LACTOSE MALABSORPTION AND DIARRHOEA IN CHILDREN WITH SEVERE ACUTE MALNUTRITION

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of

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DECLARATION

I, Britta Jane McLaren declare that this research report is my own work. It is being submitted for the degree of Master of Medicine in the branch of Paediatrics, in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree of examination at this or any other University.

…….

22nd day of September 2015.

PUBLICATIONS AND PRESENTATIONS

Presentations

- SAPA and SAAPS 2014 Congress – Registrar oral presentation
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ABSTRACT

Malnutrition and diarrhoea are major causes of childhood morbidity and mortality in the developing world. Lactose malabsorption has been associated with diarrhoea in malnourished children, but they are often managed with lactose containing feeds. This study quantified the prevalence of lactose malabsorption in children with severe acute malnutrition (SAM) and diarrhoea admitted to an urban South African hospital. Sixty-three Children with SAM and diarrhoea were included in the study and had their stool tested for reducing substances using the Benedict's test. Fifty-nine percent had stool positive for reducing substances (≥0.5g%). After multivariate analysis, age of <12 months was the only factor found to significantly predict positive reducing substances (LR 4, p=0.046). Death was 4 times more likely in children with positive reducing substances (p=0.035). The role of lactose free feeds in children with SAM and diarrhoea has not been adequately explored.

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TABLE OF CONTENTS

LIST OF FIGURES

Page

LIST OF TABLES

Page

1.0 INTRODUCTION

Malnutrition is a major contributor to the global burden of under-five disease and mortality (1). Wasting and severe wasting, defined as weight-for-height less than -2 and -3 standard deviations of the World Health Organization (WHO) Child Growth Standards, respectively, reflect acute malnutrition and are responsive to fluctuations in health and environment such as infections and changes in food security (1). As of 20 September 2013, joint global estimates by WHO, The United Nations Children's Fund (UNICEF) and the World Bank were that 17 million children under five are severely wasted, with an estimated prevalence of just less than 3%. Approximately 71% of all severely wasted children live in Asia and 28% in Africa. In Sub-Saharan Africa, the prevalence of severe wasting was estimated to be 9%. In South Africa, the most recent estimate of the prevalence of wasting in children under five years old was 4.7% in 2008 (1).

Severe acute malnutrition (SAM) is defined in table 1.1 below. Any one of the three criteria in the table is required to diagnose SAM (2).

Table 1.1: Diagnostic criteria for SAM in children aged 6–60 months (2)

Indicator	Measure	Cut-off
Severe wasting	Weight-for-height *	< -3 SD
Severe wasting	Mid Upper Arm Circumference (MUAC)	< 115 mm
Bilateral oedema	Clinical sign	

*Based on WHO Standards (www.who.int/childgrowth/standards)

Diarrhoea is a major cause of mortality in children with SAM (3-6). A Zambian study involving children with SAM found that the risk of dying was two-and-a-half times greater in those with diarrhoea (95% CI 1.50-4.09, $p < 0.001$) (3). A Kenyan study involving 1206 children hospitalised

with SAM and managed according to WHO guidelines found 21% mortality in those with diarrhoea on admission, and 12% in those without ($p < 0.001$) (4). It is therefore important to understand the factors contributing to diarrhoea in SAM and to optimise management in order to improve the prognosis of children with SAM and diarrhoea.

The association between malnutrition and lactose malabsorption (generally reflected as stool reducing substances greater than 0.5%) is well described (3-8). However, the recommended feeding formulas widely used in the management of malnutrition (such as F75), contain lactose (9). Their use in the setting of malnutrition with diarrhoea could be delaying recovery (5).

The *aim* of this study is to assess the prevalence of lactose malabsorption in children with diarrhoea and SAM between six months and five years of age at Rahima Moosa Mother and Child Hospital, Johannesburg, South Africa.

The *primary objective* of the study is to identify the presence or absence of reducing substances, as measured by Benedict's test, in the stool water of children with diarrhoea and SAM. The *secondary objective* of the study is to identify the risk factors associated with the presence of reducing substances, as measured by Benedict's test, in the stool water of children with diarrhoea and SAM.

2.0 LITERATURE REVIEW

2.1 Impact of malnutrition on children with diarrhoea

Diarrhoea as a complication of malnutrition has been substantially researched. Research has left no doubt as to the poor outcomes in children with severe malnutrition complicated by diarrhoea. The following paragraphs summarise relevant studies on diarrhoea and malnutrition.

A descriptive, prospective study involving 355 children under six years of age in Colombia admitted with SAM or moderate acute malnutrition found that diarrhoea was the most common complication on admission (68.4%) (10).

The presence of malnutrition increases the risk of mortality in children with diarrhoea. A large longitudinal study in the Philippines evaluated the effect of malnutrition on the risk of diarrhoeal mortality in children under two years old (11). This is one of the first longitudinal studies that estimates the cause-specific risk of mortality in malnourished children. It found that malnutrition was associated with an increased risk of diarrhoeal mortality in all children under two years old, especially in children six to 11 months old; in this period, the relative risk for diarrhoeal mortality doubled for each one-unit decrease in weight-for-age Z score. The attributable risk of malnutrition for diarrhoeal mortality rose significantly from 11.5% in children zero to five months old to 37.7% in children six to 11 months old and 36% in children 12 – 23 months old. The authors speculated that the increased use of weaning foods at around six months of age put children at increased risk of diarrhoeal disease (11).

In a large cohort study in Sudan, involving 28 000 children with diarrhoea, a clear inverse relationship was found between weight-for-height Z-score and mortality (12). A relative risk of death of 1.4 (95%CI: 0.7-2.5) was found in children whose weight-for-height Z-score was minus one to minus two. The relative risk of death increased to 3.5 (95%CI: 1.8 – 6.6) when the Z-score decreased to minus two to minus three. A further increase to 26.5 (95%CI: 13.4 – 52.3) and 24.3 (95%CI: 9.1-64, 8) was found when the Z-score decreased to minus three to minus four and less than minus four respectively (12).

A longitudinal study in Bangladesh found that the strongest predictor of the duration of diarrhoea was the nutritional status of the child: the worse the nutritional status, the longer the duration of diarrhoea (13).

A large Kenyan study found that bacteraemia, hyponatraemia, low mid-upper arm circumference, hypoxia, hypokalaemia and oedema were associated with mortality in children with SAM and diarrhoea; and that in Human Immunodeficiency Virus (HIV) infected children, diarrhoea is more frequent, but the risk of death is not increased compared to HIV uninfected children with SAM and diarrhoea (4).

Despite both malnutrition and diarrhoea being common diseases in South Africa, there have been no studies that specifically assess the impact of malnutrition on children with diarrhoea in a South African setting.

2.2 Aetiological factors of diarrhoea in children with malnutrition

There are many proposed causal factors for diarrhoea in malnourished children.

2.2.1 Impaired immunity

Several studies have shown that malnutrition is a major cause of secondary immune deficiency by impairment of cell mediated immunity and secretory immunoglobulin A (sIgA) production (14, 15). Secretory IgA, secreted by B-lymphocytes of the Peyers patches, mediates the process of pathogenic antigen presentation in the gut and prevents invasion of the mucosa by neutralising pathogens. With decreased levels of sIgA, malnourished children are more susceptible to infectious diarrhoea (14, 15). Malnutrition can also cause increased lamina propria macrophages, lymphocytes and proinflammatory cytokine production in the gut mucosa which has been shown to increase gut permeability as indicated by the lactulose: mannitol absorption test (14, 15).

2.2.2 Food-antigen sensitisation

Cow's milk protein sensitisation could be part of a post-enteritis enteropathy that plays a role in prolonged diarrhoea, following an episode of acute gastroenteritis (16). Cow's milk sensitive enteropathy is thought to occur with or without the occurrence of lactase deficiency and is due to sensitisation of the damaged small intestine mucosa to milk proteins during an episode of gastroenteritis. This allergy can cause prolonged diarrhoea and growth failure. It is thought to occur because of a combination of increased entry of antigens by passive diffusion through damaged mucosa coupled with thinning of the mucosa and decreased IgA in the lamina propria of the intestinal mucosa (16).

2.2.3 Decreased intestinal villous height

The small intestine is a metabolically demanding organ. It accounts for 17-25% of the total body oxygen consumption. It is therefore very sensitive to protein/calorie malnutrition (17). Lower intestinal villus heights are seen in animals that have protein/calorie malnutrition following decreased enterocyte turnover and reduced cell migration along the crypt-villous axis. This results in lower surface area and decreased mucosal mass (17). Although it would seem logical that decreased surface area and mucosal mass would result in decreased nutrient absorption, nutrient absorption is a result of a complex interaction of hormonal signals, fluidity, composition and electrochemical gradient of the gut mucosa, transporter gene expression, turnover and type of transporters synthesised, luminal content and luminal signalling. In malnutrition, there are various protective mechanisms that increase the absorption of essential micro- and macronutrients and result in an increased fractional absorption per gram of mucosal mass. This is an adaptive mechanism that decreases the metabolic demand of the small intestine, to allow more of the diminished nutrient stores to be delivered to the organs that are essential for survival, while still enhancing the absorptive function to partially compensate for the loss of mucosal mass. There is, however, still a decreased overall capacity for nutrient absorption because the reduction in mucosal mass outweighs its ability to increase absorption rates (17).

2.2.4 Decreased disaccharidases

A result of the villous atrophy seen in malnutrition, is decreased disaccharidases (including lactase) at the brush border of the small intestine; a secondary lactase deficiency. Thus, carbohydrate absorption is reduced before that of fat or protein (18). Some of these unabsorbed carbohydrates are metabolised to organic acids by colonic bacteria, and can then be absorbed across the colonic mucosa. Carbohydrates that have not been metabolised by colonic bacteria and organic acids that are not absorbed by the colonic mucosa will remain in the colonic lumen

and exert an osmotic effect resulting in diarrhoea. Carbohydrate malabsorption can therefore cause osmotic diarrhoea that can result in severe dehydration, acidosis and shock (19). Decreased disaccharidases is the causal factor that forms the basis of this study.

2.3. Lactose malabsorption in children with SAM and diarrhoea

Lactase is found at the tip of the brush border and is therefore the first disaccharidase to be affected by mucosal damage (20). Lactase is also present in lower concentrations than the other brush border disaccharidases and is the last to recover from mucosal damage. It is therefore the most important type of secondary disaccharidase deficiency (21,22) and most relevant in the setting of malnutrition related villous atrophy. Sucrase deficiency may occur with lactase deficiency as part of a secondary disaccharidase deficiency, but does not occur as an isolated deficiency (23). A deficiency in lactase will result in lactose malabsorption, also known as lactose intolerance. Lactose intolerance specifically implies the presence of symptoms related to lactose malabsorption such as diarrhoea, bloating, flatulence, abdominal cramping (20).

A Ugandan study investigated 196 severely malnourished children with diarrhoea. Lactose malabsorption was detected by stool-reducing substances and stool acidity in 25.5% (24) . They identified young age, kwashiorkor, peri-anal skin erosion, high mean stool frequency and recent or persistent diarrhoea as associated with lactose malabsorption. HIV infection was not significantly associated with lactose malabsorption in this study, however, lactose malabsorption is commonly found in HIV-infected Southern African children (5).

Across the globe, the management of malnutrition is not entirely evidence-based and predominantly derived from expert opinion (5). The principles of malnutrition management have

been reviewed and updated based on best available evidence. However, WHO '10 steps' advocate the use of a lactose-containing formula (9).

In many settings in Southern Africa, the mortality rate for children with SAM managed in hospital settings remains above 20% (5). This contrasts with the claim that implementation of WHO guidelines for the treatment of malnutrition can result in a mortality of less than 5 % (5). Mortality is highest in areas with least access to antibiotics, nutritional supplements, good nursing care and feeding regimens (6). Malnutrition complicates many other common medical conditions in Southern African children and contributes to their mortality (7). HIV and Tuberculosis (TB) co-infection also complicate the management of malnutrition, but there is insufficient evidence to improve guidelines on the management of malnutrition in these clinical scenarios (5).

In children with SAM, physiologic adaptations occur to enable the child to survive 'starvation'. In the absence of sufficient dietary macronutrient supply, catabolism of fat and protein stores ensues. This provides glucose and ketones for energy but also causes wasting and organ dysfunction, including decreased intestinal absorptive function, particularly of lactose (5), despite the compensatory mechanism described in 2.2.3 above.

2.4 Environmental enteropathy

Environmental enteropathy (also known as tropical enteropathy) is a subclinical condition resulting from repeated faecal oral contamination that is not yet fully understood (25). Chronic inflammation of the small intestine results in increased intestinal permeability, altered gut

immunity, blunting of the intestinal villus and malabsorption. It causes poor growth, oral vaccine failure and malnutrition that is poorly responsive to nutritional therapy. It occurs among people living in areas with poor sanitation and inadequate hygiene (25). Decreased villous height, increased crypt depth, lymphocytic infiltration of the lamina propria and increased intraepithelial lymphocytes on biopsy as well as carbohydrate malabsorption have been associated with this phenomenon. Information gained from immigrants have suggested that the aetiology is most likely to be environmental, whereby constant exposure to faecally contaminated food and water results in repeated gastroenteritis and a perpetuated state of inflammation resulting in mucosal damage (25). This condition is closely related to malnutrition and the pathological processes of environmental enteropathy and malnutrition complicated by diarrhoea are likely to overlap to a large degree.

2.5 Testing for lactose malabsorption in children

Evidence for lactose malabsorption can be detected by a Clinitest®, or by the use of Benedict's solution, both of which are inexpensive, bedside investigations (26). The Clinitest ® dates back to 1964 when Kerry and Anderson investigated its use to determine the carbohydrate content of stools (27). Clinitest® tablets are no longer being manufactured by Bayer Health Care due to lack of demand (28), but the Clinitest® is based on the principles of the Benedict's test, which uses Benedict's solution to detect reducing substances in a sample.

Benedict's solution contains copper (II) sulphate, sodium carbonate and sodium citrate. The blue copper (II) ions (present in the copper (II) sulphate) are reduced by the presence of reducing substances (e.g. glucose, lactose, fructose, galactose or pentose), and an external source of heat, to copper (I) ions, which are red in colour. When testing stool water, the higher the concentration

of reducing substances in the stool water, the greater the number of copper (II) ions that will be reduced, corresponding to a greater colour change in the solution. The concentration of reducing substances in stools is therefore demonstrated by the colour that is produced, which is compared to a standardised reference chart (appendix A) to estimate the percentage of reducing substances present in the sample (27). Reducing substances are usually found in small quantities in the stool of children, but a level of more than 0.5% is considered significantly above the normal range and indicative of carbohydrate malabsorption (27). Benedict's test has been shown to be an efficient test to predict osmotic diarrhoea (23,26,27). Benedict's test does not differentiate which form of carbohydrate is present in the stool water. Stool chromatography is needed to identify exactly which carbohydrates are present in stool, but this test is cumbersome and expensive. As discussed in section 2.3 above, in secondary disaccharidase deficiency, lactase is the most important enzyme affected, other disaccharidases are only affected in combination with lactase (23) and therefore the Benedict's test or Clinitest® is a suitable proxy for the detection of lactase malabsorption in secondary disaccharidase deficiency.

The performance of these tests in the presence of malnutrition and diarrhoea is not required when following WHO guidelines on the Management of malnutrition and diarrhoea (it is not mentioned in the WHO manual (9)) and it is seldom performed in the clinical setting. Lactose malabsorption would be detected earlier if the test were routinely performed on malnourished children with diarrhoea.

The use of a Clinitest® tablet or Benedict's Solution avoids sending a stool specimen to the laboratory, which frequently results in false negative results due to the action of stool bacteria on reducing sugars (5). The hydrogen breath test is currently the gold standard for the detection of

lactose malabsorption (24). This test is however expensive and laborious. It also requires that the patient fasts and is then given a lactose 'load'. This procedure could be potentially harmful in malnourished children and therefore the hydrogen breath test is not appropriate in the routine clinical management of SAM and was not used in this study.

2.6 Current management of children with diarrhoea and SAM

The recommended feeding formulas widely used in the management of diarrhoea in children with malnutrition contain lactose. The WHO guidelines on the management of SAM recommend the use of F75 (which contains lactose) and do not distinguish between dietary management of children with and without diarrhoea (9).

Many sites do not use F75 or F100. Lack of availability of premixed F75 and F100, and problems with mixing the formula as per the WHO manual (e.g. sterility and separation of oil and water in the formula) are the main reasons given. Many of these sites are using commercial starter formulas that have high lactose contents (29).

There have been many studies on the nutritional management of severe malnutrition.

A study in South Africa in 1965 on children with malnutrition and diarrhoea found that while on a milk-containing diet, 85% of the participants had sugars (lactose, galactose and glucose) demonstrated on stool chromatography. When all the carbohydrates were removed from the diet, there was an improvement in diarrhoea. The researchers went on to demonstrate that the defect was mainly in disaccharide absorption (especially lactose) rather than monosaccharide absorption (30). This was a small study that used technical outcomes, such as stool weight and stool lactic acid. There were no clinical outcomes measured, such as improved weight gain and

decreased mortality that could be used to determine the clinical effectiveness of a carbohydrate free feed in these children.

A Guatemalan study in 1984 compared the use of intact cow's milk based formula, to a lactose free cow's milk based formula (added β-galactosidase converted more than 90% of lactose to its monosaccharide) in the management of severe protein-energy malnutrition in 20 children (31,32). It was concluded that the use of intact milk based feeds are not contraindicated in children with malnutrition. In this Guatemalan study, the authors did not specifically look at children with diarrhoea and the incidence of lactose malabsorption (18%) was lower than what has been found in other studies. No large studies have directly compared cow's milk based formulas that are lactose free, with intact milk based formulas in children with malnutrition, diarrhoea and lactose malabsorption.

A South African study in 1989 compared the use of a soya based formula to that of a standard lactose containing cow's milk formula (20 children in each group) in the management of children with oedematous malnutrition and found no disadvantages to the use of the soya formula. They also did not find significant advantages to its use, but none of the children in their study had lactose malabsorption (33). Lactose free formulas that are cow's milk based, not soya based are now available commercially. These formulas may be a more relevant comparison to lactose containing cow's milk formula than soya based formula.

Many studies have found cereal-based diets to be associated with acceptable outcomes in the management of children with malnutrition (34-36). However, a Malawian study in 1997 compared the use of a maize-soya-egg based feed to a standard milk feed in the management of children

with malnutrition in 100 participants and found milk was superior, although they still recommended low lactose milk feed (37).

A randomised control trial (RCT) done in 2002, in an Australian aboriginal population of malnourished children with diarrhoea, compared the use of a low-osmolality lactose-free formula (De-Lact), a lactose-free formula (O-Lac) and a partially hydrolysed formula (Alfaré) (38). It concluded low osmolality milk was associated with better outcome and the authors of that trial suggested that lowering the osmolality of F75 and F100 could improve outcomes in children with diarrhoea and SAM (38). It also found that the partially hydrolysed formula was associated with less improvement in mucosal recovery, suggesting that cow's milk protein sensitisation was not contributing to greater diarrhoeal severity or enteropathy in this population. There has not been enough research done, in line with this RCT, specifically on the nutritional management of malnutrition in the face of diarrhoea and more specifically, when lactose malabsorption has been demonstrated. Although it is recognised as an important factor, the impact of osmolality lies beyond the scope of this study. The osmolality and lactose content of commonly used formulas are represented in table 2.1.

Table 2.1: Lactose and osmolality content of commonly used formulas

Formula	Osmolality (mOsm/L)	Lactose Content (g/L)
Fresh Cow's Milk	260	48
Breastmilk	273	74
Nan 1	275	80
Nan 2	Not available	56
Infacare 1	Not available	60
Infacare 2	Not available	80
F 75	333	13
F100	419	42

Contrary to the Australian study mentioned above, a Zambian study done in 2002 hypothesised that food-antigen sensitisation may have a role to play in persistent diarrhoea in children with malnutrition and found that the use of an amino-acid based elemental feed resulted in better outcomes than cow's milk or soya based feeds (39). This study did not use the comparison of a *low-lactose or low osmolality* cow's milk based feed which could account for the difference in findings between this study and the Australian in terms of the suitability of elemental feeds in children with diarrhoea and the relevance of food-antigen sensitisation.

A systematic review, of the management of malnutrition in developing countries was published in 2012. All three RCTs included, that investigated the impact of diet on outcome in children with persistent diarrhoea and malnutrition, concluded that there was no significant difference in outcome between the diets (soya based versus diluted milk based feed, soya based versus chicken

or milk based feed, elemental versus skimmed-milk/soya based feed) (40). The overall methodological quality of two of the three studies in this systematic review was considered to be 'weak' by the authors of the systematic review and all three were judged to be subject to bias in a number of areas, leaving the question of optimal nutritional management in children with diarrhoea and malnutrition unanswered.

Although there have been many studies on the subject of nutritional management of children with severe malnutrition, most of these studies were small and compared cow's milk based formula to soya based formula (representing a lactose free formula). Soya is source of protein that is known to be inferior to cow's milk protein (41). It has lower methionine content and high levels of phytate, aluminum, and phytoestrogens (isoflavones), which may have deleterious effects (41). It is therefore not an ideal choice for the nutritional management of SAM. Elemental feeds are very expensive and therefore are not feasible for the management of malnutrition in resource-constrained settings. There are other lactose free/low lactose feeds available that may be better options to soya or elemental feeds. If lactose malabsorption is commonly found in children with SAM and diarrhoea this may indicate the potential benefit of more widespread use of more appropriate lactose-free feeds in their management, such as modified cow's milk based lactose free/low lactose feed.

Children who have reducing substances greater than 0.5 g% have been shown, in an observational study in 1972, to respond to a diet free of the implicated sugar (demonstrated by stool chromatography) (27). It must be noted that this study was not performed on children with malnutrition. Since then no other studies were identified that specifically investigated the treatment of children with excessive reducing substances in their stool, who were fed a lactose-

free or low lactose formula on admission. There are studies, including a meta-analysis of 29 studies, that have looked at treating children with acute gastroenteritis with low-lactose or lactose free formula which have found benefit, especially in those children with significant dehydration, malnutrition or persistent diarrhoea (42-45).

2.7 Reason for this study

Although many studies have investigated children with malnutrition and diarrhoea, few have specifically looked at the prevalence of lactose malabsorption in these children. In those studies that do report its prevalence, the figures are conflicting. This study aims to determine the prevalence of lactose malabsorption in children with SAM and diarrhoea in a current South African setting. As a secondary objective, identifying clinical risk factors for the presence of lactose malabsorption may help to identify children that could benefit from testing for lactose malabsorption. If found to be prevalent, this would warrant a future study to investigate the effect of appropriate lactose free feeds in children with SAM and diarrhoea with documented lactose malabsorption in a similar setting.

3. 0 METHODS

3.1 Study type

This Study was a prospective observational study.

3.2 Study sample

The study participants consisted of a convenience sample of patients admitted to Rahima Moosa Mother & Child Hospital, between six months and five years old who were diagnosed with SAM (according to current WHO criteria (2)), with or without diarrhoea (defined as 3 or more watery stools a day). The samples were collected between 1 December 2012 and 30 November 2013.

3.3 Inclusion criteria

Children (aged between six months and five years) with SAM, as defined by the presence of one or more of the following (2):

1. Weight-for-height Z-score according to the WHO 2006 growth charts of less than – 3 standard deviations.
2. MUAC < 115mm.
3. Bilateral pitting oedema at least of the feet, excepting those with other conditions causing pitting oedema (e.g. nephrotic syndrome or congestive cardiac failure), regardless of the weight-for-height Z-score or MUAC.

3.4 Exclusion criteria

Parent or legal guardian declined to provide consent.

3.5 Methods

1. The participants' demographic, clinical and laboratory findings, including HIV status were noted in the questionnaire (see appendix B) after informed consent (see appendix C) was obtained. This information was often already obtained as it was usually part of the routine history and investigation of these patients at the hospital, but it was verified (and completed when necessary) with the caregiver for the purposes of the study.

2. A fresh stool sample was taken from the study participant as soon as possible after enrolment (when necessary, a small feeding tube was inserted into the anus to a depth of 1 cm in order to obtain the sample). Treatment already instituted was noted.

3. The stool sample was immediately tested for the presence of reducing substances, using Benedict's solution. The solution was prepared by the Department of Pharmacy and Pharmacology of the University of the Witwatersrand. The reagent contained 100g Sodium carbonate, 173g tri-sodium citrate dehydrate, 17.3g copper (II) sulphate pentahydrate in 1000mL distilled water. Benedict's solution was added to an equal volume of stool water. The combined solution was heated by submerging the mixture in boiling water (boiling point of water in Johannesburg is 91°C) for 15 minutes. The level of reducing substances was then determined by the presence of a colourimetric change. A standardised chart (see appendix A) was used to compare the colour change observed with the relevant level of reducing substances in the stool water. The study participant

was considered to have lactose malabsorption if the colourimetric change corresponded with 0.5% or more reducing substances.

4. The result of the Benedict's test was communicated to the treating physician for further management by them.

3.6 Data analysis

Data was captured into REDCap electronic data capture tools hosted at The University of the Witwatersrand (46). Analysis was performed using Stata software (47). Comparisons of proportions were done using chi-squared tests with Fisher exact where appropriate. Variables were compared using Student's t-test or Wilcoxon rank test when the data was not normally distributed. Risk factors with p-values below 0.2 or those that had been found to be of significance in previous studies were included in logistic regression analysis to identify independent risk factors. P-values less than 0.05 were considered significant. Anthropometrics were analysed using WHO Anthro 2005® software.

3.7 Ethical considerations

The protocol was approved by the Committee for Research in Human Subjects at the University of the Witwatersrand (Medical) before data collection commenced. The study was conducted in line with the Declaration of Helsinki principles.

As this is a prospective study, no data collection was done without informed consent from the parent or legal guardian. A copy of the participant information sheet and informed consent (appendix C) was given to the parent or legal guardian. The sheet had contact information of the researcher and of the Committee for Research in Human Subjects at the University of the Witwatersrand from whom ethical clearance was granted (reference: M121045, appendix D). The study was completed anonymously.

There was no incentive for participation besides that of gaining information that could help other children who may have similar medical conditions to the participant.

The only direct identifiable risk to the participants was one of trauma to the anus caused by insertion of the tip of a thin lubricated feeding tube 1 centimetre into the anus. This was a theoretical risk as a literature search did not find any reports of significant trauma caused by stool collection using this technique. There were no incidents of trauma to any of the study participants caused by this procedure during the study. The result of the Benedict's test was communicated to the treating clinicians and they could modify treatment of the participant if they deemed necessary.

3.8 Study Setting

The study was performed at Rahima Moosa Mother and Child Hospital, a district level hospital in Coronationville, that serves a socioeconomically poor urban community in Johannesburg, Gauteng. It offers paediatric and maternity services and admits over 3000 paediatric patients annually. There is no specific dietary management protocol for children with SAM and diarrhoea and they are managed at the discretion of the treating clinicians. They are mostly commenced on a commercial starter formula and the caloric content is increased after the stabilisation phase.

4.0 RESULTS

4.1 Demographics

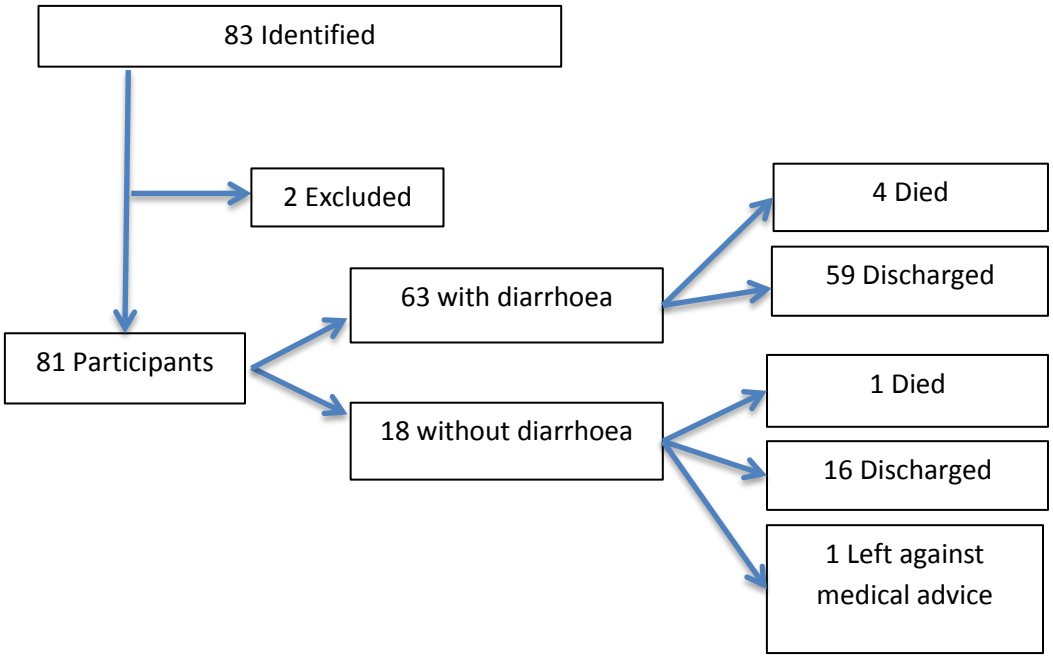


Figure 4.1: Study participants

The study participants are described in figure 4.1. All identified, eligible participants consented to inclusion in the study. One was excluded for not meeting the anthropometric criteria and one was excluded for not meeting the age criteria. The median age was 12 months (range: 6 – 38 months). The age of the study population is demonstrated in figure 4.2. The demographics of the sample population are described in table 4.1. The housing and amenities of the study population are compared to provincial and national data in table 4.2.

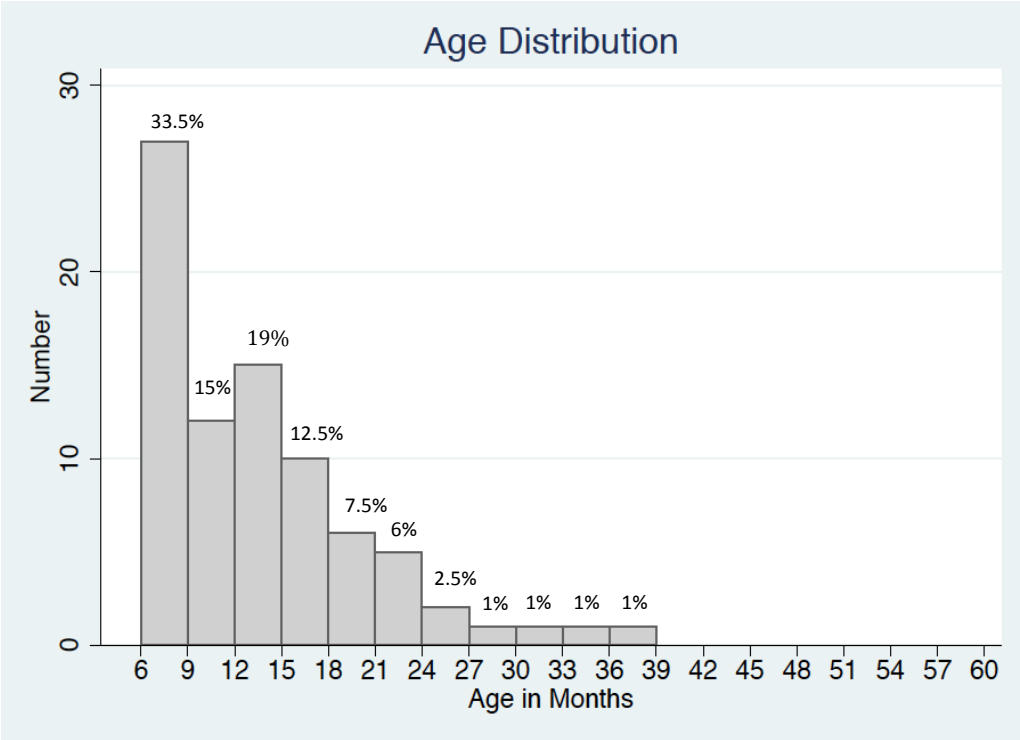


Figure 4.2 : Age distribution

Table 4.1: Demographics

Variable	Total study population n (%)
Sex	
Male	39 (48)
Female	42 (52)
Housing	
Brick	41(51)
Iron Sheeting	40 (49)
Water source	
Indoor tap	42 (52)
Communal tap	37 (46)
River water	1 (1)
Water tank	1 (1)
Toilet type	
Flush	57 (70)
Pit latrine	19 (24)
Bucket system	5 (6)
Electricity in house	
Yes	48 (59)
No	33 (41)
Over Crowding	
Yes	22 (59)
No	19 (23)

Table 4.2: Housing and amenities of the study population compared to Gauteng and South African statistics

	Study	Gauteng*	South Africa*	p value 1 **	p value 2 ***
Formal Housing	51%	80.6%	74.0%	<0.005	<0.005
Adequate Sanitation	70%	91.6%	69.1%	<0.005	0.861
Adequate Water	52%	93.3%	66.3%	<0.005	0.006
Electricity	59%	93.3%	66.4%	<0.005	0.01
Overcrowding	77%	20.8%	21.0%	<0.005	<0.005

* All statistics for Gauteng and South Africa, except for electricity statistics, were sourced from The Child Health Gauge 2013 (48). Electricity statistics were sourced from The Child Health Gauge 2007/2008 (49) as they were last included in the annual Child Health Gauge then. Definitions used for each parameter in the table are found in appendix E.

** p value 1 – compares the difference between the study results and Gauteng statistics

*** p value 2 – compares the difference between the study results and South Africa statistics

4.2 Severe acute malnutrition

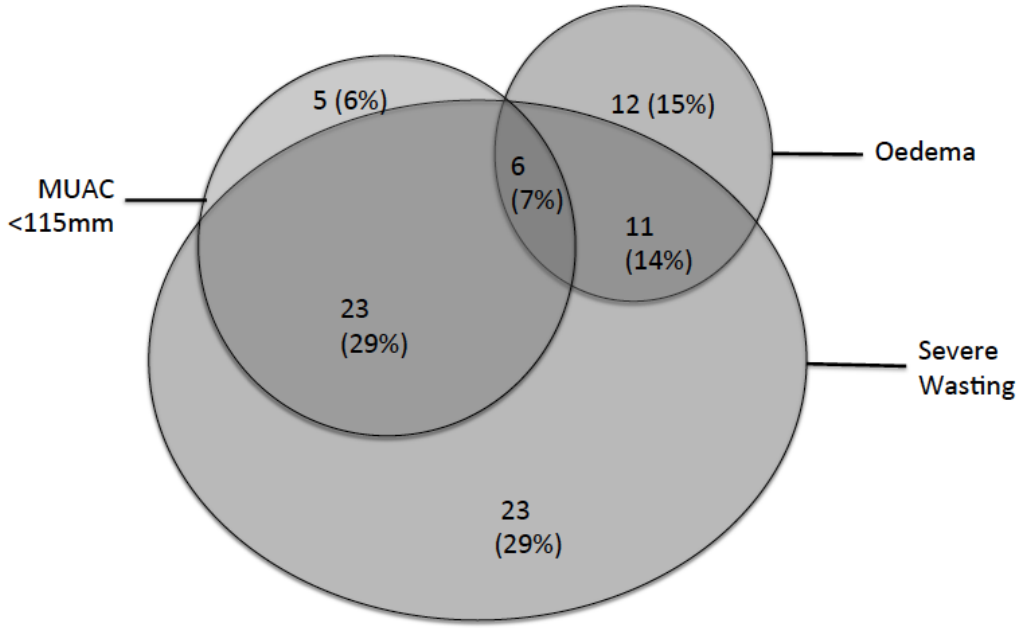


Figure 4.3: Parameters of SAM in Study Population

Figure 4.3 represents the clinical criteria of SAM with which the study population presented. Fifty percent of participants presented with only one of the three criteria.

4.3 Diarrhoea

4.3.1 Presence of lactose malabsorption

Seventy eight percent (63/81) of the study participants had diarrhoea. Fifty nine percent (37/63) of these had reducing substances 0.5% or greater (positive for lactose malabsorption). A further 13% (8/63) had reducing substances of 0.3 or 0.4%, which is considered suggestive for lactose malabsorption. Twenty nine percent (18/63) had reducing substances of 0.2% or less, which is considered negative for lactose malabsorption. For the purpose of discussing predictive factors for lactose malabsorption in children with SAM and diarrhoea, only children with reducing sugars greater than or equal to 0.5% were considered to have lactose malabsorption.

4.3.2 Risk factors for lactose malabsorption

Table 4.3 Risk factors investigated for their association with the presence of reducing substances in stool of children with diarrhoea and SAM

		Lactose Malabsorption (Reducing Substances ≥0.5%)	No lactose Malabsorption (Reducing Substances <0.5%)	Test and Test Statistic	p value
Age in months median (range)		9 (6 – 32)	14 (6 – 38)	Wilcoxon rank test	0.033
Age <12 months n (%)		25 (69%)	11(31%)	*OR: 2.84 95%CI: (1.01 – 7.99)	*0.046
Episodes of loose stool in past 24 hours n (IQR)		6 (5-8)	5 (3-6)	Wilcoxon rank test	0.038
Stool frequency >7/24 hours n (%)		10 (83%)	2 (17%)	*OR 4.44 95%CI: 0.96 – 20.56	*0.054
Median duration of diarrhoea on presentation (range)		3 (1- 16)	6 (2 – 25)	Wilcoxon rank test	0.004
Duration of diarrhoea >7 days n (%)		10 (43%)	13 (57%)	*OR: 0.53 95%CI: 0.15 – 1.80	*0.301
Presence of perianal skin excoriation n (%)		15 (71%)	6 (29%)	*OR: 2.38 95% CI: 0.77-7.32	*0.127
Fever n (%) (temperature >38.0°C)		11 (55%)	9 (45%)	*OR: 0.799 95%CI: 0.27-2.35	*0.682
Severity of dehydration n (%)	**None**	7 (19%)	8 (31%)		
	Mild	12 (32%)	8(31%)	*OR: 1.52 95%CI: 0.54-4.23	*0.423
	Moderate to severe	18 (49%)	10 (38%)		
HIV Infected n (%)		9 (14%)	6 (10%)		
HIV Exposed Uninfected n (%)		15(24%)	12 (20%)	Pearson Chi^2: 0.21	0.901
HIV Uninfected n (%)		13 (21%)	8 (13%)		
Oedema (%)		16(25%)	7(11%)	*OR: 2.07 95%CI: 0.67-6.11	*0.185

* Odds ratio, test statistic Chi Square

Potential risk factors that are related to the presence of reducing substances are represented in Table 4.3. The data in this table only represents the 63 participants who had diarrhoea.

The duration of diarrhoea on presentation was found to be significantly shorter in the group positive for reducing substances. The number of loose stools was found to be marginally, but statistically significantly increased in the group that was positive for reducing substances. Age was significantly lower in the group with positive reducing substances. The rest of the risk factors were not found to have a significant association with reducing substances.

Table 4.4: Multivariate analysis of risk factors associated with positive reducing substances

Variable	Category	Bivariate Odds Ratio (95%CI)	p Value	Multivariate Odds Ratio (95%CI)	p Value
Age	< 12 months (vs ≥ 12 months)	2.8 (1.01-8.0)	0.049	3.3 (1.01-10.5)	**0.047**
Stool Frequency	> 7 per 24hours (vs ≤ 7 per 24 hours)	4.4 (0.9-22.3)	0.070	3.7(0.7-20.1)	0.131
Dehydration	Moderate-Severe (vs None – Mild)	1.5 (0.5-4.2)	0.424	1.9 (0.6-5.9)	0.294
Perianal Skin Excoriation	Present (vs Absent)	2.43 (0.87-7.30)	0.132	2.1 (0.6-7.4)	0.264
Oedema	Present (vs Absent)	2.1 (0.7-6.1)	0.189	2.2 (0.6-10.2)	0.131

The factors for the multivariate analysis were chosen because they have been shown to be associated with positive reducing substances in stool in previous studies and/or they had a p value of <0.2 on univariate analysis. Dehydration was included because it has been shown to be a significant feature of osmotic diarrhoea and of lactose malabsorption specifically (20,50). After multivariate analysis, children younger than 12 months were 3.3 times more likely to have positive reducing substances than children older than 12 months (95% CI: 1.1 – 10.5, p= 0.047), when corrected for stool frequency, dehydration, perianal skin excoriation and oedema.

4.4 HIV

Table 4.5: HIV Prevalence

HIV Status	**n (%)**
Infected	21 (26%)
Exposed and uninfected	33 (41%)
Not exposed and confirmed negative	27 (33%)
Unknown	0 (0%)

*HIV status was confirmed with an HIV PCR or antibody test (antibody test performed in children 18 months or older)

The table includes all the study participants – those with and without diarrhoea.

4.5 Participant outcome

Table 4.6 shows the variables that were investigated for their association with participant outcome. Ninety three percent (75/81) of the study participants were discharged home. Six percent (5/81) died. One participant's parents refused further hospital treatment and left the hospital against medical advice. The participant who was removed from hospital against medical advice was excluded from the study for the purposes of participant outcome calculations.

The median number of days in hospital for those who were successfully treated with positive reducing substances was 10 days (range: 1 – 58). The median number of days in hospital for those who were successfully treated with negative reducing substances was 11 (range 5 – 38, p=0.63 Wilcoxon rank test).

Table 4.6: Variables investigated for their association with participant outcome

	Death n = 5	Discharged n = 75	Test and Test Statistic	P value
HIV infected n (%)	0 (0%)	21 (28%)	Pearson Chi^2: 0.210	0.901
Age <12 months (%)	3 (60%)	42 (56%)	*OR: 1.18 95%CI: 0.18-7.54	*0.861
MUAC <115mm n (%)	3 (60%)	31 (41%)	*OR: 2.13 95%CI 0.34-12.18	*0.424
WFA Z Score less than – 4 SD n (%)	3 (60%)	29 (39%)	*OR: 2.52 95%CI: 0.41-15.35	*314
Oedema n (%)	2 (40%)	26 (34%)	*OR: 1.26 95%CI:0.20 – 8.07	*0.808
Diarrhoea n (%)	4 (80%)	59 (79%)	*OR: 1.08 95%CI: 0.11-10.54	*0.943
Reducing substances ≥0.5% n (%)	4/4(100%)	33/59 (56%)	*OR undefined #	***0.035**
Reducing substances <0.5% n (%)	0/4 (0%)	26/59 (44%)		
Anaemia (Haemoglobin <10g/dl) n (%)	4 (80%)	35 (47%)	*OR: 4.57 95%CI: 0.57 – 36.45	*0.149
Hypoxia (Saturations < 89% in room air) n (%)	2 (40%)	12 (16%)	*OR: 3.5 95%CI: .057 – 21.32	*0.712
Severe hypokalaemia (Potassium < 2.5 mmol/L) n (%)	0 (0%)	18 (24%)	*OR undefined #	*0.104
Severe Acidosis (CO_2 <16mmol/L) n (%)	3 (60%)	42 (75%)	* OR: 1.18 95%CI 0.18 -7.54)	*0.861

(all variables were measured on admission)
OR could not be defined as denominator is 0
* Odds ratio, test statistic Chi Square

The median reducing substances of children who were treated successfully was 0.5% (range 0.0 – 1), while of those who demised, the median reducing substances were 0.55% (range 0.5 – 0.6). Of the 5 participants who died, 4 had diarrhoea, all of whom had lactose malabsorption (stool reducing substances of 0.5%, 0.5%, 0.6% and 0.6%). One participant who died had no diarrhoea, but had a patent ductus arteriosus, which could have been a significant contributing factor to their death. Of the participants with diarrhoea, 4/4 (100%) of those who died had lactose

malabsorption and of those who were discharged, 42/75 (56%) had lactose malabsorption. Children with SAM and diarrhoea were more likely to die if they had lactose malabsorption (p= 0.035). The small number of deaths in the study did not allow for variance within that group. All the participants who died had lactose malabsorption and therefore an odds ratio and logistical regression analysis could not be done. There were no other factors that were found to be significantly associated with death.

5.0 DISCUSSION AND CONCLUSION

5.1 Demographics

The study hospital is a district level hospital that serves a socioeconomically poor urban community in Johannesburg, Gauteng, South Africa. The hospital also serves surrounding informal settlements. The poor socioeconomic circumstances of the study population is reflected by the low prevalence of an indoor tap, a flushing toilet and electricity as well as the low prevalence of a formal dwelling and a high prevalence of overcrowding. In the annual South African Child Gauge, the prevalence of these amenities, housing type and overcrowding, across the different provinces of South Africa, including a national average is investigated (among many other parameters that impact on child health). Table 4.2 compares results of our study participants with data from the 2013 and 2007/2008 South African Child Gauges.

As represented in the table, there was significantly less formal housing within the study population, more overcrowding and less access to basic amenities than the provincial averages. Unfortunately, similar figures for the districts served by the hospital are not available. This indicates that the participants in this study had poorer than average access to these amenities, compared to available provincial and national estimates. Malnutrition is generally more prevalent in poorer communities (as indicated by the World Bank estimates on malnutrition (1)) making this a relevant setting in which to conduct this study.

5.2 Severe acute malnutrition

Most of the study participants had a weight-for-height Z-score of less than minus three, a third had nutritional oedema and almost half had a MUAC of less than 115mm. As demonstrated in figure 4.3, half of children with SAM will only present with one of the three WHO criteria (2) and in almost half of these, the single defining criteria will be nutritional oedema or a MUAC of less than 115mm. It is therefore important to examine for nutritional oedema and measure the MUAC when assessing nutritional status, as a large proportion will be missed if only weight-for-height Z-scores are used.

5.3 Diarrhoea

5.3.1 Presence of lactose malabsorption

The Benedict's test was performed after initial hospital treatment was started (which for none of the participants included lactose free feeds, but rather no feeds when the participant was severely ill, or lactose containing feeds when more stable). More than half of the study participants with diarrhoea had lactose malabsorption. This is higher than the prevalence of lactose malabsorption in children with SAM and diarrhoea found in other studies (24, 50-52). In light of this information and that these children also appear to be at increased risk of mortality (see section 5.5), consideration should be given to the routine testing of stool for reducing substances in children presenting with SAM and diarrhoea.

The high prevalence of lactose malabsorption demonstrated in this study, supported by the previous similar findings should encourage future RCTs comparing F 75 (and other formulas

commonly used in the acute management of diarrhoea in children with SAM) with the best available alternative, which is believed to be a low osmolality, lactose free milk based formula (38) to examine their impact with respect to recovery rates, time to resolution of diarrhoea, weight gain and mortality and thereby optimise the management of these high risk children. The existing research on nutritional management of children with malnutrition does not include substantial research specifically on children with malnutrition *and* diarrhoea or lactose malabsorption and has therefore not been successful in influencing policy change in existing management guidelines in these circumstances.

5.3.2 Risk factors for lactose malabsorption

Both young age and a stool frequency of more than seven per 24 hours were found to be associated with lactose malabsorption. Once corrected for other possible associated variables in the multivariate analysis, age was the only significant predictive factor for positive reducing substances. Young age has previously been found to be associated with lactose malabsorption, therefore this finding is in keeping with previous studies (5), however other factors previously found to have an association with lactose malabsorption, did not have any predictive value in this study.

The reported duration of diarrhoea was unexpectedly found to be shorter in children with lactose malabsorption than in those without. A longer duration of diarrhoea has previously been found to be associated with lactose malabsorption (53). This is not of major clinical importance, but suggests that lactose malabsorption should be considered regardless of the duration of diarrhoea reported.

The presence of perianal skin excoriation, severity of dehydration and HIV infection were expected to be associated with lactose malabsorption but there was no significant association found. This may be due to small sample size (type II error).

It was expected that fever might be negatively associated with lactose malabsorption, rather indicating an infective cause for the diarrhoea (although the two may co-exist). It however did not have a significant association.

5.4 HIV

HIV prevalence in pregnant mothers, from National HIV Survey 2011 (55), was 29.5% in South Africa and 28.7% in Gauteng. In this study, confined to children with SAM, the prevalence of children exposed to HIV was 67%. In the 2012 South African Child Gauge, the prevalence of HIV in children was 2.9% both in Gauteng and South Africa (56). The prevalence of HIV in this study was almost 25%, but was confined to children with SAM. HIV has previously been found to be more prevalent in children with malnutrition than in those without, which is reflected in our results that show higher HIV exposure and infection rates than the national and regional averages. A study done at the same hospital 5 years prior to this study, also on children with malnutrition demonstrated an HIV prevalence of 51% (57). The reduction in HIV prevalence, by half, in the same group of patients is evidence of effective HIV treatment and prevention, especially, improved prevention of mother to child transmission. A systematic review and meta-analysis of 17 studies (4891children) in Sub-Saharan Africa (58) found that the prevalence of HIV in children with SAM was 29%, similar to our sample.

5.5 Participant outcome

In-hospital mortality rate in the study was close to the 5% mortality rate that WHO claims can be achieved by implementation of their guidelines for the treatment of malnutrition, and lower than the 20% (and greater) mortality rate seen in many settings in Southern Africa (5). Between 2005 and 2007, in-hospital mortality rates in South Africa for children under 18 years old, ranged from three to 15 deaths per 100 admissions, 40% of these deaths were in children who were classified as either marasmic, kwashiorkor or marasmic-kwashiorkor (58). The latest (2012) in-hospital mortality rate for children managed at the study hospital was 1 per 100 admissions in children aged 28 days to 1 year. In comparison to these rates, the in-hospital mortality rate in this study falls on the lower end of the spectrum of the national data, but the high risk for mortality of patients with diarrhoea and SAM is demonstrated by the increased in-hospital mortality rate of children in the study as compared to the hospital statistics ($p<0.005$).

There was no significant difference found in the duration of hospital stay of participants with lactose malabsorption compared to those without. At the time of the study, there was no standard treatment protocol at RMMCH for the management of children with reducing substances found in their stools. The Benedict's test result was communicated to the treating clinicians and the decision to change to a lactose-free formula depended on their personal preference, experience and on the availability of lactose-free formula in the hospital at that particular time. This inconsistency in the treatment of these children could have affected the duration of their hospital stay and further studies would need to be done to evaluate the impact of treating children with stool positive for reducing substances with lactose-free or low lactose formula.

Death was more likely in the presence of detected stool reducing substances (p=0.035). This may indicate the clinical importance of screening children with SAM and diarrhoea for lactose malabsorption. They could then be identified as being at higher risk for mortality and managed accordingly. Although it has already been suggested that all children with malnutrition and diarrhoea be managed with feeds that are low in lactose (or lactose free) and have a low osmolality (5,8,38), further studies on the appropriate management of children with SAM and diarrhoea with positive reducing substances still need to be conducted.

5.6 Limitations

The parents were asked questions from the questionnaire and this subjected the study to recall bias, but this effect should have been uniform across all the study participants.

The Benedict's test relies on a colourimetric change which is compared to a standardised chart (appendix A), the interpretation of the colour is somewhat subjective. Inter-observer bias was minimised by only one researcher conducting the Benedict's test.

Reducing substances were only tested for once, on or shortly after admission. This was usually after initial medical and dietary hospital treatment (including lactose containing feeds) had been commenced. The hospital treatment was not thought to affect the result of the Benedict's test. Timing or composition of last meal was not considered. It is possible that some participants, who did not have lactose malabsorption when tested, developed lactose malabsorption during their hospital stay. These patients would not have been identified.

The study was conducted in a single hospital over a one year period and the study sample was therefore small. This could have resulted in decreased variability in the study results, particularly in the group that died (only five participants). Other associations with lactose malabsorption may have been evident in a larger study population, specifically a longer duration of diarrhoea, the presence of perianal skin excoriation and dehydration.

5.7 Conclusion

Malnutrition contributes significantly to the burden of disease in South Africa and globally. Diarrhoea is a frequent and serious complication of malnutrition. Lactose malabsorption was found to be common in children with SAM and diarrhoea in this study. Age of less than 12 months was found to be the only significant factor predicting lactose malabsorption when correcting for other variables. The presence of lactose malabsorption was associated with higher mortality in children with SAM and diarrhoea. Clinicians treating children with SAM and diarrhoea should consider testing stool for reducing substances as those with a positive result could be at increased risk for mortality. While studies have investigated the dietary management of malnourished children with diarrhoea, there have been no studies that specifically investigate the management of malnourished children who have documented lactose malabsorption. This study highlights the need for a future study investigating the impact of the use of the most appropriate lactose free feeds on the recovery of children with SAM whose stool is positive for reducing substances.

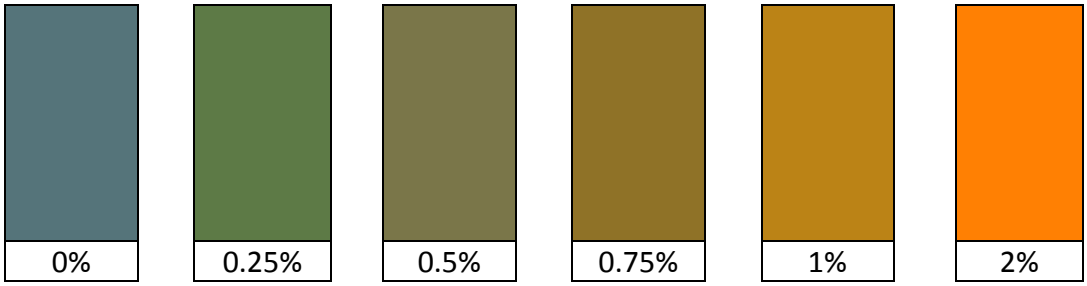
Funding and Conflict of interests

The Benedict's solution was funded by the researchers. No relevant conflicts of interest to declare.

Appendix A

Benedict's test colour chart

(With corresponding level of Reducing Substances)



Appendix B

Questionnaire

Study Number:

Date completed:

Date of admission:

Demographics and housing:

Age:					
Sex	Male	Female			
Housing type	Brick	Iron Sheeting	Mud	Other	
Number of Rooms for sleeping					
Number of people in household					
Usual water source	Indoor tap	Communal tap	River	Borehole	Other
Toilet Type	None	Pit latrine	Bucket system	Flush toilet	
Electricity	Yes	No			

SAM Indicators:

Admission weight:			
Admission length/height:			
Z Scores:	Actual measurement:	Z score:	
Weight for age:			
Height for age:			
Weight for height:			
MUAC :		<115mm	>115mm
Oedema (bilateral pitting involving at least the feet)		Present	Absent
Skin fold thickness			

Diarrhoea:

Diarrhoea	Present	Absent		
Consistency	Watery	Explosive	Blood/Mucus	
Duration	<14 days	>14 days	Date of onset:	
Episodes in a 24 hour period (max)				
Vomiting:	Present	Absent	Date of onset:	
Perianal skin erosion:	Present	Absent		

Feeding:

Breast exclusive	Formula Exclusive Type of formula:	Mixed Feeding Type of formula: Estimated date of introduction:	Complementary Food (list details)

Medical:

HIV Status	Positive	Negative	Exposed	Not exposed	
	On ART Date of initiation:		On NVP		
	Not on ART		Not on NVP		
	Co-trimoxasole Prophylaxis		Co-trimoxasole Prophylaxis		
	WHO Clinical Stage:				
	CD4 Count: CD4 %:				

	Date:				
Co morbidity	UTI	Pneumonia/LRTI	TB	Meningitis	Other:
Health care before current admission	None	Clinic	Traditional Healer	General Practitioner	Other:
Previous admission	Yes	No	Date discharged:	Number of admissions in the last year:	
Antibiotics	Used more than 3 weeks ago	Used in last 3 weeks	Used in last 2 weeks	Used in last week	Name of Antibiotic
Antimicrobials used during this admission, prior to stool collection:					
Highest Recorded Temperature					
Dehydration on admission	Mild	Moderate	Severe	Not assessed	
Hypoxia during admission	Yes	No	Lowest recorded sats:		
Vaccinations Missed:					
Rotavirus vaccination dates:					
Final Outcome:	Death	Discharged Duration of stay:	Absconded or refused hospital treatment	Transferred: Name of facility:	Referred to step down facility: Name of facility:

Investigations:

Hb	
CRP	
WCC	
Neutrophil %	
Lymphocyte %	
Monocyte %	
Eosinophil %	
Platelets	
Blood Culture	
Total protein	
INR	
PTT	
Sodium	
Potassium	
Urine: Leucocytes	
Urine: Culture (and sensitivity)	
Stool Reducing substance %	
Stool Microscopy	
Stool Culture	
Stool Electron Microscopy	

Appendix C

Participant information sheet and informed consent

Introduction and Invitation to participate:

Hello. My name is Britta McLaren, I am a master's student and I am doing a research project for my degree. I would like to ask for some of your time to explain the research that I am doing and to ask for you and your child's help to do this research. Research is the way that we find information that can help us to answer certain questions.

Please feel free to ask any questions during our discussion.

What is the reason for this study?

This study is not part of the normal care that your child will receive in hospital, but it is being done to learn more information that will help us to treat children like yours. There are many children in South Africa who are very underweight for their age (whose weight is much lower than other children their age) and have diarrhoea (having watery stools). The presence of diarrhoea in a child who is very underweight for their age can make health of the child worse than if the child did not have diarrhoea. We are trying to identify some of the causes of diarrhoea in underweight children, especially lactose intolerance (which is when the body does not absorb the sugar found in breast milk, cow's milk and most formulas) and infections (germs that may be causing diarrhoea – this is not for the master's research, but for other research). Only children under the age of 5 who are admitted at this hospital are invited to participate in this study

What is involved in the study?

Should you decide to join the study, I would like to ask you a few questions(face-to-face, in a private space), about your child's past and present health, previous medicines used and vaccines received, HIV status and living conditions. I would need to assess your child's weight, height, measure around the upper part of their arm and I would like your permission to have a look at your child's hospital file and blood test results (and to record this information). I would then need to take a little bit of your child's stool (poo). The stool sample will be taken from your child's nappy or bed pan. If this is not possible, a small, soft tube will be inserted into your child's bottom and some stool will be sucked out. This is not a painful procedure and you are welcome to watch while it is being done. I have done this many times before and have never caused any harm when doing it. It is the normal way that doctors take a sample of stool if they need one and they can't get it from the nappy.

I will tell you the results of the testing for sugar in the stool immediately, but the testing for germs takes a few days to weeks. If you want to know these results I can arrange that. If it is found that your child does not actually fit the definition of malnutrition (very underweight for age) that I am using then I will no longer need you to participate in this study.

Expected duration of participation:

In total I will take about 30 minutes of your time. If your child does not pass any stool before I am finished, I may need to come back later in the day to collect the stool sample.

What are the risks of this study?

I do not for see any risks to you or your child should you decide to participate in the study. The tube that we use to suck out some stool is thin and will cause very little pain or discomfort. It is a procedure that has been done many times and there are no likely complications from this procedure. If anything unexpected does occur as a result of this procedure, I will take the responsibility of making sure that it is fixed as best as possible.

What will be benefits of this study?

Your child will not directly benefit from the study, but if I do gain any information from the stool testing, that I think will help your doctors to treat your child better, I will (with your permission) tell them the information. Your child's treatment however will not change if you agree or don't agree to participate in the study, your doctors will still offer you the same services. The information that we collect may be used to help other children who have a similar condition to your child. You may ask me any questions, which may help you to understand your child's condition better and I am available to discuss any concerns that you have about your child if you wish.

What are your rights in this study?

It is your right to give permission for me to include your child in the study or not. If you choose to participate, you may change your mind at any stage or choose not to answer any question(s). If you choose not to participate or decide to leave the study once you have agreed to be a part of it, your child will continue to receive treatment and will not lose out on any health services.

Confidentiality of participant information:

I will make every effort to keep all your information confidential and anonymous. Absolute confidentiality cannot be guaranteed. Personal information may be disclosed if required by law. Organizations that may inspect and/or copy research records for quality assurance and data analysis include groups such as the Research Ethics Committee but all your information will be put under a study number and not your name. None of the study personnel or the people who access this study will know which information (including HIV status) belongs to you or your child.

What are the costs?

There will be no cost involved for you if you choose for your child to participate in the study. You will also not receive any payment for taking part.

Contact details:

If you have any questions about the study, your child's rights within the study or any concerns that you have to do with the study, you may contact me at any time:

Dr Britta McLaren: 0114709127/0832256282.

This study has received ethical approval from the Human Research Ethics committee of the University of the Witwatersrand (M121045), Johannesburg (011 7171234). If you have any questions about the rights of you or your child as a participant in this study or the ethics of this study, please contact the office on the above number.

Informed Consent for Participation:

Parent or legal guardian declaration section:

I, _____ (name of parent/guardian), agree that the nature of the study questions, examination and stool collection have all been explained to me and that I agree that my child may participate in the following study procedures:

I agree to be interviewed and allow information from my child's hospital records and laboratory results (including the HIV status) to be collected for this study. I agree for my child's weight, height and mid upper arm circumference to be measured I agree to the testing of my child's stool for the presence of germs and lactose intolerance	Yes	No

The signature of the parent or legal guardian below means that the study has been explained to them and that they understand what it entails and agrees that that their child may participate.

Name of parent or legal guardian:
Signature of parent or legal guardian:
Date:
Name of witness:
Signature of witness:
Date:

Name of researcher:	
Signature of researcher:	
Date:	

Storage of samples for future testing:

Patient information section:

Once your child's stool sample has been tested for the purpose of this study, the sample may be stored anonymously, for an indefinite period of time in a general bank in the laboratory for testing in the future. This is a normal procedure that is done in the laboratory. It is done so that if new germs are discovered in the future, the laboratory can look back at old samples to see if they can find the germ in older samples which would show that the 'new germ' was actually always there, but they didn't know how to find it in the old samples. If the germ is not found in old samples, then they will know that this germ is really a new germ that has developed. The samples in the bank are also used when new tests are developed, to verify the new tests by testing old samples with the new test. No genetic testing (testing for your child's DNA or genes) will ever be done.

You may give permission for the laboratory to store the sample or not.

Parent or legal guardian declaration section:

I, _____ (name of parent/guardian), agree that the nature and purpose of stool storage for future testing has been explained to me.

I agree for my child's stool sample to be stored for possible testing the future	Yes	No

The signature of the parent or legal guardian below means that the storage of the stool sample has been explained to them and that they understand what it entails and agrees that that their child's stool sample may be stored for future testing.

Name of parent or legal guardian:
Signature of parent or legal guardian:
Date:
Name of witness:
Signature of witness:
Date:

Name of researcher:	
Signature of researcher:	
Date:	

Appendix D

Ethical clearance certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Dr Britta McLaren

CLEARANCE CERTIFICATE	**M121045**
PROJECT	Diarrhoea and Malnutrition: What Factors are at Play?
INVESTIGATORS	Dr Britta McLaren.
DEPARTMENT	Department of Paediatrics
DATE CONSIDERED	26/10/2012
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 26/10/2012 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor : Dr Tim de Maayer

DECLARATION OF INVESTIGATOR(S)
To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

Appendix E

Definitions

[1] Formal housing: a dwelling or brick structure on a separate stand, flat or apartment, town/cluster/semi-detached house, unit in retirement village, room or flatlet on a larger property. 'Informal' housing: The following housing types: informal dwelling or shack in backyard, informal dwelling or shack in informal settlement, dwelling or house/flat/room in backyard, caravan or tent (45).

[2] Adequate toilet facilities (used as proxy for basic sanitation): Includes facilities that are safe, reduce odours and are within or near a house. Inadequate sanitation includes a wide range of poor toilet facilities including pit latrines that are not ventilated, chemical toilets, buckets, or no facilities at all(45).

[3] Adequate drinking water: Access to a clean and reliable water supply that is at their house. All other water supplies, including rivers and communal taps, are considered inadequate (45).

[4] Electricity: Households that have an electricity connection include households that are connected to the mains electricity supply (46).

[5] Overcrowding: a ratio of more than two people per room (excluding bathrooms but including kitchen and living room). There is no standard measure of overcrowding in South Africa, but there are many international definitions. The definition used here is derived from the United Nations Human Settlement Program (UN-HABITAT) definition, which is a maximum of two people per habitable room. 'Habitable' excludes bathroom and toilet (45).

Appendix F

Plagiarism Report

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ORIGINALITY REPORT

5%	2%	4%	%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Talbert, Alison, Nahashon Thuo, Japhet Karisa, Charles Chesaro, Eric Ohuma, James Ignas, James A. Berkley, Christopher Toromo, Sarah Atkinson, and Kathryn Maitland. "Diarrhoea Complicating Severe Acute Malnutrition in Kenyan Children: A Prospective Descriptive Study of Risk Factors and Outcome", 10.1371, 2012. Publication	1%
2	www.nt.gov.au Internet Source	1%
3	Banda, Theresa, Vivian Pribram, Margaret Lawson, Catherine Mkangama, and Gertrude Nyirenda. "Malnutrition, Infant Feeding, Maternal and Child Health", Nutrition and HIV, 2013. Publication	<1%
4	Korpe, P.S.. "Environmental enteropathy: critical implications of a poorly understood condition", Trends in Molecular Medicine, 201206 Publication	<1%

5	brilliantbiologystudent.weebly.com Internet Source	<1%
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7	Maartens, Gary, Connie Celum, and Sharon R Lewin. "HIV infection: epidemiology, pathogenesis, treatment, and prevention", The Lancet, 2014. Publication	<1%
8	Biggs, C.. "Clinical dietetic practice in the treatment of severe acute malnutrition in a high HIV setting", Journal of Human Nutrition and Dietetics, 2013. Publication	<1%
9	V. Cheryl Nikodem. "Do Cabbage Leaves Prevent Breast Engorgement? A Randomized, Controlled Study", Birth, 6/1993 Publication	<1%
10	Jung Yeon Chin and Ho-Joong Youn. "The Effect of Ablation for Paroxysmal Atrial Fibrillation on Left Atrial Volume and Function: A One-Year Follow-Up Study", Yonsei Medical Journal, 2014. Publication	<1%
11	www.wjem.org Internet Source	<1%
12	Linda Vidulich. "Infant programming of bone size and bone mass in 10-year-old black and	<1%

white South African children", Paediatric and Perinatal Epidemiology, 7/2007
Publication

13	www.aitoolkit.org Internet Source	<1%
14	apps.who.int Internet Source	<1%
15	Hofmeyr, G Justus, A Metin Gülmezoglu, Cynthia Pileggi, and G Justus Hofmeyr. "Vaginal misoprostol for cervical ripening and induction of labour", Cochrane Database of Systematic Reviews Reviews, 2010. Publication	<1%
16	www.ichrc.org Internet Source	<1%
17	Isanaka, S., R. F. Grais, A. Briend, and F. Checchi. "Estimates of the Duration of Untreated Acute Malnutrition in Children From Niger", American Journal of Epidemiology, 2011. Publication	<1%
18	pastprimates.net Internet Source	<1%
19	Sara E. Wuehler. "Accelerating improvements in nutritional and health status of young children in the Sahel region of Sub-Saharan Africa: review of international guidelines on infant and young child feeding	<1%

and nutrition : IYCN in the Sahel", Maternal and Child Nutrition, 04/2011
Publication

20	Arinaitwe, Emmanuel, Anne Gasasira, Wendy Verret, Jaco Homsy, Humphrey Wanzira, Abel Kakuru, Taylor G Sandison, Sera Young, Jordan W Tappero, Moses R Kamya, and Grant Dorsey. "The association between malnutrition and the incidence of malaria among young HIV-infected and -uninfected Ugandan children: a prospective study", Malaria Journal, 2012. Publication	<1%
21	www.cmamforum.org Internet Source	<1%
22	Onis, Mercedes, and Zita Weise Prinzo. "Managing children with severe acute malnutrition — what's new? : A Health Policy Perspective", Indian Pediatrics, 2014. Publication	<1%

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EXCLUDE BIBLIOGRAPHY	ON		

6.0 REFERENCES

(1) UNICEF,WHO,World Bank. 2012. UNICEF-WHO-World Bank Joint Child Malnutrition Estimates. Available: www.who.int/nutrition/publications/childgrowthstandards_estimates2011/en/index.html (Accessed 8 October 2014)

(2) WHO, UNICEF. 2009. WHO growth standards and the identification of severe acute malnutrition in infants and children. A joint statement by the World Health Organization and the United Nations Children's Fund. Available: www.who.int/nutrition/publications/severemalnutrition/9789241598163/en/index.html (Accessed 8 October 2014).

(3) Irena A, Mwambazi M, Mulenga V. Diarrhea is a major killer of children with severe acute malnutrition admitted to inpatient set-up in Lusaka, Zambia. Nutr J 2011;10(1):110.

(4) Talbert A, Thuo N, Karisa J, et al. Diarrhoea complicating severe acute malnutrition in Kenyan children: A prospective descriptive study of risk factors and outcomes. PLos ONE 2012 2012;7(6):1-8. doi:10.1371/journal.pone.0038321

(5) Brewster D. Inpatient management of severe malnutrition: time for a change in protocol and practice. Ann Trop Paediatr 2011;31(2):97-107.

(6) Golden M,H. Evolution of nutritional management of acute malnutrition. Indian Pediatr 2010;46:667-678.

(7) Bejon P, Mohammed S, Mwangi I, et al. Fraction of all hospital admissions and deaths attributable to malnutrition among children in rural Kenya. Am J Clin Nutr 2008;88:1626-1631.

(8) Brewster D. Critical appraisal of the management of severe malnutrition: 2 Dietary management. J Paediatr Child Health 2006;42:575-583.

(9) WHO. 1999. Management of severe malnutrition: a manual for physicians and other senior health workers. Available: http://www.who.int/nutrition/publications/severemalnutrition/9241545119/en/index.html (accessed 8 October 2014)

(10) Bernal C, Velásquez C, Alcaraz G, et al. Treatment of severe malnutrition in children: experience in implementing the World Health Organization guidelines in Turbo, Colombia. J Pediatr Gastroenterol Nutr 2008;46(3):322-328.

(11) Yoon PW, Black RE, Moulton LH,et al. The effect of malnutrition on the risk of diarrheal and respiratory mortality in children< 2 y of age in Cebu, Philippines. Am J Clin Nutr 1997;65(4):1070-1077.

(12) Fawzi WW, Herrera MG, Spiegelman DL, et al. A prospective study of malnutrition in relation to child mortality in the Sudan. Am J Clin Nutr 1997;65(4):1062-1069.

(13) Black RE, Brown KH, Becker S. Malnutrition is a determining factor in diarrheal duration, but not incidence, among young children in a longitudinal study in rural Bangladesh. Am J Clin Nutr 1984;39(1):87-94.

(14) Rodríguez L, Cervantes E, Ortiz R. Malnutrition and gastrointestinal and respiratory infections in children: a public health problem. Int J Environ Res Publ Health 2011;8(4):1174-1205.

(15) Welsh FK, Farmery SM, MacLennan K, et al. Gut barrier function in malnourished patients. Gut 1998;42(3):396-401.

(16) Walker-Smith J. Cow's milk intolerance as a cause of postenteritis diarrhoea. J Pediatr Gastroenterol Nutr 1982;1(2):163-174.

(17) Ferraris RP, Carey HV. Intestinal transport during fasting and malnutrition. Ann Rev Nutr 2000;20(1):195-219.

(18) Greene HL, McCabe DR, Merenstein GB. Protracted diarrhea and malnutrition in infancy: Changes in intestinal morphology and disaccharidase activities during treatment with total intravenous nutrition or oral elemental diets. J Pediatr 1975;87(5):695-704.

(19) Kukuruzovic R, Robins-Browne RM, Anstey NM, et al. Enteric pathogens, intestinal permeability and nitric oxide production in acute gastroenteritis. Pediatr Infect Dis J 2002;21(8):730-739.

(20) Hammer HF, Hammer J. Diarrhea caused by carbohydrate malabsorption. Gastroenterol Clin North Am 2012;41(3):611-627.

(21) Dahlqvist A. Method for assay of intestinal disaccharidases. Ann Biochem 1964;7(1):18-25.

(22) Plotkin GR, Isselbacher KJ. Secondary disaccharidase deficiency in adult celiac disease (nontropical sprue) and other malabsorption states. N Engl J Med 1964;271(20):1033-1037.

(23) Gracey M, Burke V. Sugar-induced diarrhoea in children. Arch Dis Child 1973;48(5):331-336.

(24) Nyeko R, Kalyesubula I, Mworozi E, et al. Lactose intolerance among severely malnourished children with diarrhoea admitted to the nutrition unit, Mulago hospital, Uganda. BMC Pediatrics 2010;10(1):31.

(25) Korpe PS, Petri Jr WA. Environmental enteropathy: critical implications of a poorly understood condition. Trends Mol Med 2012;18(6):328-336.

(26) Castro-Rodriguez JA, Salazar-Lindo E, Leon-Barua R. Differentiation of osmotic and secretory diarrhoea by stool carbohydrate and osmolar gap measurements. Arch Dis Child 1997;77(3):201-205.

(27) Soeparto P, Stobo EA, Walker-Smith JA. Role of chemical examination of the stool in diagnosis of sugar malabsorption in children. Arch Dis Child 1972;47(251):56-61.

(28) Chong CK, Bunn J, Newland P. Lactose intolerance in infants: harder to investigate now following discontinuation of Clinitest. Arch Dis Child 2011;96(6):611-611.

(29) Biggs C. Clinical dietetic practice in the treatment of severe acute malnutrition in a high HIV setting. J Hum Nutr Diet 2013;26(2):175-181.

(30) Bowie MD, Brinkman GL, Hansen J. Acquired disaccharide intolerance in malnutrition. J Pediatr 1965;66:1083-91.

(31) Solomons NW, Torun B, Caballero B, et al. The effect of dietary lactose on the early recovery from protein-energy malnutrition. I. Clinical and anthropometric indices. Am J Clin Nutr 1984;40(3):591-600.

(32) Torun B, Solomons NW, Caballero B, et al. The effect of dietary lactose on the early recovery from protein-energy malnutrition. II. Indices of nutrient absorption. Am J Clin Nutr 1984;40(3):601-610.

(33) Pettifor J, Rajah R, Venter A, et al. A double-blind control trial of the efficacy of a soya or milk based formula in the treatment of kwashiokor. SA j Food Sci Nutr 1989;1(2):11-15.

(34) Brown KH. Appropriate diets for the rehabilitation of malnourished children in the community setting. Acta Pediatr 1991;80(s374):151-159.

(35) Baker R, Baker S, Margo G, et al. Successful use of a soya-maize mixture in the treatment of kwashiorkor. S Afr Med J 1978;53(17):674-677.

(36) John C, Gopaldas T. Evaluation of the impact on growth of a controlled 6-month feeding trial on children (6-24 months) fed a complementary feed of a high energy-low bulk gruel versus a high energy-high bulk gruel in addition to their habitual home diet. J Trop Pediatr 1993;39(1):16-22.

(37) Brewster DR, Manary MJ, Menzies IS, et al. Comparison of milk and maize based diets in kwashiorkor. Arch Dis Child 1997;76(3):242-248.

(38) Brewster D, Kukuruzovic R. Milk formulas in acute gastroenteritis and malnutrition: a randomized trial. J Pediatr Child Health 2002;38(6):571-577.

(39) Amadi B. Role of food antigen elimination in treating children with persistent diarrhea and malnutrition in Zambia. J Pediatr Gastroenterol Nutr 2002;34:S54-S56.

(40) Picot J, Hartwell D, Harris P, et al. The effectiveness of interventions to treat severe acute malnutrition in young children: a systematic review. Health Technol Assess 2012;16(19):1-316.

(41) Agostoni C, Axelsson I, Goulet O, et al. Soy protein infant formulae and follow-on formulae: a commentary by the ESPGHAN Committee on Nutrition. J Pediatr Gastroenterol Nutr 2006;42(4):352-361.

(42) Wall C, Webster J, Quirk P, et al. The nutritional management of acute diarrhea in young infants: effect of carbohydrate ingested. J Pediatr Gastroenterol Nutr 1994;19(2):170-174.

(43) Brown KH, Peerson JM, Fontaine O. Use of nonhuman milks in the dietary management of young children with acute diarrhea: a meta-analysis of clinical trials. Pediatrics 1994;93(1):17-27.

(44) Brand JC, Miller JJ, Vorbach EA, et al. A trial of lactose hydrolysed milk in Australian Aboriginal children. Med J Aust 1977;2(4 Suppl):10-13.

(45) Mitchell J, Brand J, Halbisch J. Weight-gain inhibition by lactose in Australian aboriginal children: A controlled trial of normal and lactose hydrolysed milk. The Lancet 1977;309(8010):500-502.

(46) Harris P, Taylor R, Thielke R et al. Research electronic data capture (REDCap) - A metadata-driven methodology and workflow process for providing translational research informatics support. J Biomed Inform 2009;42(2):377-381.

(47) StataCorp. 2009. *Stata Statistical Software: Release 11*. College Station, TX: StataCorp LP.

(48) Berry L, Biersteker L, Dawes A, et al. South African Child Gauge 2013. Cape Town: Children's Institute, University of Cape Town. 2013. Available: http://www.ci.org.za/index.php?option=com_content&view=article&id=509:sa-child-gauge&catid=40&Itemid=81&showall=&limitstart=3 (accessed 8 October 2014)

(49) Proudlock P, Dutschke M, Jamieson L, et al. South African Child Gauge 2007/2008.Cape Town: Children's Institue, University of Cape Town. 2008. Available: http://www.ci.org.za/index.php?option=com_content&view=article&id=509:sa-child-gauge&catid=40&Itemid=81&showall=&limitstart=3 (accessed 8 October 2014)

(50) Castro-Rodriguez JA, Salazar-Lindo E, Leon-Barua R. Differentiation of osmotic and secretory diarrhoea by stool carbohydrate and osmolar gap measurements. Arch Dis Child 1997;77(3):201-205.

(51) Beau JP, Fontaine O, Garenne M. Management of malnourished children with acute diarrhoea and sugar intolerance. J Trop Pediatr 1989;35(6):281-4.

(52) Fagundes-Neto U, Viaro T, Lifshitz F. Tolerance to glucose polymers in malnourished infants with diarrhoea and disaccharide intolerance. Am J Clin Nutr 1985;41(2):228-34.

(53) Fajardo LF, Leal H, Victoria F, et al. Milk intolerance in Colombian children, its prevalence and relation to lactose malabsorption. Arch Latinoam Nutr 1979;29(3):329-39.

(54) Lifshitz F, Coello-Ramirez P, Gutierrez-Topete G, et al. Carbohydrate intolerance in infants with diarrhea. J Pediatr 1971;79(5):760-767.

(55) South Africa. National Dept. of Health. 2011. The National Antenatal Sentinel HIV and Syphilis Prevalence Survey, South Africa. Available: http://www.sanac.org.za/resources/cat_view/7-publications/9-reports (accessed 8 October 2014)

(56) Hall K, Woolard I, Lake L, et al. South African Child Gauge 2012. Cape Town: Children's Institute, University of Cape Town. 2012. Available:

http://www.ci.org.za/index.php?option=com_content&view=article&id=509:sa-child-gauge&catid=40&Itemid=81&showall=&limitstart=3 (accessed 8 October 2014)

(57) De Maayer T, Saloojee H. Clinical outcomes of severe malnutrition in a high tuberculosis and HIV setting. Arch Dis Child 2011;96(6):560-564.

(58) Fergusson P, Tomkins A. HIV prevalence and mortality among children undergoing treatment for severe acute malnutrition in sub-Saharan Africa: a systematic review and meta-analysis. Trans R Soc Trop Med Hyg 2009;103(6):541-548.

(59) Patrick M, Stephen C. Child PIP: Making mortality meaningful by using a structured mortality review process to improve the quality of care that children receive in the South African health system. S A J Child Health 2008;2(2):38-42.