The Aetiology of Hypophosphatemia in Children Recovering from Kwashiorkor

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DECLARATION:

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Date: **15th August 2015**

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Abstract

Background

The aetiology of hypophosphatemia in children recovering from kwashiorkor is poorly understood. Current theory of the pathophysiology of hypophosphatemia due to refeeding syndrome in adults describes intracellular phosphate trapping, mediated by a metabolic shift from lipid-based catabolism to carbohydrate metabolism. Treatment of hypophosphatemia associated with severe acute malnutrition (SAM) presents a challenge to the control of serum phosphate levels.

Hypothesis

This study explores the hypothesis that hypophosphatemia is caused by impaired renal tubular phosphate reabsorption in children recovering from oedematous malnutrition.

Study Design

A prospective pilot study was based at the Tygerberg Children's Hospital Gastroenterology Unit, a tertiary referral unit in Cape Town, South Africa. Written informed consent was obtained from the legal guardian and ethical approval was obtained from Stellenbosch University's Human Research Ethics Committee.

Ten children between the ages 6-59 months admitted to the Gastroenterology Unit and classified as having kwashiorkor or marasmic kwashiorkor were included. Children were excluded if they were known with pre-existing renal or endocrine disease involving phosphate, magnesium or calcium metabolism. Additionally patients transferred from referring hospitals who had already initiated a refeeding and treatment regimen, any patient with a haemoglobin of ≤6g/dl on admission or any child known to be HIV positive were also excluded from the study.

Methods

Biochemical and anthropometric variables were monitored during the course of treatment and refeeding. Management of the patients was standardised as per the Tygerberg Children's Hospitals protocol for refeeding in severe acute malnutrition, which is in accordance with the WHO 10 step protocol. Treatment of hypophosphatemia included oral administration of 75-100 mg/kg/day of 0.8g Na_2HPO4 + 0.2g KH_2PO4 + 10 ml H_2O providing a solution of 100 mg PO_4/ml (7.8 mmol of phosphate per 10 mls solution). If patients were unable to tolerate oral feeds intravenous KPO_4 was administered at 1 mmol/kg/day of phosphate.

Results

On admission 70% of children were assessed as severely underweight for age (median WAZ: -2.77, IQR: -5.07; -1.10) and 60% as stunted (median HAZ: -2.52, IQR: -5.23; -1.14) based on the WHO Z-score classification. All children were oedematous. Serum phosphate levels fell within the reference range for age (median: 1.3 mmol/l, IQR: 0.9; 1.4) and decreased to a nadir on day 7 (median: 1.15 mmol/l, IQR: 0.82; 1.5) despite routine phosphate supplementation. Low serum ionised calcium concentration at baseline (median: 1.8, IQR: 1.6; 1.88) reached a nadir at day 3 of treatment (median: 1.71, IQR: 1.53; 1.98), associated with a peak in PTH secretion on day 7 (median: 11.35, IQR: 9.1; 13.6), and an increased urinary phosphate (median: 3.85 IQR: 0.9; 37.85) on day 14. Renal threshold for phosphate reabsorption remained low throughout the course of refeeding and none of the patients developed biochemical evidence of refeeding syndrome.

A significant positive correlation between ionised calcium and phosphate (p=0.004) was determined when calculating the Spearman Rank co-efficient; indicating that low serum ionised calcium concentration contributed to hypophosphatemia. This finding was confirmed by appropriate physiologic response to ionised serum hypocalcaemia by the parathyroid hormone axis. Although a positive correlation between urinary and serum phosphate; and a negative correlation between urinary phosphate and serum calcium were observed as expected; these were not found to be statistically significant, possibly due to the limited sample size and missing variables. However, a significant negative correlation (p=0.0012) was demonstrated between ionised calcium levels and PCT; this finding is previously undescribed in the setting of SAM.

Conclusion

This study demonstrated that in children recovering from SAM, low serum ionised calcium levels are a potential driver for phosphaturia in the face of hypophosphatemia. This is mediated via an appropriate PTH response. Further investigation of calcium supplementation and the contribution of vitamin D to phosphate homeostasis in SAM should be undertaken.

Opsomming

Agtergrond

Die etiologie van hipofosfatemie in kinders met kwasjiorkor is onbekend. Die huidige teorie vir die patofisiologie van hipofosfatemie in volwassenes met hervoedingsindroom is dat fosfaat intrasellulêr vasgevang word weens die verskuiwing van ‘n oorheersend lipied afhanklike energie metabolisme na een wat koolhidraat oorheersend is. Die behandeling van hipofosfatemie en in die wangevoed kind is uitdagend.

Hipothese

Die studie ondersoek die etiologie van hipofosfatemie in kinders wat van kwasjiorkor herstel met spesifieke verwysing na kalsiumhomeostase, tendense in urinêre fosfaat verliese en merkers van voedings rehabilitasie.

Studie-Ontwerp

Die studie het plaas gevind in die gastroënterologie eenheid van Tygerberg Kinder Hospitaal, 'n tersiêre verwysing eenheid in Kaapstad, Suid-Afrika.

Tien kinders tussen die ouderdomme van 6-59 maande met kwasjiorkor of marasmiese kwasjiorkor is in die gastroënterologie eenheid opgeneem. Toestemming is van die wettige ouer verkry. Etiese goedkeuring vir die studie is verkry van die Navorsingsetiekkomitee van die Universiteit van Stellenbosch. Kinders bekend met bestaande nier of endokriene siektes wat fosfaat, magnesium of kalsium metabolism kon beinvloed was uitgesluit. Pasiënte wat reeds in ander hospitale behandel is, of enige pasiënt met 'n hemoglobien van ≤6g / dl met toelating; of enige kind bekend met MIV infeksie is nie ingesluit nie.

Methode

Biochemiese en antropometriese veranderlikes is tydens toelating aangeteken. Die kinders is behandel volgens die Tygerberg Kinder Hospitaal protokol vir die behandeling van ernstige wanvoeding, hierdie protokol stem ooreen met die WGO riglyne. Behandeling van hipofosfatemie sluit die orale toediening van 75-100 mg / kg / dag van 0.8g Na_2HPO_4 + 0.2g KH_2PO_4 + 10 ml H_2O in deur ‘n oplossing van 100mg PO_4 / ml (7.8 mmol fosfaat per 10ml oplossing). Indien pasiënte nie die orale oplossing kon inneem nie is binneaarse KPO_4 (1mmol / kg / dag) toegedien.

Resultate

Met opname was 70% van die kinders ernstig ondergewig (mediaan WAZ: -2.77, IKR: -5.07; -1.10) en 60% as groeivertraag (mediaan HAZ: -2.52, IKR: -5.23; -1.14) gebaseer op die WGO klassifikasie. Serum fosfaat vlakke het binne die normale verwysing waardes vir ouderdom geval (mediaan: 1.3 mmol/l, IKR: 0.9; 1.4 mmol / l) en het verminder tot ‘n laagte punt op dag 7 (mediaan: 1.15mmol / l, IKR: 0.82; 1.5) ten spyte van roetine fosfaat supplementasie. Die serum geïoniseer kalsiumkonsentrasie was laag met toelating (mediaan: 1.8, IKR: 1.6; 1.88) en het verder gedaal tot 'n laagtepunt op dag 3 van behandeling (mediaan: 1.71, IKR: 1.53; 1.98). Na dag 3, het die PTH konsentrasie gestyg tot ‘n hoogtepunt op dag 7 (mediaan: 11.35, IKR: 9.1; 13.6). Urinêre fosfaat het ‘n hoogtepunt bereik op dag 14 (mediaan: 3.85, IKR: 0.9; 37.85). Nie een van die pasiënte het enige biochemieise tekens van hervoedingsindroom ontwikkel nie.

Die Spearman Rang koëffisiënt het aangedui dat daar 'n hoogs beduidende positiewe korrelasie tussen geïoniseerde kalsium en fosfaat (p= 0.004) is. Hierdie bevinding bevestig dat lae serum geïoniseerde kalsiumkonsentrasie die behandeling van hipofosfatemie mag beinvloed. Hierdie bevinding is bevestig deur die toepaslike fisiologiese reaksie op die lae geïoniseerd kalsium deur paratiroïed hormoon. Daar was nie 'n beduidende positiewe korrelasie tussen urinêre en serum fosfaat, of 'n beduidende negatiewe korrelasie tussen urinêre fosfaat en serum kalsium nie. Die belang van hierdie bevindinge is onseker aangesien die datastel nie volledig was nie. 'n Hoogs beduidende negatiewe korrelasie (p= 0.0012) is gevind tussen die geïoniseerd kalsium vlak en PKT, hierdie bevinding is nie voorheen beskryf in kinders met wanvoeding nie.

Gevolgtrekking

Fosfaaturie in die teenwoordigheid van hipofosfatemie tydens die behandeling van wangevoede kinders is waarskynlik gedryf deur 'n lae geïoniseerde serum kalsium vlak met ‘n toepaslike fisiologiese PTH reaksie. Kalsium aanvullings en die bydrae van vitamiene D in fosfaat homeostase moet verder ondersoek word.

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Abbreviations and Terminology

ATP:	Adenosine triphosphate
BMI:	Body mass index
CRP:	C-reactive protein
DNA:	Deoxyribonucleic Acid
FGF23:	Fibroblast Growth Factor 23
HAZ:	Height Age Z-score
ICU:	Intensive Care Unit
IQR:	Interquartile range
Na:	Sodium
NAFLD:	Non-alcoholic Fatty Liver Disease
PCT:	Procalcitonin
PEM:	Protein Energy Malnutrition
Pi:	Inorganic Phosphate
PTH:	Parathyroid hormone
SAM:	Severe acute malnutrition
SD:	Standard deviation
WAZ:	Weight Age Z-score
WFH:	Weight for height
WHO:	World Health Organisation

1. Introduction

The homeostatic role of phosphate has been well described; it is an integral part of energy metabolism, forms part of the basic structure of DNA, glycoproteins and second messengers, and is required for bone mineralization. As such, it is vital to normal function on a molecular, cellular and tissue level [1]. When the balance between phosphate absorption, utilization and excretion is disturbed, the resulting hypophosphatemia may lead to severe complications such as respiratory failure and rhabdomyolysis [1].

Hypophosphatemia (serum phosphate of ≤ 1 mmol/l) is commonly associated with malnutrition in both children and adults. Studies report a 20% prevalence of hypophosphatemia in critically ill adults and 10.4% prevalence in a subpopulation of malnourished adults [2, 3]. Similarly, in children admitted to a Bangladeshi paediatric unit with acute malnutrition and sepsis, the prevalence of hypophosphatemia in moderate to severe acute malnutrition was 25% [4].

As described by Kimutai, children with malnutrition and severe hypophosphatemia have an increased risk of mortality. Kimutai found that mortality was higher in children with a low serum phosphate nadir during refeeding [5]. Manary described a similar association reporting a 63% morality rate in children with serum phosphate concentrations of <0.32mmol/l during refeeding [6].

While studies investigating hypophosphatemia in the setting of a paediatric intensive care unit have failed to demonstrate an increased risk of mortality, malnutrition has been demonstrated to be a risk factor for development of hypophosphatemia in this setting [7]. In a study describing the prevalence and risk factors for development of hypophosphatemia in critically ill children, Santana noted that 39.1% of children who developed hypophosphatemia during admission to ICU were malnourished. In addition, this subpopulation demonstrated significantly lower serum phosphate levels during admission as compared to well-nourished controls (p=0.01) [8].

A study from the Tygerberg Children's Hospital gastroenterology unit revealed a declining serum phosphate level until day 5 post admission despite aggressive supplementation [9]. Yoshimatsu confirmed similar results, describing a nadir in serum phosphate concentration between days 2 and 4 post-admission, in 21 of 48 patients with severe acute malnutrition [4]. Despite congruence in persistence of hypophosphatemia on supplementation, neither study identified any possible factors attributable to this pattern.

While the aetiology of hypophosphatemia in the setting of acute malnutrition is poorly understood, the complex interplay between vitamin D and calcium homeostasis with

additional catabolic shifts similar to those seen in adult refeeding syndrome may all contribute. Hypophosphatemia has been well described in children with early chronic renal disease; risk factors associated with deranged bone homeostasis and resultant hypocalcaemia and hypophosphatemia include disturbances of the endocrine gonadal and growth hormone axes, calcium and vitamin D metabolism, metabolic acidosis and malnutrition [10]. Additionally phosphatonins (specifically fibroblast-growth factor 23) have been associated with increased urinary phosphate excretion and inhibition of renal 1-α hydroxylation of vitamin D [10, 11]. These factors may also contribute to hypophosphatemia in the setting of severe acute malnutrition.

Renal phosphate losses in severe acute malnutrition have previously been attributed to declining glomerular filtration and tubulopathy with subsequent overflow aminoaciduria and acidosis. Furthermore, the contribution of phosphatonins, as well as selective expression of sodium-phosphate type IIa receptors in the renal tubules in animal models, may give a potential clue to the mechanism of renal phosphate handling in the setting of malnutrition [12, 13]. In addition to renal phosphate wasting, refeeding induced intracellular phosphate trapping with a shift from lipid based to carbohydrate-based metabolism may act to negatively impact serum phosphate levels [1].

Mechanistic and pathophysiologic explanation of the aetiology of hypophosphatemia in children recovering from oedematous malnutrition is lacking. Clarification of the pathophysiology may shed light on more effective means of management, hence countering the associated risk of mortality. Based on known physiologic processes, calcium homeostasis in reference to phosphate excretion, as well as the contribution of infection should be considered as a basis for possible explanation.

We conducted a pilot study in children with oedematous malnutrition to explore the contribution of urinary phosphate losses to hypophosphatemia.

2. Literature Review

Malnutrition is defined as a pathological state brought about as a consequence of inadequate or inappropriate nutrition. In severe acute malnutrition this relates both to the quantity and quality of ingested calories, and may manifest clinically as marasmus, kwashiorkor or marasmic kwashiorkor [14].

There are various methods for the classification of malnutrition these include clinical and anthropometric. The prevalence of malnutrition in any given population is defined by these classifications and criteria [15].

2.1. Classifications of malnutrition in children

The Gomez classification describes the weight of a child as a percentage compared to an "average" child within a population (this is ascribed as the 50th percentile on standardised growth charts). These values are further sub-divided to provide an objective assessment; with mild malnutrition considered to be 75-89%, moderate malnutrition between 60-74%, and severe malnutrition at less than 60% of the expected weight for age. This classification is largely used as a screening tool within a population [16].

The Wellcome classification is a modification of the Gomez classification and includes the presence or absence of oedema to further delineate severe acute malnutrition. Kwashiorkor is classified as a weight for age of 60-80% with oedema, marasmic kwashiorkor as less than 60% with oedema, and in the absence of oedema marasmus is diagnosed. This classification is commonly used within the context of clinical practice [16].

The Waterlow classification describes the effect of chronic malnutrition on body proportions, specifically height. Weight for height is used to describe wasting and height for age is used to describe stunting. This classification grades chronic malnutrition as mild, moderate or severe by comparing the percentage of a patient's weight to the weight of an average child of the same height within a given population [17].

The WHO classification of severe acute malnutrition utilises a weight for height index (WFH) expressed as a standard deviation (SD) score to describe the probability of malnutrition. The threshold for malnutrition is defined as a SD score below -2, with a WFH between -3 and -2 defined as moderate and less than -3 defined as severe malnutrition. Recent studies have correlated Z-scores with body mass index (BMI), determining that a value of 17 kg/m2 approximates the -2 SD [18].

The latest revision of the WHO malnutrition classification stratifies mortality risk by anthropometric indices in children aged 6 to 60 months. Following epidemiologic data extrapolated from the 2005 WHO multicentre growth reference study, increased risk of mortality was demonstrated in children with a weight for height (WFH) below the -3SD and a mid-upper arm circumference (MUAC) of <115mm. Bilateral pedal oedema was identified as an independent indicator of severe acute malnutrition. Similar prevalence of severe acute malnutrition was demonstrated on assessment of both WFH (1.48%) and MUAC (1.49%), this classification is translatable on a large-scale programmatic level, enabling appropriate identification, treatment and referral of children with severe acute malnutrition [19].

Additional haematological and biochemical indicators of severe acute malnutrition include; hypoalbuminemia, lymphopenia, and electrolytes abnormalities such as hypophosphatemia, hypokalaemia, hypocalcaemia and hypomagnesaemia. These act as adjunctive markers to both clinical and anthropometric parameters in the assessment of severe acute malnutrition.

2.2. Epidemiology of malnutrition

A large cross-sectional study conducted by the WHO tracked global trends in the prevalence of childhood stunting between 1980 and 2000; this study considered stunting as a surrogate marker for child health. Despite an observed decrease in African prevalence from 40.5% in 1980 to 35.2% in 2000, West Africa showed no improvement at 34% while East Africa's prevalence worsened from 12.9 million to 22 million stunted pre-schoolers. The cumulative improvement in percentage points per year was 0.26%, and lagged far behind other continents [20].

Recent South African data ranks malnutrition as a leading cause of childhood mortality. In 2013 severe acute malnutrition was attributed as the cause of mortality in 2.7% of infants younger than one and 4.6% of children between 1-14 years of age, indicating a significant disease burden [21]. However, with mortality often attributed to an infectious aetiology (diarrhoeal disease, pneumonia, etc.) there is under reporting of severe acute malnutrition as a cause of mortality [22].

A pending report by Dr L Swanson describes the patient population at Tygerberg Children's Hospital as having a mortality rate of 28.9% in the setting of severe acute malnutrition and hypophosphatemia. This clearly illustrates mortality associated with severe acute malnutrition and hypophosphatemia within the context our patient population.

2.3. Aetiology and outcome of malnutrition

The aetiology of malnutrition in children is multifactorial, involving inadequate and/or inappropriate caloric intake coupled with intercurrent infection. This observation, made by

Waterlow described the peak prevalence of wasting at two years of age, associated with an increased incidence of diarrhoeal disease. Additionally, children have higher energy requirements per unit body mass and limited energy reserves. Poverty and food insecurity further perpetuate malnutrition and place a vulnerable child at increased risk for poor outcome [23].

Longitudinal studies investigating the sequelae of malnutrition in children have demonstrated multi-system morbidity. Malnutrition in infancy has been found to be associated not only with poor growth, healing and immune function but also poor psychosocial development. Furthermore, malnutrition has been associated with an increased risk for development of cardiovascular disease, type 2 diabetes mellitus and hypertension in later life [18].

2.4. Hypophosphatemia and phosphate physiology

Hypophosphatemia is well described in children with severe acute malnutrition. At Tygerberg Children's Hospital, worsening serum hypophosphatemia has regularly been observed in the face of phosphaturia, despite phosphate supplementation. Falling serum phosphate levels in children with severe acute malnutrition have largely been attributed to electrolyte redistribution and may be accompanied by other electrolyte abnormalities found in refeeding syndrome [24]. However, whether or not these children have a normal capacity for renal phosphate reabsorption has yet to be investigated. Reviewing existing literature on the topic did not yield any salient information.

Phosphate is pivotal to the maintenance of homeostasis; it is integral to cellular function as a component of energy production, cell membrane structure as well as comprising constituents of enzymes and nucleic acids. Under physiologic conditions phosphate metabolism is tightly regulated [1]. The recommended daily allowance of phosphate for a healthy child is 800mg/m^2; with 85-90% of ingested phosphate being stored in bone in conjunction with calcium as hydroxyapatite, the remaining 10-15% form organic phosphate compounds and inorganic phosphate with 14% of these located in the intracellular and 0.03% in the extracellular compartments [25]. Approximately 10% of plasma phosphate is protein bound and hence unavailable for glomerular filtration. In the context of severe acute malnutrition with hypoalbuminemia as a cardinal feature, this may contribute to increased renal phosphate loss [26].

Dietary phosphate is readily obtainable in food sources rich in protein as well as cereals and nuts [27]. Phosphate is absorbed primarily in the jejunum and duodenum by transcellular and paracellular mechanisms [28,12]. Transcellular absorption occurs via intestinal type IIb sodium-phosphate (Na/Pi) cotransporters, whereas paracellular absorption occurs by

concentration gradient dependent diffusion. Furthermore, under the action of Na/Pi cotransporter type III, phosphate is transported to tissue and bone [12].

Recent studies have identified additional factors that play a role in phosphate homeostasis; these have collectively been called phosphatonins [28]. Phosphatonins' mechanism of action includes the down regulation of proximal renal tubular type IIa Na/Pi cotransporter expression, and 1-α hydroxylation of vitamin D, with resultant renal phosphate loss [10,12,29]. Phosphaturia in the presence of a dietary phosphate load was found to occur in thyro-parathyroidectomised rats, indicating that this mechanism is parathyroid hormone independent. Renal phosphate excretion did not affect serum phosphate levels [28]. In the setting of long standing hypophosphatemia, 1,25-dihydroxy-cholecalciferol and parathyroid hormone secretion may thus make a more significant direct contribution to the maintenance of phosphate homeostasis [12].

Renal proximal tubular reabsorption of inorganic phosphate by Na/Pi cotransporters plays a vital role in maintenance of phosphate homeostasis. Type I Na/Pi transporters act as non-specific anion channels. Type II Na/Pi cotransporters are responsible for the majority of phosphate transportation and are influenced by a variety of hormones; including vitamin D, parathyroid hormone, insulin like growth factor and growth hormone. Animal models have demonstrated a higher expression of type IIa Na/Pi cotransporters in the proximal renal tubules of juveniles. The basis for this high density of type IIa Na/Pi expression is due to low intracellular phosphate concentrations with high levels of growth hormone and insulin like growth factor; suggesting this may also be the case in children [13]. Reabsorption of phosphate is improved by an alkaline urinary pH; alternatively in severe acute malnutrition, reduced reabsorption of free amino acids results in aminoaciduria, which may favour phosphate excretion [30, 31].

Parathyroid hormone acts to increase renal phosphate excretion in favour of calcium reabsorption. This action is achieved via phosphorylation of renal tubular type IIa Na/Pi cotransporters, with a resultant reduction in cotransporter expression. This results in decreased phosphate reabsorption and occurs in the apical brush border membrane of the proximal renal tubule [28]. The primary role of parathyroid hormone is mobilisation of calcium. This is achieved by osteoclastic breakdown of hydroxyapatite to liberate both phosphate and calcium. PTH also increases 1,25-dihydroxy-choelcalciferol, thereby increasing intestinal calcium reabsorption [27].

Animal studies have demonstrated up-regulation and increased expression of type IIa Na/Pi cotransporters in the setting of inadequate phosphate intake. However, in a diet chronically deficient of phosphate, the expression of these cotransporters is related to protein synthesis

and not dietary phosphate levels [26]. This factor may be relevant in the setting of severe acute malnutrition.

The parathyroid hormone axis is driven by pre-existing serum hypocalcaemia, with resultant renal phosphate loss. In the setting of severe acute malnutrition, Frenk observed a decreased total calcium concentration both on admission and refeeding with a normal serum ionised calcium concentration; this finding was attributed to hypoalbuminemia [32]. Similar results were obtained in South African children, whereby Freiman observed low serum calcium concentrations during refeeding in a cohort of children with kwashiorkor; these were also attributed to hypoalbuminemia [33]. Barbosa Dias described risk factors for development of hypocalcaemia in a cohort of Brazilian children admitted to ICU, malnutrition was determined to not significantly contribute to the of development of hypocalcaemia during admission (OR = 1, 95% CI: 0.6-1.8, p=0.89) [34].

Refeeding syndrome is well described in the context of adult anorexia nervosa; this is defined as the triad of hypophosphatemia, hypomagnesaemia and hypokalaemia [35]. Refeeding syndrome occurs as a consequence of a metabolic shift from a state of starvation, wherein nutrition is predominately lipolysis driven, to a state of refeeding, wherein carbohydrate metabolism is favoured. This shift leads to intracellular phosphate trapping with resultant clinical manifestations; these include low adenosine triphosphate (ATP) levels in red blood cells causing hypoxia and haemolysis, reduced myocardial contractility and respiratory muscle paralysis. Supplemental phosphate is required to ensure adequate oxidative phosphorylation and protein synthesis [35].

Severe hypophosphatemia is a common finding in children with oedematous malnutrition. Manary described severe hypophosphatemia in 12% of a Malawian kwashiorkor cohort, with an associated increased mortality rate of 63% in severe hypophosphatemia (i.e. a phosphate less than 0.32 mmol/l, $p<0.02$) [6]. In a South African study, Freiman found that children presenting with kwashiorkor with or without acute diarrhoeal disease had lower serum phosphate levels on admission and refeeding as compared to healthy controls ($p<0.0005$). Mortality was exclusive to those with kwashiorkor, with 10 out of 60 malnourished children demising during refeeding [33].

A study performed in Tygerberg Children's Hospital gastrointestinal unit in 2000 demonstrated a 77% prevalence of worsening hypophosphatemia, despite phosphate supplementation within the first four days following admission. This study did not demonstrate any significant correlation between morbidity, mortality and hypophosphatemia [9]. These findings were consistent with a study done by Kimutai in children admitted with severe acute malnutrition between June 2005 and February 2006; this study showed an 86% prevalence of

hypophosphatemia, increasing to 90% on day one and 93% on day two of treatment. Kimutai's study reported a positive correlation between hypophosphatemia and mortality (p=0.028); with a mortality rate of 21% in patients with exaggerated drops in serum phosphate levels; however it is worth noting that the study included children with marasmus [5].

Adult studies have shown a correlation between early infection and hypophosphatemia [36,37]. Hypophosphatemia in the context of systemic inflammatory response syndrome has been attributed to the catabolic state induced by stress hormones and interleukins, and as such as been suggested as a marker of sepsis in adult patients [36, 38].

The role of phosphate in the maintenance of homeostasis and normal cellular function is complex. In the setting of severe acute malnutrition, hypophosphatemia has been shown to increase the morbidity and mortality of patients. The pathophysiology of this condition is poorly understood and the role of renal phosphate handling through endocrine, phosphatonin and inflammatory mediators should be further examined.

3. Aims

This study aims to investigate the relationship between phosphaturia and hypophosphatemia in children with oedematous malnutrition (i.e. kwashiorkor and marasmus kwashiorkor).

4. Objectives

To determine:

1. Serial serum phosphate and calcium levels in infants with kwashiorkor or marasmic kwashiorkor during admission and refeeding
2. Renal phosphate excretion on admission and weekly during the first 3 weeks of refeeding
3. Serial serum magnesium and potassium concentrations during refeeding
4. Parathyroid hormone concentrations during refeeding
5. Evidence of infection during the first 3 weeks following admission
6. Associations between anthropometric indices, hormonal concentrations, infection and serum phosphate concentration

5. Hypothesis

This study explores the hypothesis that hypophosphatemia is caused by impaired renal tubular phosphate reabsorption in children recovering from oedematous malnutrition.

6. Assumption and Limitations

The sample size of this pilot study was 10 patients. All of the patients were recruited from the Tygerberg Children's Hospital Gastroenterology Unit, a referral centre for regional hospitals in the Eastern Sub-district of the Cape Metropole. This unit only admits children who have complicated disease, thus introducing selection bias towards the extreme disease spectrum of patients with severe acute malnutrition.

Although the vitamin D status would have provided additional insights, vitamin D assays were not routinely performed in the unit at the time this study was conducted and thus constitute an additional limitation that should be addressed in future research.

7. Methods

7.1. Study population

This study was conducted over a period of 12 months, from the 1st January 2013 to the 1st January 2014.

Infants presenting with oedematous malnutrition to Tygerberg Children's Hospital Gastroenterology Unit between 6 and 59 months of age were recruited. These children were classified according to the WHO classification with anthropometry expressed as Z-scores.

7.2. Inclusion criteria

1. Age 6 - 59 months (6 months to 4 years 11 months)
2. Kwashiorkor or marasmic kwashiorkor as classified by the WHO classification
3. Informed written consent given by the legal guardian

7.3. Exclusion criteria

1. Children with known pre-existing renal disease
2. Children with known pre-existing endocrine disease affecting phosphate, calcium or magnesium metabolism
3. Children transferred from surrounding hospitals who had already initiated treatment for severe acute malnutrition
4. HIV infection: The presence of HIV associated nephropathy is a confounding factor as children may have abnormal renal function and tubulopathy. (All parents of children admitted to our ward undergo counselling for HIV testing due to the strong association with malnutrition)
5. Children with a laboratory determined haemoglobin on admission of less than 6 g/dl
6. Children whose legal guardian did not give informed consent to enrol in this study

7.4. Enrolment procedure

The legal guardian of the each child admitted with kwashiorkor or marasmic kwashiorkor was approached within 24 hours of admission to Tygerberg Children's Hospital and invited to enrol in the study. Written informed consent was obtainted from the legal guardian. Assent was not sought from the patients, as all were younger than 60 months of age.

7.5. Sample size

Ten patients were enrolled in this pilot study. As per Johanson and Brooks, a pilot sample size of 10 allows an estimate of the mean with a 95% confidence interval of 0.042 either side of the mean [39].

Due to the nature and complexity of treating children with hypophosphatemia and kwashiorkor in addition to the prohibitive cost of a larger study, a pilot study was chosen to test the hypothesis. The results from this study will be used to motivate for a larger study closely examining phosphate metabolism in this population of children

7.6. Management of kwashiorkor

Treatment followed standard treatment guidelines for the management of severe acute malnutrition in Tygerberg Children's Hospital [Appendix B]. Management of the patients was at the discretion of the attending physician.

Electrolytes were routinely measured: on admission, daily for the first week, and twice weekly thereafter.

7.7. Diagnostic Criteria for SAM in children 6-60 months of age

The table below summarises the new WHO criteria for diagnosis of severe acute malnutrition [19].

Indicator	Measure	Cut-off
Severe Wasting	WFH	< -3 SD
Severe Wasting	MUAC	< 115mm
Bilateral Oedema	Clinical	

7.8. Parameters recorded

1. Date of birth

2. Sex

3. Anthropometry during hospitalisation: (performed weekly, expressed as WHO Z-scores)
    - Weight
    - Length
    - Mid upper arm circumference
4. Clinical features during hospitalisation:
    - Oedema
    - Skin lesions
    - Liver span

5. Infections during hospitalisation:
    - Blood cultures
    - Urine cultures
    - Stool cultures
    - Gastric aspirate cultures for Mycobacterium Tuberculosis
      (Performed routinely on all children with severe malnutrition)

6. Routine biochemistry and serum electrolytes:
    - Serum phosphate, calcium and magnesium concentrations
    - Parathyroid hormone
    - CRP and PCT
    - Liver biochemistry
    - Serum Albumin
    - Serum sodium, potassium, urea and creatinine concentrations
    - Urinary phosphate and creatinine concentrations

7. Daily feeds: type and total daily volume

8. Daily fluids: type and total daily volume

9. Vitals during hospitalisation:
    - Temperature
    - Random finger prick glucose

8. Ethical considerations

8.1. Consent

Informed consent was obtained from the legal guardian prior to enrolment in the study. Treatment of the patients was in accordance with the “Tygerberg Children’s Hospital: Severe Malnutrition Refeeding Regimen”.

8.2. Benefits to the community

A better understanding of the pathophysiology of hypophosphatemia in young children recovering from oedematous malnutrition will allow for adaptation of the treatment protocol, potentially decreasing the mortality rate.

8.3. Benefits to the patients

Individual patients did not benefit directly from participation in the study.

8.4. Risks to the patients

The additional risks conferred by participation in this study were minimal.

Routine special investigations were performed as per the treatment protocol with additional blood samples not exceeding 1 ml (average weight of patients admitted with kwashiorkor in the last four years = 4.4 kg). This aliquot was in keeping with current ethical guidelines for venepuncture (maximum of 1% of total blood volume per procedure) and as such did not compromise the clinical wellbeing of the patient [40].

Blood was drawn after application of local anaesthetic to the patients’ skin (EMLA cream), thus minimizing discomfort. Parents were counselled regarding the bloodletting procedure. As per the exclusion criteria, any patient with a haemoglobin concentration of less than 6 g/dl was excluded from this study due to the risk of repeated venepuncture.

Urine specimens were collected using urine bags (no catheterization); these bags were removed promptly after the urine was passed.

8.5. Anonymity

The data was recorded directly into an electronic database; patients were each assigned a unique identifier independent of their name and hospital number. The names and hospital numbers of the patients linked to the study numbers were held in a separate database once data entry was completed. This database has been kept secure in a separate location.

9. Sample Analysis Methodology

Samples were collected and analysed at the NHLS laboratory at Tygerberg Children's Hospital. The laboratory is accredited by SANAS (South African National Accreditation System) and participates in external quality control. Analytes were measured using an ADVIA Centaur 1800, an automated bench-side chemistry assay system providing assay testing electrolyte, biochemical and nutritional parameters. Siemens Medical Solutions Diagnostics provided the reagents and calibrators with external quality control performed by Thistle.

9.1. Serum Sodium

The ADVIA Sodium (Na) method is based on an indirect potentiometric procedure using an ion selective electrode (ISE). The sodium ion selective electrode responds selectively to sodium ions according to the Nernst equation.

9.2. Serum Potassium

The ADVIA Potassium (K) method is based on an indirect potentiometric procedure using an ion selective electrode (ISE). The potassium ion selective electrode responds selectively to potassium ions according to the Nernst equation.

9.3. Serum Urea

The ADVIA Urea Nitrogen (UN) method is based on the Roch-Ramel enzymatic reaction using urease and glutamate dehydrogenase. Urea is hydrolysed in the presence of water and urease to produce ammonia and carbon dioxide. The ammonia reacts with 2-oxoglutarate in the presence of glutamate dehydrogenase and NADH. The concentration is determined using spectrophotometry.

9.4. Serum Creatinine

As described in the original procedure of Jaffe, creatinine reacts with picric acid in an alkaline medium to produce a red-coloured creatinine-picrate complex. The concentration is determined using spectrophotometry.

9.5. Serum Phosphate

The inorganic phosphorus (IP) method is based on the Daly and Ertinghausen procedure, this relies on the formation of a UV absorbing complex between phosphorus and molybdate. Inorganic phosphorus reacts with ammonium molybdate in the presence of sulphuric acid to form an unreduced phosphomolybdate complex. The concentration is determined using spectrophotometry.

9.6. Serum Calcium

The calcium (CA) method is based on the work of Gitelman. Calcium ions form a violet complex with o-cresolphthalein complexone in an alkaline medium and the concentration is then determined using spectrophotometry.

9.7. Serum Magnesium

The magnesium (MG) method is based on the modified xylidyl blue reaction. The reagent was modified to eliminate the use of organic solvents. The concentration is determined using spectrophotometry.

9.8. Serum Albumin

The albumin (ALB) method is based on the method of Doumas, Watson, and Biggs and uses bromocresol green solution (BCG) as a binding dye. The concentration is determined using spectrophotometry.

9.9. Serum Total protein

The Total Protein II (TP) method is based on the method of Weichselbaum, using a biuret reagent (cupric sulphate in an alkaline solution). Protein peptide bonds interact with the cupric ions to form a purple complex that is measured as an endpoint reaction. The concentration is determined using spectrophotometry.

Analytes measured using a Beckman SYNCHRON CX7. Beckman supplied reagents and calibrators; external quality control was performed by Bio-Rad.

9.10. Urinary Phosphate

The phosphorus concentration is measured by a timed endpoint method. Inorganic phosphorus reacts with ammonium molybdate in an acidic solution to form a coloured phosphomolybdate complex. The concentration is determined using spectrophotometry.

9.11. PTH

The ADVIA Centaur Intact PTH assay is a two-site sandwich immunoassay using direct chemiluminometric technology. It uses constant amounts of two anti-human PTH antibodies in the Lite reagent. The first antibody is a polyclonal goat anti-human PTH (N-terminal 1–34) antibody labelled with acridinium ester. The second antibody is a biotinylated polyclonal goat anti-human PTH (39–84 regions) antibody. Streptavidin in the solid phase is covalently coupled to paramagnetic latex particles.

9.12. Calculation of TmP/GFR

The maximal threshold for phosphate reabsorption (TmP/GFR) was determined using the phosphate/creatinine clearance ratio (C_P/C_{Cr}), in order to establish the fractional tubular reabsorption of phosphate (TRP). In all cases the TRP was greater than 0.86 and the formula below was utilised to calculate the TmP/GFR [41].

$$\frac{C_P}{C_{Cr}} = \frac{\text{Serum creatinine}}{\text{Urine creatinine}} \times \frac{\text{Urine phosphate}}{\text{Serum phosphate}} \qquad (1)$$

$$TRP = \frac{C_P}{1 - C_{Cr}} \qquad (2)$$

$$\frac{TmP}{GFR} = \text{Serum Phosphate} \times \frac{(0.3 \times TRP)}{1 - (0.8 \times TRP)} \quad [\text{mmol/l}] \qquad (3)$$

10. Statistical Analysis

10.1. Descriptive Statistics

Recorded variables were summarised using standard descriptive statistical methods.

1. Continuous variables that follow a normal distribution were described by a mean and standard deviation

2. Continuous variables that did not follow a normal distribution were described by a median and inter-quartile range

10.2. Inferential Statistics

Due to the small sample size, non-parametric techniques were used to assess associations and differences between groups.

1. The following categorisation of patients were used for comparison of the variables analysed:
   - Survivors versus non-survivors
   - Severe hypophosphatemia (< 0.3 mmol/l) versus non-severe hypophosphatemia

2. Associations between variables were analysed by non-parametric methods e.g. the Spearman rank correlation

3. Correlation of non-parametric independent variables was determined using Restricted Maximal Likelihood (REML) analysis. REML determines the likelihood of correlation of independent variables to a fixed outcome.

11. Results

11.1. Demographics and baseline biochemistry

11.1.1. Demographics of the population

Ten patients were enrolled in this pilot study: six completed three weeks of nutritional rehabilitation; two demised; and two were discharged early, one at day 14 and one at day 20. On admission the mean age was 12.5 months (range: 7-18, SD: 3.50). Of the cohort, 7 patients (70%) were male. Baseline demographic information is summarised in table 1.

Baseline Characteristics (n=10)			
Sex	70% male		
	Mean	**Range**	**Standard deviation**
Age (months)	12.50	7 - 18	3.50
Weight (kg)	7.05	3.86 - 9.92	1.70
Height (cm)	66.76	57 - 74	5.38
	Median	**IQR**	
Mid-Upper-Arm-Circumference (cm)	12.25	10.90; 14.40	
Weight-For-Length (Z score)	-2.00	-4.00; 0.12	
Weight-Age-Z-score	-2.77	-5.07; -1.10	
Height-Age-Z-score	-2.52	-5.23; -1.14	
Phosphate (1.00-1.95 mmol/l)	1.30	0.90; 1.40	
Ionised calcium (2.19 -2.64 mmol/l)	1.80	1.60; 1.88	
Corrected Calcium (2.19-2.64 mmol/l)	2.21	1.91; 2.36	
Albumin (32 – 47 g/l)	14.00	12.25; 24.00	
Magnesium (0.70 – 0.95 mmol/l)	0.79	0.70; 0.88	

Table 1: Baseline demographics and electrolytes

11.1.2. Anthropometry

Malnutrition was diagnosed and classified according to the WHO classification [19], and anthropometry plotted on age appropriate WHO Z-score charts. On admission 70% (n=7) of children were classified as severely underweight for age (Weight-Age-Z-score median: -2.77, IQR: -5.07; -1.10) and 60% (n=6) were classified as stunted (Height-Age-Z-score median: -2.52, IQR: -5.23; -1.14). All patients were oedematous on admission. Three patients fulfilled the WHO criteria for severe acute malnutrition, two of which met both the Weight-for-Length (WFL) and Mid-Upper-Arm-Circumference (MUAC) criteria; the third patient met only the Weight-for-Length criterion. Trends in MUAC and WFL during refeeding are summarised in figures 1 and 2.

On age stratification, children ≤ 12 months old had a median Weight-Age-Z-score (WAZ) on admission of -2.90 (IQR: -5.30; -1.10) while children > 12 months had a median score -2.40

(IQR: -4.40; -0.50). The trends in WAZ and Height-Age-Z-score (HAZ) are summarised in table 2.

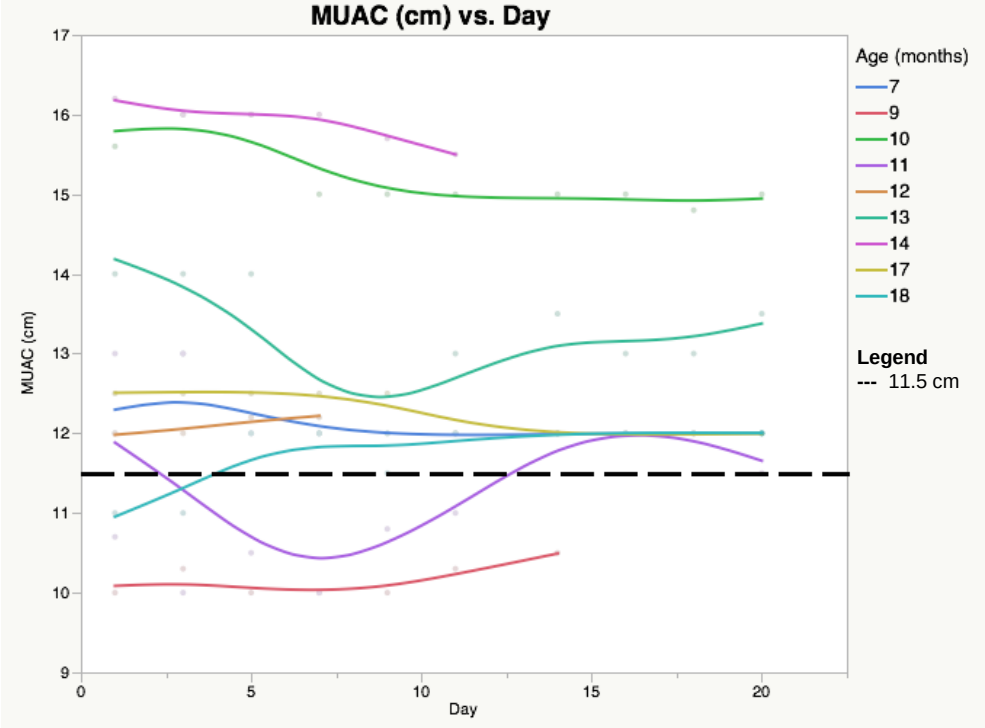


Figure 1: Mid-Upper-Arm-Circumference during refeeding

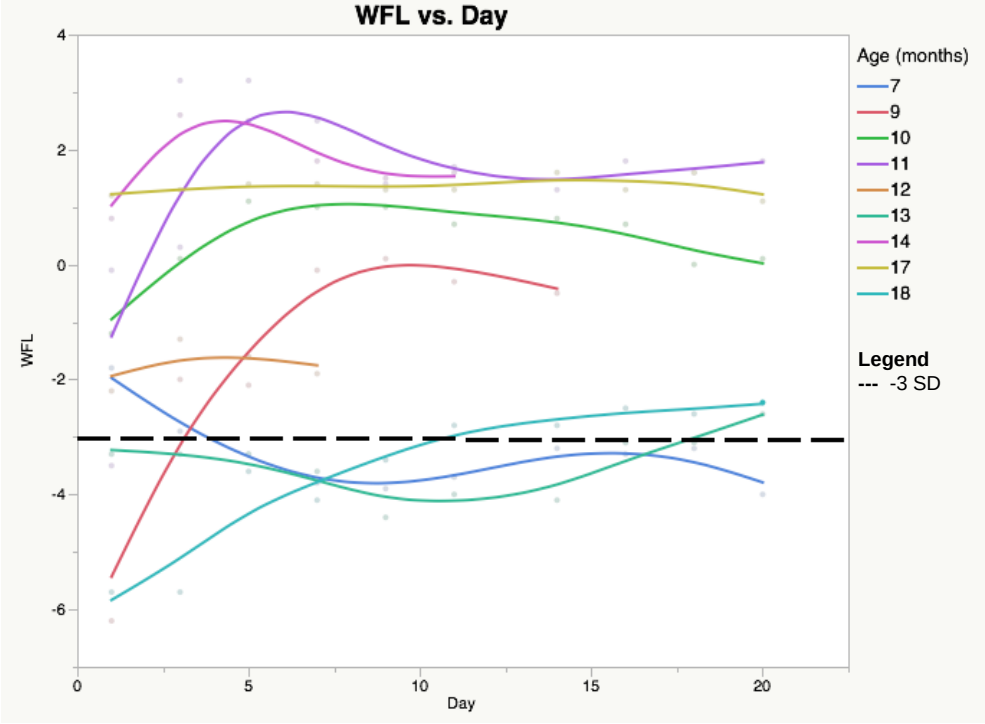


Figure 2: Weight-For-Length during refeeding

	Day	Admission	3	5	7	9	11	14	16	18	20
WAZ	**≤ 12 months (n = 6)**										
	Median	-2.90	-2.70	-3.40	-3.80	-3.80	-3.90	-3.80	-3.30	-3.70	-3.70
	IQR	-5.30; -1.10	-4.80; -0.30	-4.80; 0.80	-4.40; -1.30	-3.90; -0.70	-4.00; -0.90	-4.10; -0.90	-3.60; 0.00	-3.80; 0.00	-4.00; 0.00
	> 12 months (n = 4)										
	Median	-2.40	-1.60	-1.50	-2.00	-2.30	-2.20	-3.00	-2.40	-2.40	-2.40
	IQR	-4.40; -0.50	-4.40; -0.10	-3.80; 0.00	-4.00; -0.30	-3.60; -0.40	-3.70; -0.40	-4.00; 1.00	-3.80; 0.00	-3.90; -0.10	-3.80; -0.20
HAZ	**≤ 12 months (n = 6)**										
	Median	-1.90	-4.20	-5.32	-5.30	-4.10	-4.10	-4.00	-2.90	-2.90	-3.00
	IQR	-6.30; -0.90	-6.60; -1.10	-6.80; -1.90	-6.80; -2.00	-6.80; -1.60	-7.10; -1.70	-6.80; -1.70	-7.30; -1.40	-7.40; -1.40	-7.40; -1.40
	> 12 months (n = 4)										
	Median	-3.40	-3.40	-3.40	-2.30	-3.50	-3.50	-2.50	-2.50	-2.50	-2.60
	IQR	-4.90; -1.80	-4.50; -1.80	-4.50; -1.50	-3.90; -0.40	-4.60; -1.60	-4.60; -1.6-	-4.60; -1.30	-4.60; -1.40	-4.50; - 1.40	-4.60; -1.40
WFL	**≤ 12 months (n = 6)**										
	Median	-2.00	-0.6.00	-1.60	-0.80	0.50	0.20	0.15	0.70	0.00	0.10
	IQR	-4.10; -0.90	-2.20; 1.00	-2.70; 2.10	-2.80; 1.70	-2.90; 1.30	-2.80; 1.40	-2.50; 1.10	-3.30; 1.80	-3.20; 1.60	-4.00; 1.80
	> 12 months (n = 4)										
	Median	-1.25	-1.00	-0.95	-1.10	-1.05	-0.75	-2.80	-2.50	-2.60	-2.40
	IQR	-5.10; 1.10	-5.10; 2.27	-3.52; 2.22	-3.97; 1.70	-4.15; 1.45	-3.70; 1.52	-4.10; 1.60	-3.10; 1.30	-3.10; 1.60	-2.60; 1.10

Table 2: WAZ, HAZ & WFL during refeeding

11.1.3. Oedema

The Z-score classification in isolation does not account for oedema and interstitial fluid shifts during refeeding. As such, the WHO classification for severe acute malnutrition utilises oedema as a clinical identifier [19]. All patients in our cohort were diagnosed with bipedal oedema on admission. Resolution of oedema occurred in 50% of children between day 9 and 11 of refeeding (figure 3). This correlated with the nadir of WAZ in children younger than 12 months (table 2). Trends in weight and albumin as well as WAZ and HAZ during refeeding are represented in figures 4 and 5.

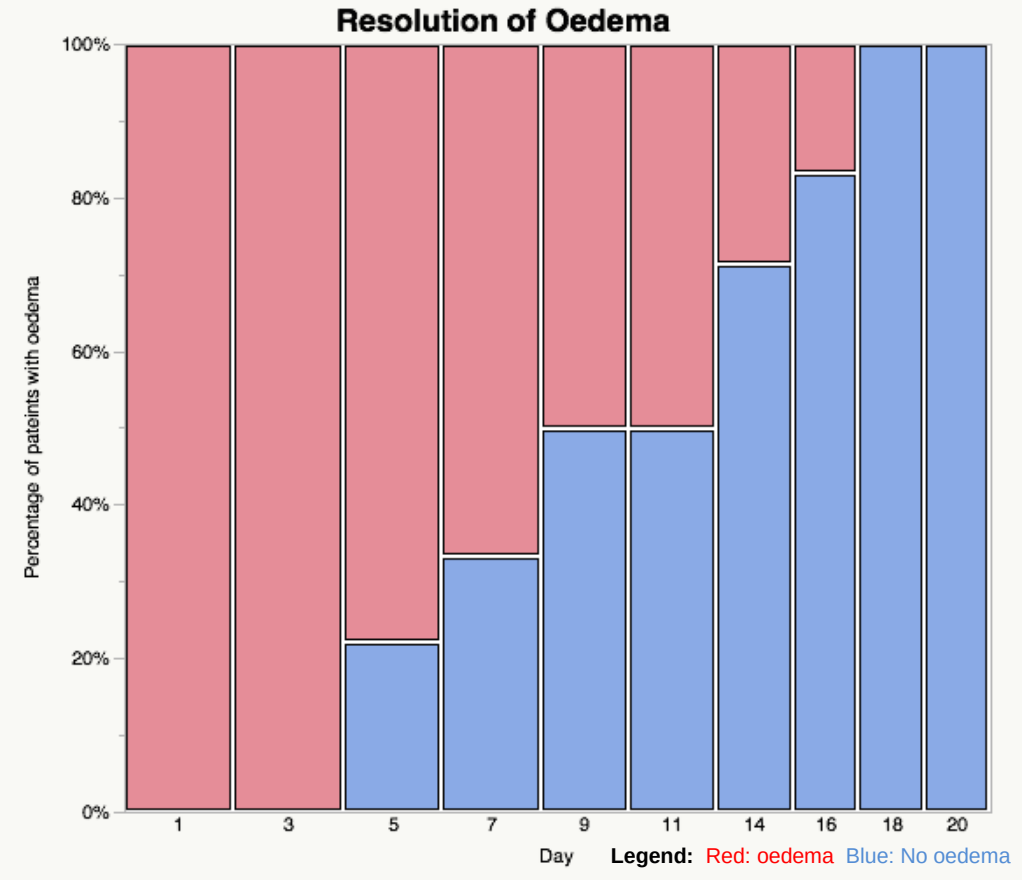


Figure 3: Course of oedema resolution during refeeding

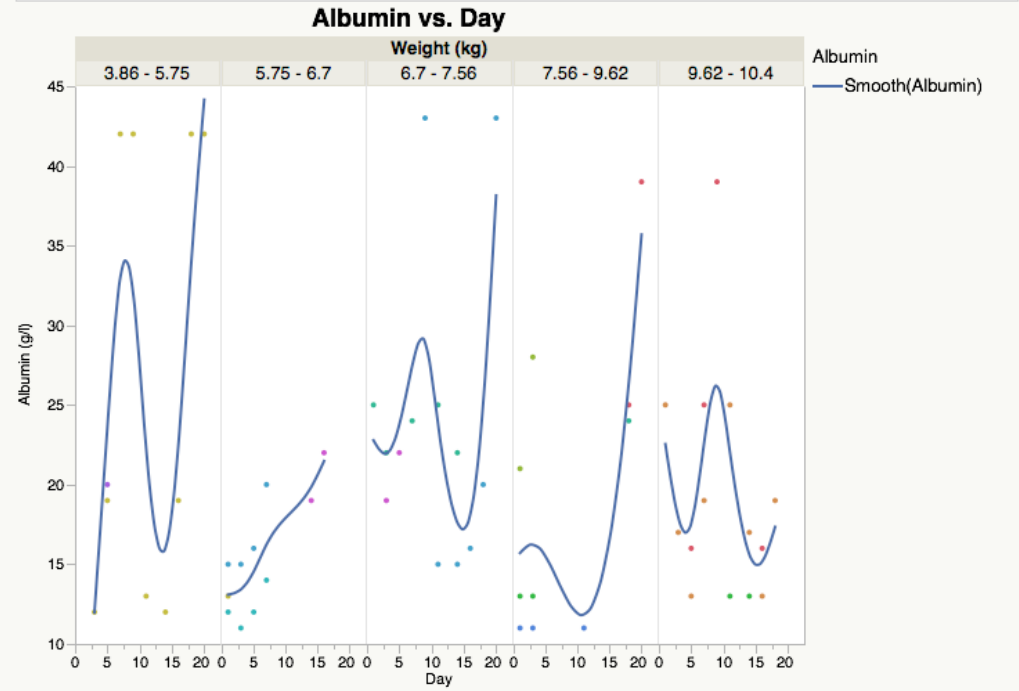


Figure 4: Serum albumin during refeeding stratified by weight

Figure 4 depicts the course of serum albumin during refeeding stratified by weight. In children with acute severe malnutrition hepatic synthesis of albumin has been determined to be 101mg/kg/day. This improves to 148mg/kg/day during refeeding and nutritional recovery [42].

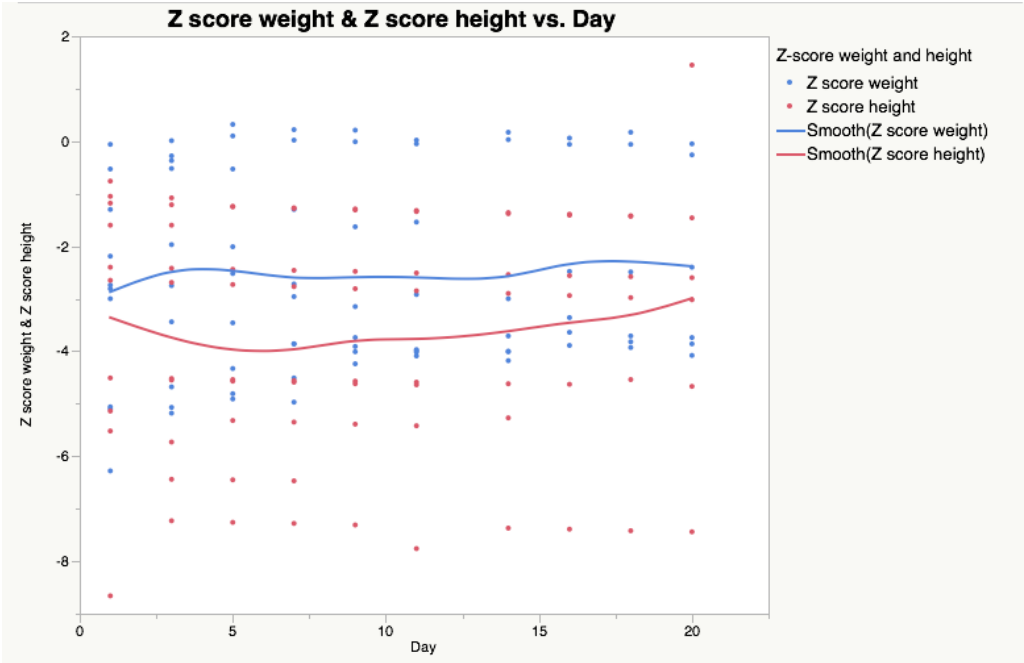


Figure 5: Weight-for-Age & Height-for-Age Z-Scores during refeeding

11.1.4. Clinical Features

On admission 60% (n=6) of patients had associated co-morbid infections. Three patients presented with pneumonia (one with confirmed pulmonary tuberculosis identified by direct smear and culture), and three with septicaemia and acute diarrhoeal disease. Two out of three patients admitted with sepsis demised during refeeding, one with proven gram-positive septicaemia. Shock was diagnosed in 20% (n=2) of patients on admission, necessitating fluid resuscitation and inotropic support.

Skin manifestations were present in 90% of the patient sample; the most common dermatologic diseases being desquamation (40%), hypopigmentation (30%) and dermatitis (20%). All patients had resolution of their dermatologic disease by day 18, which corresponded with a median serum albumin of 40.5g/l (IQR: 25.75; 41.75) on day 20 and a mean weight gain of +0.345 kg from baseline. As shown in figure 6; two patients (aged nine and fourteen months) had no recovery in serum albumin concentrations, one of which (aged nine months) demised during refeeding.

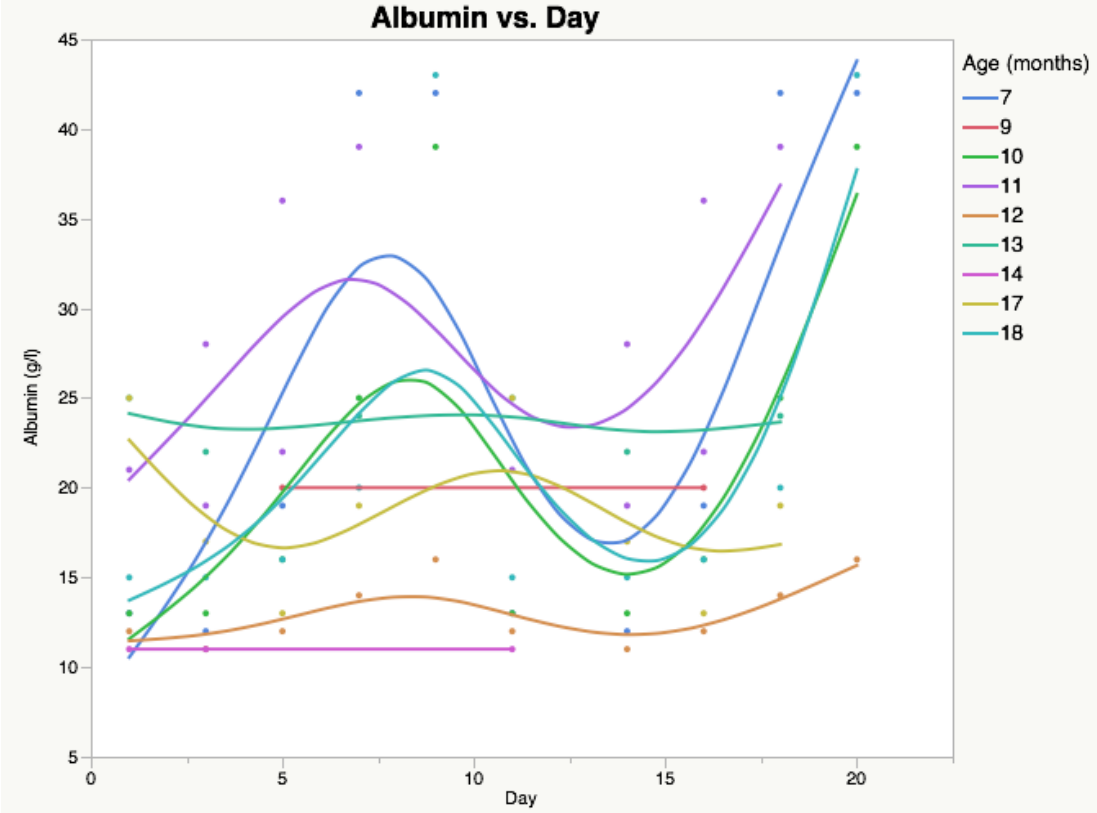


Figure 6: Age stratified serum albumin trends

Age stratified liver span on admission is displayed in figure 7. According to paediatric aged based reference ranges, 33.3% (n=2) of children younger than 12 months had a hepatic span on palpation/ percussion greater than the 50th centile on admission [43]. In children older than 12 months only one child had a hepatic span of 8 cm corresponding to the 75th centile.

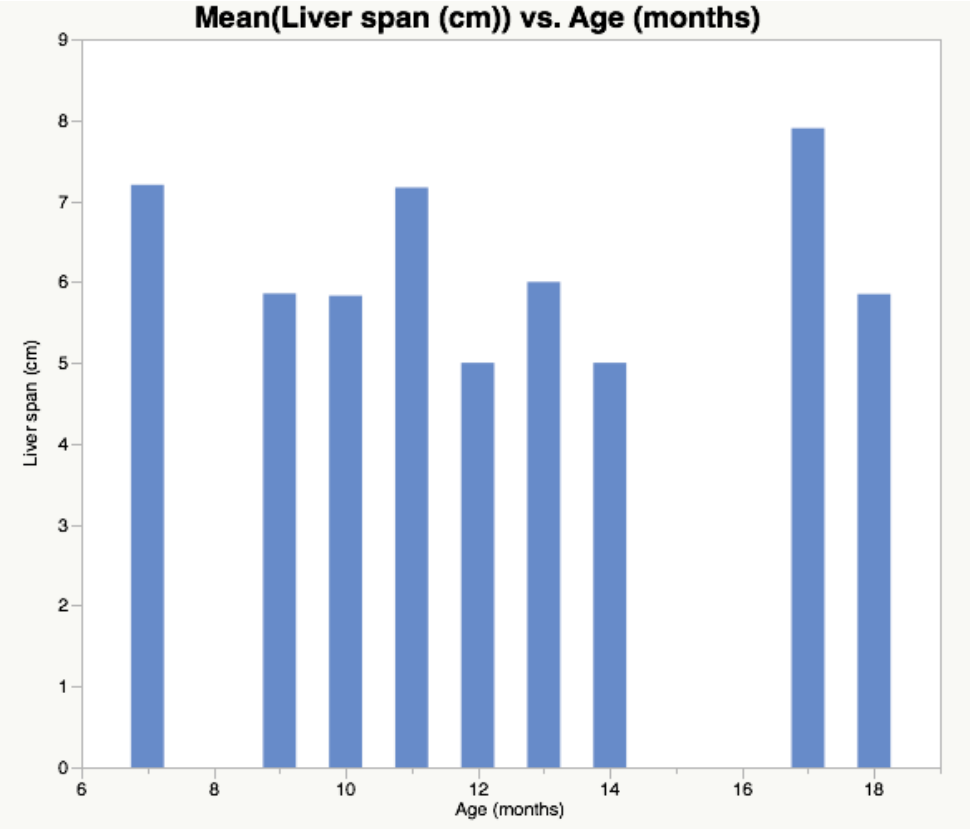


Figure 7: Liver span on admission

Within the first 24 hours of admission 30% (n=3) of patients developed pyrexia (temperature ≥ 37.5 °C), with 90% developing hypothermia (temperature ≤ 35 °C) during the course of treatment. This is in keeping with the temperature instability described in severe acute malnutrition.

Median early morning glucose (HGT) on admission was 6.3 mmol/l (IQR: 4.7; 10.7) as tested by a ward glucometer and fell within the euglycaemic range (3.8 - 7.7 mmol/l). There was a single episode of hypoglycaemia in a patient on day 2 with a random serum glucose level of 2 mmol/l, this patient subsequently died within 24 hours onset of hypoglycaemia. Glucose trends during refeeding are summarized in figure 8.

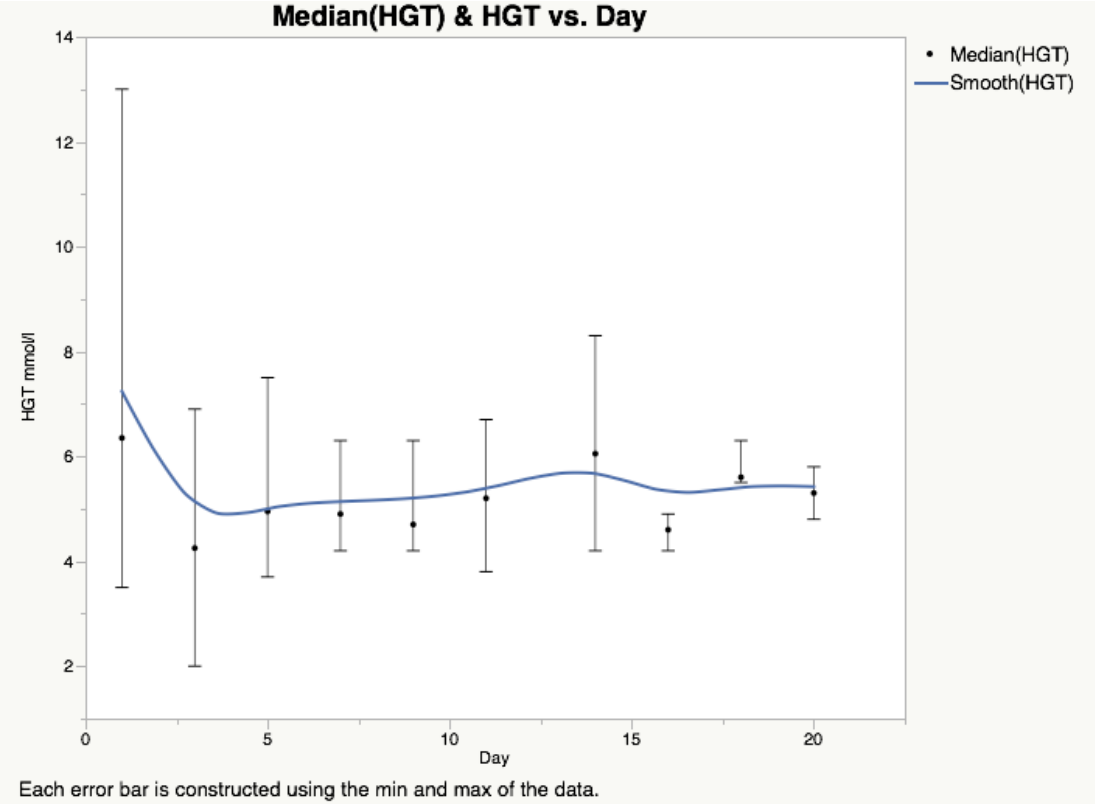


Figure 8: Serum glucose concentrations during refeeding

11.1.5. Baseline biochemistry

At baseline, serum phosphate concentration fell within the age appropriate reference range (median: 1.3 mmol/l, IQR: 0.9; 1.4). Hypoalbuminemia (median: 14g/l, IQR: 12.25; 24) shown in figure 9 and low serum ionised calcium (median: 1.8 mmol/l, IQR: 1.6; 1.88) were noted on admission. Serum total protein concentration was also low (median: 46, IQR; 40; 53), but normalised by day 20 (median: 69, IQR: 66; 75).

Liver biochemistry during refeeding is summarised in table 3 and figure 10. At baseline, alanine transaminase (ALT median: 64.5, IQR: 45.9; 45) and aspartate transaminase concentrations (AST median: 60, IQR: 49; 140.5) were both elevated. Serum ALT concentration continued to increase to a median of 84 U/l on day 3 of refeeding (IQR: 32; 126). The elevation of ALT in conjunction with clinical hepatomegaly in the setting of malnutrition may be indicative of hepatic steatosis. Adult studies have demonstrated an association between hepatic steatosis and an AST/ALT ratio greater than 1 [44]. On admission the median AST/ALT was 1.8 (IQR: 0.81; 2.7), which improved with refeeding, this trend is demonstrated in figure 11.

Additional baseline characteristics and electrolytes are summarized in table 1.

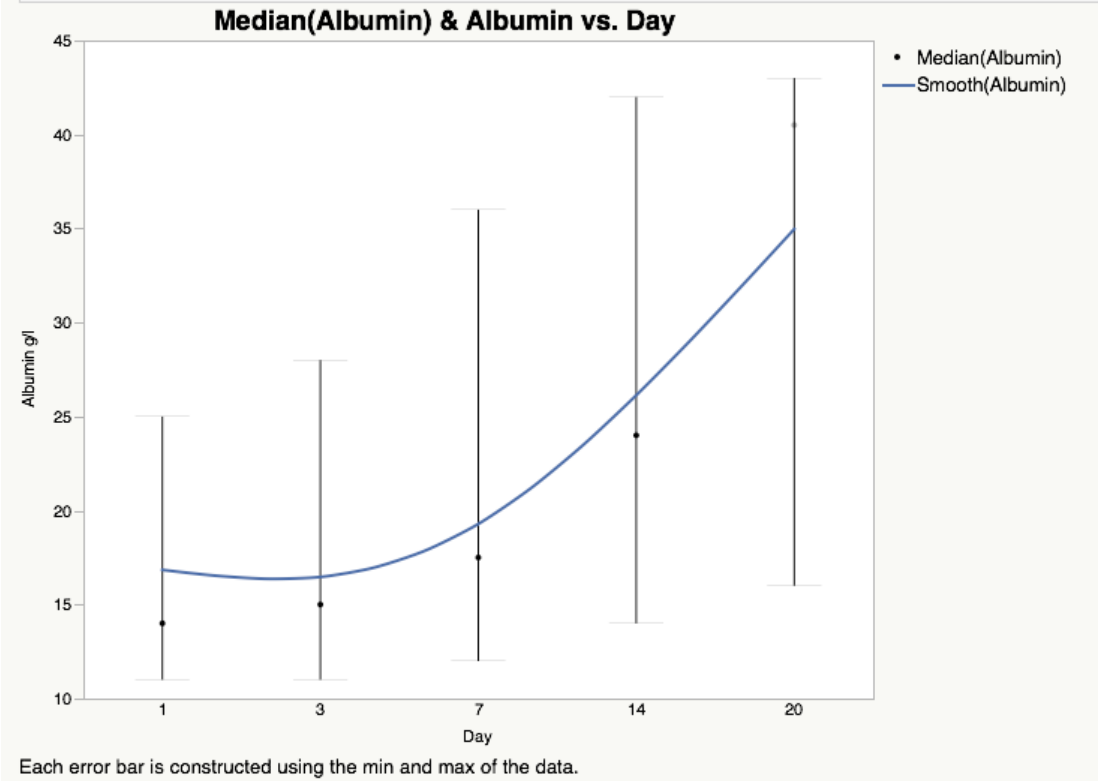


Figure 9: Serum albumin median and range during refeeding

	Admission		Day 3		Day 7	
	Median	IQR	Median	IQR	Median	IQR
Bilirubin Total (0-21 µmol/l)	14.5	5.5; 31	19	4; 24	12	10; 14
Alanine Transaminase (5-30 U/l)	64.5	45; 149	84	32; 126	72	55; 161
Aspartate Transaminase (0-38 U/l)	60	49; 140.5	55.5	36.5; 295.75	48	38; 141

Table 3: Liver biochemistry during first week of refeeding

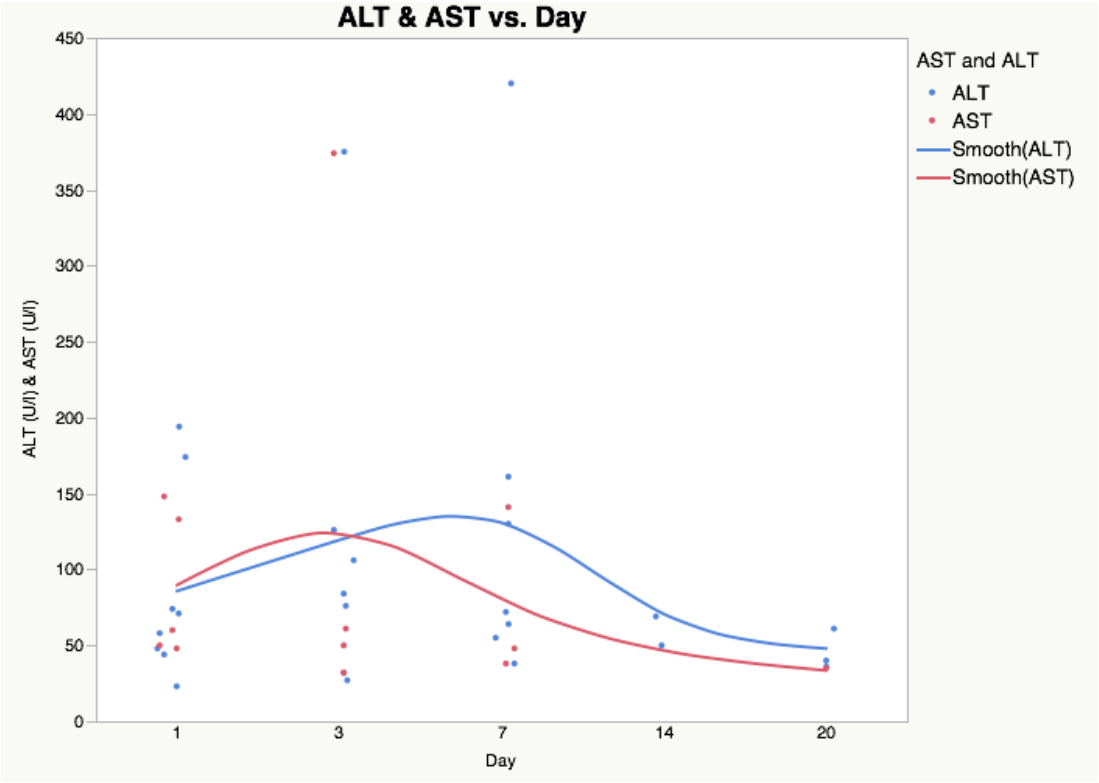


Figure 10: Liver biochemistry trends during refeeding

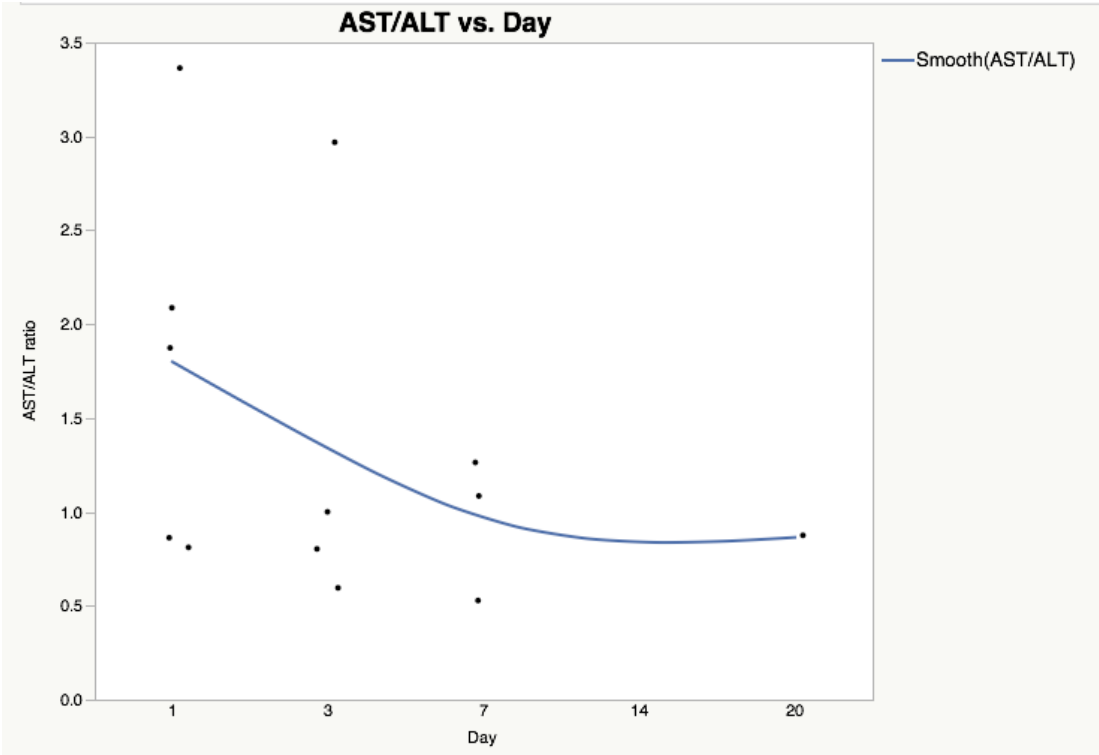


Figure 11: Alanine Transaminase & Aspartate Transaminase ratio during refeeding

11.1.6. Refeeding protocol

The nutritional rehabilitation and refeeding protocol used in this study (Appendix B) advocates a “start low and go slow“ approach for reintroduction of feeds. This policy is consistent with WHO severe acute malnutrition refeeding recommendations. The median millilitre per kilogram per day (ml/kg/day) feed reintroduced to patients younger that 12 months on day 1 was 98 ml/kg/day (IQR: 84.25; 103). This ensured a median energy intake in kilocalories per kilogram per day (kCal/kg/day) of 71.3 kCal/kg/day (IQR: 62.68; 75.25). In children 12 months and older, milk feeds were started at a median of 68 ml/kg/day (IQR: 60; 106) with a median energy intake of 45.56 kCal/kg day (IQR: 45; 71). Escalation of milk feeds and controlled introduction of solids proceeded as per protocol. Rate of feed escalation stratified by age is summarised in figures 12 and 13. On admission 40% (n=4) of the cohort were started on age appropriate standard infant formula, with an additional 30% (n=3) being kept nil per mouth. Distribution of feeds over the course of refeeding is summarized in figure 14.

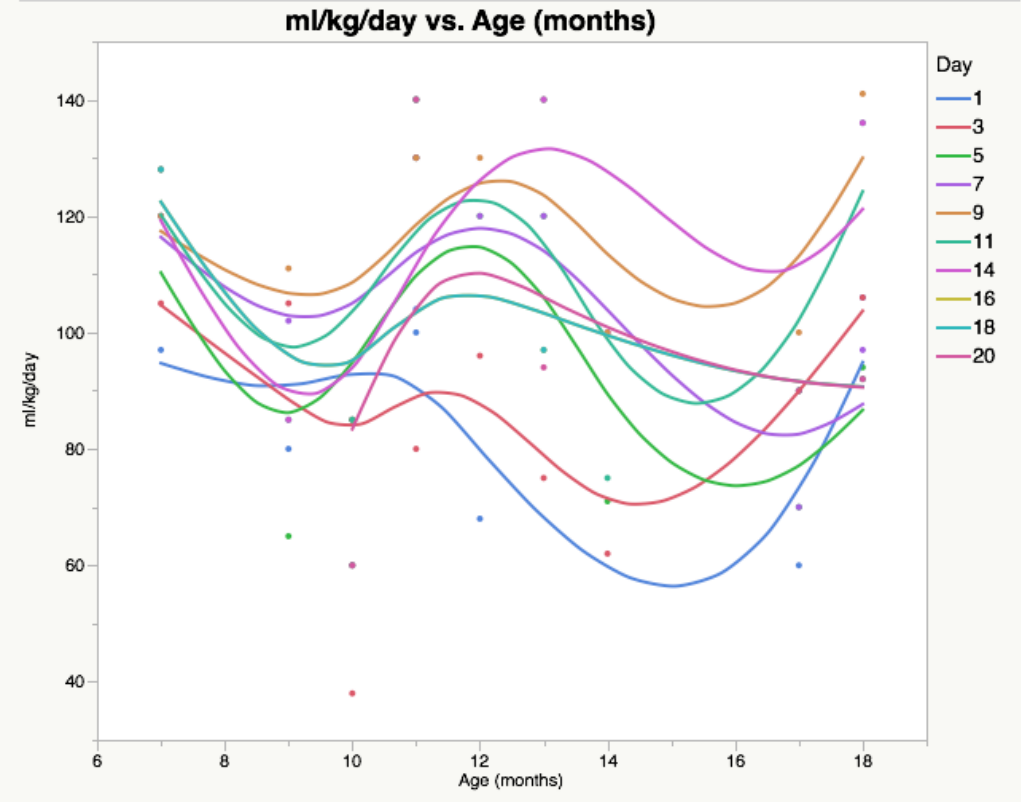


Figure 12: Feed volume during refeeding

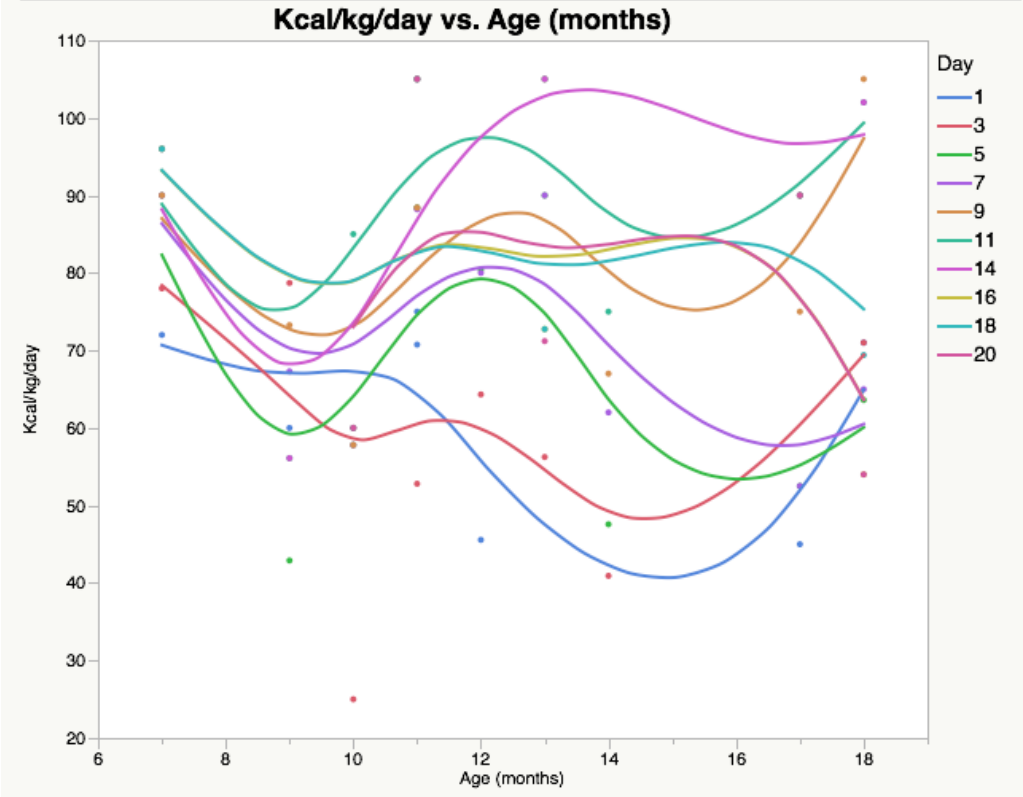
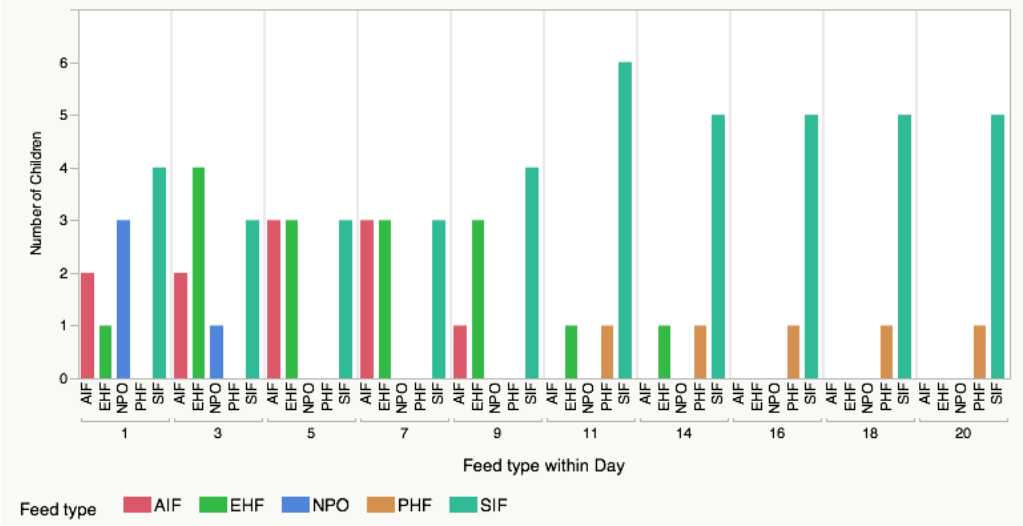


Figure 13: Caloric intake during refeeding



Histogram Legend

NPO:	Nil per os
EHF:	Extensively hydrolyzed formula
PHF:	Partially hydrolyzed formula
SIF:	Standard Infant formula
AIF:	Acidified Infant formula

Figure 14: Milk feed types during refeeding

11.2. Phosphate and Calcium characteristics

11.2.1. Serum phosphate during refeeding

On admission serum phosphate level fell within the defined normal range for age with a median value of 1.3 mmol/l (IQR: 0.90; 1.40). Serial serum phosphate levels were tracked over the course of refeeding, revealing a nadir of 1.15 mmol/l in the median serum phosphate at day 7 of treatment (IQR: 0.82; 1.50). It is important to note that despite this value not fulfilling the definition of hypophosphatemia, 70% (n=7) of patients were being supplemented with phosphate at this time with 42.8% (n=3) of these patients requiring an increased dose adjustment at a mean of day 5.6 (SD: 0.57) during refeeding. Serum phosphate levels continued to trend in an upward direction after day 7 with a median phosphate of 1.90 mmol/l on day 20 (IQR: 1.25; 2.25). No patient developed severe hypophosphatemia (serum phosphate < 0.3 mmol/l) during refeeding. The median serum phosphate concentration over the course of refeeding is summarised in figure 15 and table 4.

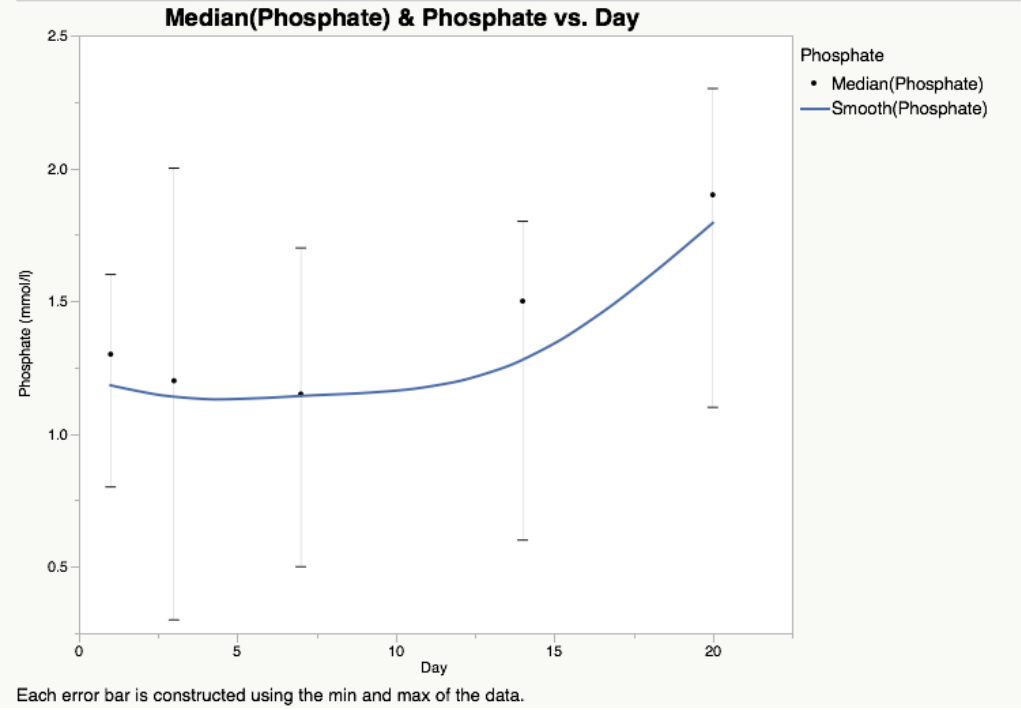


Figure 15: Serum Phosphate during refeeding

Electrolytes	Admission		Day 3		Day 7		Day 14		Day 20	
	Median	IQR	Median	IQR	Median	IQR	Median	IQR	Median	IQR
Phosphate (1.00 - 1.95 mmol/l)	1.30	0.90; 1.40	1.20	0.72; 1.42	1.15	0.82; 1.50	1.50	0.90; 1.60	1.90	1.25; 2.25
Ionised calcium (2.19 - 2.64 mmol/l)	1.80	1.60; 1.88	1.71	1.53; 1.98	1.89	1.66; 2.15	2.08	1.89; 2.26	2.47	2.25; 2.54
Corrected Calcium (2.19 - 2.64 mmol/l)	2.21	1.91; 2.36	2.28	2.04; 2.34	2.31	2.09; 2.46	2.35	2.30; 4.60	2.42	2.37; 2.54
Magnesium (0.70 - 0.95 mmol/l)	0.80	0.70; 0.88	0.74	0.68; 0.88	0.84	0.51; 0.90	0.79	0.65; 0.85	0.87	0.82; 0.90
Albumin (32 - 47 g/l)	14.00	12.25; 24	16.00	12.25; 25.25	17.5	13.76; 21.5	24.00	19.00; 39.00	40.5	21.75; 42.75
PTH (1.20 - 8.50 pmol/l)	2.75	1.90; 3.60	2.10	0.29; 3.30	11.35	9.10; 13.6	0.95	0.90; 1.00	2.30	0.14; 2.60
Urinary Phosphate (mmol/l)**	1.60	0.00; 1.20	3.90	0.85; 16.02	1.38	0.90; 7.15	3.85	0.90; 37.85	2.21	0.60; 22.70
TmP/GFR (1.15 - 2.60 mmol/l) [45]			0.46	0.4; 0.52	0.38	0.27; 0.50	0.27	0.18; 0.37		

Table 4: Phosphate metabolism during refeeding

** Tygerberg Hospital NHLS Chemical Pathology has no validated reference range for spot urinary phosphate.

11.2.2. Phosphate initiation

70% (n=7) of patients required phosphate supplementation at a mean day of 1.14 (SD: 0.37) of treatment; the median dose of phosphate given (both oral and intravenous) was 0.53 mmol/kg/day (IQR: 0.37; 0.90). This dose was at the lower limit of the recommended protocol dosing range. A further three patients required an increase in their phosphate dose at a mean day of 5.6 (SD: 0.57) with a mean change in dose from baseline of +0.16 mmol/kg/day (SD: 0.05).

11.2.3. Calcium, PTH and urinary phosphate

Serum ionised calcium levels were low at baseline (median: 1.80 mmol/l, IQR: 1.60; 1.88), reaching a nadir on day 3 of treatment (median: 1.71 mmol/l, IQR: 1.53; 1.98). This was followed by a peak in parathyroid hormone (PTH) secretion on day 7 (median: 11.35 pmol/l, IQR: 9.10; 13.60) and an increased urinary phosphate excretion on day 14 (median: 3.85 mmol/l, IQR: 0.90; 37.85) (figure 16). Magnesium levels remained within the normal range throughout refeeding, negating the effect of hypomagnesaemia on PTH secretion. Similarly, corrected calcium levels also remained within the normal range. Although limited, the data available for determination of the TmP/GFR revealed a persistently low maximal renal threshold for phosphate reabsorption. In keeping with renal phosphate wasting and possible tubulopathy, the day 3 TmP/GFR median was 0.46 mmol/l (IQR: 0.4; 0.52) and the day 14 median was 0.27 mmol/l (IQR: 0.18; 0.37). Trends in TmP/GFR did not mirror the normalisation in serum albumin, possibly indicating non-recovery in Na/Pi IIa renal receptors. The internationally validated reference range for children of 6 months of age is 1.15 - 2.60 mmol/l [45]. The aforementioned parameters are summarised in table 4.

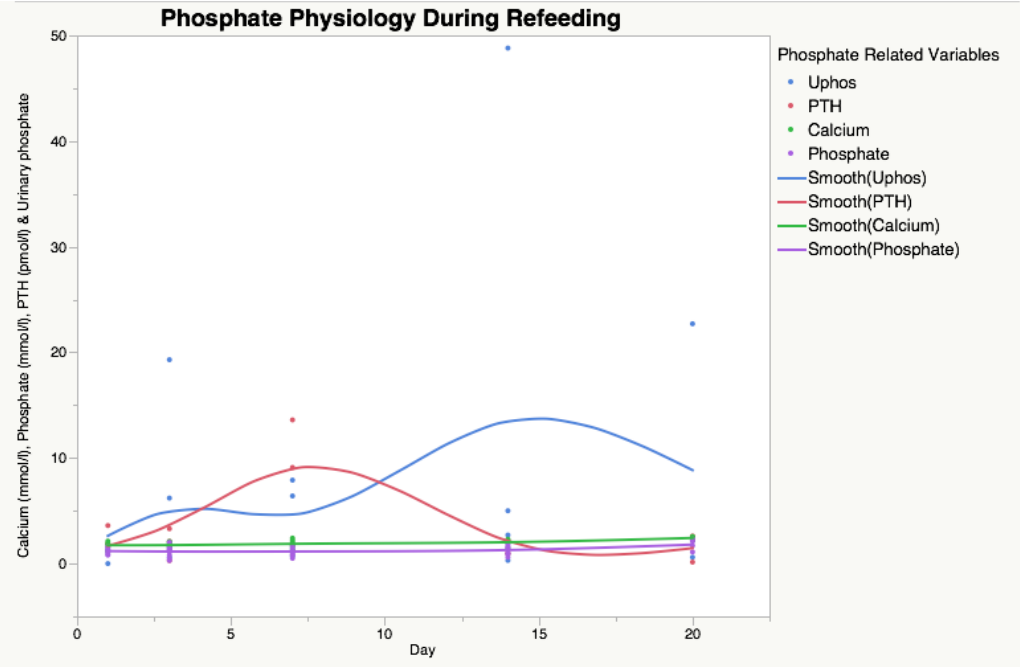


Figure 16: Phosphate physiology during refeeding

11.2.4. Metabolic acidosis during refeeding

30% (n=3) of patients were diagnosed with metabolic acidosis: one patient on admission, the other two on day 5 and 12 respectively. The mean pH was 7.13 (SD: 0.17) with a mean base excess (BE) of -17 (SD: 6.06). One patient demised within 96 hours of developing acidosis, the other two patients had resolution of their metabolic disturbance within 24 hours. Sodium bicarbonate infusions were not used during the resuscitation and treatment of acidosis in these patients, negating the possible confounding effect on ionised hypocalcaemia.

11.2.5. Indicators of refeeding syndrome

Refeeding syndrome is characterised by a triad of hypophosphatemia, hypokalaemia and hypomagnesaemia; and occurs during the nutritional rehabilitation of a malnourished patient. Additional cardinal features may include renal retention of sodium and water, thiamine deficiency and hyperglycaemia with osmotic diuresis [46]. In our study cohort, no patient developed hypomagnesaemia or hypokalaemia associated with refeeding (figure 17). These findings are not in keeping with refeeding data from adult anorexic patients, however routine supplementation may partially account for this.

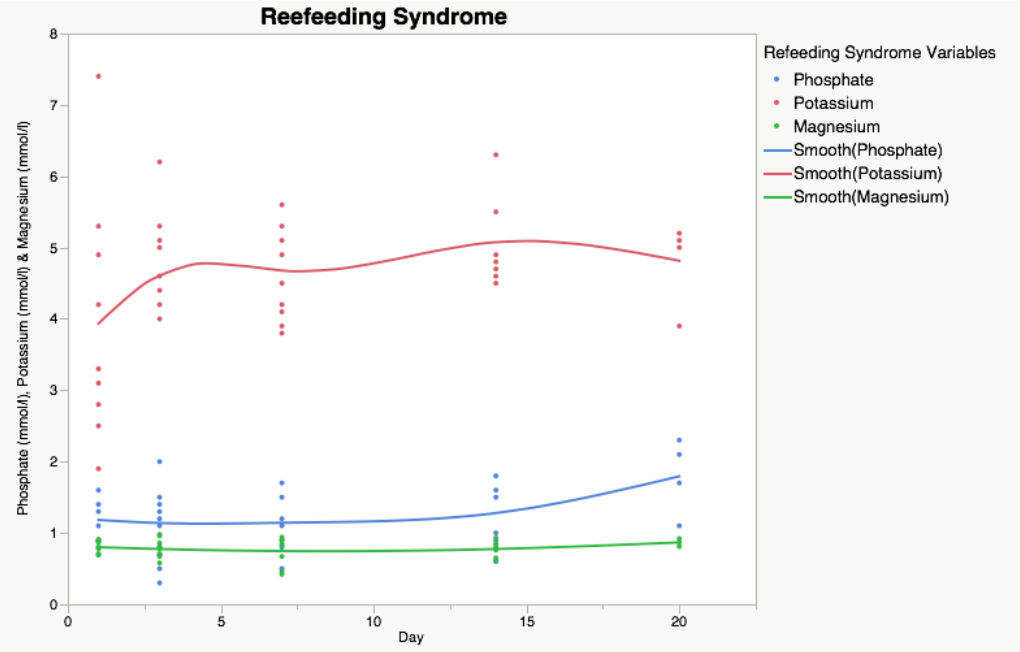


Figure 17: Potassium, Magnesium and Phosphate during refeeding

Supplementation of potassium (15% KCl) was initiated on admission in all children with a mean dose of 2.45 mmol/kg/day (SD: 1.13). Magnesium chloride was not routinely initiated on admission, however 6 patients required oral magnesium chloride supplementation at a mean of 3.5 days (SD: 4.46) post admission. The mean magnesium chloride dose administered was 1.45 mmol/kg/day (SD: 0.68). Both potassium and magnesium chloride doses were within the recommended protocol dosing ranges.

11.2.6. Infective parameters and phosphate

Hypophosphatemia has been associated with an increased risk of sepsis in adult patients treated in intensive care units [36, 38]. In our cohort, a peak median C-reactive protein (CRP) of 74 mg/l (IQR: 94; 236) was recorded on day 1, declining to a median CRP of 18 mg/l on day 7 (IQR: 9; 27) with subsequent normalisation by day 14. Procalcitonin followed a bimodal distribution with mean peaks of 33 ng/ml (SD: 45.90) on day 1 and 24 ng/ml (SD: 43.00) on day 7; possibly indicating nosocomial sepsis. One patient was found to be bacteraemic and cultured staphylococcal aureus within 24 hours of admission; the organism was isolated from a blood culture and was sensitive to both cloxacillin and clindamycin.

Assessment of Spearman rank correlation (ρ) between phosphate and both PCT (ρ = -0.15, p= 0.490) and CRP (ρ = -0.41, p= 0.200) showed a non-significant negative correlation, as displayed in figures 18 and 19 respectively. Similarly, figure 20 shows a non-significant negative correlation between ionised calcium and CRP (ρ = -0.59, p= 0.055).

A significant negative Spearman rank co-efficient was however revealed on assessment of the correlation between PCT and serum ionised calcium (ρ = -0.67, p= 0.0012) (figure 21). This is previously undescribed in the setting of paediatric malnutrition.

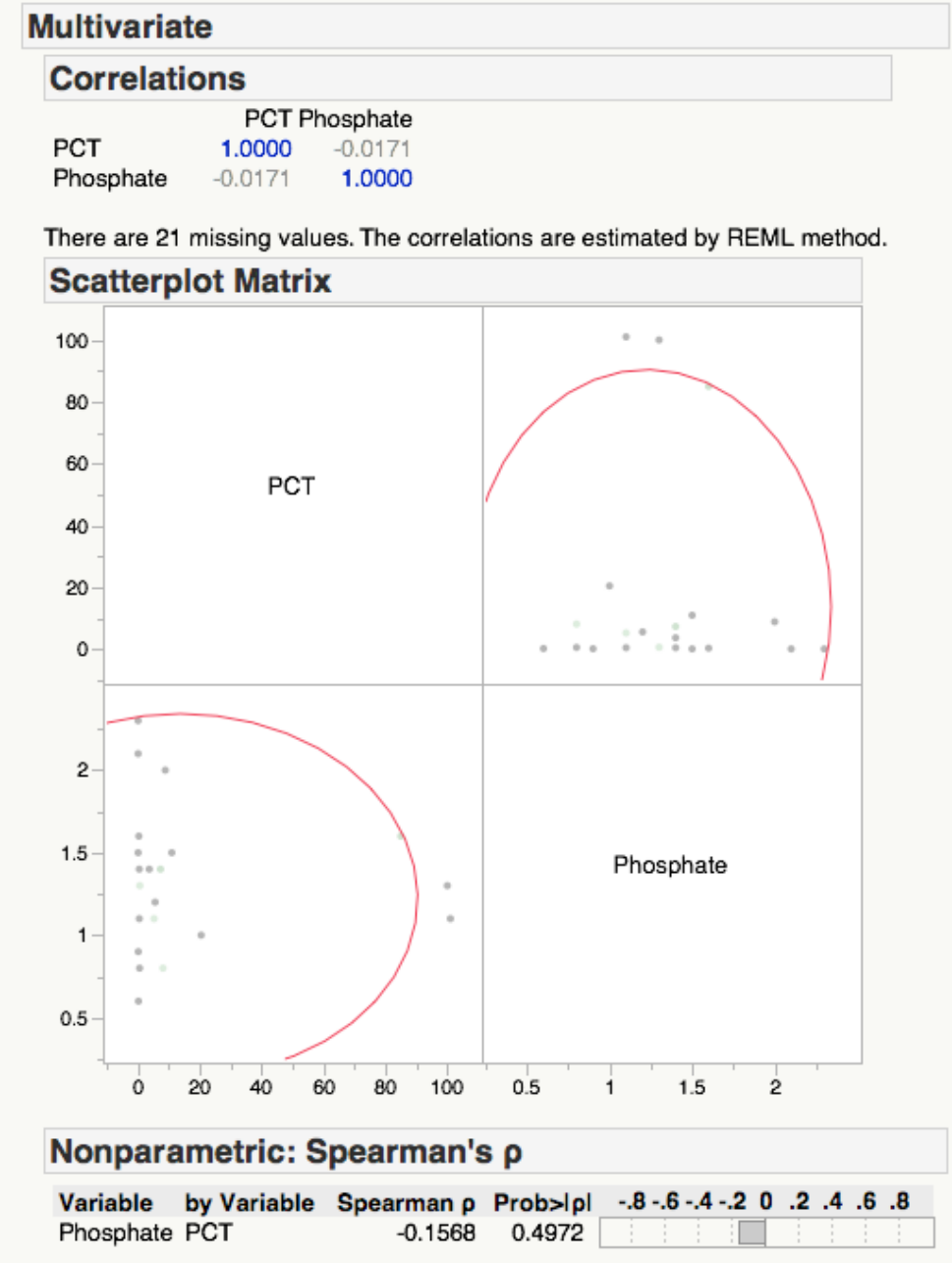


Figure 18: Multivariate analysis of Phosphate and PCT

Figure 18 depicts the correlation between serum phosphate and PCT concentration. The Spearman rank analysis of this cohort indicated that low serum phosphate levels correlated with an elevation in PCT.

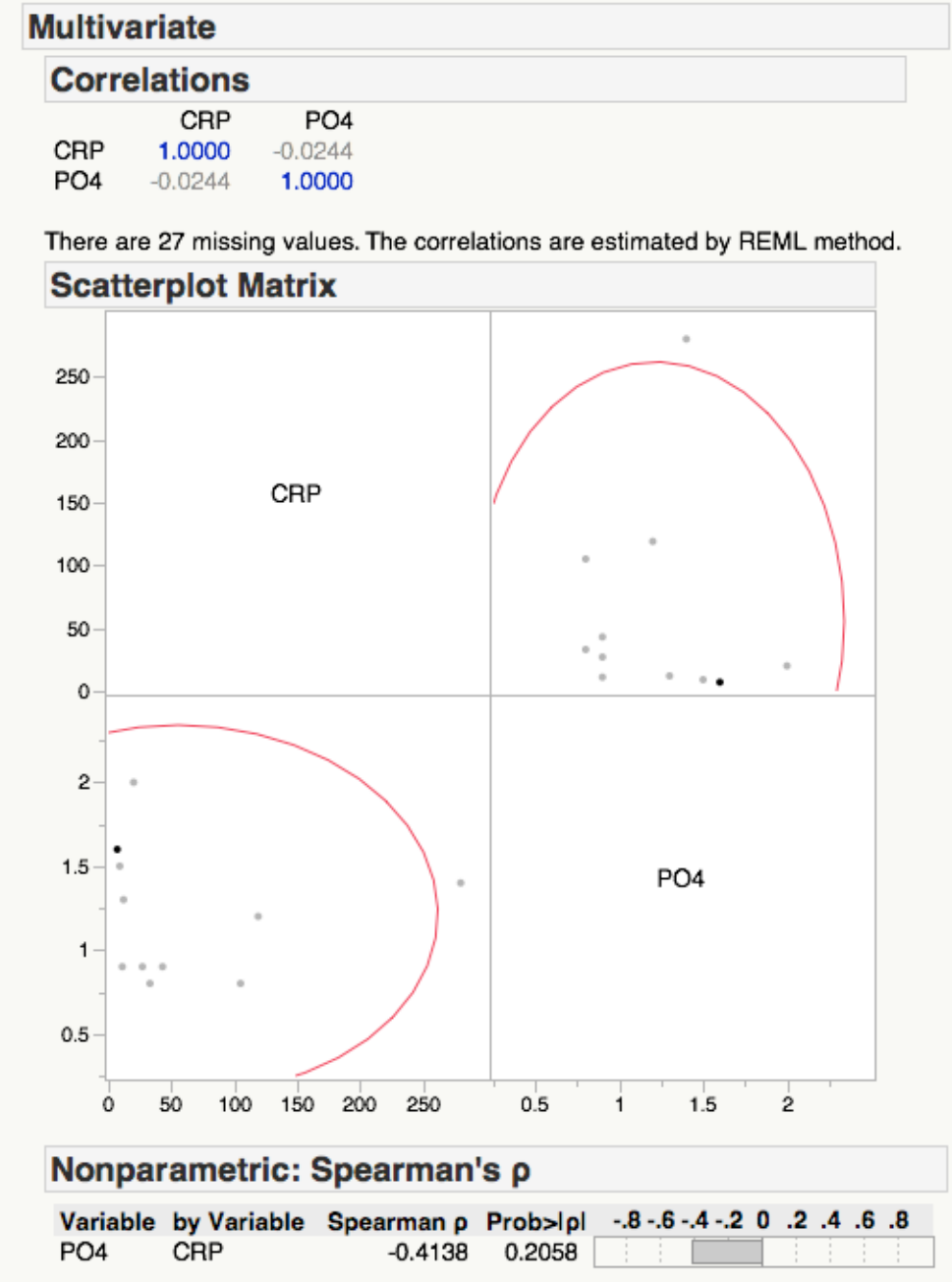


Figure 19: Multivariate analysis of Phosphate and CRP

Figure 19 depicts the correlation between serum phosphate and CRP. In this analysis, the Spearman rank analysis indicated that hypophosphatemia correlated with an elevation in CRP.

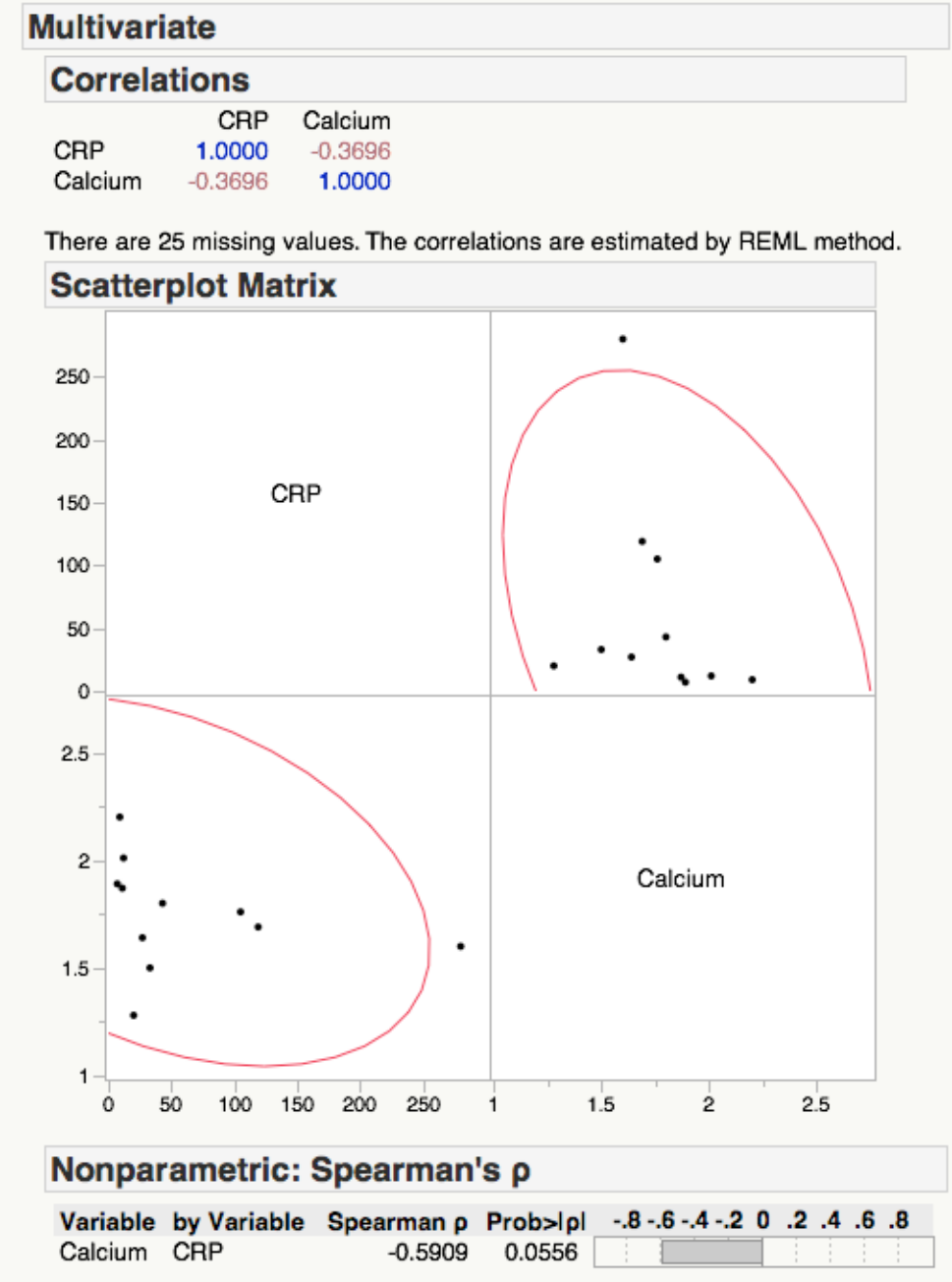


Figure 20: Multivariate analysis of Ionised Calcium and CRP

Figure 20 depicts the correlation between serum ionised calcium levels and CRP. As demonstrated, serum ionised hypocalcaemia showed a non-significant correlation with an elevation in CRP.

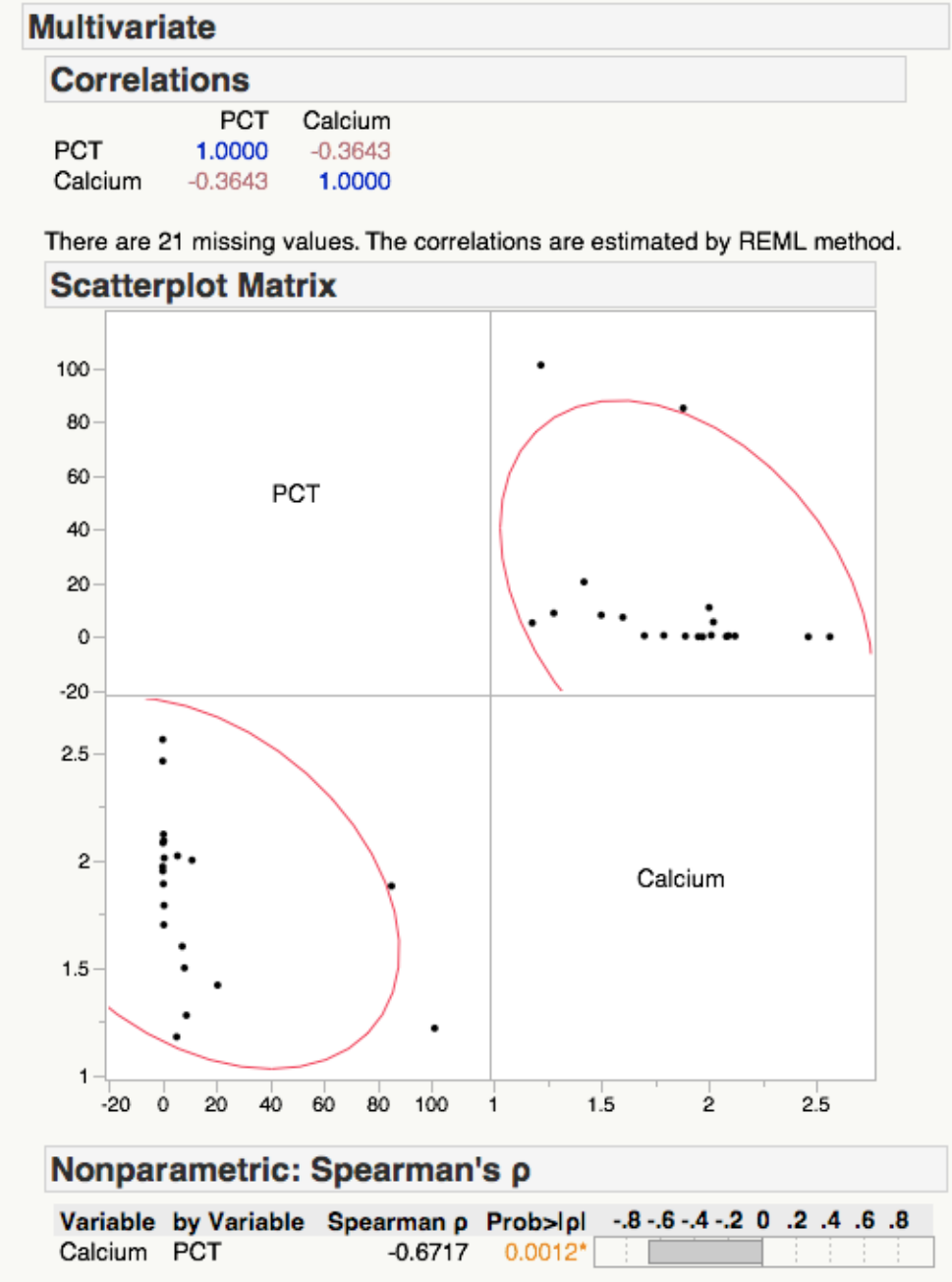


Figure 21: Multivariate analysis of Ionised Calcium and PCT

Figure 21 depicts the significance of correlation between serum ionised calcium levels and PCT. In contrast to previous non-significant findings, this analysis produced a significant negative correlation; indicating that ionised hypocalcaemia was significantly correlated to an elevated serum PCT.

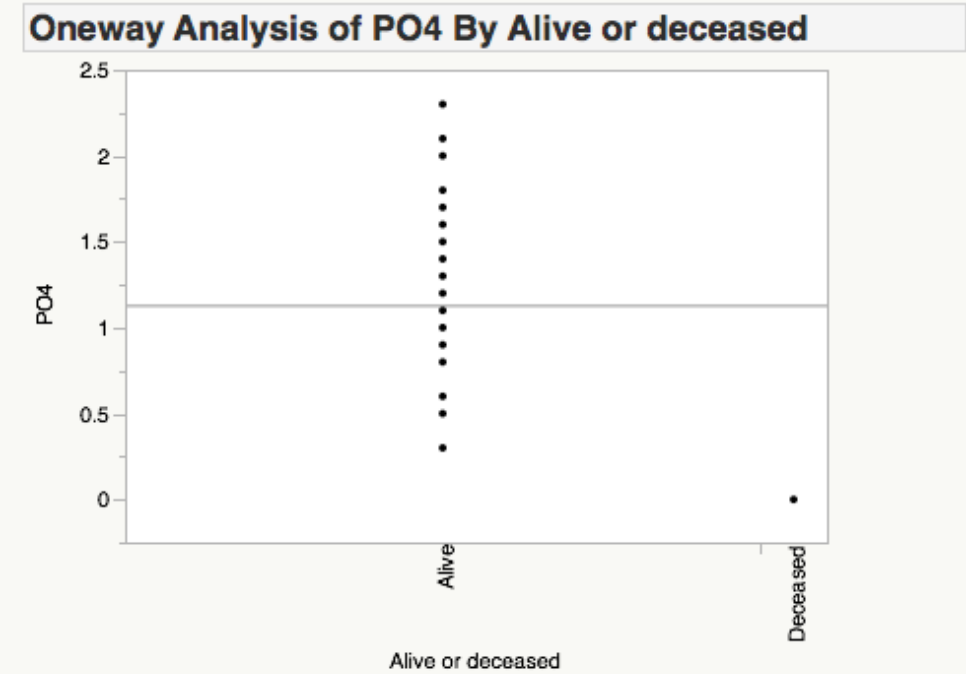
11.2.7. Survivors and non-survivors

There was a case fatality rate of 20% (n=2) during the study period. As detailed in Table 5, Patient A was hypophosphatemic on admission; Patient B had a normal serum phosphate on admission however the serum phosphate fell sequentially reaching a nadir on day 14, the patient demised 48 hours thereafter.

	Patient A	Patient B
Age (months)	11	9
Phosphate on admission	0.9 mmol/l	1.6 mmol/l
Phosphate nadir (day)	0.5 mmol/l (day 3)	1.0 mmol/l (day 14)
Calcium on admission	1.8 mmol/l	1.88 mmol/l
Calcium nadir (day)	1.72 mmol/l (day 3)	1.22 mmol/l (day 7)
Co-morbid infections	Sepsis and acute diarrhoeal disease	Sepsis and acute diarrhoeal disease
CRP on admission	43 mg/l	
PCT on admission		85 ng/ml
Positive Bacterial Cultures	None cultured	Staphylococcus Aureus
Day of demise during refeeding	Day 4	Day 16

Table 5: Characteristics of deceased patients

Utilising non-parametric Wilcoxon rank sum, differences in median serum phosphate concentrations were significant between survivors and non-survivors (p= 0.0012) (figure 22). Similarly significant differences were detected when considering median ionised calcium levels (p= 0.0013), median CRP (p= 0.0046) and median PCT (p= 0.0017) as shown in figures 23, 24 and 25 respectively. The subsequent 3-month follow up of survivors revealed no late mortality following nutritional rehabilitation.



Missing Rows 8

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
Alive	38	893.000	817.000	23.5000	3.244
Deceased	4	10.000	86.000	2.5000	-3.244

2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
10	-3.24414	0.0012*

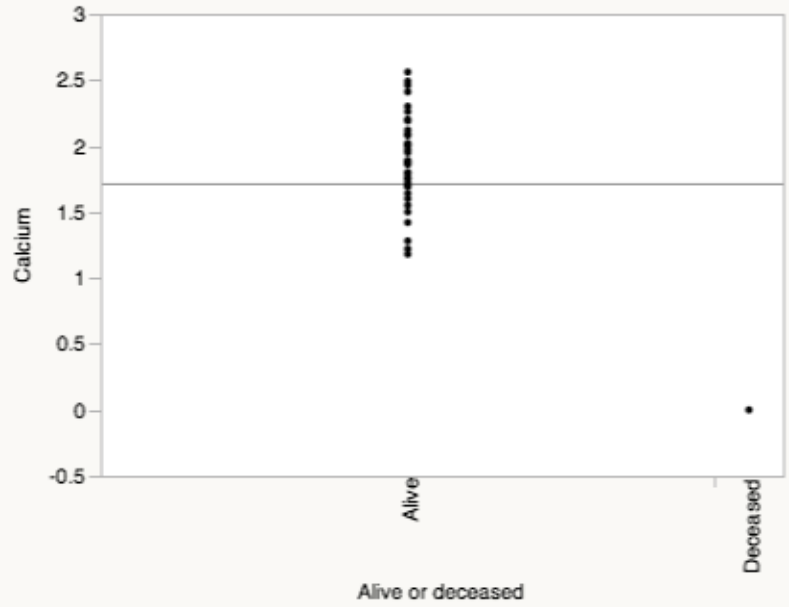
1-way Test, ChiSquare Approximation

ChiSquare	DF	Prob>ChiSq
10.6643	1	0.0011*

Figure 22: Serum Phosphate in survivors and non-survivors

Fit Group

Oneway Analysis of Calcium By Alive or deceased



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Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
Alive	36	810.000	738.000	22.5000	3.225
Deceased	4	10.000	82.000	2.5000	-3.225

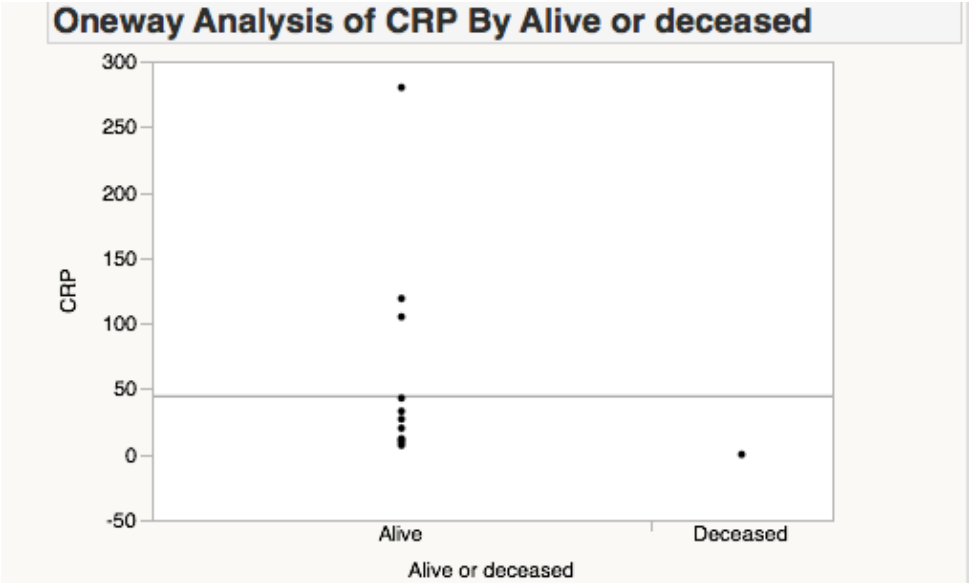
2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
10	-3.22513	0.0013*

1-way Test, ChiSquare Approximation

ChiSquare	DF	Prob>ChiSq
10.5475	1	0.0012*

Figure 23: Serum Ionized Calcium in survivors and non-survivors



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Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
Alive	11	110.000	88.000	10.0000	2.832
Deceased	4	10.000	32.000	2.5000	-2.832

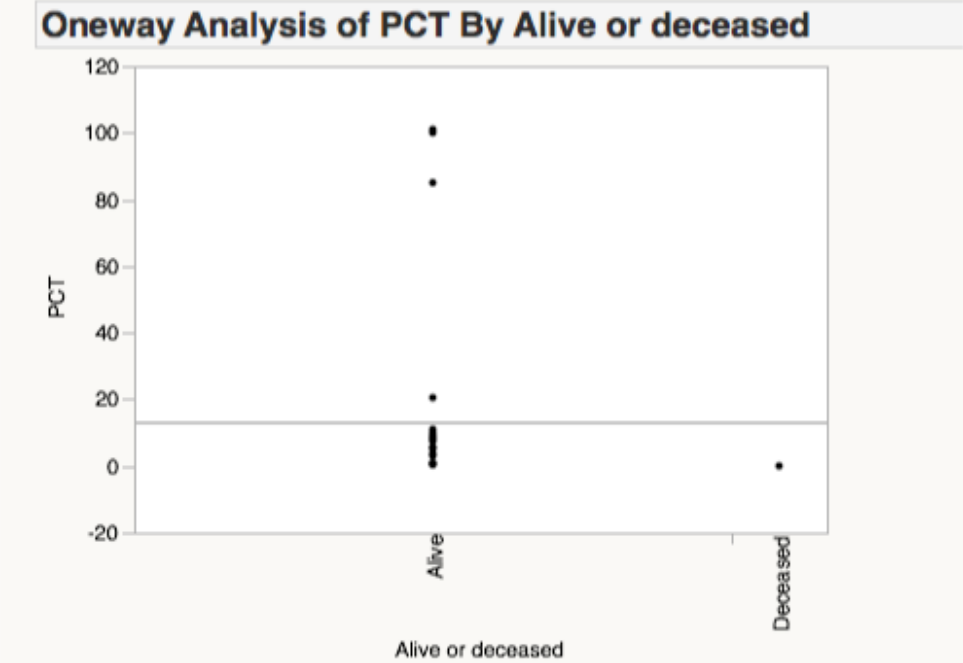
2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
10	-2.83241	0.0046*

1-way Test, ChiSquare Approximation

ChiSquare	DF	Prob>ChiSq
8.4000	1	0.0038*

Figure 24: CRP in survivors and non-survivors



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Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
Alive	25	425.000	375.000	17.0000	3.135
Deceased	4	10.000	60.000	2.5000	-3.135

2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
10	-3.13490	0.0017*

1-way Test, ChiSquare Approximation

ChiSquare	DF	Prob>ChiSq
10.0272	1	0.0015*

Figure 25: PCT in survivors and non-survivors

11.3. Measure of correlation and association

Assessment of different non-parametric measures of correlation were used to further clarify the relationship between serum ionised calcium and phosphate concentrations. The Kendall Tau co-efficient was utilized to ascertain the measure of association; and the Hoeffdigs D co-efficient used to determine if the two variables were independent from one another.

A significant positive correlation was demonstrated between ionised calcium and serum phosphate level on determination of the Spearman rank co-efficient (ρ = 0.566, p= 0.0004). Similarly, the Kendall Tau co-efficient analysis revealed a significantly positive association between the two variables (τ= 0.427, p= 0.0004) and the Hoeffdigs D co-efficient showed significant dependence (D= 0.12, p= 0.0001). These findings are summarised in figure 26. As shown in figure 27, none of the of other variables analysed demonstrated a significant correlation; this might be due to limited sample size and missing data.

In summary, these non-parametric measures of correlation clearly illustrated both the dependency and association between hypophosphatemia and low serum ionised calcium levels. Noteworthy is the unanticipated role of serum ionised hypocalcaemia in the context of severe acute malnutrition and refeeding as it has significant implications for the management of hypophosphatemia in this patient population.

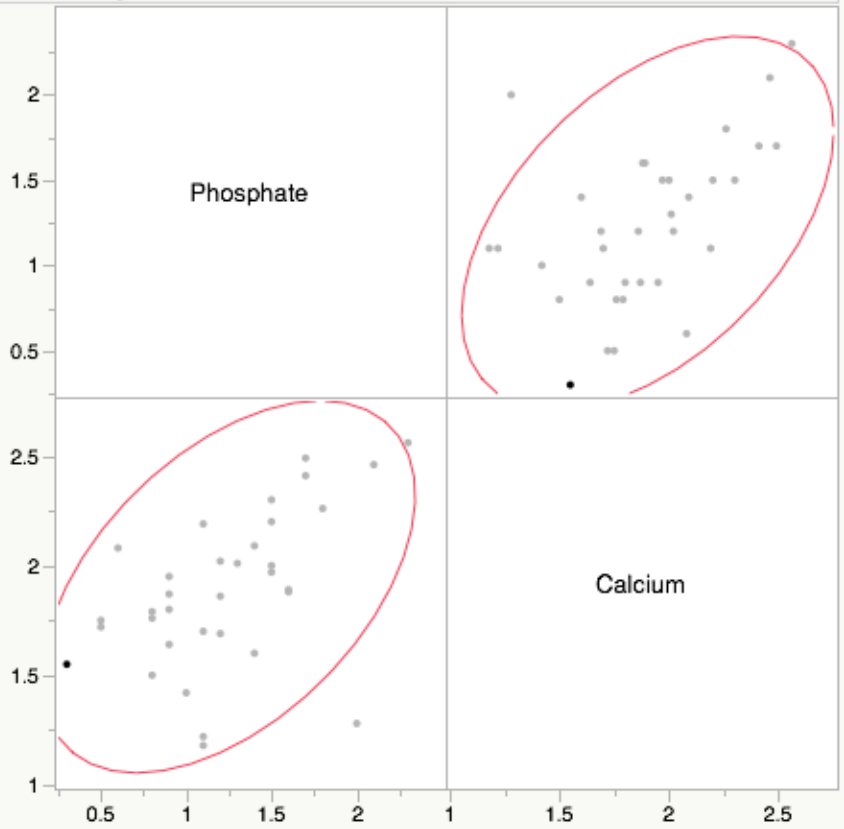
Multivariate

Correlations

	Phosphate	Calcium
Phosphate	1.0000	0.4932
Calcium	0.4932	1.0000

There are 4 missing values. The correlations are estimated by REML method.

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Calcium	Phosphate	0.5661	0.0004*	

Nonparametric: Kendall's τ

Variable	by Variable	Kendall τ	Prob>|τ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Calcium	Phosphate	0.4271	0.0004*	

Nonparametric: Hoeffding's D

Variable	by Variable	Hoeffding D	Prob>D	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Calcium	Phosphate	0.1243	0.0001*	

Figure 26: Multivariate analysis of Phosphate and Ionised Calcium

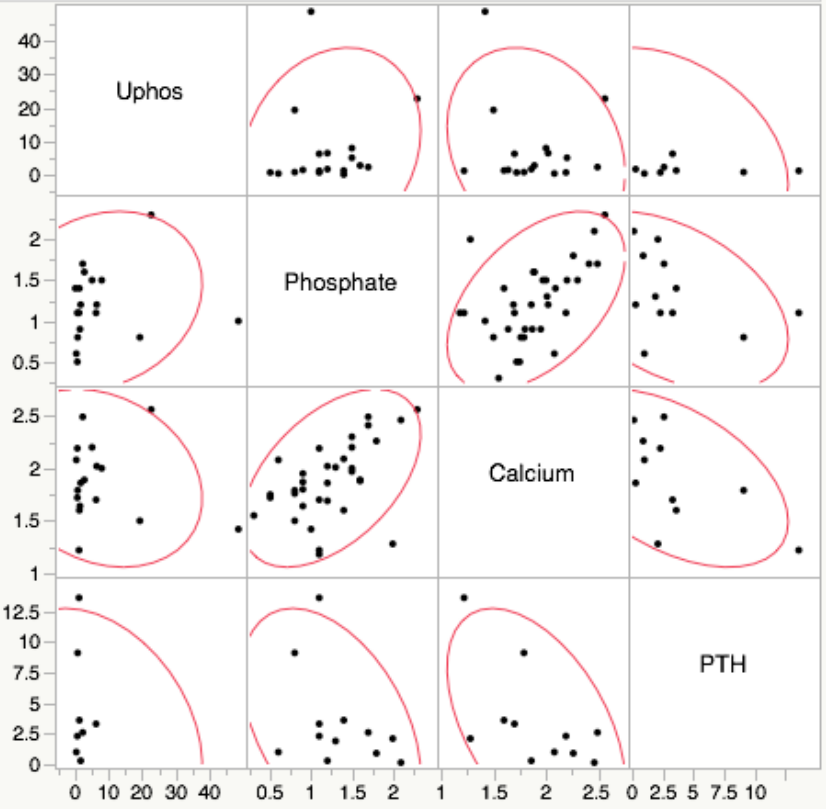
Multivariate

Correlations

	Uphos	Phosphate	Calcium	PTH
Uphos	1.0000	0.1889	-0.2271	-0.3414
Phosphate	0.1889	1.0000	0.4959	-0.4307
Calcium	-0.2271	0.4959	1.0000	-0.4894
PTH	-0.3414	-0.4307	-0.4894	1.0000

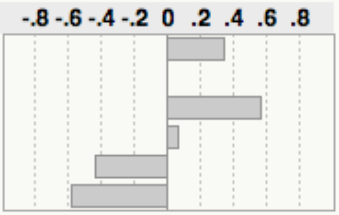
There are 31 missing values. The correlations are estimated by REML method.

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|
Phosphate	Uphos	0.3515	0.1526
Calcium	Uphos	-0.0037	0.9888
Calcium	Phosphate	0.5661	0.0004*
PTH	Uphos	0.0714	0.8665
PTH	Phosphate	-0.4366	0.1558
PTH	Calcium	-0.5818	0.0604



Nonparametric: Kendall's τ

Variable	by Variable	Kendall τ	Prob>|τ|
Phosphate	Uphos	0.2618	0.1372
Calcium	Uphos	0.0221	0.9016
Calcium	Phosphate	0.4271	0.0004*
PTH	Uphos	0.0714	0.8046
PTH	Phosphate	-0.3257	0.1463
PTH	Calcium	-0.4182	0.0734

Figure 27: Multivariate analysis of variables associated with Phosphate homeostasis

12. Discussion

In this pilot study we utilised known physiologic models to clarify factors associated with hypophosphatemia during refeeding of children with severe acute malnutrition. Measurements of homeostatic parameters were used to expand upon known trends and indicators of calcium and phosphate pathophysiology in severe acute malnutrition. Our hypothesis of urinary phosphate loss as mediated by parathyroid hormone was confirmed. However, the significant correlation and association between low serum ionised calcium and low serum phosphate was an unanticipated finding.

The demographics of our study population were in keeping with children admitted to our gastroenterology unit with oedematous malnutrition, indicating good generalizability within our greater patient population. The Gastroenterology Unit at Tygerberg Children's Hospital is a tertiary referral unit, thereby potentially introducing a selection bias. However, the need for micronutrient and electrolyte support is more pronounced in the severely malnourished patient, necessitating close biochemical monitoring.

A disparity between suggested feed initiation and escalation as per the treatment protocol was identified. In this patient population feeds were initiated between 60 - 90 ml/kg/day, with inconsistent feed build up. Current unit recommendations suggest feed initiation at 40 - 60 ml/kg/day with escalation in aliquots of 20 ml/kg/day. Caloric intake mirrored this trend. Careful scrutiny and monitoring of feed prescription should be undertaken to minimise the risk for development of refeeding syndrome in children with severe acute malnutrition.

Of note in this cohort was the presence of baseline hypocalcaemia in the absence of hypophosphatemia. It is our experience that while malnourished patients are often admitted with normal serum phosphate levels, many develop hypophosphatemia during refeeding. This has resulted in a policy of pre-emptive phosphate supplementation, both by intravenous and oral means. The majority of patients (70%, n=7) in our study were initiated on phosphate supplementation at a mean day of 1.14 (SD: 0.37) despite a cohort median serum phosphate of 1.30 mmol/l (IQR: 0.90; 1.40) on admission.

The guidelines for phosphate supplementation in severe acute malnutrition recommend a phosphate dose of 75 - 100 mg/kg/day. The solution used for phosphate supplementation at Tygerberg Children's Hospital is a formulation comprising of 0.8 g Na_2HPO_4 + 0.2g KH_2PO_4 + 10 ml H_2O equating to 100 mg/ml of elemental phosphate or 0.78 mmol/ml. Based on mmol/kg/day dosing this equates to a dosage range of 0.58 - 0.78 mmol/kg/day. In our study the mmol/kg/day dose of phosphate was found to be a median of 0.53 mmol/kg/day (IQR: 0.37; 0.9). The median dose administered in our population was lower than the recommended dosing range, indicating a predilection by the treating physicians. The fact that the median serum phosphate level on admission was within normal limits may account for the dosage selection.

Despite phosphate supplementation and the normal median serum phosphate level on admission, serial serum phosphate measurements revealed a downward trend confirming our prior experience. Phosphate levels reached a nadir on day 7 with a low to normal median serum phosphate of 1.15 mmol/l (IQR: 0.82; 1.5). This finding alludes to a mechanism resulting in phosphate loss, in spite of supplementation.

As proposed in the hypothesis, renal tubular phosphate losses via tubulopathy and/or phosphatonin action may be contributory in the development of hypophosphatemia in children with severe acute malnutrition. Corroborating evidence for potential phosphatonin mediated phosphate wasting was provided through analysis of the threshold for renal phosphate reabsorption (TmP/GFR). However, what has been clearly demonstrated is that low serum ionised calcium is a significant driving force behind parathyroid hormone induced urinary phosphate loss.

A study conducted in Mexican children with severe acute malnutrition demonstrated hypocalcaemia as a consequence of decreased albumin bound fraction with normal serum ionised calcium concentrations, there was also preservation of total serum calcium levels [32]. Hypocalcaemia has also been described in Indian marasmic children wherein the total ionised serum calcium levels were only moderately reduced [34]. Both studies found that serum calcium levels were maintained during refeeding. These findings are in contradiction to our sample that maintained low median serum ionised calcium until day 20 of refeeding.

The contribution of hypovitaminosis D to low serum ionised calcium levels was not addressed in this study and is thus a limitation. There is conflicting evidence regarding the prevalence of vitamin D deficiency in children with oedematous malnutrition. The prevalence of vitamin D deficiency in healthy rural Ethiopian school children was described by Herrador; showing a mean 25-hydroxy D level of 80.1 +/- 26.24 nmol/l, in keeping with vitamin D insufficiency [47]. Earlier studies in Brazilian children showed no difference in vitamin D levels when malnourished children were compared to healthy controls [48].

In the setting of severe acute malnutrition the use of surrogate markers for vitamin D deficiency is limited. Alkaline Phosphatase concentrations have been demonstrated to have low sensitivity and specificity for metabolic bone disease in this population [49]. Further examination of vitamin D status and contribution to calcium and phosphate homeostasis would be needed in order to better clarify treatment options. Additionally impaired hepatic hydroxylation of vitamin D secondary to hepatic steatosis and phosphatonin-mediated derangements in renal 1-α hydroxylation may also play a role; necessitating additional supplementation with vitamin D.

The Spearman rank co-efficient indicated a significant positive correlation between serum ionised calcium and serum phosphate concentration (p= 0.004). This confirms the expected physiologic

model and also indicates that this homeostatic relationship is preserved in severe acute malnutrition. Additionally, the strength of correlation may allude to hypophosphatemia being driven by low serum ionised calcium levels.

Although this study demonstrated non-significant correlations between urinary and serum phosphate as well as between urinary phosphate and serum ionised calcium, their nature confirmed what is known to be physiologically accurate. The negative correlation between urinary phosphate and serum ionised calcium was observed as expected, as rising serum calcium concentrations provide negative feedback to the parathyroid gland resulting in inhibition of PTH secretion. The anticipated positive correlation between urinary and serum phosphate was observed, consistent with phosphatonin mediated lowering of the renal tubular threshold for phosphate excretion [10, 12, 29]. The persistently low TmP/GFR during refeeding further supports the hypothesis of phosphatonin and PTH contribution in this setting.

Phosphatonins are proteins associated with negative phosphate balance; of particular interest to renal phosphate handling is fibroblast growth factor 23 (FGF23). This recently discovered phosphatonin is secreted by osteoclasts and is known to be significant in tumour mediated, x-linked and renal failure associated hypophosphatemia. FGF 23 acts on Na/Pi type IIa renal cotransporters resulting in urinary phosphate wasting; with the inhibition of 1-α hydroxylase activity resulting in exacerbation of urinary phosphate loss due to secondary hypocalcaemia [10, 29]. In this study, the possible contribution of FGF23 is supported by the body of evidence encompassing: the persistence of baseline hypocalcaemia, the positive correlation between urinary and serum phosphate concentrations, and the low TmP/GFR values that did not improve on nutritional rehabilitation.

Despite baseline ionised hypocalcaemia, parathyroid hormone levels on admission fell within the age appropriate reference range. A rise in PTH secretion was observed secondary to a negative trend in ionised calcium levels; this could be attributed to an altered threshold for PTH secretion. Mathematical modelling of PTH secretion in response to changes in serum ionised calcium levels have demonstrated an asymmetric reverse sigmoid relationship in healthy adult patients. Analysis of steady state dynamics revealed a threshold for ionised calcium of 1.2 mmol/l, above which PTH secretion spiked [50]; this delay in PTH response may account for the normal levels on admission. The effect of serum magnesium concentrations on PTH secretion was also considered. While hypomagnesaemia may either impair or promote PTH secretion via complex interactions with the alpha-unit of parathyroid gland G-coupled proteins [51], it was not observed in our cohort.

Metabolic acidosis has been show to cause renal tubular dysfunction; this may result in phosphaturia [30, 31]. However, the three patients in our study who developed metabolic acidosis did not exhibit worsening phosphaturia. Additionally sodium bicarbonate infusions were not prescribed in the treatment of these patients, negating the effect on hypocalcaemia.

Serum phosphate levels have been proposed as a surrogate marker for sepsis and systemic inflammation in critically ill adult patients [36, 38]. Sauerwein demonstrated a tendency towards elevated levels of C-reactive protein in children with severe acute malnutrition, despite no clear source of infection [52]. Similarly, Page confirmed that at baseline both PCT and CRP are elevated in malnourished children; with CRP found to have the best positive predictive value with regards to mortality (sensitivity 81%, specificity 58%) [53]. In the setting of systemic inflammation and infection, procalcitonin is primarily produced by the neuroendocrine cells of the lungs and intestines, not the parafollicular C cells of the thyroid [54]. This mechanism has been confirmed by studies of systemic inflammation in thyroidectomised patients.

Although not statistically significant, our analysis confirmed the negative correlation between serum phosphate and inflammatory markers, as found in adult studies. However, a significant negative correlation was detected between ionised calcium and PCT (ρ = -0.67, p= 0.0012). Bearing in mind the origin of PCT in systemic inflammation and the role played by calcium in lymphocyte activation and gene transcription, hypocalcaemia could also be considered as a surrogate marker for infection [55]. As there is some epidemiologic evidence of the role in vitamin D and infectious risk, vitamin D status should not be disregarded in this context [56]. The role of both vitamin D and calcium concentrations in relation to infectious risk requires further exploration in this clinical context.

Median serum ionised calcium (p= 0.0013) and median phosphate concentrations (p= 0.0012) differed significantly between survivors and non-survivors, with similar findings observed on consideration of CRP (p= 0.0046) and PCT (p= 0.0017). These results confirm paediatric mortality data regarding children with severe acute malnutrition and elevated inflammatory markers, as well as adult mortality data regarding hypophosphatemia [53, 2]. Surprisingly, a significant difference in median PCT was detected between survivors and non-survivors; this has not been demonstrated in paediatric studies involving malnourished children.

In light of our findings, the relationship between low serum ionised calcium as well as vitamin D status and hypophosphatemia warrants further investigation. The role of parathyroid hormone and renal tubular phosphate excretion mirrored an expected physiologic response in relation to low serum ionised calcium levels. This is suggestive of an intact physiologic response to hypocalcaemia, however the aetiology of the low serum ionised calcium requires further clarification. Vitamin D deficiency has been described in healthy African children; the contribution of vitamin D may have a significant impact on the treatment of hypocalcaemia and hypophosphatemia in children with oedematous malnutrition. In addition, hepatic steatosis may impair the capacity of hydroxylation of vitamin D3, necessitating supplementation with vitamin D. In 2011, utilising the US NHANESII database (National health and nutritional examination survey), Liangpunsakul observed a correlation between adult patients with elevated ALT and hypovitaminosis D [57]. Targher confirmed this with histologic evidence of hepatic steatosis in vitamin D deficient adults [58].

In adults, an AST/ALT ratio greater than one occurs in patients with hepatic steatosis (in addition to patients with muscle disease and alcoholic hepatitis). Limited adult studies have demonstrated a significant correlation between an AST/ALT ratio of >1 and histologic evidence of hepatic steatosis [44]. Studies in children have failed to demonstrate a similar correlation. The aetiology of hepatic steatosis in childhood malnutrition is poorly understood, however impairment of VLDL synthesis and exportation of free fatty acids have been implicated [44]. This mechanism differs from NAFLD in the obese child, which is largely attributed to insulin resistance. Elevation of AST and ALT in the context of severe acute malnutrition has previously been demonstrated in a cohort of Bangladeshi children; whereby the AST and ALT were correlated with malnutrition severity based on the Gomez classification. When compared to healthy controls, all children with severe acute malnutrition had significantly increased AST and ALT serum concentrations ($p < 0.001$). It is hypothesized that the origin of these transaminases may be indicative of either hepatic injury or tissue breakdown (striated muscle and myocardium) [59]. This finding may be indicative of hepatic steatosis in this specific population but would require further investigation for confirmation.

Whilst not confirmed, the initial hypothesis regarding the aetiology of hypophosphatemia in children recovering from oedematous malnutrition has shed light on previously unconsidered factors. This pilot study has highlighted the necessity to investigate calcium and vitamin D homeostasis more closely in malnourished children. The contributions of hepatic steatosis as well as limitations in inflammatory markers are areas that could benefit from further investigation. It is reassuring to note the preservation of normal physiologic response to calcium and phosphate homeostasis in children with severe acute malnutrition. Extending the current treatment regimen of hypophosphatemia by inclusion of calcium and vitamin D may provide a significant reduction in the morbidity and mortality associated with severe acute malnutrition.

13. Conclusions and Recommendation

13.1. Conclusion

The aim of this pilot study was to investigate the relationship between hypophosphatemia and phosphaturia in children recovering from severe acute malnutrition. In the context of this study the relationship has been shown to be appropriately physiologic in the face of prolonged serum ionised hypocalcaemia. The PTH driven loss of phosphate resulted in hypophosphatemia secondary to hypocalcaemia. In addition, alterations in the renal threshold of phosphate reabsorption secondary to possible phosphatonin action may also be contributory. If hypocalcaemia is addressed and treated, resultant phosphate normalisation may follow.

Derangements of calcium homeostasis were linked to abnormal phosphate metabolism, inflammation, and potentially vitamin D metabolism. Implications of normalisation of serum ionised calcium, and possibly vitamin D may have systemic and survival benefits in children with severe acute malnutrition.

13.2. Recommendations

Current local and WHO recommendations regarding the treatment of hypophosphatemia in children with oedematous malnutrition may need revision to include calcium supplementation. The addition of vitamin D warrants further investigation. Further areas of interest for future investigation generated from this pilot study include:

1. Determination of vitamin D levels in children with severe acute malnutrition
2. Investigation of the prevalence of hypovitaminosis D in children with severe acute malnutrition and biochemical/radiologic evidence of hepatic steatosis
3. The sensitivity and specificity of CRP and PCT in the setting of severe acute malnutrition
4. Phosphate as a surrogate marker of infection in children

13.3. Implications and limits of generalizability of findings

The sample population investigated in this pilot study was representative of the children admitted to the Tygerberg Children's Hospital gastroenterology unit. Although the results differ from studies done at other centres, this may be accounted for by the limited sample size. A larger study would further clarify the mechanism of hypocalcaemia and other contributory factors; this would also enable more appropriate comparison with other studies.

Appendices

Appendix A: WHO guidelines for treatment of Severe Acute Malnutrition

GENERAL PRINCIPLES FOR ROUTINE CARE

(The '10 Steps')

There are ten essential steps:

1. Treat/prevent hypoglycaemia
2. Treat/prevent hypothermia
3. Treat/prevent dehydration
4. Correct electrolyte imbalance
5. Treat/prevent infection
6. Correct micronutrient deficiencies
7. Start cautious feeding
8. Achieve catch-up growth
9. Provide sensory stimulation and emotional support
10. Prepare for follow-up after recovery

These steps are accomplished in two phases:

1. An initial **stabilisation phase:** Management of acute medical conditions
2. A longer **rehabilitation phase**

Note that treatment procedures are similar for marasmus and kwashiorkor

Ashworth A et al, Ten steps to recovery: Child health dialogue, issue 3 and 4 1996

Appendix B: Malnutrition refeeding regimen of Tygerberg Children's Hospital

Classification

1. Use the Welcome-Trust Classification to distinguish between UWFA and SAM

The Underweight Child	SAM
1. Screen for Tuberculosis • CXR • Mantoux • Tine 2. Check Haemoglobin • If ≤ 8g/dl do formal FBC • Consider iron supplementation 3. Deworm 6 monthly 4. Multivitamin syrup	1. Bloods (twice weekly initially) • FBC • Blood culture, CRP • U&E, LFT's, CMP 2. Screen for Tuberculosis • CXR • Mantoux • Tine 3. Urine for MC&S 4. Stool for MC&S and parasites

Management

1. Follow SAM feeding regimen
   - Because of dangers of refeeding rather go slow than fast
2. Refer the patient to the dietician as soon as possible
3. Start with the following as soon as possible:
   - Deworming
   - Antibiotics
   - Follow gastro regimen for treatment of diarrhoea
4. Give micronutrient supplementation
5. When the patient is ready for discharge, refer him/her to the nutritional support programme to ensure that he/she will be followed up in the community

Micronutrient supplementation

1. **Vitamin A**
   - 0-5 months: 50 000 IU orally
   - 6-11 months (<10 kg): 100 000 IU orally for 1 day
   - 12-60 months (>10 kg): 200 000 IU orally for 1 day

   In case of measles or xeropthalmia repeat dose age appropriate dose after 24 hours

2. **Folate**
   - 2.5 mg daily for 7 days thereafter 2.5 mg every second day until discharge or 21 days
3. **Potassium**
   - 3-6 mmol/kg/day until oedema has cleared
4. **Magnesium**
   - 1-2 mmol/kg/day according to values
5. **Phosphorous**
   - 75-100 mg/kg/day TBH (0.8 g Na_2HPO_4 + 0.2 g KH_2PO_4 + 10 ml H_2O) provides 100 mg/ml
6. **Zinc**
   - 1-2 mg/kg/day until discharged or for +/- 21 days
7. **Multivitamins (without iron)**
   - Give a standard dose e.g. Vidaylin or abidec 0.6 ml/day until discharged
8. **Iron**
   - 6 mg/kg is started once threat of infection has passed, usually after 2 weeks of hospitalization

Resuscitation

No milk or solids while resuscitating

If in shock: Give 20 ml/kg Haemacel, human serum or crystalloids intravenously
Once shock has resolved rehydrate and administer fluids via nasogastric tube (NGT)

If not in shock: Resuscitate with oral rehydration fluid and avoid unnecessary IV fluids
Excess fluid may precipitate cardiac failure and must only be used when the patient is severely dehydrated or in shock.

Start with the medication and micronutrient supplementation

Total Volume ORS (e.g. Sorol)= (Rehydration Fluid) + (Maintenance Fluid) + (On-going losses)

<table>
<tr><td>Maintenance Fluid (MF)</td><td colspan="2"><10 kg: 120 ml/kg/day
10-20 kg: 1000 ml + 50 ml (weight: -10 kg)</td></tr>
<tr><td>Rehydration Fluid (RF)</td><td colspan="2">5% dehydrated = 50 ml/kg ORF
10% dehydrated = 100 ml/kg ORF</td></tr>
<tr><td rowspan="3">On-going losses (OL)</td><td>Moderate diarrhoea</td><td>50 ml/kg/day ORF extra</td></tr>
<tr><td>Severe diarrhoea</td><td>100 ml/kg/day ORF extra</td></tr>
<tr><td colspan="2">The method of using ORF after each loose stool should only be used when the diarrhoea is starting to clear, give 10-20 ml/kg after each loose stool</td></tr>
</table>

General aspects regarding refeeding

1. **Route:** If oral intake is poor use a NGT (nasogastric tube)

2. **Frequency:** 3 hourly (8 bolus feeds/day) 2 hourly feeds are only Needed in cases of severe hypoglycaemia/hypothermia.

3. **Type of milk:** Always start with a relatively low protein formula. Don't use a follow up milk (e.g. lactogen II)

- If the period of diarrhoea is very short or if not proven to be lactose intolerant, breast milk or a normal starter formula (e.g. Nan I or Lactogen I) can be used
- If lactose intolerant use a low lactose (e.g. Nan Pelargon) or a lactose free formula (Isomil or infasoy)
- Consider the use of a semi-elemental feed (e.g. Alfare) in cases of severe SAM (e.g. a very low albumin) or in suspected protein sensitivity

Volume of milk: Follow the SAM regimen

Usually the starting volume is 60ml/kg/day that is increased y 20ml/kg/day more than what was given the previous day up to a maximum of 120ml/kg/day.
In severe cases the recommended starting dose is 40ml/kg/day of a semi-elemental feed.

Always remember
"A little nutritional support is good but too much is lethal"

The SAM Feeding Regimen of Tygerberg Hospital

Day 1

- **Solids:** None
- **Milk feeds:** 60ml/kg/day (start with 40ml/kg in severe cases)
- **Sorol:** None
- **Frequency:** Small frequent feeds (e.g. 3 hourly)
- **Route:** NGT if oral intake is poor

Day 2

- **Solids:** None
- **Milk feeds:** 80ml/kg/day (give 20ml/kg/day more than the actual intake of the previous day)
- **Sorol:** Total volume= (MF + OL) – milk feeds
- **Frequency:** Frequent feeds 3-4 hourly
- **Route:** NGT if oral intake is poor

Day 3

- **Solids:** **Only** if there are clear signs of improvement
- **Milk feeds:** **100ml/kg/day**
- **Sorol:** Total volume = (MF + OL) – milk feeds
- **Frequency:** Adapt as needed
- **Route:** Adapt as needed

Day 4

- **Solids:** If rice-porridge was started on day 3 and taken well, consider giving rice porridge twice daily
- **Milk feeds:** If solids were taken well keep the milk volume the same. If solids not yet given (e.g. baby too young) or if solids are not taken well, increase the milk to 120ml/kg.
- **Sorol:** Total volume = (MF + OL) – milk feeds

Day 5

- **Solids:** Adapt as needed e.g. order puree/baby diet if the porridge was eaten well
- **Milk feeds:** Order the milk depending on the intake of solids
    1. If under 6 months or in not yet able to eat, order at least 120 ml/kg/day this may be gradually increased up to a maximum of 150 ml/kg only if the patient is improving
    2. If only rice-porridge is taken, give at least 120ml/kg milk
- **Sorol:** On-going losses

Day 6 and onwards

- **Solids, milk feeds & sorol:**
    1. If not yet eating order 120-150ml/kg (same guideline as day 5)
    2. Once eating rice porridge well, continue with about 120ml/kg milk and gradually change the diet (e.g. from a puree to a baby diet) until the appropriate diet for age or weight-age

Ensure that the diet provides a total of 150 to 200 kCal/kg/day by supplementing the diet with energy in the form of carbohydrate and fat (e.g. using polycose, oil and/or margarine)

Note: Supplementation of energy

1. Energy in the form of carbohydrate (e.g. polycose) may be supplemented from day 3 of feeding to provide a maximum of 10kcal/kg/day extra.
2. Solids are not introduced, neither is oil supplemented if the patient is not improving (e.g. oedema not resolving, is still apathetic and irritable and albumin is not increasing)

Day 14

Iron supplementation (6mg/kg/day) is started on day 14 if resolved oedema, diarrhoea and infections (the CRP level must be less than 10)

Discharge Criteria

1. Appetite improved
2. No infection
3. Oedema resolved
4. Normal weight-height ratio/child is thriving
5. Mother understands reasons for SAM and its prevention
6. Follow up has been organised at malnutrition clinic in the community.

Tygerberg Children's Hospital SAM Feeding Regimen, revised in 2004, Dr E Nel

Appendix C: Participant Information Leaflet and Consent Form

Title of Research Project:

The Aetiology of Hypophosphatemia in Children Recovering from Kwashiorkor

REFERENCE NUMBER: N11/10/312
PRINCIPAL INVESTIGATOR: Dr Simone Nicol
ADDRESS: Tygerberg Hospital, Belville, Cape Town
CONTACT NUMBER: 021 938 4911

Your child is being invited to take part in a research project. Please take some time to read the information presented here, which will explain the details of this project. Please ask the study staff or doctor any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how you could be involved. Also, your participation is entirely voluntary and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do agree to take part.

This study has been approved by the Health Research Ethics Committee (HREC) at Stellenbosch University and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines for Research.

1. What is this research study all about?

1.1. This study will take place in Tygerberg Children's Hospitals paediatric gastrointestinal ward and will involve ten patients.

1.2. This study is looking at children with who swell up because of an imbalanced diet that causes kwashiorkor and the way in which an important salt in the blood called phosphate is lost. Kwashiorkor is a condition that children develop when they do not get the correct type or amount of food. We are going to look at the amount of phosphate in the urine and blood as well as hormones and signs of infection, which may affect the amount of phosphate in the blood. Phosphate is a salt, which is important for normal growth and development of many parts of the body including muscle and bone. Children that have too little phosphate develop problems with their bones and are often sick with infections. The kidney is important in keeping the phosphate levels normal because it prevents phosphate from being lost in the urine.

1.3. All patients with kwashiorkor are admitted to the ward (G7) and are started on vitamins and milk feeds or a drip. We usually draw blood every day for the first week and then twice a

week after that to make sure that your child does not develop serious complications. For this study we will also take a small amount of extra blood to do more tests. We will not draw blood from your child unless the doctors who are looking after your child are drawing blood that they would normally have taken.

1.4. Your child will receive vitamins and supplements as well as antibiotics as needed. This will be done as part of the routine treatment of all patients with malnutrition.

2. Why have you been invited to participate?

2.1. You have been asked to participate in this study because your child has kwashiorkor.

3. What will your responsibilities be?

3.1. This study will be conducted whilst your child is in hospital and will end once your child has been discharged.

4. Will you benefit from taking part in this research?

4.1. Your child will receive the treatment for their illness that they would normally receive. The results obtained from this study will give us some answers as to why children with this condition have low phosphate in their blood and will help us treat children in future.

5. Are there in risks involved in your taking part in this research?

5.1. The amount of extra blood that is taken during the study is so little that it will not be dangerous for your child.

6. If you do not agree to take part, what alternatives do you have?

6.1. If you do not agree to take part in the study your child will still get the same care.

7. Who will have access to your medical records?

7.1. The information will be collected by Dr Simone Nicol and will be kept in a safe place. Your child's name will not be used and nobody else will be able to look at the information. When we publish the study we will not use your child's name or identify him/her in any way.

8. Will you be paid to take part in this study and are there any costs involved?

8.1. No you will not be paid to take part in the study. There will be no costs involved for you, if you do take part.

8.2. You can contact Dr Simone Nicol at 083 533 6564 if you have any further queries or encounter any problems.

8.3. You can contact the Health Research Ethics Committee at 021-938 9207 if you have any concerns or complaints that have not been adequately addressed by your study doctor.

8.4. You will receive a copy of this information and consent form for your own records.

Declaration by participant

By signing below, I _____ agree to take part in a research study entitled

"The Aetiology of Hypophosphatemia in Children Recovering from Kwashiorkor"

I declare that:

1. I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable
2. I have had a chance to ask questions and all my questions have been adequately answered
3. I understand that taking part in this study is voluntary and I have not been pressurised to take part
4. I may choose to leave the study at any time and will not be penalised or prejudiced in any way
5. I may be asked to leave the study before it has finished, if the study doctor or researcher feels it is in my best interests, or if I do not follow the study plan, as agreed to.

Signed at (place)_____ on (date) _____20

_____ _____

Signature of participant Signature of witness

Declaration by investigator

I (name) _____ declare that:

1. I explained the information in this document to _____
2. I encouraged him/her to ask questions and took adequate time to answer them
3. I am satisfied that he/she adequately understands all aspects of the research, as discussed above
4. I did/did not use an interpreter. (If an interpreter is used then the interpreter must sign the declaration below

Signed at (place) _____ On (date)_____20

_____ _____

Signature of investigator Signature of witness

Declaration by interpreter

I (name) _____ declare that:

1. I assisted the investigator (name) _____ to explain the information in this document to (name of participant) _____ using the language medium of Afrikaans/Xhosa
2. We encouraged him/her to ask questions and took adequate time to answer them
3. I conveyed a factually correct version of what was related to me
4. I am satisfied that the participant fully understands the content of this informed consent document and has had all his/her question satisfactorily answered

Signed at (place)_____On (date)_____20.

_____ _____

Signature of interpreter Signature of witness

Appendix D: Data Collection Sheets

Biochemical and infective parameters

Patient:

	U&E	CMP	Urinary Creatinine	Urinary Phosphate	Total Protein	LFTs	Albumin	PTH	PCT	CRP	Blood culture	Stool Culture	Urine Culture	TB
Admission														
Day 3														
Day 7														
Day 14														
Day 20														

Anthropometric parameters

Patient:

DOB & sex:

	Weight (kg)	Length/height (cm)	Mid upper arm circumference	Z-score weight	Z-score height
Admission					
Day 3					
Day 5					
Day 7					
Day 9					
Day 11					
Day 14					
Day 16					
Day 18					
Day 20					

Clinical Parameters

Patient:

	Random Glucose	Hypothermia	Pyrexia	Liver Span	Skin lesions	Oedema	Infections
Admission							
Day 3							
Day 5							
Day 7							
Day 9							
Day 11							
Day 14							
Day 16							
Day 18							
Day 20							

Feeds and fluids

Patient:

	Type of feed	Amount of feed	Type of fluids	Amount of fluids
Admission				
Day 3				
Day 5				
Day 7				
Day 9				
Day 11				
Day 14				
Day 16				
Day 18				
Day 20				

Appendix E: Anthropometry

1. Weight measurement

- Patients will be weighed on the same standardised scale
- The scale will be calibrated before every patient is weighed
- Patients will be weighed without clothing, nappies or shoes
- Patient will be weighed three times in order to ensure accuracy and an average will be taken of these three weights
- Weights will be plotted on a standardised growth chart as Z- scores [60]

2. Length measurements

- Patients length will be measured using the same length board
- Length will be measured without clothing, nappies or shoes
- Patients length will be measures three times in order to ensure accuracy and an average will be taken [61].

3. Mid-Upper arm circumference (MUAC)

- MUAC is determined by measuring the circumference of the upper arm by using a standardised tap.
- The midpoint of the child's arm will be located by measurement from the shoulder to the elbow
- The MUAC will then be measured and recorded to the nearest 0.1cm [61]

Weight and height are both good indicators of nutritional status and wellbeing; however they fail to differentiate between fluid, fat and muscle. MUAC is a better reflection of lean muscle mass and is less affected by fat and fluid [61].

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