias in assessing the adherence to RUTF in this study.

The long process of obtaining ethical approvals from the different ethic and review committees meant that a considerable amount of time was lost in this process. It took more than 9 months to get all the necessary ethical approvals to conduct the study. The study needed approval from four different committees including the faculty of Medicine ethics committee at Trinity College Dublin; the scientific review committee at the Infectious Diseases Institute at Makerere; the higher degrees and ethics committee of the school of biomedical sciences at Makerere University and the Uganda National Council of Science and Technology. All these committees had different requirements responding to their requirements to get the approvals affected the available time for data collection.

Finally, taking on this volume of work single handedly as a PhD student was no small task especially during the data collection. Tasks ranging from identification of participants from the HIV clinics, giving them the study information, enrolling them into the study and collecting all the study specific data and procedures, to collecting blood samples and isolation of PBMCs all in the same day was quite challenging. However, the student managed to carry out all these activities and conduct the study to a good standard.

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9.0 APPENDICES

9.1 APPENDIX ONE: LITERATURE REVIEW TABLE

Sno	Author, year , type of article, country	Title of article & Study design	Study population	Intervention	Control	Duration	Main outcome measure	Major findings	Comments
1	McDonald Ndekha 2009 Original research Malawi	Nutritional status of Malawian adults on antiretroviral therapy 1 year after supplementary feeding in the first 3 months of therapy- Prospective Cohort study	336 HIV infected adults on ART who had received supplementation for 3 months	RUTF	Corn Soy Blend	9 months	BMI at 3 and 9 months after supplementation	BMI gain during the supplementation period was not maintained later	It's a cohort analysis Comparison arm was CSB Different outcome measure
2	Ronald A Cantrell, 2008 Original Research Zambia	A pilot study of food supplementation to improve adherence to ART among food insecure adults in Lusaka Cluster Non-Randomized study	636 Food insecure HIV positive patients initiating ART.	Micronutrient fortified CSB, oil, maize meal and beans	Standard of care	12 months	Adherence and weight gain	Improved adherence to ART in group receiving that supplementation No significant gain in CD4 count or weight	It's an evaluation of a program Different outcomes and intervention Probably PLWHIV came to the clinic to get food not the ART

Sno	Author, year , type of article, country	Title of article & Study design	Study population	Intervention	Control	Duration	Main outcome measure	Major findings	Comments
3	McDonald Ndekha, 2010 Original Research Malawi	Supplementary feeding with either ready-to-use fortified spread or corn-soy blend in wasted adults starting antiretroviral therapy in Malawi: randomized, investigator blinded, controlled trial Randomized Control Trial	491 HIV positive adult patients initiating ART BMI<18.5	RUTF Vs Corn Soy Blend	Corn Soy blend	3.5 months	Changes in BMI and fat-free body mass .	RUTF participants had a 0.5 higher change in BMI compared to the CSB arm and 0.7 kg greater increase in fat free mass	Had a big loss to follow up (15%) Comparator almost similar to intervention
4	Paluku Bahwere, 2009 Original Research Malawi	Acceptability and effectiveness of chickpea sesame based RUTF in malnourished HIV positive adults Cohort Study	66 adult HIV positive patients and majority were stage 4 disease	Locally made RUTF	Not mentioned	5 months	Patient acceptability and weight gain	High acceptability Increased weight and physical activity	It is a cohort study measuring different outcomes in terms of acceptability of the intervention
5	Denise Evans 2013, Original Research South Africa	Impact of nutritional supplementation on immune response, BMI and bioelectrical impedance in HIV-positive patients starting ART Pilot Randomized Control Trial	36 HIV positive adults initiating ART with self-reported unintentional weight loss	Future life porridge + ART 100g daily equivalent to 400Kcal	ART only	6 months	Absolute and percentage change in BMI, CD4, Body mass composition physical activity	Higher absolute and percentage change in all outcomes	High loss to follow up (28%) Very few numbers 26 completed Self-reported weight loss could not hard inclusion criteria

Sno	Author, year , type of article, country	Title of article & Study design	Study population	Intervention	Control	Duration	Main outcome measure	Major findings	Comments
6	RD Semba Original Research 2007 Malawi	Micronutrient supplements and mortality of HIV infected adults with PTB Randomized Control Trial	829 HIV + and 573 HIV- adults all with smear positive TB BMI<19.0	Micronutrients	Placebo	24 months	Mortality	No difference in Mortality b/n comparison groups	ART naïve participants Co infected with TB Micronutrient supplement HIV –ves were masks Non-randomised study High mortality Loss of power due to mortality
7	Louise Ivers 2010, Original research Haiti	Food assistance is associated with improved body mass index, food security and attendance at clinic in an HIV program in central Haiti: observational cohort study	600 HIV infected adults (BMI<18.5, or TB infected or Poor)	Food supplementation	Not mentioned	12 months	Food Security, BMI Adherence, Quality of life	Lesser decrease in BMI, better food security & adherence improved in food assisted group,	Observational cohort Food assistance not supplementation
8	Laurence Ahoua 2011 Original research Uganda/Kenya	Nutrition outcomes of HIV-infected malnourished adults treated with ready-to-use therapeutic food in sub-Saharan Africa: Retrospective Cohort Study	1340 Patients aged 15 years or older with a body mass index of less than 17 kg/m2	RUTF	Not mentioned	4 months	Clinical status, BMI, CD4 increase morbidity and mortality	Increased nutritional recovery and TB and diarrhea increased chances of failure	Programmatic data and was retrospective Not all were on ART

Sno	Author, year , type of article, country	Title of article & Study design	Study population	Intervention	Control	Duration	Main outcome measure	Major findings	Comments
9	Lategan R, 2010 Original research South Africa	Nutritional status of HIV-infected adults on ART and the impact of nutritional supplementation in the Northern Cape Province, South Africa Prospective non-randomized trial	158 patients on ART	Enriched porridge with macro and micro nutrients	Standard of care	4 months	weight changes	No net change in weight	High loss to follow-up 38%.
10	Swaminathan 2010 Original research India	Nutritional Supplementation in HIV-Infected Individuals in South India: A Prospective Interventional Study Cluster non-randomized trial	636 ART-naive patients, 362 completed study	The high-calorie, high-protein Macronutrient supplement vs Nutritional counselling	Nutritional counselling	6 months	Changes in anthropometry blood chemical analysis immune status body composition	Significant increases in weight, BMI, MUAC, fat-free mass, and body cell mass in the supplement group No changes in lipid levels	Over 40% loss to follow-up. With differential loss to follow-up in the control group. No sample size considerations given. Not among Malnourished participants

Sno	Author, year, type of article, country	Title of article & Study design	Study population	Intervention	Control	Duration	Main outcome measure	Major findings	Comments
11	Andrea M. Rehman, 2015, Original Research Tanzania, Zambia	Effects on Anthropometry and Appetite of Vitamins and Minerals Given in Lipid Nutritional Supplements for Malnourished HIV-Infected Adults Referred for Antiretroviral Therapy Randomized Control Trial	1815 patients. 20% died 45% completed study with complete data Inclusion BMI<18.5 Initiating ART	LNS with added vitamins and minerals (LNS-VM) equivalent 150 kcal/day for 2 weeks then 1400g/day	Lipid-based supplements without (LNS)	12- weeks	vitamin and mineral supplementation would improve appetite and permit nutritional recover	Anthropometric measures were consistently higher at 12-week ART in the LNS-VM than in the LNS group but statistically significant. No differences in Appetite	No statistical significance in differences Intervention and comparator quite similar. Initial sample was 2800, recruitment stopped due to high mortality
12	T. D. Sudarsanam, 2011- Original research India	Pilot randomized trial of nutritional supplementation in patients with tuberculosis and HIV–tuberculosis coinfection receiving directly observed short-course chemotherapy for tuberculosis Randomized Control Trial	103 (81 TB & 22 HIV/TB) Patients: Inclusion Newly diagnosed with TB, with or without HIV BMI<19.0	locally prepared cereal–lentil mixture providing 930 kcal and a multivitamin micronutrient supplement	Standard of care	6 months	The primary outcome Outcome of TB treatment, as classified by the national program.	Statistically significant increase in caloric intake, proteins and fats in the supplemented group.	Nutritional outcomes were secondary outcomes No details of sample size calculation. Also did a 24-hour dietary recall

Sno	Author, year , type of article, country	Title of article & Study design	Study population	Intervention	Control	Duration	Main outcome measure	Major findings	Comments
13	Suzanne Filteau 2015 Original Research Tanzania and Zambia	Effects on mortality of a nutritional intervention for malnourished HIV-infected adults referred for antiretroviral therapy: a randomized controlled trial Randomized control trial	1815 ART naïve adults BMI<18.5 Initiating ART	LNS with added vitamins and minerals (LNS-VM) equivalent 150 kcal/day for 2 weeks then 1400g/day	Lipid-based supplements (LNS)	12-weeks	Decrease the early mortality of malnourished adults starting ART	Supplementation with LNS-VM did not reduce mortality, but improved CD4 count	High mortality, and loss to follow-up. No difference in intervention and comparator
14	Mette F Olsen, 2014 Ethiopia	Effects of nutritional supplementation for HIV patients starting antiretroviral treatment: randomized controlled trial in Ethiopia Randomized Control Trial	318 adults initiating ART, BMI>16.0	200 g (4600 kJ) of supplement containing whey or soy during either the first three or the subsequent three months of ART	Placebo	3 months	Lean body mass Hand grip strength Physical activity	Increase in lean mass by 0.85 kg (whey) or 0.97 kg (Soy) more than controls Hand grip strength increased by 0.68 kg for whey and 0.93 kg for soy	Patient's BMI >19.5

Sno	Author, year , type of article, country	Title of article & Study design	Study population	Intervention	Control	Duration	Main outcome measure	Major findings	Comments
15	Adama Diouf, 2016 Senegal	Daily consumption of ready-to-use peanut-based therapeutic food increased fat free mass, improved anemic status but has no impact on the zinc status of people living with HIV/AIDS: a randomized controlled trial Randomized Control Study	65 hospitalized HIV infected persons	100g of RUTF	Standard hospital diet	9 weeks	Changes in body composition, anemia and zinc	11% increase in fat free mass, and weight. Increase in energy and zinc intake	Hospitalized patient population, follow-up for 9 weeks
16	Marianna K. Baum 2013, Botswana	Effect of Micronutrient Supplementation on Disease Progression in Asymptomatic, Antiretroviral-Naive, HIV-Infected Adults in Botswana A Randomized Clinical Trial (Factorial design)	878 HIV infected participants	Daily multivitamins (B vitamins and vitamins C and E), selenium alone, or multivitamins with selenium	Placebo	24 weeks	Reaching a CD4 cell count less than 200/ μ l until May 2008; after this date, reaching a CD4 cell count of 250/ μ l or less	The combined supplement of multivitamins had a significantly lower risk vs placebo of reaching CD4 cell count 250/ μ l or less.	Micronutrient supplementation study. Done in pre-ART era

9.2 APPENDIX TWO

NOURISH ADULT STUDY PARTICIPANT INFORMATION LEAFLET

Study investigators:

Dr Bernard Kikaire

Phone: 0772 669396

INTRODUCTION

We are conducting a study on HIV and nutrition and we invite you to join this study; it will be conducted by Dr Bernard Kikaire, who is a student and this study is part of his studentship.

It is up to you to join or not to join the study. Please ask questions and you have at least 7 days to decide whether to participate in this study or not

WHY ARE WE DOING THIS RESEARCH?

We want to find out what happens to the ability of the body to break down drugs (drug metabolism) as well as cells that protect our bodies against infection (cells of immunity) when HIV infected people are malnourished, and how these two effects change after these people have been given food supplements.

We will also study the effect of nutritional supplement on Body Mass Index (weight for height) as well as the acceptability and adherence to Plumpy nut among malnourished HIV infected adults starting anti HIV medicines.

Studies have showed that giving food supplements is beneficial to HIV infected malnourished people in terms of improving their nutritional status, and general wellbeing. However, they have not shown a clear benefit in terms of CD4 cells (cells that help us fight infection), and no studies have been done to show how food supplementation affects the drug metabolism and cells that protect against infection among HIV infected malnourished people.

WHO WILL PARTICIPATE IN THIS STUDY

The people who will participate in this study are infected with HIV, and will be grouped into four (4) groups as follows:

- A- These will be 50 moderately malnourished adults who are going to start receiving anti HIV medicines.

- B- 25 Severely malnourished adults who are going to start receiving anti HIV medicines
- C- 50 moderately Malnourished adults who have been on anti HIV medicines for at least one year
- D- 25 Well Nourished adults who are going to start anti HIV medicines

GROUP A and C:

Participants in group A will be randomised as below:

- The **NUTRITIONAL SUPPLEMENT** group will receive a nutritional supplement (Plumpy nut) as well as their anti HIV medicines and nutritional counselling.
- **NUTRITIONAL COUNSELLING ONLY** group (half of those who join the study) will receive their anti HIV medicines as well as nutritional counselling but not nutritional supplementation.

GROUP B

All participants in group B will be given the nutritional supplement and will not be randomized.

GROUP D

Participants in group D will not be given nutritional supplement but will be followed up together with the other participants.

All participants will be given nutritional counseling and education.

The nutritional supplement to be given is plumpy nut and is recommended by World Health Organisation for use in malnourished HIV infected people. This food is not to replace your daily food but is to eaten on top of your daily food that you use at home. All participants receiving nutritional supplementation will receive two (2) sachets of plumpy nut per day for the study period.

All participants will be given water containing a drug called Midazolam 0.2mg on the second and on the last visit of the study.

PLUMPY NUT

Plumpy nut is a nutrition supplementation food that has been approved by World Health Organisation for use among people that are malnourished. It is a paste and is rich in many nutrients required by the body.

We advise that you take plumpy nut with a lot of water as it can cause dehydration.

MIDAZOLAM

The drug that will be used to estimate the ability of your body to breakdown drugs is called Midazolam. It is one of the drugs used for this purpose and is completely safe. The drug has other

purposes such as an anaesthetic (making patients sleep) during surgery; treatment of epilepsy among others. The dose of the drug used for these purposes is very large and we shall not be using this dose. We shall be using a dose 50 times smaller than the dose used in treating of epilepsy or during surgery. This dose will be dissolved in 50 mls of water that you will be given to drink.

The drug starts to work within 30 minutes of taking it, and its effects last for up to 1 ½ hours. Almost all the drug (90%) will be removed from your body within one day through urine.

You will remain in the clinic for at least one hour after taking the drug and this will help the research team to monitor you for any possible adverse events that have been mentioned below.

WHAT WILL HAPPEN DURING THE RESEARCH?

You will be expected to attend a total of 5 visits in a period of 3 months. Details of procedures to be done on each visit are shown in the table below.

First visit

A counsellor will talk to you and explain the study to you before you decide whether to join the study or not.

If you accept to join the study, you will be asked questions and examined to ensure you are eligible to participate in the study.

Second Visit

On the day you come to get your anti HIV medicines, we request not to take anything except water before you come to the clinic.

You will be given breakfast after the blood samples have been collected

Monthly Visits

You will return to the research clinic every time you come to pick your anti HIV medicines. This should be at the end of a month from the day you are given your anti HIV medicines.

Month 3 visit

This will be the last visit of the study, and we request not to take anything except water before you come to the clinic. We also request that you do not take your night dose of anti HIV medicines until you come to the clinic

You will be given breakfast after the blood samples have been collected.

Table of study procedures for the NOURISH Project

First Visit	Second Visit	Monthly Visits	Last Visit (12 Weeks)
Detailed Medical history and Examination	Nutritional assessment	Assessment of adherence to plumpy nut and ART	Medical history and examination
24 hour dietary recall	24 hour dietary recall	24 hour dietary recall	Nutritional counseling and assessment
Assessment of nutritional status	Nutritional counseling and education on use of plumpy nut	Nutritional counseling and assessment	Assessment of adherence to plumpy nut and ART
Collection of blood samples	Midazolam given	Monthly refill of plumpy nut	
Collection of Urine	Plumpy nut supplied		
Collection of Quality of Life information	Collection of blood samples		Collection of Quality of life information

All your medical care will be provided by your parent clinic but the study doctor will work together with the clinic team to ensure good health care provision.

AMOUNT OF BLOOD TO BE COLLECTED

We will collect a total of up to 30mls of blood from you. This is equivalent to 6 table spoons of blood. This blood will be used to carry out several tests that will help us answer the study questions.

PERMISSIONS AND APPROVALS

This study has been reviewed and approved by the committee that protects the rights and welfare of study participants, the Biomedical Sciences Research and Ethics committee at Makerere College of Health Sciences and the Uganda National Council of Science and Technology, the body that oversees research in Uganda. It was also reviewed and approved by the committee that protects the rights and welfare of study participants in Trinity College Dublin in Ireland where the some of the researchers are based.

PREGNANCY AND BREASTFEEDING

You cannot join if you are pregnant or breastfeeding. To be in the study you must be willing to use appropriate birth control methods. If you do become pregnant during the study, please tell the study doctor *right away* because your HIV medicines may need to be changed and if you will need to start receive nutritional supplementation (if you are randomised to not receiving it). You may continue to participate in the study.

WHAT ARE THE RISKS OF BEING IN THIS STUDY?

Possible risks of being in the NUTRITIONAL SUPPLEMENTATION group:

The most common side effects associated with taking plumpy nut include nausea, vomiting abdominal pain and change in taste in the mouth. However these are not common and are usually mild. If you are allergic to any of the components of plumpy nut, you may develop allergy signs and symptoms.

Possible risks of being in the NUTRITIONAL COUNSELLING ONLY group:

- Your nutritional status may not recover as quickly as those in the supplemented group and you may experience more illnesses but this has not been proven by research.

Risks of taking Midazolam

Midazolam has been associated with several side effects which include but not limited to Nausea, vomiting, dizziness, or drowsiness. It has also been associated with breathing difficulties and forgetfulness.

In case you experience any of these effects or any other, please notify the research team and they will offer appropriate medical care.

In case you experience such effects, do not hesitate to contact the research team for care and management of these effects.

WHAT ARE THE BENEFITS OF BEING IN THIS STUDY?

All participants will benefit from nutritional counselling and assessment which may improve on their dietary habits, as well as have effect on their health. They will also benefit from the routine tests that will be offered by the study and this will help to inform their medical care.

The participants in the nutritional supplementation group may benefit from the additional effects of supplementation but this is for a short period and we do not know how significant this will be.

WHAT CHOICES DO YOU HAVE OTHER THAN BEING IN THIS STUDY?

You do not have to join this research study if you do not want to. If you join, you can quit at any time. If you choose not to join or to quit, it will not affect your regular medical care. If you decide not to join, please continue with the medical care offered at your health facility. If you decide to withdraw from the study, you may choose to also withdraw your stored samples if you wish and this shall not affect your medical care.

CAN YOUR STUDY PARTICIPATION BE STOPPED EVEN IF YOU DON'T AGREE?

The study doctor can take you out of this study if continuing in the study would harm you, if you go to prison, or if the study is stopped by the study sponsor, review committees (IRB/REC) or government authorities, or for other administrative reasons.

WHAT WILL WE PAY FOR?

The study will provide the plumpy nut, and will pay for the laboratory tests, and other tests that are part of this study. During the study, your anti HIV medicines and all other medicines will be provided to you by the government through your health facility.

We will refund the costs of your transport to the clinic for study scheduled visits that are not part of the regular HIV care related visits and this will not be more than ten thousand (10000) Uganda Shillings.

WHO WILL BE ABLE TO SEE YOUR MEDICAL INFORMATION?

The study investigators will ask for your permission to access your medical information from the medical files in the clinic you attend.

We will protect the privacy of your medical information as much as legally possible, and release your records only with your written permission. We will label your study records with a code and you will not be identified in any publications about this research. However, your records may be seen by independent groups (IRBs) that make sure the study is ethically acceptable.

WHAT WILL HAPPEN AT THE END OF THE STUDY?

If you stop participating or the study ends, you will continue to receive all the medical care and anti HIV medicines from government health facility. We will not continue supplying the plumpy nut after the study ends or when you decide to stop participating in the study.

STORAGE OF SPECIMEN AND GENETIC TESTING

Some of the blood and urine that will be collected from you will be stored for future testing. The tests to be done are to help the researchers answer the questions they are trying to answer.

Part of these tests will be genetic tests that will help them understand how the enzymes that break down drugs in the body work.

If you do not wish to have your samples stored, please indicate this on the consent form provided.

WHO CAN YOU TALK TO ABOUT THIS STUDY?

Please contact Dr Bernard Kikaire, Tel: 0772 – 669 396, if you have any questions or concerns about this research study or contact Dr Eria Mwaka , Tel: 0752-575 050Chairman of School of Biomedical Sciences Higher Degrees and Research Ethics Committee.

9.3: APPENDIX THREE:

CONSENT FORM

Effect of Nutritional supplementation on immune responses and drug metabolism among malnourished HIV infected adults initiating antiretroviral treatment in Uganda.

PRINCIPAL INVESTIGATORS: Dr BERNARD KIKAIRE

BACKGROUND:

Below is an outline of the major points about your participation in the NOURISH study. Please indicate by ticking in the box which aspects you understand and agree to participate in. If there is an aspect you do not agree to participate, please do not tick the box.

1. I have read/been read the information sheet for the NOURISH study and I understand what will be required of me if I participate in the study.

☐

My questions concerning this study have been answered by:

2. I understand that I may be given plumpy nut for three months while I am in the NOURISH study, but my healthcare will continue to be provided by the national health system through IDI clinic.
3. I understand that I will participate in the study for three months and will return to the research clinic at monthly intervals
4. I understand and that I will be given a small dose of midazolam to take while at the clinic.
5. I understand that I may withdraw from the study at any time, without giving a reason and without affecting my normal care and management, and that I may withdraw permission to use my stored samples
6. I may allow blood and urine samples to be taken and stored for testing later. I understand that I may not be given results of tests performed on stored
7. I agree to genetic testing on my blood
8. **I agree to take part in the NOURISH study.**

9.4: APPENDIX FOUR

DECLARATION

I have read, or had read to me, the information leaflet for this project and I understand the contents. I have had the opportunity to ask questions and all my questions have been answered to my satisfaction. I freely and voluntarily agree to be part of this research study, though without prejudice to my legal and ethical rights. I understand that I may withdraw from the study at any time and I have received a copy of this agreement.

PARTICIPANT'S NAME:

SIGNATURE/ Thumbprint:

Date:

If participant thumb prints, an independent witness should sign the consent form

NAME OF INDEPENDENT WITNESS:
.....

SIGNATURE:
.....

Date:

Statement of investigator's responsibility: I have explained the nature and purpose of this research study, the procedures to be undertaken and any risks that may be involved. I have offered to answer any questions and fully answered such questions. I believe that the participant understands my explanation and has freely given informed consent.

INVESTIGATOR'S SIGNATURE: **Date:**
.....
Date:

9.5: APPENDIX FIVE:

TRINITY COLLEGE IRB APPROVAL



**COLÁISTE NA TRÍONÓIDE, BAILE ÁTHA TRINITY COLLEGE
CLIATH DUBLIN**

Dámh na nEolaíochtaí Slaínte,

**Faculty of Health
Sciences,**

Foirgneamh na Ceimice

Chemistry Building,

Colaiste na Tríonóide,

Bernard Kikaire,

School of Medicine,

Trinity College Dublin,

Dublin 2.

25/07/14

Re: Effect of a nutritional supplementation on immune responses and drug metabolism among HIV infected adults initiating antiretroviral treatment in Uganda.

Dear Bernard,

Further to a meeting of the Faculty of Health Sciences Ethics Committee, we are pleased to inform you that the above project has been approved without further audit required.

Yours sincerely,

pp. Sonya McConnell

Dr. Ruth Pilkington

Chairperson

Faculty Research Ethics Committee

9.6: APPENDIX SIX

The focus group guide

The focus group guide that was used is detailed below:

Introductory questions (important for breaking the ice and building rapport)

1. What issues come to mind when you think about your diet?

Transition question

2. If you had a choice to supplement your diet, what are the things that you would consider in selection of the supplement?

Specific Questions on Plumpy’Nut

3. What do you know about Plumpy’Nut?

PROBE: Why was it included in your diet?

4. What is your experience with regard to consumption of Plumpy’Nut?

PROBES:

- How delectable/palatable do you find Plumpy’Nut?
 - What can you say about the taste, smell and texture?
 - How does Plumpy’Nut compare with other staple foods that you are accustomed to and enjoy to eat?
 - Are you always able to access Plumpy’Nut whenever you need it?
5. Do you share Plumpy’Nut with anyone else? (Further probe: who and why?)
 6. How comfortable are you with other people seeing you with Plumpy’Nut/knowing that you use Plumpy’Nut?
 7. What kinds of questions/concerns have other people close to you ever asked/expressed about Plumpy’Nut?

8. How have you benefited from Plumpy’Nut?
9. Overall, what kind of difficulties have you experienced with your use of Plumpy’Nut?

PROBE:

- Difficulties relating to taste/texture.
- Availability and access.
- Stigma of using Plumpy’Nut.

10. How have you overcome/dealt with the difficulties you identified above?

11. In what ways would you like Plumpy’Nut to be changed/improved to make it better for you/those who take it?

Closing

What questions do you have for me regarding the use of Plumpy’Nut?

9.7: Appendix SEVEN:

Materials used in immunology experiments:

Biosafety level three laboratory

The BSL3 enforces the use of personal protective equipment (PPE) to minimize the risks of exposure of the staff to potentially infectious pathogens. PPE included the wearing of disposable laboratory coats that are used for a limited period and eventually replaced by a new coat, in addition, “double gloving” where two pairs of gloves are worn when one is going to work in the laboratory for extra protection and for easy discarding in case the outer most glove is soiled. Over shoes and protective eye wear are also used in this laboratory to protect against spills to the shoes and eyes respectively.

Biosafety Cabinet Class II

To ensure sterility of samples, personnel and the environment from contamination, the work was performed in a biosafety cabinet. Biosafety Cabinet Class II operates under the principle of air flow around the work area and through High Efficiency Particulate Arrestance (HEPA) filters, creating a safe “Air curtain” around the work area resulting protection of user, sample and environment from contamination.

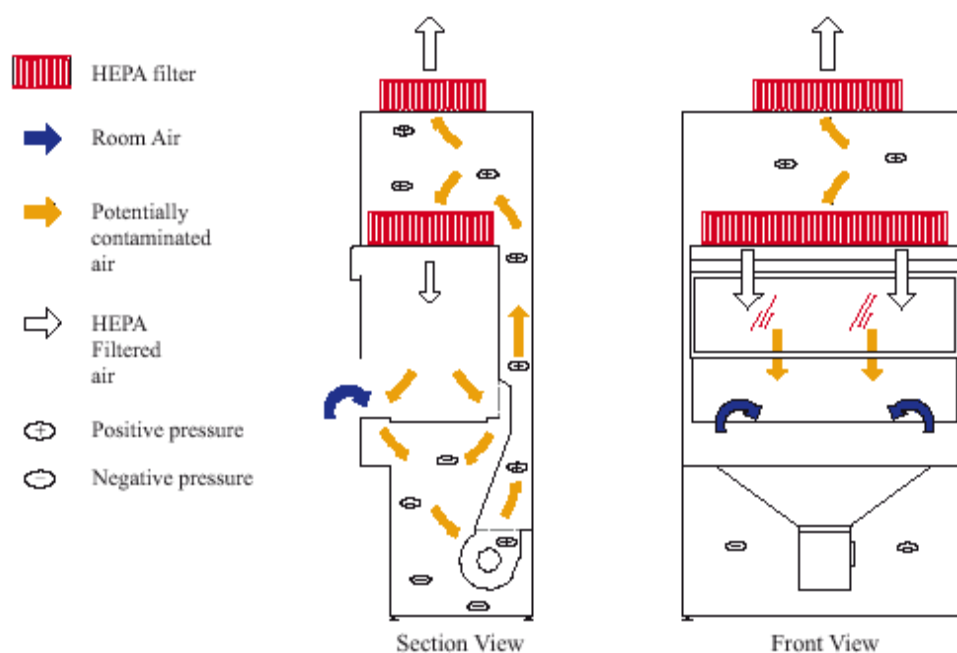


Figure 5.5: The biosafety cabinet level II. During operation, the motor driven blowers mounted in the cabinet protect the operator by drawing directional mass airflow around the user and into the air grill. This air then flows underneath the work surface circling back up to the top of the cabinet and through the HEPA filters. 70% of the HEPA filtered sterile air is blown downward over products and processes to prevent contamination. The remaining 30% goes through the HEPA filter by the pull of an

The immunology experiments involved the use of several materials. General laboratory reagents for PBMCs isolation and flow cytometry (Table 5.2), plastic ware (Table 5.3) and equipment (Table 5.4) were used during the experiments.

Antibodies that bind to specific surfaces of clusters of differentiation (CDs) were used in the all flow cytometry experiments during immunophenotyping and the functionality assays (Table 5.5). The antibodies used were commercially conjugated to fluorochromes such as Fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridine chlorophyll protein (PerCP), Pacific blue (PB) and allophycocyanin (APC). Some antibodies used where conjugated with tandem fluorochromes and these included PE.Cy7, PerCP.Cy5.5 and APC.Cy7; these are derived from the association

of PE, PerCP and APC with cyanine 5.5 (Cy5.5) or cyanine 7(Cy7). These are shown in table 5.5

Table 5.2. General reagents used in experiments

Reagent	Supplier
Phosphate buffered saline	Gibco Life Technologies
RPML glutamax medium	Gibco Life Technologies
HEPES	Gibco Life Technologies
Penstrep	Gibco Life Technologies
Fungizone	Gibco Life Technologies
Trypan Blue	Gibco Life Technologies
Lymphoprep	Axis-Shield, Norway
Dimethyl sulphoxide (DMSO)	Sigma-Aldrich
One Comp Beads	eBioscience
Bovine serum albumin	Fisher Scientific
Sodium azide	Sigma-Aldrich
4% paraformaldehyde	Santa Cruz Biotechnology
Foetal calf serum	HyClone or other company
Antibodies – see Table 4	
FACSCanto II reagents – CS&T Beads, FACS flow, FACS Clean.	

Table 5.3. Plasticware

Item	Supplier	Catalogue Number
3.5 ml transfer pipettes	Sarstedt	86.1171.010
Automatic pipette tips	local supplier	
EDTA blood tubes	local supplier	
Centrifuge tubes - 30 ml Universal tubes	Fisherbrand	FSH30APS
- or 15 ml Falcon tubes	Sarstedt	62.554.502
Cryovials	Thermo Scientific	
	Or Nalgene	5000-0020
Flow cytometry tubes (5 ml polystyrene)	BD Falcon	352008

Table 5.4. Equipment

Equipment type
Automatic pipettes – 2-20 µl, 20-200 µl and 200-1000 µl or equivalent
Bench centrifuge
Laminar air flow cabinet
Haemocytometer slide
Microscope
Centrifuge tube and flow cytometry tube racks
“Mr. Frosty” 1°C freezing container – Nalgene 5100-0001
+4°C fridge
-20°C freezer
-80°C freezer
Liquid nitrogen
FACSCanto II flow cytometer

Table 5.5: Antibodies

Cell	Antibody marker	Fluorochrome Clone	source
CD3	Pacific Blue	UCHT1	BD-Pharmingen
CD4	PE/Cy7	SK3	BD-Pharmingen
iNKT cell	PE	6B11	BD-Pharmingen
TCR Vδ1	FITC	TS8.2	Thermo Fisher
CD8α	APC	RPA-T8	BD-Pharmingen
CD3	Pacific Blue	UCHT1	BD-Pharmingen
TCR Vδ2	FITC	B6	BD-Pharmingen
TCR Vδ3	APC	PI1.5B	Beckman Coulter

CD19	PE/Cy7	SJ25C1	BD-Pharmingen
CD56	PE	BI59	BD-Pharmingen
CD3	Pacific Blue	UCHT1	BD-Pharmingen
CD4	PE/Cy7	SK3	BD-Pharmingen
CD3	PE	HIT3a	BD-Pharmingen
CD8 α	APC	RPA-T8	BD-Pharmingen
CD8 α	FITC	HIT8a	BD-Pharmingen
Dead Cells	FVD	efluor 506	Thermo Fisher
