MMED DISSERTATION

HYPOPHOSPHATEMIA IN REFEEDING SYNDROME AS A CAUSE OF EARLY MORTALITY IN PATIENTS INITIATING ANTIRETROVIRAL THERAPY IN ZAMBIA

BY

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(School of Medicine)

THE UNIVERSITY OF ZAMBIA

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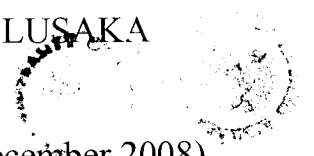

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APPROVAL PAGE

This dissertation of **CHRISTOPHER NYIRENDA** has been approved as fulfilling the requirements or partial fulfillment of the requirements for the award of Master of Medicine in **INTERNAL MEDICINE** by the University of Zambia.

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DECLARATION

I declare that this dissertation represents my own work and that it has not previously been submitted for a degree, diploma or other qualification at this or another University.

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ABSTRACT

Despite the numerous benefits seen with the introduction of antiretroviral therapy among people living with HIV/AIDS in low-income countries, there has been a surprisingly high mortality rate, especially in the first 90days of therapy. Causes of this early ART mortality are not well known in Zambia.
It is probable that the high prevalence of malnutrition in sub-Saharan Africa, exacerbated in persons with HIV disease, is a major factor in early ART mortality.Refeeding syndrome in particular may be responsible for a portion of the early deaths. This syndrome occurs in persons with severe cachexia from any cause who experience sudden increases in food intake and nutrient absorption that exceeds the body's ability to meet the high phosphorous demands for metabolism of carbohydrate. The resulting hypophosphatemia could lead to adverse outcomes including cardiopulmonary failure and death.

This study looked at a group of Zambians, who were HIV positive and just about to initiate antiretroviral therapy (ART) and the expected mortality in this group would be high. The aims of the study were:
1. To determine whether refeeding syndrome occurs, and estimate its incidence.
2. To determine whether persons who develop refeeding syndrome are at higher risk of early ART mortality or near mortality than persons who do not develop refeeding syndrome.

Study participants were recruited from Chawama Clinic in Lusaka, Zambia and included 148 ART naïve adult men and women initiating treatment and at high risk for early mortality.
After obtaining consent, participants were scheduled for routine initiation of ART with usual 2-, 4-, 8-, and 12-week follow up visits after initiation of therapy. Because initial immunologic and clinical responses, intestinal changes, and refeeding syndrome could all occur in the first 1-2 weeks after ART begins, a 1week post-ART visit was added for the purpose of this study.
If eligible, in addition to routine procedures, including obtaining demographic and socioeconomic factors, history and physical exam, disease stage classification, and adherence evaluation, blood samples for hematology, biochemistry and CD4+cell counts were drawn.

Serum phosphorus was used as the primary identifying parameter of refeeding syndrome for which hypophosphataemia is a dominant feature.
Routine physical examination with emphasis on clinical features of refeeding syndrome and adherence evaluation were also done during the visits.

148 participants were seen of whom 90(61%) females and 58(39%) males. Of these 17(11%) met the criterion for refeeding syndrome. In 7 it occurred at two or more visits despite supplementation. There were 28(19%) participants who had mild hypophosphatemia (serum phosphate 0.65-0.8mmol/l) at least once.

The prevalence of hypophosphatemia was highest after 1 week of ART. Phosphate levels decreased significantly in the first 1 week in the whole cohort before recovering in week 2 and thereafter. Male gender was found to be a significant risk factor (OR 4.2, 95%CI 1.3-13).

Overall 28 deaths were recorded in 30.1 person years of follow up (median 86days).Of these 4 (15%) had refeeding syndrome and 3 had persistently low phosphate despite oral or IV supplementation. Thirteen subjects who met our criteria refeeding syndrome survived after phosphorus supplementation.

Mortality was not significantly associated with refeeding syndrome. However; correlation may have been influenced by phosphate supplementation and multiple other independent causes of mortality.
Hypophosphatemia occurs early in ART in some Zambian subjects exhibiting major risk factors for early mortality and may be responsible for a portion of the early deaths.

This dissertation is dedicated to my dear mum and dad for all their support towards my education

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Acronyms used.

AIDS	Acquired Immunodeficiency Syndrome
ALT	Alanine Aminotransferase
ART	Antiretroviral Therapy
AST	Aspartate transaminase
ATP	Adenosine triphosphate
BMI	Body mass index
BUN	Blood Urea Nitrogen
CBC	Complete blood count
CC	Cohort collaboration
CPK	Creatinine phosphokinase
CIDRZ	Center for infectious Disease Research in Zambia
HAART	Highly active antiretroviral therapy
HIV	Human immunodeficiency syndrome
IRB	Institutional Review Board
LDH	Lactate Dehydrogenase
LINC	Lower income countries
PCP	Pneumocystis Carini Pneumonia
PID	Patient Identification number
QA	Quality assurance
RS	Refeeding Syndrome
STD	Sexually Transmitted Diseases
UAB	University of Alabama at Birmingham
SD	Standard deviation

Chapter 1

1.0 Background and Significance

Early ART mortality. Despite the numerous benefits seen with the introduction of antiretroviral therapy among people living with HIV/AIDS in low-income countries, there has been a surprisingly high mortality rate, especially in the first 90days of therapy. In the large multinational Antiretroviral Therapy in Lower income Countries and ART Cohort Collaboration (ART-LINC/ART-CC) comparison of low and high income countries, patients starting antiretroviral therapy(ART) in low income countries had lower CD4+ counts (median 108cells/ul vs.234cells/ul).[9] Although immunological and viral load responses to ART were similar in low and high income countries, the hazard ratio for mortality was 4.3times higher in the former in the first month of ART, and fell to 1.5 in months 7-12. Baseline CD4+ counts (especially those less than 50cells/ul) and World Health Organization (WHO) HIV stage (III/IV) were the major identified risk factors. Data from the Centre for Infectious Disease Research in Zambia (CIDRZ) confirm these findings in Lusaka.[9] the mortality rate in the first 90days of therapy was 29.9 per 100 patient years, but thereafter it dropped to 4.9 per 100 patient-years, comparable to that among patients treated at the University of Alabama 1917 Clinic in Birmingham, Alabama. More than half of the early mortality occurred in the first 30 days. In multivariate analyses, the risk factors for early mortality mirrored those of the ART-LINC/ART-CC Study, with the addition of BMI<16kg/m2 and hemoglobin<8.0g/dl as significant predictors. At present the causes of early ART mortality are unknown in Zambia. Hypotheses include the advanced stage of HIV disease being treated; overwhelming immune reconstitution inflammatory syndrome (IRIS); undiagnosed opportunistic infections, malaria; drug toxicities; and endocrinopathies such as hypothyroidism or adrenal insufficiency.

Refeeding syndrome. It is also probable that the high prevalence of malnutrition in sub-Saharan Africa, exacerbated in persons with HIV disease, is a major factor in early ART mortality. The refeeding syndrome in particular may be responsible for a portion of the early deaths. This syndrome has been tragically observed and poignantly described in repatriated prisoners of war who had been starved and re-fed rapidly, but it occurs in

persons with severe cachexia (marasmus) from any cause who experience sudden increases in macronutrient intake due to improved food supply or therapeutic feeding. Severe hypophosphatemia,which is potentially the most serious complication of refeeding,results from aggressive feeding with carbohydrate as the predominant energy source.[10]Because energy expenditure and glucose oxidation are low during starvation(fatty acids are the predominant fuel),the need for phosphorous(which is used in glycolysis and Adenosine Triphosphate production)is relatively low. When carbohydrate is provided, the demand for phosphorous increases dramatically and can exceed the body's ability to mobilize it from bone. When phosphate levels fall below 0.65mmol/l, and particularly below 0.5mmol/l, the risk of adverse clinical effects is high, especially if there is concurrent hyperglycemia which may reflect intracellular phosphate depletion and interruption of glycolysis and the Krebs cycle. The complications of severe hypophosphatemia include weakness, muscle paralysis, decreased cardiac output, respiratory failure, decreased oxygen release from red blood cells and decreased white blood cell bacterial activity.[12]Refeeding hypophosphatemia can cause cardio respiratory failure and death within several days in patients who are chronically starved but metabolically stable. Peripheral or pulmonary oedema, hypokalemia and hypomagnesaemia may also accompany Refeeding syndrome, but they are less specific than hypophosphatemia.

While food supply and macronutrient intake do not necessarily change in African patients with HIV who start ART, an alternative pathophysiology is conceivable. HIV enteropathy which is common in patients with HIV disease, impairs gasterointestinal function and nutrient absorption.[13]With rapid reductions in viral load after ART is instituted, improvements in HIV enteropathy occur even in the first week of ART.[14]This is likely to increase absorption of many nutrients, supplying the body with a sudden increase in nutrient flux such as starving individual's tissues have not seen for some time. This could in turn trigger all the responses both adaptive and maladaptive which occur in classic refeeding syndrome. Manifestations of the refeeding syndrome could occur within the first week or two of ART, producing the high 30-and 90-day mortality rates reported by ART-LINC/ART-CC[9] and by Stringer et al.[1].

To my knowledge there are no other investigations of potential nutritional causes for early ART mortality underway in Zambia. Findings from this study could therefore have a significant impact on People Living with HIV/AIDS starting ART in Lusaka and worldwide. It is also hoped that there will be increased awareness about HIV/AIDS and access to ARVs through sensitization techniques for the study to the community in which the study is conducted.

The treatment of more than 80,000 people living with HIV/AIDS, with comprehensive prospective data collection at Zambian government sites receiving support from the centre for infectious Disease Research in Zambia, provided an outstanding opportunity to conduct this study.

1.1 Statement of the problem

Despite its benefits the introduction of anti-retroviral therapy among persons with HIV/AIDS in low-income countries has been attended by a surprisingly high early mortality rate. Data from the Centre for Infectious Disease Research in Zambia,(Stringer et al; early outcomes in patients on ART.2006)shows that 13.3% of patients in the highest risk group died in the first 90 days of therapy, and most of these deaths occurred in the first 30 days.[1]Causes of this early mortality are still not well understood.

1.2 Specific aims

Specifically, in a cohort of ART-naïve Zambians who are starting ART and exhibiting a risk factor for early mortality (defined by BMI<18.5kg/m2 or CD4+ count<50cells/μl), the **Aims** of the study were:

1. To determine whether refeeding syndrome occurs, and estimate its incidence

   Refeeding syndrome was defined as serum phosphorus<0.65mmol/l at any time in the first 90 days of ART.

2. To determine whether persons who developed refeeding syndrome were at higher risk of early ART mortality or near-mortality than persons who did not develop refeeding syndrome.

1.3 Hypothesis

Signs of refeeding syndrome will occur in the first 90 days and particularly in the first 30 days, among patients initiating ART and at high risk for early mortality.

Chapter 2

2.0 Literature review

Refeeding syndrome has been used to describe those phenomena, such as severe hypophosphatemia and other metabolic complications, seen in malnourished patients receiving concentrated calories via oral or total parenteral nutrition.

Severe hypophosphatemia and its associated complications in patients being refed with total parenteral nutrition after severe weight loss have been termed the refeeding syndrome.[11]However; this narrow definition does not encompass the broad range of interrelated metabolic derangements that can occur during the refeeding stage of an individual at risk. In addition to phosphorous depletion, changes in potassium, magnesium, and glucose metabolism, occurrence of vitamin deficiency and need for fluid resuscitation may all have a significant bearing on the metabolic milieu of the patient and should be included in the broader definition of refeeding syndrome. The potential adverse effects of therapeutically refeeding a severely malnourished patient were recognized even before the advent of parenteral nutrition.[18-21] Refeeding syndrome has also been well known to the paediatritian and has been described in the setting of marasmus and kwashiorkor.

2.1 History

Some of the adverse consequences of refeeding were described in the medical literature in the 1940s.Keys et al[18] reported a classic study, the Minnesota Experiment, which studied the effect of drastic food restriction and subsequent oral refeeding in previously healthy subjects. Subjects who had undergone 6 months of starvation showed no evidence of dyspnoea, increased venous pressure, or cardiac dilatation.However, during the recovery phase as the volunteers were refed, the cardiovascular reserve was diminished to the point that cardiac failure occurred in some. The observation in this intentional experiment correlated with the prior unintentional refeeding experiments undergone by victims of world war II.Hypertension and cardiac insufficiency increased markedly in the populace of Leningrad after the post siege restoration of normal food and liquid intake, often in patients apparently healthy up until the time acute syndromes appeared.[19]Peripheral oedema was also seen in the Leningrad patients, as well as

hospitalized, recovering Japanese prisoners of war.[20]Neurological complications coincident with refeeding including coma and convulsions, were noted in victims of war in the Netherlands.[21]

These severe cardiopulmonary and neurological complications of refeeding,along with other phenomena were rediscovered with the introduction of total parenteral nutrition(TPN)for chronically ill, essentially starved hospitalized patients in the 1970s and 1980s.In 1972 Silvis and Paragas[22] reported three cases with paraesthesias,weakness,and seizures occurring within 4-5days of beginning total parenteral nutrition for patients with weight loss secondary to regional ileitis or prolonged gastric outlet obstruction. In 1981, Weinsier et al[11] reported two cases of refeeding syndrome. The first case was a severely malnourished patient with anorexia nervosa who suffered an acute myocardial infarction and subsequent death which was associated with the institution of TPN 44h prior. The second patient developed acute respiratory failure after having begun TPN 48hr earlier for malnutrition secondary to malabsorption.It is important to note that both patients had normal serum phosphorus levels before beginning TPN therapy, but had extremely low phosphorous levels (0.36mmol/l and 0.23mmol/l, respectively) at the time of their initial decompensation.

2.2 Pathogenesis

The mechanism of hypophosphatemia associated with refeeding has been elucidated in the past 30yrs.In the starved individual the catabolism of fat and muscle leads to loss of lean muscle mass, water and minerals.[23,24]The serum concentrations of these depleted components including phosphorus, generally remains normal due to adjustments in renal rates of excretion.[23,25]With conversion to carbohydrate as the major source of energy during refeeding, insulin release is stimulated. Carbohydrate repletion and insulin release together enhance uptake of glucose, phosphorus, water, and other components into cells, as well as stimulate anabolic protein synthesis.[23]

The combination of depletion of total body phosphorus stores during catabolic starvation and increased cellular influx of phosphorus during anabolic refeeding leads to severe

extra cellular hypophosphatemia.Low serum phosphorus levels are directly related to depletion of phoshorylated intermediates and compounds such as red cell Adenosine Triphosphate ,2,3Diphosphoglycerate and G-3-PD,which are important to metabolism.[24]Also the deficiency of these compounds has been implicated in the cardiac,neuromuscular,hematologic,respiratory dysfunction and in other complications of hypophosphatemia.

2.21 Cardiac dysfunction.

Recent work suggests a correlation between hypophosphatemia and observed cardiac decompesation phenomenon; cardiac output as measured via thermo dilution was impaired in 7 critically ill hypophosphatemic patients, but improved significantly with correction of single variable of serum phosphorus.[26]Another study reported a syndrome of congestive heart failure in three patients who had ingested large quantities of phosphorus binding antacids on a chronic basis.[27]This syndrome, as in other complications of hypophosphatemia, was reversible with phosphorus repletion. Similar depression and restoration of cardiac function with hypo and normo-phosphatemia respectively, have been seen experimentally in dogs.[28]It has been postulated again that reduced phosphorus levels lead to depleted ATP levels, and in turn, depressed myocardial sarcomere contractility.[26]Another theory is that severe hypophosphatemia actually can cause acute myocardial damage,[11]as seen in the myofibrillar fragmentation at autopsy in starved refed swine[29] and in fasted refed young female who died of sudden ventricular fibrillation.[30]

2.22 Neuromuscular dysfunction. Acute areflexic paralysis, diffuse sensory loss, cranial nerve palsies,paresthesias,weakness,seizures,rhabdomyolysis,GuillainBarre-likesyndromeand respiratory insufficiency represent a diffuse spectrum of neuromuscular complications reported with refeeding associated hypophosphatemia.[11,22,31,32]The mechanism of neurological deficit is uncertain, but it has been suggested that the physiological effects of hypophosphatemia,including increased oxyhemoglobin affinity and hemolytic anemia could lead to tissue hypoxia and hence altered tissue function.[31]A reduction in available ATP to enhance respiratory muscle contraction has been suggested

as a mechanism for acute respiratory failure.[33]Infact measured diaphragmatic contractility appears to be severely depressed in hypophoshatemic patients with acute respiratory failure on ventilators, with a corresponding improvement in contractility with phosphorus repletion.[34]Rhabdomyolysis in the setting of refeeding hyper alimentation has been seen experimentally in dogs[35] and in chronic severe alcoholism in man, presumably secondary to severe ATP depletion in muscle tissue.

2.23 Hematologic RBC dysfunction.

The hematological consequences of hypophosphatemia have been studied extensively. Patients who became hypophosphatemic during TPN were found to have reduced production of 2,3DPG, erythrocyte ATP and other phosphorylated intermediates of RBC glycolysis.[36]ATP and 2,3DPG have an important role in promoting release of oxygen from hemoglobin.[37,38]The decline in 2,3 DPG has the effect of increasing the RBC affinity for oxygen,impairing its release and decreasing the delivery of oxygen to the tissue.[36]Reduced levels of RBC ATP and 2,3 DPG were also present in a patient with severe hypophosphatemia and a hemolytic anemia characterized by microspherocytic morphology, increasing rigidity and diminished survival of the cell;[39]the RBC abnormalities corrected with phosphorus repletion. Similar morphologic alteration in RBCs has been seen in dogs with refeeding hyperalimentation and hypophosphatemia,and an association between the survival of RBCs and their ATP content has been demonstrated experimentally.[40,41]It has been suggested that the rigid, poorly deformable RBC seen in hypophosphatemia has a reduced capability for capillary transit, further contributing to tissue hypoxia.[24]Thus hypophosphatemia appears to lead to RBC dysfunction via alterations in cell shape survival, and physiologic capacity.

2.24 Hematologic-WBC dysfunction.

White blood cell (WBC) dysfunction has also been seen as a complication of hypophosphatemia of parenteral nutrition.[42]Dogs undergoing TPN refeeding with induced hypophosphatemia and a patient with hyperalimentation and coincident hypophosphatemia were shown to have depressed chemotactic, phagocytic and bacteriocidal activity of granulocytes. Measured granulocyte ATP content was also

reduced. The WBC abnormalities were corrected in both dogs and patient with phosphorus repletion. It has been suggested that reduced WBC ATP content adversely affects the sub cellular microtubular contraction of the cells which interfered with such functions as pseudopod and vacuole formation involved in chemotaxis and phagocytosis,respectively.[24]It has further been postulated that the increased incidence of septicaemia seen in patients receiving hyperalimentation may be related to WBC dysfunction, in addition to the central line catheter as a foreign body and to the patients underlying debilitating state.[40]

2.25 Patient series-hypophosphatemia.

Hypophosphatemia has been reported in patients being repleted both parenterally[22,43-48] and enterally[49-51].Thompson and Hodges[48] retrospectively reviewed 68 courses of TPN in 61 patients and reported that 12% were hypophosphatemic before initiation of TPN;88% were initially normophosphatemic at institution of TPN,but 42% of patients in this latter group became hypophosphatemic during the initiation phase.They concluded that chronically malnourished patients require a slower rate of infusion to prevent hypophosphatemia.Patients identified at greatest risk included those with alcoholism,chronic weight loss,hyperglycemia,exogenous insulin requirements,or on chronic antacid or diuretic therapy.

In 1980 Silvis et al[49] reported hypophosphatemia and neurologic changes with oral refeeding in an alcoholic patient whose phosphorus dropped to 0.1mmol/l.A retrospective review over 3 years uncovered another 47 patients with phosphorus levels less than 0.32mmol/l with seven cases that were clinically evaluable.All of these patients were noted to have paraesthesia and alcoholism;three patients were receiving TPN in addition to oral feeding.This report and others[52,53] underscore the frequency of refeeding hypophosphatemia in chronic alcoholism.

Hayek and Eisenberg[51] recently reported severe hypophospatemia (<0.32mmol/l) in 25 prospective SICU patients. The phosphorus levels dropped dramatically after oral feedings in these patients and were corrected by enteral supplementation of an oral phosphate mixture. The report re emphasizes the importance of checking serum

phosphorus levels before refeeding patients and daily during the first week of refeeding in this ICU population. It should be noted that most of these reports have focused on hypophosphatemia without a more detailed investigation of other electrolytes that may be synergistic in the refeeding syndrome.

2.26 Cardiac Dysfunction

Reduction in cardiac mass and output during starvation was first observed in starvation in the 1940s.[54]In 1978 Heymsfield et al[55] studied hospitalized severely malnourished patients with modern techniques of cardiac assessment, including echocardiography before and during hyperalimentation refeeding. At baseline (pre-refeeding), these patients were found to have reduced total heart volume end diastolic volume, and left ventricular mass.With refeeding, ventricular volume returned rapidly toward normal, while left ventricular mass was still reduced. Of note two out of five repleted patients developed congestive heart failure during rapid repletion. This finding suggested that hyperalimentation could produce cardiac decompesation by creating a repleted circulatory demand on a still nutritionally depleted cardiac mass that had no time to 'catch up'. Furthermore, over hydration was reported by Heymsfield et al[56] in as much as 20 to 25% of patients refed with enteral nutrition.

Sodium fluxes may play a separate, additional role in cardiac overload with refeeding. Patrick[57] has implicated sodium shifts in sudden death during refeeding of starved children with oral formulas. Leukocyte intracellular sodium concentration and sodium pump activity, without specific recording of serum phosphorus and other electrolyte levels, were measured before refeeding. Children who suffered cardiopulmonary compromise and sudden death were found to have normal leukocyte intracellular sodium and sodium pump activity; whereas children who did not develop compromise had high intracellular sodium levels and low sodium pump activity. Two children were identified to have high sodium pump activity and prospective risk for cardiopulmonary compromise and subsequently survived with digoxin and diuretic therapy when congestive heart failure developed.Although the relationship between leukocyte sodium pump activity and other organ systems was not elucidated,it was theorized that inhibition of the cellular sodium-potassium ATPase activity by digoxin helped facilitate survival in the two

children with predicted refeeding complications. In light of this study, it has been suggested that digoxin be employed therapeutically in patients who develop congestive heart failure early in refeeding.

2.27 Patients at risk.

While the historical background of the refeeding syndrome described thus far has occurred most commonly in patients fitting the pattern of chronic cachexia due to prolonged starvation or marasmus, it does not appear to have occurred exclusively in this category of malnutrition. The phenomenon has not only been seen in patients with anorexia nervosa[58] who fit the category of classic marasmus, but also in patients who are hypoalbuminemic with apparently the more acute starvation pattern of kwashiorkor protein-calorie malnutrition.[11]The syndrome has also been seen in starved children in Jamaica with well defined kwashiorkor or marasmus .[57]In addition Patrick[57] reported death in four severely malnourished children after they were placed on 'high energy feeding' by mouth. This again illustrates that the refeeding syndrome does not occur just in patients placed on parenteral nutrition for severe malnutrition, but in any patient who has been chronically deprived of adequate nutrition. Table 1 lists those patients who should be considered especially 'at risk'.

Anorexia nervosa is perhaps the classic modern potential setting for refeeding syndrome. The disease is characterized by intentional starvation and extreme weight loss, along with psychological disturbances.[59]The cardiac disease component of anorexia nervosa is particularly noteworthy. Like other patients with severe malnutrition and weight loss,[55]anorexics develop loss of cardiac mass and chamber size.[60]Mitral valve prolapse may be seen due to size disproportion between the mitral valve and a shrunken left ventrical.[61]Cardiac response to exercise is blunted,probably secondary to reduced cardiac mass.[62]Electrocardiographic changes,particulary prolongation of the Q-T interval,are prominent.[58]

With a combination of total body and cardiac mass depletion, patients with anorexia nervosa become prone to complications due to electrolyte shifts and volume repletion in refeeding.In fact cardiopulmonary failure and seizures[23] have been reported in anorexia

nervosa patients associated with hypophosphatemia while patients were receiving TPN. Sudden death due to cardiac arrhythmia has been seen in three patients.[58]It should be noted that patients with anorexia nervosa can be successfully refed with either enteral[63] or parenteral[64] nutrition. However the disease serves as a sobering model for the possible calamity inherent in refeeding severely malnourished hospitalized patients. With the coincident existence of chronic illness and secondary chronic malnutrition in today's hospital population, the potential for the refeeding syndrome is greater than ever and one setting in which this could occur is HIV wasting syndrome. It is possible that the high prevalence of malnutrition associated with HIV disease, could result in refeeding syndrome (RS) and be responsible for a portion of the early deaths and perhaps even a substantial portion at initiation of antiretroviral therapy. Patients are at particular risk of RS when feeding is introduced too rapidly,when calories come predominantly from carbohydrate,and when serum phosphorous levels drop significantly.[2]Within several days HAART can improve appetite, potentially resulting in increased food intake and/or nutrient absorption that exceeds the body's ability to meet the high phosphorous demands of carbohydrate metabolism .The resulting hypophosphatemia could lead to adverse outcomes, including cardiopulmonary decompensation and death.

2.3 Patient risk profile for refeeding syndrome

Table (I)

Anorexia nervosa[58,59]
Classic kwashiorkor[57]
Classic marasmus[57]
Chronic malnutrition-underfeeding[2]
Chronic alcoholism[49,52,53]
Morbid obesity with massive weight loss[2,30]
Patient unfed in 7-10days with evidence of stress and depletion[65]
Prolonged fasting[57]
Prolonged intravenous hydration[43]
Chronic diarrhea?
HIV wasting syndrome?

2.4 Clinical features of refeeding syndrome

Table (II)

Body weakness
Muscle paralysis
Peripheral or pulmonary edema
Convulsions
Coma
Cardio respiratory failure
Death

Chapter 3

3.0 Research design and methods

Study Type: Longitudinal

Study participants were recruited from Chawama Clinic in Lusaka, Zambia. We were aiming at 200 ART naïve adult men and women initiating ART and with malnutrition.

Qualification for ART by Zambian national guidelines requires:

1. CD4+count less than 200cells/mm^3; or
2. WHO stage IV disease; or
3. WHO stage III disease and CD4+ lymphocyte count less than 350cells/mm^3

Duration: Each participant was followed up for 12weeks (6 visits, 5 of which coincide with routine ART program visits at the clinic. The total duration of the study was dependant upon the time required to recruit the 200 participants.

The following were monitored at each visit:

Review of systems and physical examinations

Serum samples (5mls of blood) for electrolytes and nutrients were drawn, labeled and sent to the lab. Parameters measured included phosphorus, potassium, magnesium, glucose and albumin. Since hypophosphataemia is a predominant feature for refeeding syndrome, serum phosphorus was used as the primary identifying parameter.

Refeeding syndrome was considered present when a subjects serum phosphorus fell below 0.65mmol/l at any time in the first 90days of ART and severe when a subject's serum phosphorous fell below 0.5mmol/l. Peripheral or pulmonary oedema, hyperglycemia, hypokalemia and hypomagnesaemia was assessed and recorded so that various combinations of them could be stratified, at least for descriptive purposes. They were not included in the case definition because they are not *sine qua non* for RS and they are prone to confounding from other conditions.

3.1 Inclusion criteria

1. Persons with HIV/AIDS, 16years of age or older, who are starting ART and were able and willing to provide informed consent.
2. ART-naïve (had never received ART)
3. High risk of early ART mortality (BMI<18.5kg/m^2 or CD4+ count<50cells/μl).[1, 9] We did not include HIV stage in the risk assessment because of challenges in making definitive diagnoses of stage defining illnesses.
4. Intended to remain in the current geographical area for the duration of the study.
5. Willing to adhere to a stepped up clinic visit schedule in the first 90days and to be followed up at home in the event of missed clinic visits.

3.2 Exclusion criteria

1. BMI>18.5kg/m2
2. Use of ART before
3. Conditions such as Alcohol abuse or Psychosis
4. Clients who were not likely to remain in the geographical area for the duration of the study.

3.3 Sample size

According to data from CIDRZ (Stringer et al; early outcomes of ART 2006), there were 215 deaths in the first 90days of ART among 1,613 persons in the high-risk group, making the raw mortality 13.3%. A similar mortality rate at 95% confidence interval gives a population of about 200 subjects. This sample size did not give us statistical power to test the effect of RS on mortality but we hoped to be able to make sufficient observations to draw reasonable conclusions regarding an association.

Sample size calculation, $n=z^2pq/d^2$, where n=sample size, p=13.3%, q=1-p, z=1.96 and d=0.05 at 95% confidence interval.

3.4 Data management

All 80,000+patients receiving HIV care and treatment in government institutions in Lusaka are followed in an electronic patient tracking system developed by CIDRZ.Sites collect clinical data in real time and update a central data base via a high speed wireless network. This study utilized this network, using CIDRZ secure data entry, error checking, QA, and backup procedures. The SAS (SAS Institute, Inc., Cary, NC) was used to perform statistical analyses.

3.5 Statistical analysis

Aim1: The incidence of the refeeding syndrome was estimated as the number of refeeding syndrome events per person-time of follow-up. The prevalence was estimated as the total number of refeeding syndrome events over the total number of participants enrolled. Statistical analyses were carried out with serum phosphorus as both a categorical and a continuous variable.

Aim2: The association between the refeeding syndrome (coded yes or no) and mortality/near mortality within 90days coded yes or no, (with and without including near mortality) was assessed using multivariate unconditional logistic regression (UCLR).Statistical analyses with mortality/near mortality as an outcome and serum phosphorus as a continuous independent variable, were also performed to determine whether they are associated and whether the association is linear or curvilinear.

Statistical power

We are not aware of the existence of data on the incidence or prevalence of refeeding syndrome that would allow to effectively compute statistical power. The purpose of this study was to produce such data so that future studies, particularly intervention studies to reduce the incidence of and mortality from RS in people living with HIV and AIDS can be adequately powered.

Data and Safety Monitoring Plan.

An experienced HIV medicine investigator who was not directly involved in this project was named Data Monitoring Officer to monitor progress of the evaluation with respect to enrolment, follow up, drop outs, and interim analyses.

3.6 Ethical considerations

Human Subjects

This proposal and consent forms were approved by the University of Zambia Research Ethics Committee. Once a study candidate was identified, details were carefully discussed with the participant. The participant was asked to read and sign the consent form. If the participant and legal guardian were unable to read, the process for consenting illiterate participants, as defined by the University of Zambia Research Ethics Committee, was followed. Written informed consent was obtained from all participants. The consent was translated and back translated into the local language prior to evaluation initiation.

Risks to the subjects. The level of risk associated with this research was expected to be minimal. Minor bleeding and bruising could be experienced from blood draws.

Protection against risks. While HIV status knowledge could be stigmatizing, we recruited from persons who had already learned their status through voluntary counseling and testing.

Potential benefits to subjects and others. The subjects benefited from detection of causes for early ART mortality and their appropriate interventions. Findings from this study could also have a significant impact on People Living with HIV/AIDS and initiating ART in Lusaka and worldwide.

Chapter 4

4.0 Study procedures

This study made use of the routinely measured parameters at the ART Clinic, such as height, weight, BMI, Blood Pressure, heamatology, biochemistry, CD4+ count and HIV staging/antibodies.

Physical examination with attention to signs of refeeding syndrome; serum phosphorus, glucose, potassium, magnesium and albumin were measured at each visit. The ART program routinely includes visits with a nurse or clinical officer 2, 4, 8 and 12 weeks after ART initiation. Because initial immunologic and clinical responses, intestinal changes, and refeeding syndrome could all occur in the first one to two weeks after ART begins a one week post initiation of ART visit was added for the study.

4.1 Distribution of Responsibilities.

Enrolments: Performed by either the study nurse, clinical officer or the investigator. This was based on the subject's eligibility to initiate ART and the inclusion criteria for the study.

Systemic Review and Physical Examination. This was performed by the investigator and the Clinical Officer. History taking and physical examinations payed particular attention to signs and symptoms such as pulmonary oedema, dyspnea on exertion or at rest, orthopnea, paroxysmal nocturnal dyspnea, tachypnea, tachycardia, jugular venous distention, abnormal heart and lung sounds, and pitting pedal edema.

Other potential causes for these findings e.g. congestive heart failure, Pulmonary Tuberculosis, Kaposis Sarcoma or PCP were recorded in order to control confounding in statistical analyses.Subjects were also examined for hair changes and skin integrity which are abnormal in protein energy malnutrition of the kwashiorkor type and also in advanced AIDS,[2] which could be an additional nutritional cause for early mortality.

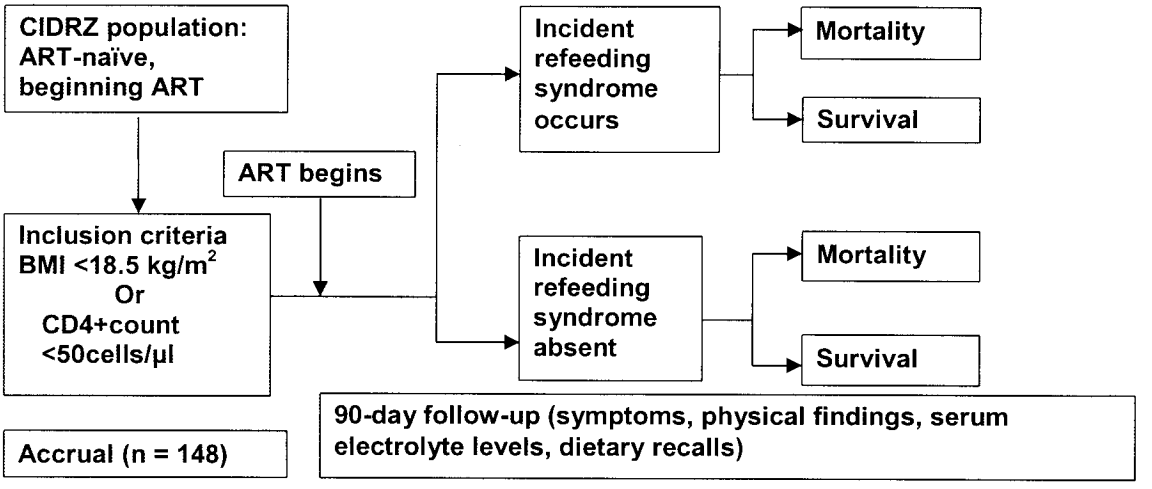
Biochemical measures.

Blood samples were collected by the investigator and the clinical officer at each visit, and transported to the CIDRZ Central Laboratory.

4.2 Follow up

Each participant was followed up for 12weeks in which 6 visits were required. When clinical assessments at scheduled visits indicated worsening illness or serious decompesation, subjects were admitted for inpatient care. Those who were late for the 1-, 2-, or 4-week clinic by more than 4 days or for the 8-or 12-week visit by more than 7 days were followed up and called for review. The following is a schematic presentation of the follow up events.

Figure 1.



Chapter 5

5.0 Results

5.1 Baseline characteristics

148 participants of whom 58(39%) men and 90(61%) women were enrolled and followed up over 12 weeks (6visits for each participant).The total duration was 1year.

The age range was 20-65yrs of which the mean age (33.5years) and the mean BMI was 17.0kg/m^2.Refer to (Table 1) for the baseline characteristics.

Table 1.

Baseline Characteristics of Cohort

N = 148 (61% women, 39% men)	Mean (SD)
Age, years	33.5 (7.5)
Height, cm	165 (9)
Weight, kg	46.1 (7.4)
BMI, kg/m^2	17.0 (2.3)
CD4+ cells/μl	50 (52)
Follow-up, person-days	74.4 (26.3)

Table 2.Baseline Symptoms and Signs

Diarrhoea	15%
Vomiting	9%
Cough	41%
Dyspnoea	14%
Oedema	10%

5.2 Participants with severe hypophosphatemia

There were 16(11%) of the total number of participants, who met the criterion for refeeding syndrome (Table3). In 7 of them, it occurred at two or more visits despite supplementation.28 (19%) had mild hypophosphatemia (serum phosphate0.65-0.87mmol/l)at least once. Prevalence of hypophosphatemia was highest after 1week of antiretroviral therapy. Phosphate levels decreased significantly in the first 1week in the whole cohort before recovering at week 2 and thereafter (figure.2).

Male gender was found to be the only significant risk factor OR4.2, 95%CI 1.3-13.3.

Table 3. Crude Incidence of Hypophosphatemia

Serum phosphate	Number	Percent
< 0.65 mmol/L*	16	11%
0.65-0.87 mmol/L	28	19%
Total	44	30%

Serum phosphate categories:

- Severe hypophosphatemia (<0.65mmol/l)*
- Mild hypophosphatemia (0.65-0.87mmol/l)
- Normophosphatemia (>0.87mmol/l)
- Our criterion for refeeding syndrome*

Figure 2. Prevalence of hypophosphatemia by Visit

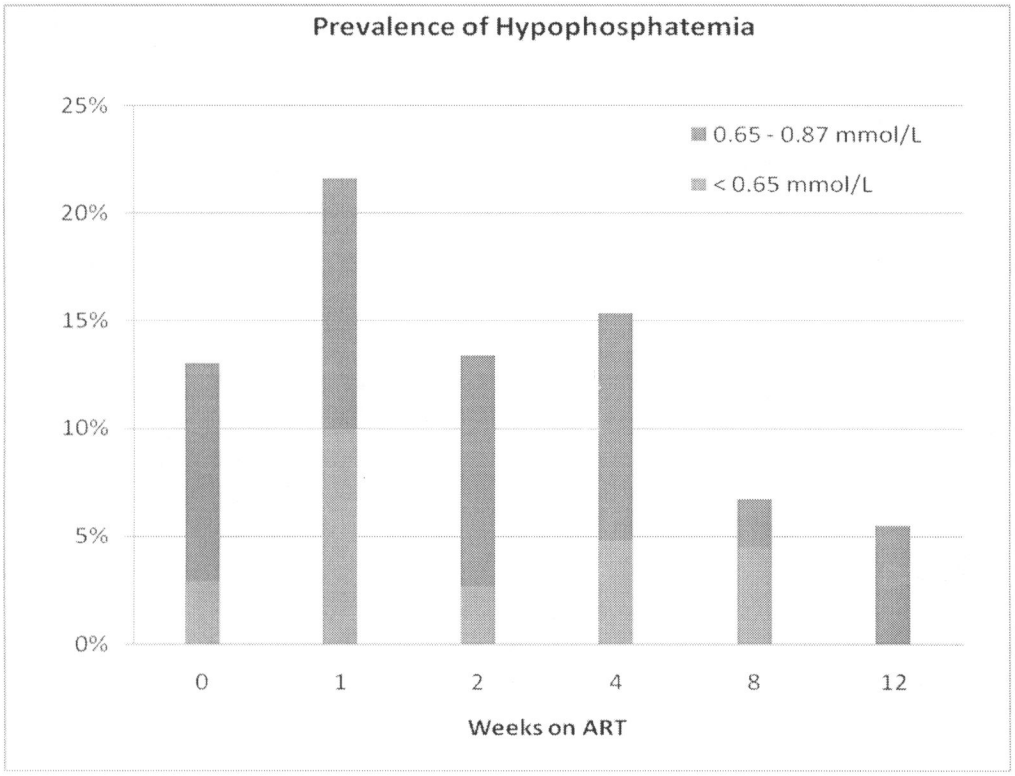


Figure3

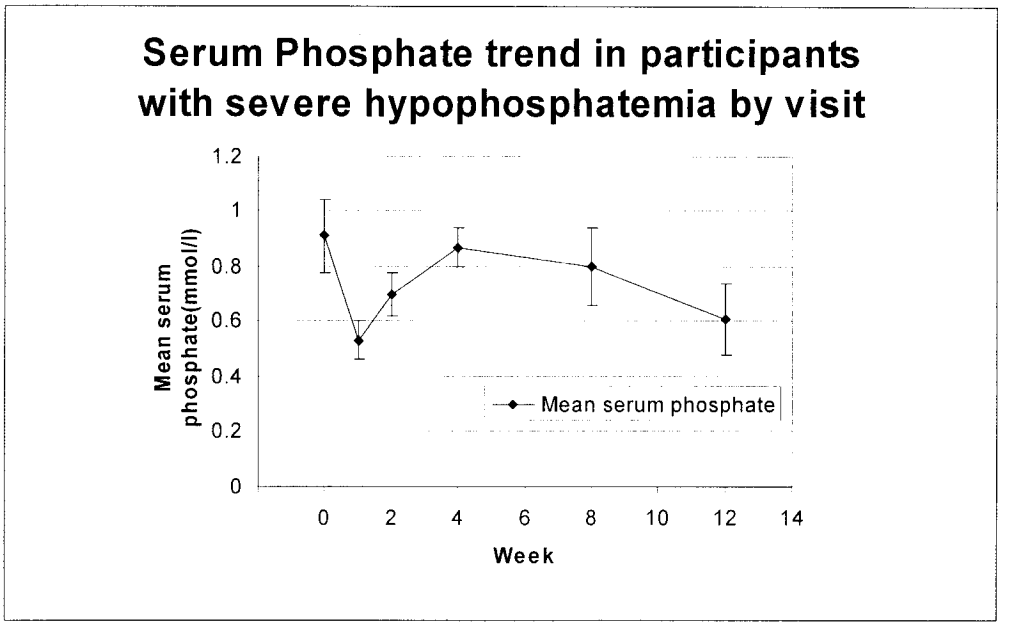


Figure 4.

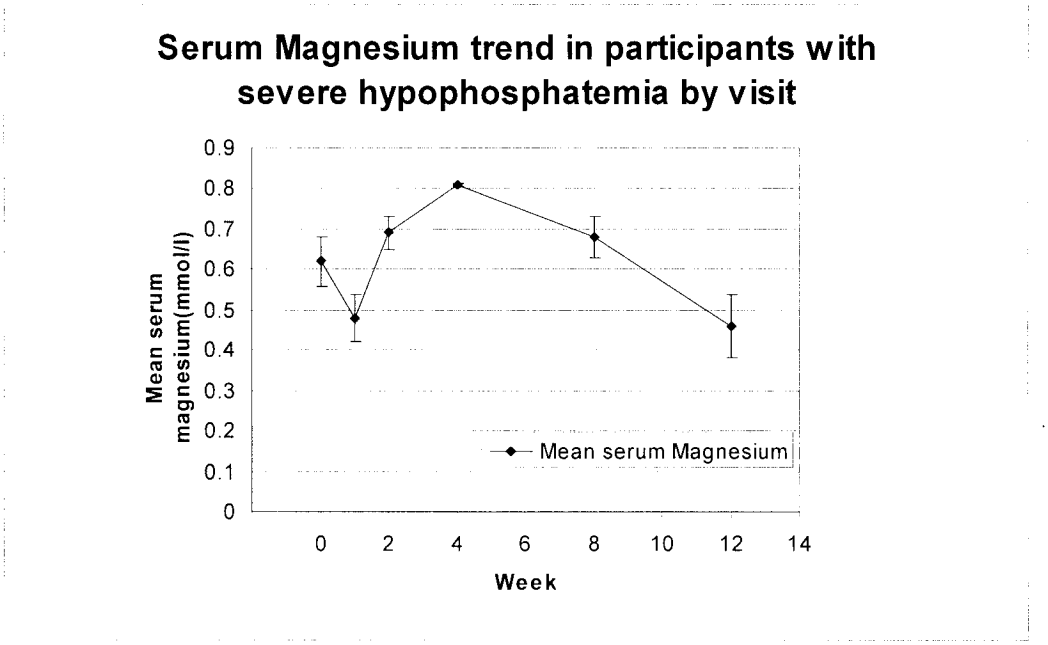


Figure 5.

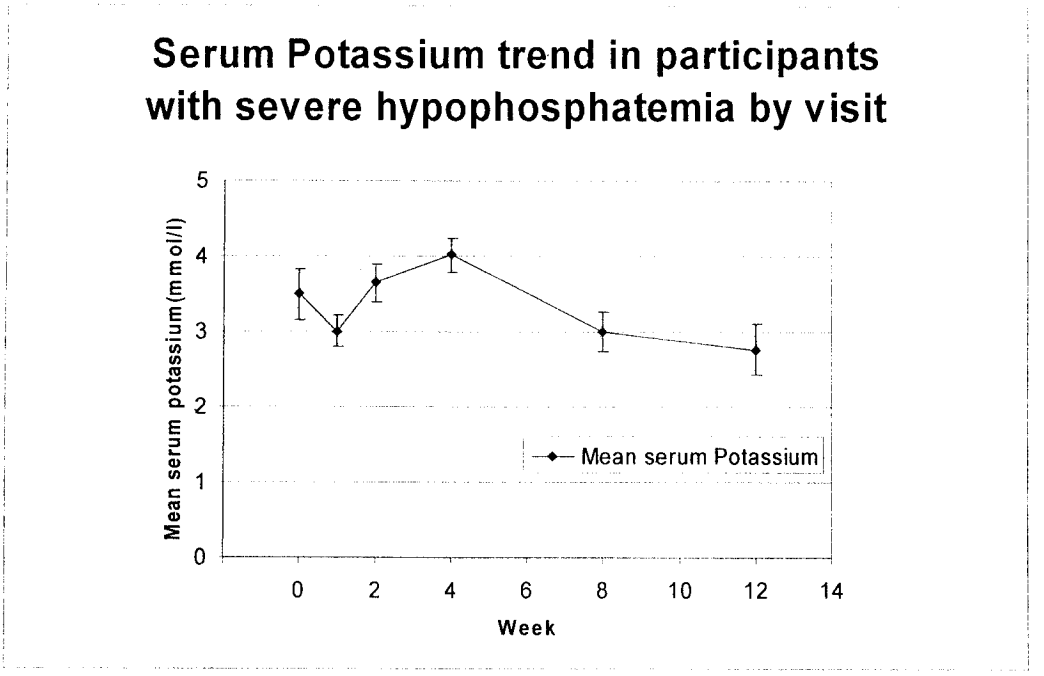


Figure 6.

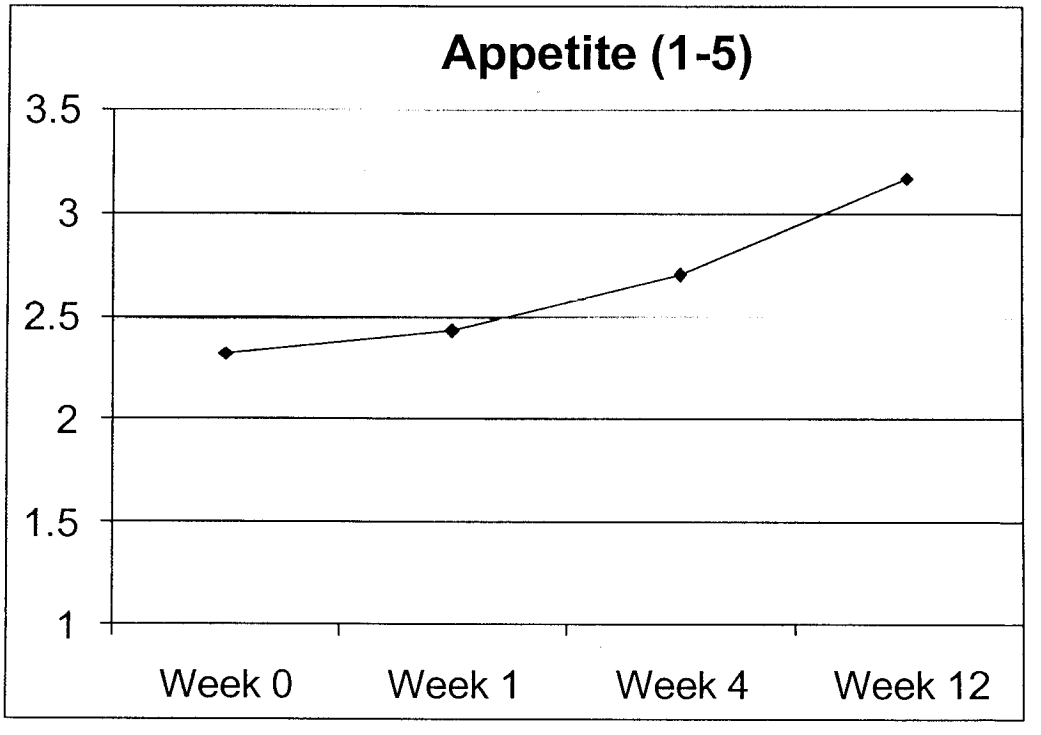
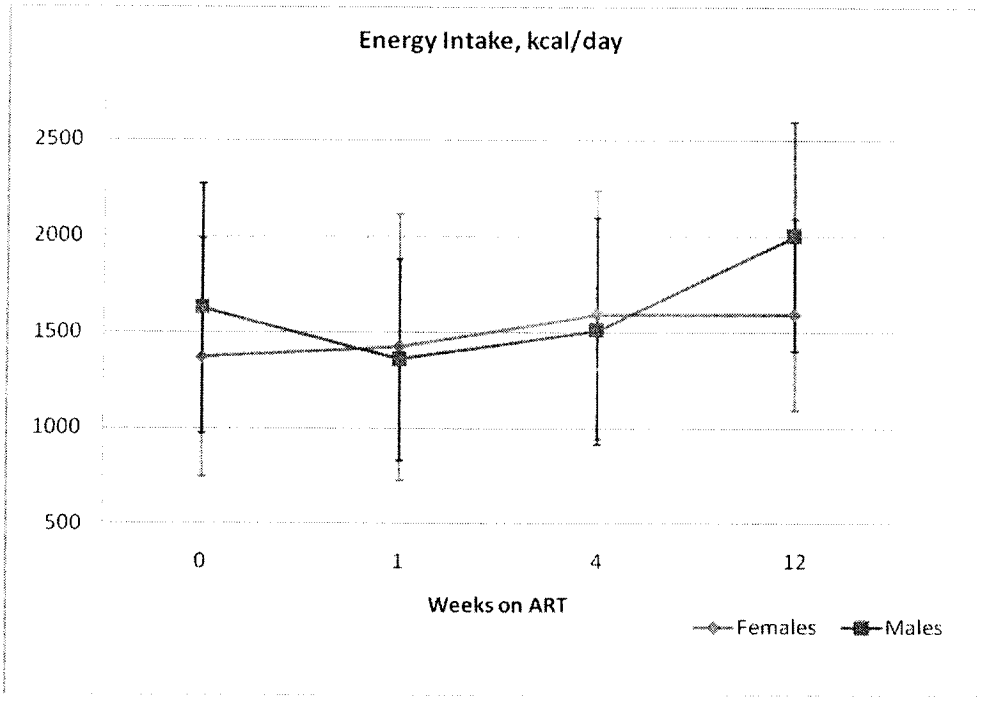


Figure 7.



5.3 Mortalities

There were 28 deaths in 30.1 person years of follow up (median 86days). Of these 4 (15%) met our criteria for refeeding syndrome, including 3 with serum phosphate that were persistently low despite oral and/or iv supplementation. One participant died before iv supplementation could be given. Six additional participants died with low serum phosphate levels, but fell short of our definition of refeeding syndrome (Table 4.). Mortality was not significantly associated with severe hypophosphatemia.

Table 4.Mortalities

- **Overall 28 deaths in 30.1 person-years of follow up (median 86 days)**

Serum Phosphate	Deaths	Survivors
< 0.65 mmol/l	4	13
0.65-0.87mmol/l	6	26
>0.87 mmol/l	16	81
Total	28	120

Table 5.Characteristics by BMI categories

Variable	BMI>18.5 n=36		16-18.5 n=51		<16 n=61	
	Mean	SD	Mean	SD	Mean	SD
Age, years	34.03	7.97	33.85	8.61	32.54	6.89
Baseline CD4+	30.31	12.46	26.40	11.76	84.89	69.56
Baseline BMI	20.14	1.13	16.95	0.68	14.94	0.98
Baseline Weight,kg	52.57	6.38	47.54	4.79	40.47	5.20
Baseline Height	1.61	0.09	1.67	0.08	1.65	0.08
Phosphorous at V1,mmol/l	1.26	0.27	1.20	0.36	1.26	0.31
Phosphorous at V2,mmol/l	1.08	0.24	0.93	0.32	1.08	0.22
Phosphorus at V3,mmol/l	1.14	0.30	1.09	0.26	1.22	0.21
Phosphorus at V4,mmol/l	1.16	0.36	1.14	0.31	1.19	0.26
Phosphorus at V5,mmol/l	1.18	0.21	1.14	0.34	1.34	0.37
Phosphorus at V6,mmol/l	1.17	0.23	1.28	0.21	1.22	0.26

5.4 Surviving participants with severe hypophosphatemia

Thirteen subjects with severe hypophosphatemia survived after phosphorous supplementation. A classic example was that of a 35year old male participant who

developed signs and symptoms of refeeding syndrome following severe hypophosphatemia but recovered after IV and oral phosphorous supplementation (Table 6).

This participant with WHO clinical stage3 HIV disease presented to the clinic for initiation of antiretroviral therapy 32weeks after testing HIV-positive. He had recently completed a 6-month course of treatment for pulmonary tuberculosis. He reported anorexia and diarrhoea, and he was severely cachectic (height165cm and weight 40kg {body mass index (BMI)14.9kg/m2}).His CD4+count was 326cells/ul.He was started on zidovudine, lamivudine, and efavirenz, and cotrimoxazole was provided for standard prophylaxis against pneumocystis pneumonia.

The patient's clinical and biochemical course are outlined in Table 6.Biochemical results are reported after 5 to 7days, introducing delays in correcting abnormalities. He experienced periodic diarrhoea and persistent hypokalemia in the first 3weeks despite oral KCL supplementation .In the second week of ART his condition markedly deteriorated. He reported shortness of breath, diarrhoea, and vomiting, and he appeared volume depleted and extremely weak. Results from the 1-week visit revealed severe hypophosphatemia.He improved after 24hours'treatment with intravenous hydration and infusion of sodium phosphate, and was discharged on oral potassium phosphate powder. Although he reported full adherence to prescribed supplements, at week 3 the serum phosphate had decreased again to critical level. Because he declined readmission for intravenous intervention, further oral supplementation was provided. At week 4 he reported recurrence of diarrhoea and loss of appetite, and appeared volume depleted, but his serum phosphate and potassium were normal. Intravenous fluids were administered and oral rehydration solution was provided. At week 6, he reported swelling and shortness of breath but also extreme hunger and consumption of substantial quantities of food. He had gained 15kg and exhibited anasarca, tachycardia and pulmonary oedema.Serum chemistries were normal. Oral furosemide was prescribed. At weeks 8 and 14, he reported hunger but otherwise asymptomatic, and he progressively gained weight even as his oedema resolved. Serum chemistries except albumin remained normal.

Table 6.Serum chemistry results and clinical features by visit (Surviving participant).

Date	Normal range	23 Nov 06	30 Nov 06	7 Dec 06	15 Dec 06	21 Dec 06	2 Jan 07	18 Jan 07	27 Feb 07
Weeks on ART		0	1	2	3	4	6	8	14
Symptoms		Diarrhoea	Stiffness of fingers	Dyspnoea, diarrhoea, vomiting	None	Diarrhoea, anorexia	Anasarca, exertional dyspnoea	None	None
Appetite[a]		None	Little	None	Little	None	Very Hungry	Hungry	Hungry
Energy intake, kcal		740	1023	–	–	630	–	–	2590
Weight, kg		40	40	38	42	37	52	56	63.5
Physical findings		Cachexia, lung rales	Unchanged	Volume depletion, extreme weakness	Stable	Lung rales, volume depletion	Anasarca, tachycardia, lung rales	Mild pedal oedema	Stable
Serum phosphate, mmol/L	0.87-1.45	1.38	0.55	1.04 after IV phosphate infusion	0.37	1.54	0.95	1.84	1.1

Chapter 6

6.0 Discussion

This study was conducted over the period, November 2006 to November 2007.148 participants of which 58(39%) males and 90(61%) females were enrolled and followed up over 12weeks (6 visits) for each participant. All participants were black Zambians.

6.1 Baseline characteristics

The age range was 20-65 years of which the mean age (33.5years) and the mean BMI was 17.0kg/m^2.The mean CD4+ count was 50cells/ul.With the given BMI mean, most of the participants were wasted and potentially at risk of developing refeeding syndrome and ultimate early mortality upon initiating antiretroviral therapy.

All participants in the study underwent physical examination at each visit, looking for features of refeeding syndrome. Symptoms presented included, coughing, dyspnoea, orthopnoea, paroxysmal nocturnal dyspnoea, vomiting and diarrhea. Signs included oedema (peripheral and pulmonary), tachycardia, tachypnoea, petechiae, ascites, dry cracking skin and atrophic lingual papillae.

There were 148 participants at the close of the study which fell short of the targeted sample size of 200 due to resource constraints. Of these, 28 deaths were reported. Eight participants were lost to follow due to transfers to other localities outside the catchment for the clinic. Five were lost to follow up for unknown reasons while six participants were discontinued for failing to adhere to the study requirements.

File reviews were done from the site clinic as well as progress reports given by community health workers to determine cause of death and reasons for loss to follow up. In some cases the study physician (author) would make physical follow-ups to health facilities such as private clinics and the University Teaching Hospital were some participants might have been referred for further management of various complications.

6.2 Mortalities

Of the 28 deaths recorded,3 were reported to have severe diarrhoea ,5 had pneumonia,1 meningoencephalitis, 2 septicemia, 2 severe anemia, 1 PCP, 1cervical cancer related complications and 1 severe gastroenteritis with anemia prior their deaths. In 12 participants possible cause of death remained unknown.

Four, (15%) of the 28 deaths met the criteria for refeeding syndrome. These included 3 participants with persistently low serum phosphate (≤0.5mmol/l) despite oral and/or IV supplementation. One participant died before IV supplementation could be given while six (6) additional participants died with low serum phosphate levels but fell short of the criteria for refeeding syndrome. There was no significant association between mortality and severe hypophosphatemia. This outcome could have been influenced by phosphorous supplementation and multiple other independent causes of mortality. Also the small number of deaths in participants presenting with severe hypophosphatemia gives low statistical power.

Out of the 148 participants enrolled, 16(11%) met the criterion for refeeding syndrome. In seven of them it occurred at two or more visits despite supplementation. In (28) (19%) mild hypophosphatemia (serum phosphate 0.65-0.87mmol) was reported at least once. The prevalence of hypophosphatemia was highest after 1 week of antiretroviral therapy. Phosphate levels decreased significantly in the first 1week of antiretroviral therapy in the whole cohort before recovering at week 2 and thereafter as illustrated in fig 3.Figures 4 and 5 illustrate the mean serum magnesium and the mean serum potassium by visit respectively in participants who also presented with severe hypophosphatemia.The trend in these electrolytes is also characterized by a fall in levels by week 1 and then a steady rise on subsequent weeks with a common fall by week 4.The initial correlation in these trends could suggest part of a broad range of interrelated metabolic derangements that might occur in refeeding syndrome. The gradual recovery of appetite by week on antiretroviral therapy did not seem to correspond with energy intake in the first 1week (figures 6 and 7) and might be suggestive of other possible mechanisms for the drop in the phosphate concentrations early in the course of antiretroviral therapy. Some individual participants had dramatic decreases after 1-4 weeks on antiretroviral therapy (~1.4-1.7 down to ~0.5-0.6) in 6 individuals. Some subjects had sustained hypophosphatemia despite

supplementation. Male gender was found to be the only significant risk factor; odds ratio4.2, at 95% confidence interval. It is not clear to us yet why this outcome. Could it be related to higher consumptions of meals in this gender proportionate to higher energy demands for the type of activities men are likely to perform?

6.3 Surviving participants who presented with severe hypophosphatemia

Out of the 120 participants who lived up to the end of the study, 13 were reported to have met the criteria for refeeding syndrome and survived after IV and oral phosphorous supplementation. Twenty six subjects who had mild hypophosphatemia survived on oral phosphorous supplementation, World Food Programme ration and nutritional counseling.

The results of a 35year old male participant who developed signs and symptoms of refeeding syndrome following severe hypophosphatemia but recovered after phosphorous supplementation represents a series of clinical and biochemical changes typical of refeeding syndrome as observed in patients with severe wasting of any cause who experience sudden increases in nutrient intake due to improved food supply or therapeutic feeding.[11]Hypophosphatemia is a hall mark of refeeding syndrome,[10]but a broad range of metabolic derangements involving potassium,magnesium,glucose,and vitamins have also been described, including weakness, muscle paralysis,cardiorespiratory failure with pulmonary and peripheral oedema,leukocyte dysfunction and long term effects such as osteomalacia.[12,22,23,24,26]It is probable that diarrhoea and volume depletion could cause some electrolyte deficiencies in our patient ,but we believe his acute hypophosphatemia,which developed in his first week on ART and after volume repletion and was followed by anasarca and pulmonary oedema could represent an exaggerated metabolic response to refeeding and/or to improved intestinal macronutrient absorption. His severe clinical presentation inspite of a relatively high CD4+ count at ART initiation suggests that malnutrition might have placed him at particular risk for refeeding syndrome.

Hypophosphatemia has been associated in one retrospective report with use of non nucleoside reverse transcriptase inhibitor drugs[65](such as efavirenz)and with use of tenofovir[66](which our patient did not receive).These associations are however inconsistent and the reports have not assessed nutritional status(e.g.,BMI),so some cases of hypophosphatemia may have been unrecognized RS.Other causes such as alcohol abuse,paraneoplastic syndrome and renal tubular

reabsorptive dysfunction such as Fanconi's Syndrome were highly unlikely.Infact such co morbid conditions were in the exclusion criteria for the study.

These observations were prompted by reports of high mortality in the first 90days of ART in Zambia and low income countries.[19]They suggest that refeeding syndrome may be among the risk factors for early mortality. Results of this study are comparable to baseline data obtained by Drs Paul Kelly and Beatrice Amadi from the Department of Paediatrics at the University Teaching Hospital, looking at pretreatment values for phosphate, calcium and magnesium. This data revealed that 11 out of 18 (61%) pretreatment children had hypophosphatemia (reference range was <1.45 mmol of phosphate).This is a further demonstration that hypophosphatemia may be an important cause of mortality in the Zambian population. In some settings refeeding syndrome has been prevented by gradual nutritional restoration with intake of calories, salt and fluids increasing gradually over a period of days or weeks.[21]In this study though, hypophosphatemia does not seem to have been induced by increases in dietary energy or carbohydrate intake following antiretroviral therapy initiation. How fast to reefed should not necessarily be guided by the level of appetite. However, caution has to be exercised in how fast to reefed patients who have been chronically starved to avoid complications due to volume overload and electrolyte shifts. These would be as a result of total body as well as organ atrophy such as loss of cardiac mass and chamber size, atrophy of the pancreas and the gastrointestinal tract. In the first few weeks of feeding and/or ART, at risk patients (particularly those with wasting) should not be aggressively fed substantial quantities of meals and fluids to avoid complications. Further studies would be required to determine whether these and other measures such as phosphate supplementation would reduce morbidity and mortality in wasted HIV patients initiating antiretroviral therapy.

Chapter 7

7.0 Conclusions

Hypophosphatemia occurs early among some Zambian HIV patients initiating antiretroviral therapy and exhibiting major risk factors for early mortality may contribute to high early mortality.

In this study, 11% of the participants met the criterion for refeeding syndrome and the overall crude incidence of hypophosphatemia was 30%.

There was no significant association between mortality and severe hypophosphatemia, which outcome could have been due to phosphorous supplementation in those subjects who presented with critically low phosphorous levels.

Refeeding syndrome can be prevented. Close monitoring for electrolytes and careful restoration of circulatory volume and caloric delivery improves survival in the subjects at risk.

Nutritional causes of early antiretroviral therapy (ART) mortality should be examined, studied and be considered in comprehensive HIV care and treatment.

7.1 Recommendations

1. Health education about refeeding syndrome, to the caregiver and the patients at risk is necessary to prevent its occurrence (Caregiver's guidelines, Appendix 2).

2. There is need for clinicians to closely monitor electrolytes and correct any abnormalities before initiating nutritional support, especially over the first month of treatment.

3. Studies are needed to

- Further explore the causes of hypophosphatemia
- To determine whether micronutrient supplementation will reduce morbidity and mortality in wasted HIV patients initiating antiretroviral therapy. In this case a randomized controlled trial of phosphate, potassium and magnesium supplements.

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APPENDICES

THE UNIVERSITY OF ZAMBIA

RESEARCH ETHICS COMMITTEE

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Ridgeway Campus
P.O. Box 50110
Lusaka, Zambia

5 July, 2007
Ref.: 013-05-07

Dr Christopher Nyirenda, BScHB, MBChB
Department of Internal Medicine
University Teaching Hospital
P/Bag RW1X
LUSAKA

Dear Dr Nyirenda,

RE: RESEARCH PROPOSAL ENTITLED. **"HYPOPHOSPHATAEMIA IN REFEEDING SYNDROME AS A CAUSE OF EARLY MORTALITY IN PATIENTS INITIATING ANTIRETROVIRAL THERAPY IN ZAMBIA"**

The above-mentioned research proposal was presented to the Research Ethics Committee meeting on 6 June, 2007, where changes were recommended. We would like to acknowledge receipt of the corrected version with clarifications. The proposal has now been approved. Congratulations!

CONDITIONS:

- This approval is based strictly on your submitted proposal. Should there be need for you to modify or change the study design or methodology, you will need to seek clearance from the Research Ethics Committee.
- If you have need for further clarification please consult this office. Please note that it is mandatory that you submit a detailed progress report of your study to this Committee every six months and a final copy of your report at the end of the study.
- Any serious adverse events must be reported at once to this Committee.

Yours sincerely,

Prof. J. T. Karashani, MB, ChB, PhD
CHAIRMAN

Date of approval: **5 July, 2007** Date of expiry: **4 July, 2008**

CAREGIVER GUIDELINES

Signs and symptoms that may suggest refeeding syndrome

- Extreme weakness
- Shortages of breath (difficulty breathing), especially when walking or lying flat in bed
    - Needing to have the head and chest propped up on pillows when lying down, in order to breath well
- Decreased urine production
- New swelling of any part of the body, such as the feet and ankles, or the back if (if lying down)

Recommendations for people whose blood phosphorus levels are found to be low, and to whom WFP food ration is provided.

- Reduced dietary intakes of Nshima if you are eating large quantities
    - Replace Nshima as much as possible with HEPS or Soya, Cooking Oil, and Meats
    - Boil the HEPS to make a porridge, and eat the porridge
    - Add cooking oil to any of your foods

- Increased dietary intake of foods that have high phosphorus content:

    - Pumpkin Seeds (Nyangu/Impupu)
    - Other kinds of Seeds
    - Nuts, such as Groundnuts and Almonds
    - Beans, such as Soya Beans, Red Beans, Kabulangeti Beans, Solwezi Beans, and White Beans
    - Dairy products, such as Milk, Yogurt, Cheese, and Ice Cream
    - Eggs

- Increase dietary intakes of fruits and vegetables, for general health benefits
- Return to the clinic for repeat measurement of the abnormal values about 1 week later.

February, 2007

CONSENT FORM **PID #**_____

TITLE OF RESEARCH:	HYPOPHOSPHATEMIA IN REFEEDING SYNDROME AS A CAUSE OF EARLY MORTALITY IN HIV/AIDS PATIENTS INITIATING ANTIRETROVIRAL THERAPY IN ZAMBIA
INVESTIGATOR:	CHRISTOPHER NYIRENDA (BSCHB, MBCHB)
SUPERVISORS:	Prof DOUGLAS HEIMBURGER, DR. PAUL KELLY (MD, FRCP)
	DR. ISAAC ZULU (MBCHB, MPH)
SPONSORS :	NIH AND UAB CENTRE FOR AIDS RESEARCH, IN THE USA

INTRODUCTION:

You are being asked to participate in a research study to determine if a condition called refeeding syndrome occurs during HIV/AIDS treatment. Refeeding syndrome is the name for a number of dangerous changes that can happen when people, who have not been able to eat very well, are once again able to eat better. During your HIV/AIDS treatment program we hope your appetite will improve and that you will be able to digest food better. Some people, however, become more seriously ill or may even die during the treatment.

This is a consent form. It gives you information about this study. We want you to know the procedures, purpose and what is expected of you if you decide to join. If you decide to take part In this study after all the explanation about it, we will ask you to sign this consent form or make a thumbprint in front of a witness. Please note that participation in this study is entirely voluntary.

PURPOSE OF THE STUDY

The purpose of the study is to determine if changes that happen when you are able to eat better maybe a cause for becoming more seriously ill. This is a pilot study. That means this research has not been done before. New information from this study may be used as the basis for future research into the cause.

STUDY PROCEDURES

If you agree to participate in this research study some extra procedures will be added to the first 12 weeks of your treatment. You will make an extra visit to the clinic one week after the treatment is started. At this 1 week visit and at the routine 2- week, 4- week, 8- week and 12-week visits, about 1 teaspoon of blood will be drawn. At each visit you will be asked extra medical questions and will be given a more detailed physical examination that looks for signs of refeeding syndrome.

Patients Initials/Thumbprint _____

February, 2007

PID #:_____

RISKS AND DISCOMFORTS
When study blood samples are taken, there is a small risk of pain, bruising or infection at the blood drawing site.

BENEFITS
There may be no benefit to you for participating in this study. Because your progress will be closely monitored for refeeding syndrome, precautions can be taken as soon as dangerous changes are seen. This could reduce risk to you during your HIV/AIDS treatment. It is also possible that knowledge gained during this research could save the lives of other patients' receiving HIV/AIDS treatment.

ALTERNATIVES
There are no alternative treatments available other than receiving your HIV/AIDS treatment without the additional monitoring offered by this study.

Confidentiality

The information gathered during this study will be kept confidential to the extent permitted by law. However, your doctor, clinic staff, and study personnel will be able to inspect your medical records and have access to confidential information that identifies you by name. Information that could identify you by name may also be shared with the University of Zambia Research Ethics Committee. Before information about you is analyzed for research proposes, your name will be removed. It will be replaced with a code number so that your name cannot be identified, and it will be stored in a secure locked computer. Your name will not be used if any of information about you is published in international journals. Your name will not be shared with the Zambian Government if it uses information form the study to improve the treatment of persons with HIV/AIDS.

WITHDRAWAL FROM THE STUDY
You are free to withdraw your consent and to discontinue participation in this project at any time without prejudice against further care that you may receive at this institution.

Your participation in the study may be stopped at any time by the study doctor or the sponsor without your consent. This might be for reasons such as:

- The sponsor or investigator stops the research.

Patients Initials/Thumbprint: _____

PID # _____

SIGNIFICANT NEW FINDINGS

Any significant new findings that develop during the course of the study that may affect your willingness to continue in the research will be given to you by the clinic staff providing your treatment.

COST OF PARTICIPATION.

There will be no cost to you from participation in the research. All study related examinations and laboratory tests will be provided at no cost during the 12 weeks study period.However,you will be reimbursed for your transportation cost related to the additional 1-week visit that is made for the research study.

PAYMENT FOR RESEARCH-RELATED INJURIES

It is unlikely that you will be injured because of taking part in this study. If you are injured because of participating in this study, the clinic will give you immediate care for the injuries. This care will be to the standard available in the Government Health Institutions of Zambia. The cost of this treatment will not be charged to you.

PERSONS TO CONTACT FOR QUESTIONS OR PROBLEMS

If you have any questions about the research or a research related injury, contact Dr. Christopher Nyirenda. He can be reached at the University Teaching Hospital, Department of Medicine, P/Bag RW IX, Lusaka. The telephone number is 0152269 or mobile phone 097808749. he can also be contacted through the clinical officer or nurse of the clinic in which you are receiving your treatment. If you have questions about your rights as a research participant, you may contact, the Secretary, Research Ethics Committee at the University of Zambia. Her telephone number is 01256067.

LEGAL RIGHTS

You are not waiving any of your legal rights by signing this consent form.

Patients Initials/Thumbprint: _____

February, 2007

PID # _____

NUTRITION-RELATED CLINICAL REVIEW

Patient ID ☐☐☐ - ☐☐☐ - ☐☐☐☐☐ - ☐ (District - Facility - Serial no.)

Date ☐☐/☐☐/☐☐ (Day/Month/Year)

Visit Code ☐☐

REVIEW OF SYSTEMS

Dyspnea ◯Yes ◯No

Orthopnea ◯Yes ◯No

Diarrhea ◯Yes ◯No

Appetite ◯None ◯Little ◯Normal ◯Hungry ◯Very hungry

Decreased urine output ◯Yes ◯No

Cough ◯Yes ◯No

Paroxysmal nocturnal dypsnea ◯Yes ◯No

Vomiting ◯Yes ◯No

Gather from Initial History & Physical form at first visit: Sex: ◯Female ◯ Male

BMI ☐☐.☐ CD4 ☐☐☐

Date of birth: ☐☐/☐☐/☐☐☐☐ (Day/Month/Year)

VITALS

Height (cm) 1ST VISIT ONLY ☐☐☐ Weight (kg) ☐☐☐.☐ Wt last visit ☐☐☐.☐

BP ☐☐☐/☐☐☐ Temp. C ☐☐.☐ Heart rate/min ☐☐☐ Resp rate ☐☐

EXAMINATION

Skin dryness ◯Yes ◯No

Skin flaking/cracking ◯Yes ◯No

Neck: hepatojugular reflux ◯Yes ◯No

Abdomen: ascites ◯Yes ◯No

Corkscrew hairs on arms or legs ◯Yes ◯No

Dryness/cracking of lips or angular somatitis ◯Yes ◯No

Atrophic lingual papillae ◯Yes ◯No

Petechiae ◯Yes ◯No *If yes:* ☐Diffuse ☐Perifollicular

Lungs: rales ◯Yes ◯No

If yes, check all that apply: ☐Lower third ☐Middle third ☐Upper third

Lungs: dullness to percussion ◯Yes ◯No

If yes, check all that apply: ☐Lower third ☐Middle third ☐Upper third

Heart ◯Normal ◯Abnormal If abnormal, specify: _____

Pitting Edema ◯Yes ◯No

If yes, location: ☐Pedal ☐Pretibial ☐Thigh ☐Abdominal ☐Anasarca

LABORATORY RESULTS

Specimen collection date: ☐☐/☐☐/☐☐ (Day/Month/Year)

Phosphorus ☐.☐☐

Potassium ☐☐

Magnesium ☐☐☐

Glucose ☐☐☐☐

Albumin ☐☐.☐☐

Information, education, & communication provided on _____

Date results entered: ☐☐/☐☐/☐☐ (Day/Month/Year)

Staff signature _____

CURRENT MEDICATIONS

NRTIs

☐Zidovudine (AZT)
☐Stavudine (D4T)
☐Lamivudine (3TC)
☐Abacavir (ABC)
☐Tenofovir (TDF)
☐Didanosine (ddI)
☐Emtricitabine (FTC)

NNRTIs

◯Nevirapine (NVP)
◯Efavirenz (EFV)

PIs

◯Lopinavir/ritonavir (LPV/r)
◯Indinavir (IDV)
◯Nelfinavir (NFV)

Non-ARVs

☐Septrin
☐Fluconazole
☐Anti-malarials
☐TB medication _____
☐Traditional medicines and herbs
☐Other _____
☐Other _____
☐Other _____
☐Other _____

If on ARVs, clinical judgement of adherence:

☐ Adherent, continue
☐ Poorly adherent, needs counseling
☐ Poorly adherent stop treatment

Comments

Return in: ◯1 wk ◯2 wks ◯1 mo ◯2 mos Date of next visit: ☐☐/☐☐/☐☐ (Day/Month/Year)

Staff Signature _____