Gaining insight into the determinants of mortality in hospitalized severely malnourished children

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CHAPTER 1

General introduction and thesis outline

Childhood malnutrition is amongst one of the most challenging global health problems. The complexity of childhood malnutrition has intrigued countless health care professionals, scientists and policy makers for decades and many have committed to solve this problem to improve child survival. Unfortunately, childhood malnutrition still threatens the lives and future of far too many young children; malnutrition increases the risk of death, makes children more susceptible to disease and holds them back from reaching their full physical and cognitive potential[1,2].

Substantial progress has been made in the therapeutic management and survival of severely malnourished children[1]. However, considerable disparities in survival continue to exist between severely malnourished children with and without medical complications. While community-based programmes have transformed outcomes for children with uncomplicated severe malnutrition[3], severely malnourished children with complications still face high mortality rates during treatment in hospital. In African hospitals mortality still ranges between 23 and 46%[4–7]. Even after discharge, the prognosis is poor with reported mortality rates as high as 42% in the subsequent year[5].

Many severely malnourished children suffer from diarrhoea during hospital admission, which increases their risk of death substantially[8–10]. Studies have revealed that intestinal structure and function are seriously disrupted in severely malnourished children with more severe changes reported in children with concurrent diarrhoea[11,12]. Accumulating evidence suggests that this enteropathy may persist despite treatment[13], which could contribute to poor clinical outcomes in hospital. The continuation of diarrhoea and outstandingly high mortality rates during admission, as well as high mortality rates after discharge, suggest that current treatment algorithms may not fully address the changes in the intestine of these severely ill children. Moreover, limited characterization of these potentially persistent intestinal changes and limited insights into their underlying mechanisms hamper our ability to provide the optimal treatment needed for survival and recovery.

This chapter describes the epidemiology, pathophysiology and therapeutic management of severe childhood malnutrition. Next, it discusses the current knowledge of intestinal changes in severe malnutrition, including putative underlying mechanisms. Moreover, it highlights the need for new preclinical models to study the mechanisms underlying intestinal changes in severe malnutrition.

Severe childhood malnutrition

Definition of childhood malnutrition

Malnutrition refers to an imbalance in the intake of nutrients and energy relative to the body's requirements to ensure homeostasis and, in the case of children, optimal growth. Malnutrition can be caused by nutrient deficiencies or excesses, otherwise known as undernutrition and overnutrition[14]. In this thesis, the term malnutrition refers to undernutrition in children. The current classification of childhood malnutrition is based on body measurements (e.g. body weight, mid-upper arm circumference) and compares these parameters with a reference population that the World Health Organization (WHO) designated as "healthy growers"[15].

Several forms of childhood malnutrition are recognized: stunting, wasting, underweight and deficiencies of vitamins and minerals. Stunting or reduced linear growth results from chronic or recurrent undernutrition, whereas wasting can indicate more acute severe weight loss. The term 'underweight' encompasses both stunting and wasting. A child is stunted when he or she is too short for their age, which is defined as a height-for-age that is more than two standard deviations below the WHO Child Growth Standards median (HAZ score <-2). Inadequate nutrition, recurrent infections and/or chronic diseases are some of the most direct causes of stunting. Stunting has adverse long-term consequences for survival, child and adult health, and cognitive development[16,17]. A child is wasted when he or she is too thin for their height, which is defined as a weight-for-height (> 2 years of age) or weight-for-length (<2 years of age) that is more than two standard deviations below the WHO Child Growth Standards median (WHZ/WFL score <-2).

Definition of severe acute malnutrition and historical perspective

Severe acute malnutrition (SAM), which is the focus of this thesis, is the most life-threatening form of undernutrition. SAM is currently defined as a WHZ score <-3, mid-upper arm circumference (MUAC) < 11.5 cm and/or bilateral pitting oedema in children between 6-59 months of age[18,19]. SAM classically presents as one of two phenotypically distinct forms that are marked by different metabolic responses to severe undernutrition: the non-oedematous form known as marasmus, and the oedematous forms, which include kwashiorkor and marasmic-kwashiorkor[20]. Marasmus is defined by severe wasting of adipose and muscle tissue. A child with marasmus is extremely thin ("skin over bones") and often has a wizened "old man" appearance (**Figure 1**). Oedematous

malnutrition was first described in several local publications in Latin America during the 19th century (cited in [21]) and was followed by other occasional reports[22,23]. However, it was only after the report of Dr. Cecily Williams in 1933[24] describing this disorder as "a nutritional disease of childhood associated with a maize diet" and giving the condition its present name "Kwashiorkor" in 1935[25], that the condition became widely recognized. During the early 1950s, Brock and Autret[26] demonstrated that kwashiorkor was prevalent in sub-Saharan countries and provided a clear description of the clinical features that matched the earlier description by Dr Williams[24]. Subsequent surveys demonstrated that kwashiorkor was also prevalent in Central America[27] and Brazil[28]. In these studies, intermediate cases between kwashiorkor and marasmus were also described (e.g. stunting and hair depigmentation but no oedema). Kwashiorkor was defined as a clinical syndrome, mainly seen in children under the age of 2 years. The clinical characteristics included the presence of bilateral pitting oedema, skin rashes, ulceration (e.g. flaky paint dermatosis) and hair abnormalities (e.g. sparse, depigmented hair) (**Figure 1**). In 1952, the Joint Food and Agriculture Organization (FAO)/WHO Committee on Nutrition introduced the term "protein malnutrition" referring to the concept that kwashiorkor was due to an inadequate dietary protein intake as opposed to marasmus which was the outcome of prolonged insufficient dietary intake of all macronutrients[29]. In 1960, the FAO/WHO expert committee proposed that kwashiorkor and marasmus were two main categories of 'protein-calorie malnutrition', which referred to the observation that diets in these areas are commonly low in protein, but provide calories ranging from inadequate to excessive[30]. In the years following the introduction of kwashiorkor as a clinical syndrome, some authors also used the term kwashiorkor to describe conditions when only some of the clinical manifestations of kwashiorkor were observed leading to some confusion. In 1970, a consensus was reached on the categorisation of malnutrition; kwashiorkor should be used for children with oedema, marasmus for children with a low weight-for-age (later replaced by weight-for-height[31]) and marasmic-kwashiorkor for children with both oedema and low weight-for-age. The hair, skin and liver manifestations were not included in this definition[32]. Some scientists started to use the term "oedematous malnutrition" instead of kwashiorkor. In this thesis, the terms severe wasting and oedematous malnutrition will be used to indicate marasmus and (marasmic-)kwashiorkor, respectively.

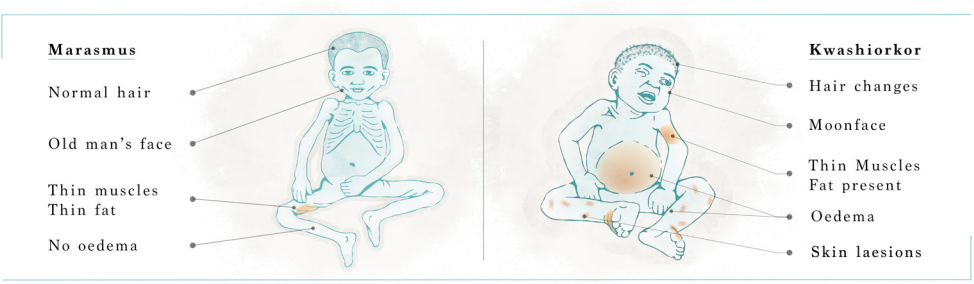

Figure 1. Severe acute malnutrition. Clinical characteristics of severely malnourished children diagnosed with marasmus/severe wasting (left) and kwashiorkor/oedematous malnutrition (right).

Epidemiology

Childhood malnutrition still affects and threatens the lives of an overwhelming number of children worldwide. Young children under the age of five bear the brunt of the problem being more susceptible due to their high nutritional demands during this period critical for child growth and development. The majority of malnourished children live in low- and middle-income countries; Africa and Asia account for the largest shares of the global burden with 27% and 69% of wasted children respectively[1]. In 2019 an estimated 144 million children under the age of five were stunted and 47 million were wasted, of whom 14.3 million children were severely wasted[1]. There are no accurate estimates on the global burden of oedematous malnutrition. This dearth of reliable data is due to the large geographical variability of oedematous malnutrition, the fact that assessment of oedema is often not included in large nutritional surveys and the transient nature of this disease (oedema spontaneously resolves or the child dies) which complicates its timely detection in surveys[33]. However, estimates from various African countries suggest that oedematous malnutrition accounts for more than a third of severe malnutrition cases; African countries such as Malawi, Rwanda and Zambia are at the top of the list where oedematous malnutrition accounts for 50%, 47.1% and 44.7% of all severe malnutrition cases respectively[34]. While oedematous malnutrition is apparently prevalent in many African countries, considerably lower numbers are reported in Asia with oedematous malnutrition accounting for less than 15% of all severe malnutrition cases[34]. The geographical differences in the prevalence of the different forms of severe malnutrition probably indicate that the distribution, causes and consequences of risk factors for malnutrition vary across regions. Risk factors for childhood malnutrition include living in areas with high burdens of infectious diseases (e.g. respiratory tract infections, diarrhoea, HIV), with endemic food insecurity (e.g. famine, suboptimal feeding practices) and with

suboptimal social and environmental conditions (e.g. poverty, limited access to health care, poor education)[35].

Severely malnourished children have severely disturbed physiology and metabolism[20]. To illustrate, hypoglycaemia due to disturbed glucose homeostasis [36,37] and diarrhoea related to disrupted intestinal homeostasis are common with negative impacts on survival[9,10,38]. In addition, severely malnourished children are not only more susceptible to infectious diseases, but also face higher disease severity and case fatality[39,40]. It is not surprising that regions with high numbers of severely malnourished children often also experience high childhood mortality rates, which implies that malnutrition may play an important role in the risk of death. The Lancet Nutrition series in 2013 reported that malnutrition directly or indirectly underlies almost half of all childhood deaths worldwide[2]. Severe malnutrition carries the highest mortality and contributes to approximately half a million deaths in children under five years of age worldwide[2]. Most deaths in severely malnourished children can be linked to infectious diseases such as diarrhoea and malaria and metabolic disturbances such as hypoglycaemia and refeeding syndrome[41,42]. Altogether, these numbers underline the significant burden of morbidity and mortality in children suffering from severe malnutrition.

Aetiology and pathophysiology

Insight into the predispositions and pathophysiological pathways that lead to wasting has increased over the years, while the aetiology of oedematous malnutrition still remains an enigma.

Wasting

Inadequate protein and energy intake is generally recognized as important contributor to loss of muscle and adipose tissue in wasting, but wasting is seldom caused by a single factor[20]. Although the onset and resolution of wasting can be relatively quick, the factors that drive wasting are often more longstanding, for example recurrent infectious insults. Risk factors for childhood wasting include maternal factors (e.g. low BMI, educational level), diseases (e.g. infectious disease, diarrhoea) and environmental factors (e.g. seasonality, household wealth)[43]. Studies have highlighted that interactions between risk factors exist and that some factors can be both a cause and a consequence of wasting. In addition, risk factors are often context-dependent; the significance and impact of particular risk factors can change over time, with the age of the child and with changes in the context (e.g. population displacement, famine). This intricate interplay of risk factors

makes the aetiology of wasting complex.

Our knowledge of the physiological mechanisms and metabolic adaptations that accompany wasting remains limited and is mainly derived from publications on long-term starvation and wasting due to a chronic illness (cachexia)[44]. During short-term starvation (fasting up to several days), the body shifts from carbohydrate metabolism to fat and protein catabolism, which provides glucose and ketones for energy[20]. After several days of starvation, body fat has been depleted and therefore muscle proteins are extensively broken down. Importantly, evidence suggests that these metabolic disturbances may continue after recovery from the wasting episode, which could increase vulnerability to relapse and also limit the growth potential of affected children[43].

Oedematous malnutrition

Research into the divergence of malnutrition phenotypes has led to many hypotheses about the origin of oedematous malnutrition. These hypothesis are extensively reviewed by André Briend[45] and include: 1) insufficient protein intake, 2) insufficient intake of particular amino acids, 3) maladaptation to a low-protein carbohydrate-rich diet, 4) oxidative stress, 5) intoxication with aflatoxins produced by *Aspergillus* species, 6) disruption of sulphated glycosaminoglycans (GAGs) and more recently, 7) dysbiosis of the microbial community in the gut, referred to as gut microbiota. However, none of these hypotheses can fully explain all clinical and pathophysiological characteristics of oedematous malnutrition. Therefore, considerable interest remains to explain the divergent malnutrition phenotypes. A recent comparative meta-analysis has systematically reported the available information on differences in pathogenic characteristics between children with severe wasting and oedematous malnutrition[46]. This is of importance as children with characteristics of oedematous malnutrition have a poor prognosis and respond differently to treatment compared to children with severe wasting. This may reflect more severe disturbances in organ physiology and metabolism[45]. The known structural and functional impairments of organ systems (e.g. altered immune function, liver dysfunction) in severe malnutrition have been reviewed by Bhutta et al.[20]. The current understanding of small intestinal changes will be discussed in the section 'malnutrition enteropathy'.

Treatment

Appropriate management of severe malnutrition is critical for child survival. The WHO developed clinical guidelines that take into account the known physiological and metabolic

changes in severely malnourished children[47,48]. In general, treatment includes correction of nutritional deficiencies with therapeutic food and treatment of underlying infections with antibiotics. A distinction in recommended treatment is made between children with complicated and uncomplicated severe malnutrition. Children who have no medical complications and who are able to feed sufficiently can be managed as outpatients. Children require inpatient treatment if they have medical complications including severe oedema, poor appetite, or when they are presenting with ≥1 Integrated Management of Childhood Illness (IMCI) danger signs (convulsions, lethargy, reduced conscious level and inability to feed sufficiently)[48].

Outpatient management of uncomplicated severe malnutrition is increasingly provided in community-based treatment centres through Outpatient Therapeutic Programs or OTPs. Children receive ready-to-use therapeutic foods (RUTF) in order to achieve adequate weight gain, which is monitored during regular visits to a local health facility. RUTFs are high-energy, fortified, ready-to-eat foods with a similar nutritional content to the F-100 therapeutic milk formula used to treat severely malnourished children. These foods were designed in a dehydrated form to reduce the risk of contamination and to allow safe use at home without preparation[49]. The rapid expansion of community treatment programmes extended the reach of treatment to previously untreated children. The outpatient approach yields good results with adequate weight gain, high recovery (approximately 80%)[3,50] and relatively low reported mortality rates of <1-7%[50,51].

Inpatient treatment of children with complicated severe malnutrition aims to treat life-threatening conditions (e.g. infections) and to correct nutritional deficiencies and metabolic disturbances. Recommended nutritional management occurs in three distinct phases with diets that are tailored to match the child's reduced metabolic capacity and to meet the estimated nutritional needs per treatment-stage[47,48]. In the first phase, the "stabilization phase", therapeutic feeding is cautiously started with a milk-based formula diet called F-75. F-75 contains low levels of protein and sodium and high levels of potassium, which should aid stabilization of physiological and metabolic functions in this stage. Feeding is started cautiously to avoid refeeding syndrome with its associated life-threatening metabolic derangements. Feeding can cause the catabolic state to rapidly switch to an anabolic state, which can lead to insulin secretion, acute hypoglycaemia and the transport of extracellular electrolytes into cells. The flux of electrolytes into cells can cause dangerously low blood concentrations of potassium, magnesium and phosphate. This in turn can lead to impaired cardiac function, respiratory failure, muscle weakness, impaired neurological function (e.g. lethargy, seizures) and sudden death[52].

The incidence of refeeding syndrome in malnourished children is unknown. When signs of clinical improvements such as regained appetite and, in cases of oedematous malnutrition, reduced oedema are observed, the child enters the second phase, the "transition phase". In this phase, the child will gradually transition to a diet with higher protein and energy content to achieve weight gain through either F-100 formula or RUTFs started with supplemental F-75 formula until the child is able to finish all the RUTF feeds. Finally, the child enters the last phase, the "rehabilitation phase", during which their daily intake of F-100 or RUTFs is increased to achieve catch-up growth. Under current WHO recommendations, the child can be discharged from hospital once clinically stable (e.g. serious complications have resolved) and tolerating RUTFs. Therapeutic feeding with RUTFs should be continued as an outpatient with regular follow-up in an outpatient clinic, where RUTFs are supplied and weight gain is monitored. Once discharged from outpatient treatment, the child should ideally be periodically monitored to avoid relapse.

Clinical outcomes for children with complicated severe malnutrition treated in hospital are considerably worse than for children with uncomplicated severe malnutrition treated as outpatients[4–7]. The factors driving persistent high inpatient mortality rates are not entirely clear. Major risk factors for mortality during inpatient treatment are reportedly diarrhoea, pneumonia, HIV infection, shock, anaemia, hypoglycaemia, MUAC<11.5cm and a low WHZ on admission[53,54]. These risk factors underscore that comorbidities and decompensation of physiological pathways are common in children with complicated severe malnutrition. WHO compliant management in hospital may reduce mortality to some extent, but the high mortality rates imply that the current management algorithms may be insufficient for treating this vulnerable population. Interestingly, the current WHO treatment guidelines have become engrained in daily clinical practice, even though the scientific basis is sparse, evidence is often "very low quality" and guidelines are mostly based on expert opinion[55]. The dearth of evidence for the treatment regimen is related to our limited understanding of many of the pathophysiological mechanisms underlying comorbidities and metabolic disturbances observed in severely malnourished children.

Severely malnourished children with diarrhoea often fail to respond to the current inpatient treatment regimen and pose one of the most difficult management challenges. Limited histological studies have shown that intestinal architecture is seriously altered[11,12,56], which can lead to nutrient malabsorption and intestinal barrier dysfunction in severely malnourished children[11,57]. More detailed knowledge is required of 1) the disruption in intestinal homeostasis, 2) the effect of treatment on intestinal structure and function, and 3) the mechanisms underlying intestinal alterations in severely malnourished

children. These insights will enable us to better tailor treatment to the intestinal needs; or in other words "design food from the inside out". In the next section, I will provide an overview of the anatomy and physiology of the small intestine, and then discuss the current understanding of small intestinal changes present in severe malnutrition.

Small intestine in health and in severe malnutrition

Intestinal anatomy

The small intestine is part of the gastrointestinal tract. The gastrointestinal tract is a term compromising all elements of the alimentary canal, stretching from the mouth to the anus, and its accessory organs including the salivary glands, liver and pancreas[58]. The overall function of the gastrointestinal tract is to digest and absorb nutrients from ingested food. In the mouth, salivary and lingual enzymes initiate the digestion of carbohydrates and lipids. The oesophagus serves as a conduit for the transportation of the food bolus to the stomach, where gastric acid and digestive enzymes (e.g. proteases, lipase) break it down further. Next, the bolus reaches the small intestine; this is the principal site of nutrient digestion and absorption and consists of three segments from proximal to distal: duodenum, jejunum and ileum[58]. Secretions from different organs aid in the digestive process here including bile acids from the liver for lipid digestion and pancreatic enzymes such as lipase, chymotrypsin and amylase for digestion of lipids, protein and dipeptidases, respectively. Enzymes on the luminal surface of the small intestine or 'brush border enzymes' (e.g. disaccharidases, dipeptidases) complete the digestion of carbohydrates and proteins. Once in the large intestine or the colon, fluids and electrolytes are reabsorbed. The colon is highly colonized by resident bacteria, viruses and fungi referred to as the gut microbiota[59]. Commensal bacteria have important functions such as vitamin B and K production as well as fermentation of carbohydrates that are not used by the host[59]. These non-digestible carbohydrates are converted to short-chain fatty acids (SCFA) (e.g. acetate, butyrate), which are mostly absorbed by the host and serve as a fuel source for epithelial cells[60]. Finally, the luminal content or faeces is expelled from the body.

The structural organization of the alimentary canal is similar throughout its length. It consists of four layers from the lumen outward: mucosa, submucosa, muscularis externa and serosa or adventitia[58]. The mucosa is the innermost layer consisting of the epithelium, the lamina propria and the muscularis mucosae. The submucosa, a connective tissue layer, connects the mucosa with the muscular layer muscularis externa; this is involved

in segmental contractions and peristalsis. The serosa and adventitia cover the external surface of the abdominal organs. The mucosa differs along the tract and is specifically adapted to the function of each specific part. The anatomy and physiology of the small intestinal mucosa, as the principal site of nutrient absorption and digestion, will be explained in more detail below.

Physiology of the small intestinal mucosa

The small intestinal mucosa has a complex task to serve as a semipermeable barrier that allows nutrient absorption, while also forming a defence against harmful substances, pathogens and foreign antigens. Regulation of this seemingly paradoxical task is supported by the structural composition of the intestinal mucosa. The intestinal mucosa consists of the mucus layer on the outside, a central single cell layer with differentiated and specialized epithelial cells, and the inner lamina propria with immune cells[61] (**Figure 2**). The intestinal epithelium and mucus layer will be discussed in this section.

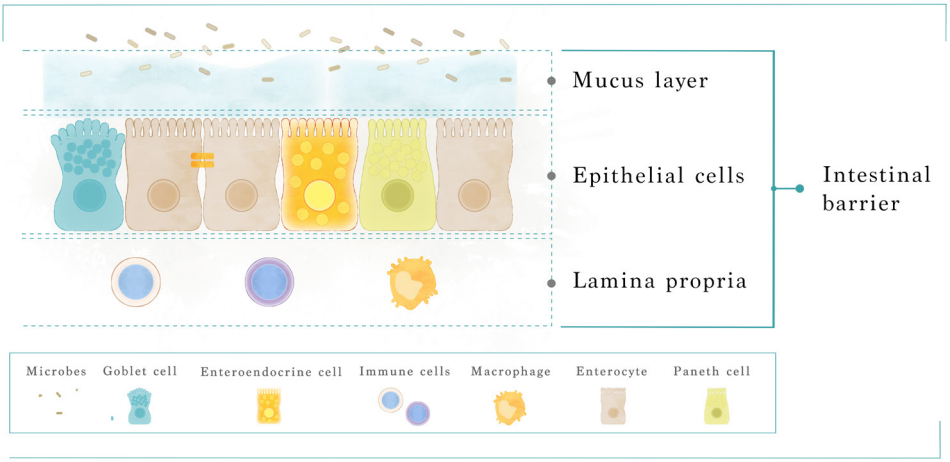

Figure 2. Intestinal mucosa serving as a semipermeable barrier.

Intestinal epithelial cells

The intestinal epithelium, composed of a single cell-layer, is arranged into a large number of crypt-villus units. A villus is a finger-shaped protrusion of the intestinal wall into the lumen and is surrounded by multiple invaginations known as crypts[62]. The intestinal epithelium is the fastest self-renewing tissue in mammals; in mice the epithelium is

completely renewed every 3-5 days, except for Paneth cells which have a life span of 3-6 weeks[63,64]. The crypt-villus architecture with its rapid turnover rate enables the small intestine to simultaneously serve as both a protective barrier and as the primary site of nutrient absorption. The small intestine owes its great nutrient absorptive capacity to the large surface area provided by its villi and microvilli, protrusion of villi into the lumen and the close relation to a dense network of capillaries underneath the epithelium to transport nutrients to the liver and the rest of the body[62]. However, this sophisticated architecture makes it vulnerable to a variety of assaults including mechanical stress, pathogens and digestive enzymes, all of which can damage the integrity of the epithelium. A continuous layer of intestinal epithelial cells and intercellular junctions are essential to preserve the integrity of the intestinal epithelium.

The layer of epithelial cells is maintained by a high epithelial turnover rate; this also reduces the time that the epithelium is exposed to harmful intraluminal conditions. Rapid epithelial turnover is supported by continuously dividing intestinal stem cells (ISCs) which reside at the base of the crypts where they are shielded from the hazards of the digestive process[62] (**Figure 3**). ISCs continuously undergo self-renewal and generate rapidly proliferating transit amplifying (TA) cells which differentiate into mature intestinal epithelial cells (IECs). IECs are pushed out of the crypts, migrate upwards the crypt-villus axis (except for Paneth cells), undergo apoptosis and are shed from the villus tip into the intestinal lumen[65,66]. Mature IECs types fulfil specialized functions and can be divided in absorptive (enterocytes and M cell) and secretory lineages (Paneth, goblet, enteroendocrine and tuft cell)[65] (**Figure 3**). Enterocytes are the most abundant IEC type, comprising about 80% of the intestinal epithelium, and are responsible for nutrient and water absorption. Goblets cells are specialized secretory cells which maintain a protective mucous layer on top the epithelium through generation and excretion of mucins. Enteroendocrine cells participate in metabolic control by acting as chemoreceptors and producing different hormones (e.g. glucagon-like peptide 1 and 2). Paneth cells reside at the base of the crypts. They play an important role in defence against invading microorganisms via the secretion of antimicrobial peptides such as defensins and lysozyme. In addition, Paneth cells also support ISC maintenance by secreting paracrine factors (e.g. WNT ligands, Notch stimuli)[67] and providing nutrients to ISCs[68]. Thus, a continuous supply of the different mature IECs in appropriate proportions is required to maintain intestinal homeostasis.

The continuous supply of new epithelial cells to the crypt-villus axis is fuelled by ISCs called crypt base columnar cells (CBCs)[69] or Lgr5+ cells based on expression of the stem cell marker 'leucine-rich-repeat-containing G-protein-coupled receptor 5 (Lgr5)'[70]. Stem

cell dynamics are regulated by various signals which come from the epithelium (e.g. Paneth cells) and from the underlying mesenchyme. Key signals that regulate stem cell renewal and intestinal fate determination (e.g. R-spondin, BMP) are extensively reviewed by Gehart and Clevers[62]. In addition to the capacity to self-renew under homeostatic conditions, the intestine has the ability to regenerate upon damage and survive acute loss of its active stem cell pool. Repopulation of the stem cell niche has been attributed to three mechanisms: mobilization of quiescent 'reserve' stem cells located at the +4 position[71,72], de-differentiation of progenitor cells [73,74] and differentiated absorptive and secretory cells that are able to revert to a stem cell state[75,76].

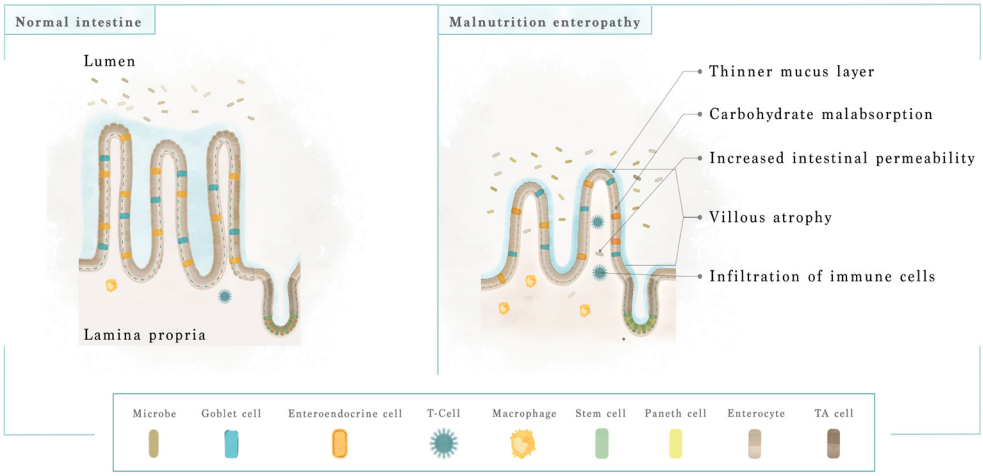

Figure 3. Morphology of the small intestine in healthy children (left) and in malnourished children (right).

Intercellular junction complexes mechanically connect intestinal epithelial cells and seal the intercellular space. These protein networks form three adhesive complexes: tight junctions (TJs), adherens junctions (AJs) and desmosomes (**Figure 4**) (reviewed in [77]). TJs, which are the most apically located in this complex, are responsible for sealing the intercellular space, whilst AJs and desmosomes are thought to be important in mechanical linkage of adjacent cells. Tight junctions are composed of several transmembrane and cytosolic proteins (e.g. occludin, claudins, zonula occludens (ZOs)) and junctional adhesion molecules (JAM), which interact not only with each other but also with the cytoskeleton[77]. Tight junctions, as dynamic structures, have the capacity to maintain barrier integrity and finely regulate paracellular transport. In addition, TJs prevent microbial translocation, maintain epithelial polarity and are involved in the regulation of cell proliferation and migration[77].

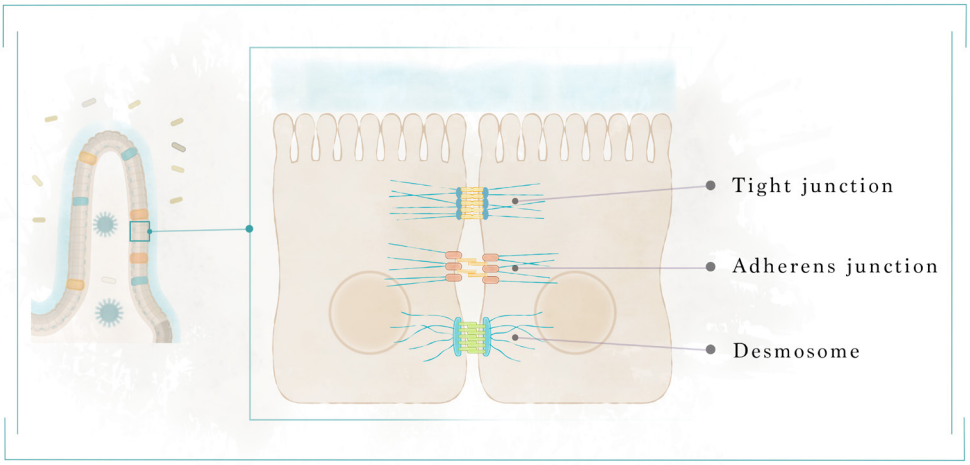

Figure 4. Cell-cell adhesion proteins between intestinal epithelial cells. Tight junctions are most apically located, followed by adherens junctions and desmosomes.

Mucus layer

The mucus layer, covering the IECs, shields them from direct contact with mechanical, biological and chemical hazards (e.g. digestive enzymes, bacteria) and protects the IECs from dehydration[78]. The thickness and structure of the mucus layer varies considerably along the intestinal tract. In the small intestine, the mucus is organised in a single layer that is easily removable and relatively porous. Whilst this enables efficient nutrient absorption, it also enables pathogens to penetrate the mucus[79]. However, the presence of antimicrobial agents in the mucus offers protection against pathogens with the highest concentrations in the crypts, as these components (e.g. defensins, lysozyme) are mainly produced by Paneth cells[80]. In the large intestine, the mucus layer consists of two layers with the inner layer virtually free of bacteria but the outer layer containing gut bacteria and dietary components[81]. The mucus layer provides an important niche for the microbial community and can be used by bacteria as binding sites and as an energy source promoting their colonisation and growth[82].

The major components that give mucus its viscous and elastic properties are large glycoproteins called mucins. Mucins are produced by goblet cells and fall into two main groups: transmembrane mucins or gel-forming mucins. Mucin 2 (MUC2) is the main gel-forming mucin in the intestine[83]. A number of different factors influence the mucus barrier, ranging from host factors, such as the gut microbiota, cytokines, to external factors such as pathogens, diet, food additives, antibiotics and pre- and probiotics[82,84–86]. Impairment of the mucus layer can be related to reduced mucus synthesis and secretion,

increased mucus degradation and penetrability, and altered mucus composition (e.g. viscosity)[84]. Importantly, mucus barrier impairment allows microbes to reach the intestinal epithelial cells and can thereby lead to infection and inflammation, as reported in intestinal diseases such as Inflammatory Bowel Disease (IBD)[87].

Malnutrition enteropathy

Histological studies have revealed that severely malnourished children suffer from severe intestinal mucosal damage, known as malnutrition enteropathy[12]. Hallmark features of malnutrition enteropathy include alterations in the small intestinal epithelium architecture with villous atrophy (shortened, leaf-shaped villi), crypt branching, narrowing of the brush border and infiltration with inflammatory cells[11,12,56] (**Figure 3**). Data on changes in the different epithelial cell types of the intestinal epithelium is scarce with only one study showing a possible reduction in the number of Paneth cells[88] and another study showing no difference in goblet cell number[89]. Notably, there is increasing recognition that the classical intestinal structure and function are almost universally abnormal in children living in low- and middle-income countries, a finding known as environmental enteropathy[90]. Continuous exposure to enteric pathogens probably underlies these changes of the small intestine as children are often living in conditions with poor hygiene and sanitation. Limited data suggests that the severity of the enteropathy may be higher in severely malnourished children especially in those with concurrent diarrhoea[11,12], but this requires further investigation.

Disruptions in intestinal structure have important implications for intestinal barrier function and nutrient absorption (**Figure 3**). Compromised intestinal barrier function has been demonstrated experimentally by increased passage of large sugar molecules (e.g. lactulose : rhamnose ratio) across the intestinal wall in severely malnourished children[11,91]. This is thought to allow microbial translocation from the intestinal lumen to the systemic circulation, potentially triggering intestinal and systemic inflammation and even sepsis[4,11]. One study reported histological lesions and disruptions of the intercellular junction proteins claudin-4 and E-cadherin in severely malnourished children, which can contribute to intestinal barrier dysfunction[11]. Nutrient malabsorption can occur due to villous atrophy, which substantially decreases the absorptive surface area of the small intestine[57]. Carbohydrate malabsorption is prevalent among severely malnourished children, especially of disaccharides such as lactose[57]. Nutrient malabsorption is possibly more severe in children with oedematous malnutrition compared to those with severe wasting[57]. When carbohydrate malabsorption occurs, the unabsorbed molecules draw

fluid into the intestinal lumen leading to osmotic diarrhoea [92]. Absorption of proteins and lipids is likely also compromised[93–96]. Important contributors include impaired hepatobiliary function[97] and exocrine pancreas insufficiency[95]. The lack of impaired digestive enzyme production by the liver and pancreas in combination with a reduced absorptive capacity can lead to protein and lipid malabsorption. The impairments in these essential intestinal functions possibly impede the nutritional recovery of severely malnourished children.

Gatekeepers of intestinal homeostasis

Accumulating data suggests that mTOR signalling, autophagy and mitochondria play key roles in maintaining proper intestinal structure and function[98–100]. Accordingly, dysregulation of these processes have been implicated in the onset and progression of intestinal diseases such as IBD and coeliac disease[101–104]. These intestinal diseases share similarities with malnutrition enteropathy such as barrier dysfunction, villous atrophy and intestinal inflammation. However, it is unknown how mTORC1, autophagy and mitochondria are affected in the intestine of severely malnourished children, and if alterations contribute to malnutrition enteropathy. In the next sections, I will describe 1) the major functions of these pathways, 2) the reported changes in these pathways in other intestinal diseases and their potential involvement in malnutrition enteropathy.

mTORC1

Organismal growth and viability require an adequate adaptation to stress and changes in nutrient availability. Cells have evolved signalling networks, which respond to various extrinsic and intrinsic stimuli, to adapt cellular metabolism and thereby promote cell proliferation and survival. A key signalling integrator in mammals is the mammalian/mechanistic target of rapamycin (mTOR).

mTOR is a serine/threonine kinase that exists in two structurally and functionally distinct multiprotein complexes, mTORC1 and mTORC2, with only mTORC1 being sensitive to rapamycin and its derivatives called rapalogs[105]. mTORC1, an important factor considered in this thesis, responds to a great number of stressors including nutritional, oxidative and endoplasmic reticulum (ER) stress[106]. Nutrients and growth factors regulate mTORC1 activity, and amino acids are indispensable for the activation of mTORC1 (**Figure 5**)[107]. In nutrient-rich conditions, mTORC1 activation promotes translation and cell growth. In nutrient-deficient conditions, mTORC1 inhibition preserves cellular homeostasis through limiting energy-consuming anabolic processes (e.g. protein synthesis) and promoting

autophagy, a process by which superfluous or damaged proteins and organelles are degraded for nutrient recycling[108]. mTORC1 is considered a master regulator of autophagy, because mTORC1 inhibition is needed to initiate the autophagy process[109]. More recent studies indicate that mTORC1 also regulates some of the subsequent steps in autophagy such as autophagosome elongation and maturation[110].

Autophagy

Autophagy is a catabolic cellular process through which intracellular components are degraded and recycled during physiological conditions as well as in response to cellular stress or nutrient shortage. Based on the mode of cargo delivery to the lysosomes, autophagy can be classified as macroautophagy, microautophagy or chaperone-mediated autophagy[111]. Macroautophagy, hereafter referred to as autophagy, has been most extensively studied and will be discussed here. The process of autophagy can be divided into different steps including initiation, maturation, fusion and degradation (**Figure 5)**.

Induction of autophagy is negatively regulated by mTORC1. mTORC1 inhibits autophagy through inactivation of Unc51-like kinase-1 (ULK1) by phosphorylating ULK1 at serine residue 757[112]. Phosphorylation of ULK1 at S757 disrupts the interaction between ULK1 and energy-sensing AMP-activated protein kinase (AMPK). AMPK is a positive regulator of autophagy[112]. Following activation of ULK, formation of ULK1 multiprotein complex facilitates the formation of the phagophore[113] and subsequently of autophagosomes[114].

Maturation and elongation of the autophagosome membrane is dependent on two ubiquitin-like steps: the conjugation of autophagy-related protein ATG12 to ATG15 and the conversion of LC3I to LC3II (reviewed in [115]). LC3-II, the lipidated form, can be incorporated into the membrane of the autophagosome and, together with LC3-I, is most commonly used in experiments as a marker for autophagy activity[116]. Upon maturation, autophagosomes fuse with lysosomes to form autolysosomes. The contents are then degraded by lysosomal hydrolases. The generated molecular building blocks (e.g. amino acids) are then exported to the cytoplasm to be reused for metabolic processes.

Autophagy can be non-selective or selective. Non-selective autophagy encompasses the usually random uptake of cytoplasm into phagophores, whereas selective autophagy involves the selective removal of superfluous or damaged cellular components[117]. Selective autophagy has been reported and further studied for different organelles including mitochondria (mitophagy)[118], peroxisomes (pexophagy)[119] and endoplasmic

reticulum (ER-phagy)[120]. This selective removal of damaged or superfluous organelles is required for the maintenance of proper organelle function, quality control and cellular homeostasis in general.

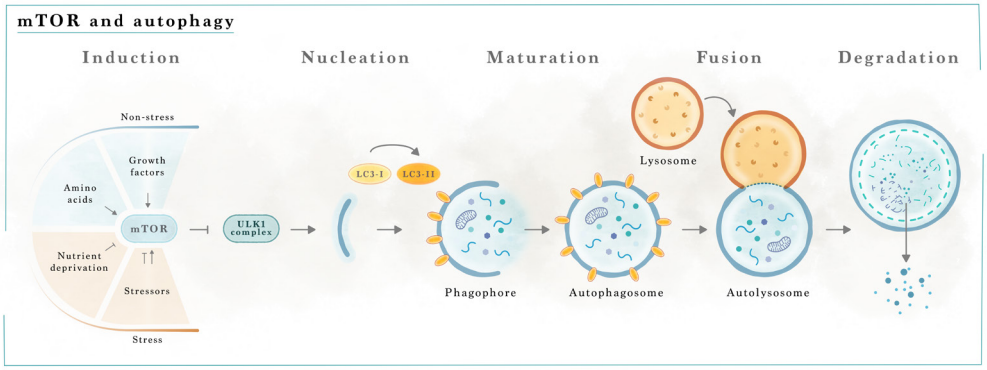

Figure 5. mTOR and autophagy pathway. mTOR, mammalian/mechanistic target of rapamycin; ULK, Unc51-like kinase-1.

Mitochondria

Mitochondria are double-membrane organelles that support cell survival through production of the energy carrier adenosine triphosphate (ATP) and enable cellular adaptation to stressors[121]. Mitochondria are frequently referred to as the 'powerhouse of the cell' as they produce the majority of the ATP required for cell metabolism[122] through oxidative phosphorylation (reviewed in [123]). In addition, mitochondria confer an array of essential cellular functions including calcium handling, signalling, maintaining redox homeostasis and synthesizing precursors for macromolecules (e.g. amino acids)(reviewed in [124,125]). Mitochondria are highly dynamic structures which constantly undergo cycles of reshaping, rebuilding and recycling, referred to as mitochondrial dynamics[126]. The processes of mitochondrial dynamics encompass the merging of two mitochondria into one (fusion), the division of one mitochondrion into two mitochondria (fission) and selective degradation of dysfunctional mitochondria (mitophagy) (reviewed in [126]). These processes are important for regulating the function of these organelles in response to environmental and metabolic changes. Mitochondria can be challenged by numerous stressors including increased reactive oxygen species (ROS)[127]. Maladaptive stress responses of mitochondria can compromise their function and can be detrimental for cell survival[127].

Dysregulated mTORC1, autophagy and mitochondrial homeostasis in intestinal diseases and malnutrition enteropathy

Alterations in mTORC1, autophagy and mitochondria have been described and implicated in the pathogenesis of various intestinal diseases. Increased activation of mTORC1 in the intestinal epithelium has been reported in patients with active coeliac disease[101] and IBD[103,128,129]. The onset or progression of IBD has been strongly associated with reductions in autophagy in the intestinal epithelium[103,130], but the evidence for impaired autophagy in coeliac disease is limited to a gene expression study in duodenal biopsies[131] and preclinical data[132]. Involvement of epithelial mitochondrial dysfunction is strongly implicated during the onset and course of IBD[104,133].

In severely malnourished children, no data about mTORC1 activity in the intestine is available. Data on autophagy is sparse, ranging from no apparent changes in autophagosomes to enlarged autophagosomes with unclear clinical implications[134–137]. Few studies have reported, sometimes opposing, mitochondrial morphological changes in the small intestine of severely malnourished children[134–137]. These morphological changes may be more evident in children with severe intestinal disease[134,136]. Dysmorphic mitochondria have also been reported in other organs of severely malnourished children[138,139]. Involvement of dysregulated autophagy and mitochondrial homeostasis is a realistic possibility in malnutrition enteropathy given the histological reports and knowledge from intestinal diseases that share similarities with malnutrition enteropathy. There is a compelling need for new information about mTORC1, autophagy and mitochondria in the small intestine of severely malnourished children.

Bridging the knowledge gaps in malnutrition enteropathy with in-vivo and ex-vivo models

Studying underlying mechanisms in severely malnourished children is challenging given the vulnerability of this population. For example, studying mechanisms behind impaired intestinal health in severe malnutrition would ideally use intestinal biopsies, which require invasive endoscopic procedures. Although limited, preclinical models have been developed to generate a better understanding of severe malnutrition and its pathophysiology.

Animal models of (severe) malnutrition

Animal models are extensively used in scientific research and have helped to unravel the pathophysiology of numerous diseases. Features of severe malnutrition have been reproduced in various species using diverse methods (e.g. caloric or protein restriction). However, no standards for defining severe malnutrition have been established for laboratory animals. Thus, the validity of a malnutrition animal model and the characteristics that need to be modelled largely depend on the specific research question. To illustrate, when the aim is to shed light on oedematous malnutrition it is a prerequisite to recapitulate essential features of oedematous malnutrition in animals such as oedema. Yet, oedema is not present in all animal models. Features of oedematous malnutrition were successfully reproduced in monkeys[140–142], pigs[143] and rats[144] that were fed a low-protein diet. Ideally, animals models should be close to human physiology to enable clinical translatability, for example through the use of monkeys or pig models. However, ethical considerations, expenses and long gestation periods make these species impractical for high turnover experiments. Therefore, rodents have been commonly used in research in general, but also for malnutrition studies specifically. In the next section, I will discuss what types of malnutrition animal models have been established and report specifically on intestinal structure and function.

Malnutrition animal models to study the impact on the intestine

As described previously, the physiology of the small intestine in severely malnourished children is disrupted with changes in the architecture, function and immune system (**Figure 3**). The impact of malnutrition on the small intestine has been evaluated in different animal models of malnutrition, which are discussed in more detail below. Malnutrition in these models was mainly induced by feeding animals deficient diets and was sometimes combined with an additional intestinal insult (e.g. pathogen). Although different forms of nutritional deprivation can induce malnutrition in animals, the impact of this on the intestine and the extent of characterization of intestinal changes in these models differs considerably (**Table 1**). Commonly used forms of nutritional deprivation include caloric restriction (CR), protein deprivation and use of a regional based diet.

Nutritional deprivation

Limitation of daily caloric intake by 15% to 30% did not impact on intestinal permeability and led to only minor changes in small intestinal morphology (reviewed in [145,146])[147].

Similarly, a recent study showed that further increasing caloric restriction to 50% for 3 weeks did not increase intestinal permeability in young mice[145]. Notably, severe forms of caloric restriction might be stressful for rodents with reports of increased plasma corticosterone levels (25% CR, juvenile rats)[148] and signs of anxiety and depression (50% CR, rats)[149]. Given the ethical considerations of increased stress levels in CR, ad libitum deficient diets could be a better alternative such as low-protein or regional based diets.

Protein-deficient diets reflect the type of diets that are common in developing countries, where staple foods, such as maize, are often high in carbohydrates and low in protein. The effects of a low-protein diet (LPD) on the intestine that have been described in the literature are highly variable (**Table 1**), which may be in part related to the severity of the protein restriction, the life stage of the animal (e.g. young or adult) and/or the animal species that was used. Importantly, the impact of protein deprivation on nutrient absorption has not been studied in these models.

As micronutrient intake is often also low in malnourished children[20], rodent diets have been formulated that reflect regional dietary patterns and mimic both macronutrient (low-protein) and micronutrient deficiencies. Studies that have used these regional diets – the Malawian diet, the Regional Basic Diet (RBD) or the Maize diet – have most extensively studied the small intestine using the RBD (**Table 1**). The RBD reflects the dietary pattern from the northeast Brazilian population[150], whereas the Maize diet is based on the publication of Dr. Cecily Williams in 1933 that noted that kwashiorkor occurred in children who were primarily fed a maize-based diet[24]. Yet, the study of nutrient absorption using these diets is very limited.

As the impact of nutritional deprivation by itself is not always sufficient to produce intestinal barrier dysfunction and intestinal inflammation, some studies have combined protein deprivation with a microbial challenge or indomethacin use, a non-steroidal anti-inflammatory drug (NSAID)(**Table 1**).

Nutritional deprivation with additional intestinal insult

High exposure to pathogens could play a role in the development and persistence of malnutrition enteropathy. In pathogen-induced enteropathy models, both parasites (*Cryptosporidium parvum*, *Giardia lamblia*) and bacteria (E. coli + Bacteroidales) have successfully induced some features of malnutrition enteropathy (**Table 1**). Indomethacin, a NSAID, was also used to induce enteropathy in malnourished mice based on the described use in enteropathy induction in mice fed a normal diet[151]. However, the

findings in these models may be pathogen(s) or NSAID specific, which decreases the reproducibility and validity.

In conclusion, nutritional deprivation (except caloric restriction) with or without an additional intestinal insult can reproduce some important characteristics of malnutrition enteropathy in animals. However, the evaluation of intestinal changes is only fragmentary (**Table 1**). This hampers a more in-depth understanding of underlying mechanisms and testing of potential therapeutic interventions. Thus, a malnutrition model is needed that comprehensively characterizes intestinal changes both in structure and function.

Table 1. Small intestinal alterations in malnutrition animal models.

Insult	Structure	Barrier function	Nutrient absorption
		Protein restriction	
0% protein, 2 weeks adult rats[152], jejunum	↓ VH	Not assessed	Not assessed
4% protein, 20 days juvenile rats[153], ileum	VH: not assessed CD: no effect	• ↑ permeability small molecules (reduced TEER) • Non-significant increase in permeability to 4 and 40 kDa FITC-dextran • ↓ protein levels TJ-protein *Ocldn*	Not assessed
4% protein, 3 weeks guinea pigs[154], jejunum	Not assessed	• ↑ permeability to small molecules (Ussing chamber), but not to macromolecules • 10% reduction of TJ strands	Not assessed
5.8% protein, 3 weeks weanling mice[145], jejunum	VH: no impact CD: no impact	• No change in permeability (Ussing chamber) or permeability to 4kDa FITC-dextran† • ↓ mRNA expression *Muc2* and TJ-protein *Ocldn*	Not assessed
7% protein, 3 weeks weanling mice[155], jejunum	VH: no impact	• ↑ permeability to 4 kDa FITC-dextran • ↓ mRNA expression TJ-protein *ZO-1* and ↑ pore-forming TJ-protein *Cldn-2*	Not assessed

Insult	Structure	Barrier function	Nutrient absorption
Regional diets			
Regional basic diet (RBD) 7% protein, 8.2% fat, reduced micronutrients			
7 days, RBD juvenile mice[156], ileum	VH: no impact ↓ CD	• No change in permeability (Ussing chamber) • No change protein levels pore-forming TJ-protein *Cldn2* or *Ocldn*	No change SGLT-1 or PEPT transporters
10 days, RBD weanling mice[157], ileum	↓ VH:CD ratio	Not assessed	Not assessed
3 weeks, RBD weanling mice[158]**,** jejunum	↓ VH, CD and VH:CD ratio	• ↑ ex vivo permeability to 4kDa FITC-Dextran • ↓ TER • ↑ TJ-protein *Cldn3* expression in crypts (IF, WB)	Not assessed
14 days, RBD weanling rats[159], jejunum	↓ VH, ↑ CD and ↓ VH:CD ratio	• ↑ bacterial translocation to spleen • ↓ goblet cell numbers	Not assessed
Maize diet, 6.4% protein, 3.4% fat, reduced micronutrients			
4 weeks juvenile pigs[160], jejunum, ileum	↓ VH	Not assessed	↓ disaccharidase and peptidase activity (only ileum)
Malawian Diet (M8), mice[161]	Impact on intestine not assessed		
Low-protein diet with a microbial challenge			
Cryptosporidium infection			
C. parvum + 2% LPD weanling mice[162], ileum	↓ VH ↑ CD	Not assessed	Not assessed
C. Parvum + 2% LPD weanling mice[157], ileum	↓ VH:CD ratio	• ↓ protein levels TJ-protein *Ocldn* • ↑ internalization of TJ-protein *Ocldn* • ↑ protein levels pore-forming TJ-protein *Cldn2*	Not assessed
Giardia lamblia infection			
G. Lamblia + 3% LPD weanling mice[163]	Not assessed	Not assessed	↓ glucose and alanine absorption
G. duodenalis + 4.3% LPD, juvenile mice[164], ileum	↓ VH and CD	Not assessed ↓ goblet cell number	Not assessed
G. lamblia, 2% LPD weanling mice[165], duodenum	↓ VH ↓ VH:CD ratio	Not assessed	Not assessed

Insult	Structure	Barrier function	Nutrient absorption
Bacterial infection			
E. coli + Bacteroidales 7% protein, weanling mice[155], jejunum	↓ VH ↓ VH:CD ratio	• ↑ permeability to 4kDa FITC-dextran	Not assessed
Low-protein diet with another gastrointestinal trigger			
Indomethacin, oral, 7 days, 5.8% protein, 3 weeks, weanling mice[145], jejunum	VH: no impact CD: no impact	• ↑ permeability to 4 kDa FITC-dextran • ↓ mRNA expression TJ-protein *Ocldn*	Not assessed

† Increased permeability after 14 days low-protein diet
CD, crypt depth; Cldn, claudin; FITC, Fluorescein isiothiocyanate; IF, immunofluorescence; LPD, low-protein diet; Muc2, mucin 2; Ocldn, occludin; SGLT1, sodium glucose cotransporters; PEPT, intestinal peptidase transporter, TEER, transepithelial electrical resistance, TER, transepithelial resistance, TJ, tight junction; VH, villous height; ZO-1, zonulin-1

In vitro models of severe malnutrition

An *in vitro*, cell-based model may be more suitable to study the impact of nutritional deficiencies on metabolic and signaling pathways. Cell culture has the advantage that many interventions can be tested rapidly and detailed time-course data can be obtained. There is currently no *in vitro* model of severe malnutrition. Yet, studies have used some form of starvation, as a proxy of malnutrition: serum starvation in intestinal cell lines to complement findings from *in vivo* malnutrition studies[166] or amino acid deprivation in isolated porcine intestinal epithelial cells[167]. In the latter study, starvation for non-essential amino acids increased permeability and decreased TJ proteins zonulin-1 and claudin-1, which indicates a decline in barrier function. However, two-dimensional cell cultures are less suitable to study functional organ properties. Advances in three-dimensional cultures, in particular organoids, have opened new avenues for the development of more physiological *in vitro* models of organ function and diseases[67,168]. Intestinal organoids are derived from self-organizing stem cells that can recapitulate the *in vivo* architecture and functionality of the original tissue (i.e. barrier function and nutrient absorption in small intestinal organoids)[169,170]. Organoids are cultured in a 3D extracellular matrix (e.g. Matrigel) and supplemented with a combination of growth factors identical to those found in the stem cells niche *in vivo* (i.e. epidermal growth factor, Noggin, R-spondin-1)[67]. These molecules allow the small intestinal organoids to continuously expand, resembling the cellular organization and self-renewal dynamics of the small intestinal epithelium. Some studies have used intestinal organoids to investigate the need of specific amino acids for proper stem cell function. Moore et al. showed that glutamine deprivation decreased epithelial proliferation and induced crypt atrophy[171]. Saito et al. specifically evaluated

methionine deprivation and demonstrated that this suppressed stem cell proliferation and promoted differentiation[172]. Although some form of amino acid deprivation, as a proxy of malnutrition, was used, these stem cell-focused studies did not fully characterize the impact of 'malnutrition' on intestinal organoids. They only removed particular amino acids and did not investigate the relation between intestinal organoid function, organelles and pathway disruption. There is a need for models like this in order to better understand pathophysiology at the intestinal epithelial level and to test interventions in a high-throughput manner. Furthermore, continuous expansion of organoids makes it possible to experiment on human cells, and it also has the potential to significantly reduce the use of laboratory animals.

Aims and outline of this thesis

The aims of this thesis were 1) to gain insight into factors that contribute to diarrhoea and poor clinical outcomes in hospitalized severely malnourished children, and 2) to study small intestinal dysfunction and identify potential underlying mechanisms in severe malnutrition.

In this thesis, clinical studies were combined with two malnutrition model systems (mouse and intestinal organoids)(**Figure 6**). In **part I** of this thesis, I investigated the role of different nutritional, microbial and inflammatory factors in diarrhoeal disease and poor clinical outcomes during admission in severely malnourished children. In **part II** of this thesis, I established and characterized a mouse model and intestinal organoid model of malnutrition enteropathy. To assess the potential of organoid models in malnutrition research in a broader perspective, I collaborated with a fellow PhD student and also added a hepatic organoid model of severe malnutrition. In the mouse and organoid models, I investigated potential mechanisms involved in intestinal dysfunction and tested interventions. In **part III** of this thesis, I discuss the future perspectives and implications of the findings for potential therapeutic interventions.

Part I – Clinical studies

Chapter 2 evaluates three commonly-used "transition-phase" diets with different carbohydrate content by comparing biochemical and clinical outcomes. Moreover, it discusses the potential role of carbohydrate malabsorption in the onset and/or continuation of diarrhoea in severely malnourished children during inpatient treatment. **Chapter 3** provides an insight into the intestinal and inflammatory mechanisms

underlying deaths in hospitalized severely malnourished children. **Chapter 4** reports on the prevalence and clearance of intestinal pathogens in severely malnourished children during hospital admission. Furthermore, it describes the contribution of these intestinal pathogens to diarrhoeal disease and other clinical outcomes.

Part II – Preclinical studies

Chapter 5 presents a novel mouse model of malnutrition enteropathy with characterization of structural and functional intestinal changes. Furthermore, it shows changes in mTORC1 activation, autophagy and mitochondria, and shows the positive impact of rapamycin on the small intestine. **Chapter 6** describes the use of intestinal organoids as a model for malnutrition enteropathy and liver dysfunction. It shows how amino acid deprivation disrupts structure, function and mitochondrial and peroxisomal health in intestinal and hepatic organoids. Moreover, it describes if interventions such as rapamycin and fenofibrate can preserve homeostasis in intestinal organoids and hepatic organoids, respectively.

Part III – Future perspectives

Chapter 7 provides a general discussion of this thesis' findings and focuses on future perspectives.

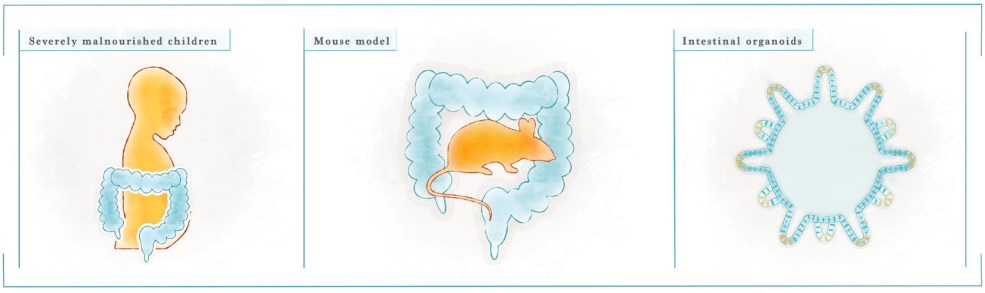

Figure 6. The approach used in this thesis. Clinical trials provide information of the patient population of interest, severely malnourished children. In the malnutrition mouse model, intestinal changes and underlying mechanisms can be studied. In intestinal organoids, the impact of severe malnutrition on the intestinal epithelium and underlying mechanisms can be studied, including barrier function, organelle dynamics and stem cell proliferation and differentiation.

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PART I

CLINICAL STUDIES