Chapter 1. General Introduction
1.1 Global prevalence of malnutrition

Childhood malnutrition is a global burden that is linked to 45% of all deaths of children under the age of five\(^1\). The World Health Organization (WHO) estimated that in 2020 149.2 million children were stunted, and 45.5 million children were wasted\(^2\) (stunting and wasting is defined in the next section). While malnutrition has been declining over the last decades, the rate of decline is not fast enough to meet the 2030 sustainable development goal of ending all forms of malnutrition by 2030\(^3\). This can be partially explained by the fact that the causes of malnutrition are multifold and complex. A common oversimplification of malnutrition is that it is caused by the lack of food. On a physiological level malnutrition is caused by a combination of inadequate macronutrients and/or micronutrients intake, malabsorption of nutrients, and increased energy expenditure, usually due to infection or disease. However, the real cause of malnutrition is a combination of environmental, social, and medical risk factors\(^4\). The United Nations Children Fund (UNICEF) report on “The State of Food Security and Nutrition in the World 2021” addresses the causes of malnutrition, the lack of food availability, discussing the impact of conflict, climate change, economic slowdown, and the unaffordability of healthy diets\(^5\). This report highlights that the underlying cause of malnutrition is poverty and inequality, with income inequality most prominently increasing the risk of malnutrition but all forms of marginalization and societal exclusions also contribute\(^5\). UNICEF’s Conceptual Framework on Maternal and Child Nutrition\(^6\) provides a clear overview of the determinates of maternal and child nutrition, highlighting areas for prevention and intervention. This framework splits the determinants into four layers as depicted as depicted in Figure 1. With good governance determining the availability of environmental, financial, social and human resources, as well as, the social norms that enables human rights, both to provide the necessary foundation for good nutrition\(^6\). The next layer includes foods and practices that ensures providing age-appropriate, sanitary and nutrient rich food and water, as well as services for education, sanitation and social protection that promotes a good diet\(^6\). The next level includes a balanced and good diet, and positive social and cultural norms, both enabling the right to nutrition\(^6\). These determinates are all needed to ensure quality maternal and child nutrition, which allows for the proper child development improving survival, health, and prosperity\(^6\). Poverty impacts malnutrition not only through the unavailability of food or poor quality of food, but also through increased risk of disease, lower access to healthcare, unhygienic environments, and lack of shelter\(^7\). As much as poverty is a risk factor for malnutrition, malnutrition is also a risk factor for poverty\(^7\). Malnutrition during pregnancy leads to lower birth weight, increased risk of wasting and stunting, which is associated with lower physical and cognitive development\(^8\)–\(^10\). Hence, the malnourished children that do survive have a lower developmental potential leading to reduced income and an increased risk of intergenerational poverty\(^11\). The Covid-19 pandemic has further negatively impacted food security and malnutrition, with the number of undernourished people increasing from 650.3 million in 2019 to 768 million in 2021\(^5\).
1.2 Definition of malnutrition

Malnutrition can refer to both under- and overnutrition, but in the scope of this thesis malnutrition will refer only to undernutrition. Malnutrition is often categorized as stunting (a low height-for-age score) or wasting (a low weight-for-height score)\(^\text{12}\). When it comes to treatment guidelines moderate acute malnutrition or severe acute malnutrition are being used as terms as these have been universally adopted. Moderate acute malnutrition is defined as a weight-for-height Z-score between –2 and –3 below the WHO child growth curve median, or by a mid-upper-arm-circumference (MUAC) of between 115-125mm\(^\text{12}\). Severe malnutrition accounts for half a million deaths in children under the age of 5 per year\(^\text{1}\) and is defined as nutritional edema (kwashiorkor) or severe wasting (marasmus): with weight-for-height score 3 standard deviations below the WHO mean growth curve or a mid-upper arm circumference (MUAC) of less than 115mm\(^\text{12}\). In this thesis severe acute malnutrition will be referred to as severe.
malnutrition, as the term acute can be misleading with severe malnutrition often being a chronic condition\textsuperscript{13}.

1.3 Management of severe malnutrition

Children with severe malnutrition are treated as either outpatients or inpatients according to the WHO severe acute malnutrition guidelines\textsuperscript{14}. These guidelines were first published in 1981 and have subsequently been updated in 1999, 2003 and 2013. The latest version recommends that children ‘who have appetite (pass the appetite test) and are clinically well and alert should be treated as outpatients’, i.e., in their community, while children ‘who have medical complications, severe edema, or poor appetite (fail the appetite test), or present with one or more Integrated Management of Childhood Illness (IMCI) danger signs’ are admitted for inpatient treatment\textsuperscript{14}. IMCI danger signs include convulsions, inability to drink, unconsciousness or drowsiness, vomiting, stiff neck, severe dehydration, stridor in a calm child, oedema of both feet, severe palmar pallor, clouding of the cornea or deep mouth ulcers in a child with measles, fever and tender swelling behind the ear in a child with an ear problem\textsuperscript{15}. Outpatient children receive ready-to-use therapeutic food (RUTF) with the exact amount determined by the child's weight. RUTF is a calorie dense, mineral and vitamin rich peanut butter paste that is provided in packages that do not require refrigeration. Inpatient treatment is divided in two phases: first the stabilization phase, which is the most fragile phase and during which acute complications of severe malnutrition are treated, and second the rehabilitation phase, which is focused on nutritional catch up\textsuperscript{14}. During the stabilization phase the children are fed a formula called F75, a low protein milk-based powder that is mixed with water and has a lower content of protein, fat, sodium, and lactose than what is given during the recovery phase. Other important components to inpatient management include antibiotics treatment, fluid management, vitamin A supplementation, and treatment of chronic co-morbidities such as HIV or tuberculosis. The rehabilitation phase is reached as children regain their appetite and are then fed RUTF or F-100 (liquid alternative to RUTF), which provides 100 kcal/100ml providing sufficient calories for the child to gain weight. This is continued after hospital discharge in the community until the severe wasting has resolved.

1.4 Whole body metabolism

To understand the pathophysiological changes occurring during malnutrition it is important to have an understanding of metabolism in both a fed and fasted state. I will first give a general introduction of metabolism on a cellular level, then discuss liver metabolism, followed by metabolic changes in severe malnutrition. Metabolism is a vast subject that comprises all chemical reactions that that place in a cell and is essential for life. It can be divided into catabolism, which is a state in which complex molecules are broken down to generate energy in a cell, or anabolism, which is a state in which complex molecules
Glucose metabolism can be divided into glycolysis, gluconeogenesis, glycogenesis, glycogenolysis and the pentose phosphate pathway (PPP). Central to all these pathways is glucose-6-phosphate (G6P), as illustrated in Figure 2. Glucose is taken up by the cell by glucose transporters and sodium glucose cotransporters and in the cell converted to G6P, which allows for the entrapment of glucose in the cell while maintaining a gradient between the cytosol and extracellular space that allows for facilitated diffusion of glucose into the cell. Depending on the cell's requirement G6P either enters the glycolytic pathway, gluconeogenic pathway, pentose phosphate pathway, or is converted to glycogen. Glycolysis converts glucose into two pyruvates, two nicotinamide adenine dinucleotide + hydrogen (NADH) and two adenosine 5'-triphosphate (ATP) within the cytosol and is the first step in the catabolism of glucose. Gluconeogenesis is an anabolic pathway that forms glucose from pyruvate and other non-carbohydrate carbon precursors. Gluconeogenesis is most prominent in the liver and kidneys and is activated during starvation when glycogen storages are depleted. Glycogen is the storage form of glucose, and hepatic glycogen stores are crucial to maintain plasma glucose levels. The PPP takes place in the cytosol along with glycolysis, as both pathways share intermediates. PPP is important in producing nicotinamide adenine dinucleotide phosphate + hydrogen (NADPH), pentose sugars needed for nucleotide synthesis, and the breakdown and generation of 5 carbon sugars.

Under aerobic conditions pyruvate generated from glycolysis is oxidized to form acetyl CoenzymeA (acetyl CoA), which enters the mitochondria and the tricarboxylic acid (TCA) cycle producing 30-32 ATP molecules. Under anaerobic conditions pyruvate is reduced to lactate generating...
2 ATP molecules. Acetyl CoA can also be formed from β-oxidation and is an intermediate between the TCA cycle, β-oxidation, and glycolysis.

**Lipid metabolism**

Lipids are mainly stored as triglycerides (TAGs) but are also found as free fatty acids, cholesterol, and phospholipids. Lipolysis is the hydrolyzation or breakdown of TAGs into fatty acid and glycerol, and this takes place in the cytoplasm. To utilize glycerol for energy, it first needs to be converted into glycerol 3-phosphate and thereafter it can enter the glycolysis pathway. β-oxidation oxidizes fatty acids into acetyl-CoA that can enter the TCA cycle. β-oxidation starts in the cytoplasm where fatty acids are converted into fatty acyl-CoA, which is combined with a carnitine through carnitine palmitoyltransferase I (CPT1) to allow for transportation into the mitochondria. In the inner mitochondrial membrane CPT2 removes the carnitine, thereby liberating fatty acyl-CoA for β-oxidation forming acetyl-CoA. β-oxidation of very-long chain fatty acids takes place in peroxisomes, while long, medium and short chain fatty acids are oxidized in mitochondria. During high availability of fatty acids, cells can also turn fatty acids into ketone bodies, acetoacetate and beta-hydroxybutyrate, and this is known as ketogenesis. This happens through the conversion of acetyl-CoA into ketone bodies, which can be oxidized in certain tissues for energy. Excess acetyl-CoA from glycolysis can also be converted into fatty acids through de novo lipogenesis. De novo lipogenesis requires that acetyl-CoA is first converted into citrate to cross from the mitochondria into the cytosol, where it can be converted back into acetyl-CoA for carboxylation into malonyl-CoA by acetyl-CoA carboxylase (ACACA) and polymerization into fatty acid. Fatty acid synthase (FASN) is the key rate-limiting enzyme that brings the conversion of malonyl-CoA to palmitate. The final step of lipogenesis is the esterification of fatty acids to form TAGs and takes places in the endoplasmic reticulum (ER).

**Amino acid metabolism**

There are 20 amino acids, and they can be subcategorized into essential and non-essential. Essential amino acids cannot be synthesized by the body and must be taken up from the diet, while non-essential
can be synthesized by the body from intermediates. Some non-essential amino acids are considered conditionally essential as the body cannot synthesize them under certain conditions such as illness and stress. Amino acids can be used as an energy source, to build proteins, and as precursors to nucleotides, neurotransmitters, and hormones. The non-essential amino acids can be synthesized from different pathways and intermediates. From the TCA cycle α-ketoglutarate can be converted into the amino acids glutamate, glutamine, proline, arginine, and oxaloacetate through aspartate can be converted into lysine, asparagine, methionine, threonine, isoleucine. From glycolysis pyruvate can be converted into alanine, valine, and leucine, and 3-phosphoglycerates into serine, glycine, and cysteine. The PPP and glycolysis pathway can form erythrose 4-phosphate that can be used to synthesize the amino acids phenylalanine, tyrosine, and tryptophan. The PPP pathway can form ribose 5-phosphates that can synthesize the amino acid histidine.

The degradation of amino acids usually yields TCA cycle intermediates or pyruvate, which can be used for gluconeogenesis. The degradation of some amino acids yields the ketone acetoacetate, which can also be converted into acetyl-CoA for the TCA cycle or for fatty acid synthesis. Amino acids are not stored in the body and are degraded predominately in the liver, where they go through transamination and oxidative deamination. Oxidative deamination consists of dehydrogenation and a hydrolysis step, which forms ammonium ions that in the liver are converted into urea for excretion.

**Hormonal control of metabolism**

Insulin and glucagon are two central hormones in metabolic regulation. Both are secreted from the islets of Langerhans within the pancreas, with insulin originating from β-cells and glucagon from α-cells. Insulin is released as blood glucose levels rise in a fed state, having both long- and short-term effects on metabolism. It stimulates the translocation of glucose transporters to the plasma membrane for increased glucose uptake for utilization or storage as glycogen. Insulin also increases fatty acid uptake and their storage into TAGs via increasing de novo lipogenesis in adipocytes and hepatocytes. Glucagon is released as blood glucose levels drop in a fasted state and has its main effect on the liver. It increases gluconeogenesis, while suppressing glucose usage in glycolysis and storage as glycogen. Other important regulators of metabolism are adrenaline, noradrenaline, thyroid hormones, cortisol, incretin hormones, and non-hormone-based regulators such as nuclear receptors, which will be discussed below in section 1.7.

**1.5 Liver metabolism**

The liver is a key metabolic organ that plays a central role in the body’s energy homeostasis. It coordinates other organs to maintain energy homeostasis through nutrient, hormonal and neuronal signaling in a tightly regulated manner. In this section, I will discuss liver metabolism in a fed and
fasted state, as well as under acute starvation, and the main regulatory pathways that guide these processes. Postprandially (after a meal), amino acids, glucose, and fatty acids are absorbed from the intestine and transported to the liver. Fatty acids are taken up by the enterocytes after bile acid micellar solubilization. In the enterocytes chylomicrons (CM) are produced which are taken up into the lymphatic system and then into the circulation for hepatic uptake. The uptake of fatty acids is mainly done by cluster of differentiation 36 (CD36) and a group of fatty acid transport proteins (FATPs). Stimulating lipolysis and fatty acid uptake lowers plasma TAG levels while increasing hepatocyte TAG content, as fatty acids are re-esterified into TAGs or with cholesterol to form cholesterol esters, both to be stored in the cytoplasm as lipid droplets. Fatty acids can also be excreted by the liver as very-low-density lipoprotein (VLDL) particles for extrahepatic tissue utilization. VLDL forms in the ER where it is combined with ApoB protein for structural stability and lipids are transferred to the particle for excretion. Lipoprotein lipase (LPL) allows the liberation of fatty acids that are taken up into the liver through fatty acid transporter proteins. Lipolysis is mainly regulated by LPL and cyclic adenosine monophosphate (cAMP)-responsive element-binding protein H (CREBH) a transcription factor that regulates lipolysis through transcriptional regulation of apolipoproteins that modulate LPL activity. Apolipoproteins C-II (ApoC-II) and A-V (ApoA-V) are both positive coactivators. ApoC-II enhances TG hydrolysis of VLDL and CM for energy delivery or storage, while ApoA-V is thought to stimulate LPL activity by reducing the inhibitory effect of angiopoetin like 3/8 (ANGPTL3/8) complexes on LPL. Inhibitory factors include ApoC-I and ApoC-III, which both negatively regulate LPL activity by inhibiting its binding to triglyceride rich lipoproteins. In the fed state, the liver mainly relies on glucose as its source of energy, and apart from TAG formation and VLDL secretion, glucose stimulates de novo lipogenesis in the liver. Glucose stimulates de novo lipogenesis through the glycolytic pathway providing a carbon source for fatty acids, but also through regulation of expression of lipogenic genes by the transcription factor carbohydrate response element binding protein (ChREBP). Insulin also induces lipogenic gene expression through activation of the transcription factor sterol regulatory element binding protein 1c (SREBP1c), while leptin has been shown to suppress SREBP1c. The released insulin stimulates glucose uptake through increased expression of hepatocyte glucose transporters (GLUTs) that allow for more glucose uptake, which is then condensed into glycogen or converted into fatty acids or amino acids. Insulin reduces glycogenolysis through the inhibition of glycogen phosphorylase and promotes glycogen synthesis by activation of the phosphoinositide-3-kinase–protein kinase B (PKB/Akt) pathway, while also suppressing gluconeogenesis. Fibroblast growth factor 19 (FGF19) is another important regulatory factor that is secreted from the enterocytes and stimulates glycogen synthesis. Amino acids that are taken up postprandially are not stored in the liver but instead synthesized into proteins, broken down for energy or converted to glucose through gluconeogenesis or to fatty acids.
During fasting the liver shifts to produce energy and the main hormone promoting this shift is glucagon. Glucagon stimulates hepatic uptake of free fatty acids, which are released from adipocytes\(^1\), as well as amino acids, lactate and pyruvate from muscle. During short-term fasting the liver relies on glycogenolysis, but as glycogen stores become depleted, hepatocytes switch to gluconeogenesis for glucose production\(^2\). The liver uses amino acids, glycerol, lactate or pyruvate as substrates for gluconeogenesis. CREB has a large role in controlling glucose production during fasting. This is done by controlling key enzymes such as G6Pase, necessary for glycogenolysis and gluconeogenesis, as well as, phosphoenolpyruvate carboxy kinase (PEPCK) which is needed to convert TCA intermediate oxaloacetate to pyruvate. Forkhead box protein O1 (FOXO1) is another transcription factor that stimulates gluconeogenesis through the upregulation of key enzymes\(^1\). Amino acids, as previously mentioned, can feed into different parts of the TCA cycle or be converted to pyruvate, while glycerol can be phosphorylated into glycerate-3 phosphate for gluconeogenesis\(^2\). CREB also regulates G6Pase and Peroxisome proliferator-activated receptor-\(\gamma\) coactivator-1\(\alpha\) (PGC-1\(\alpha\))\(^3\), which are both important in promoting gluconeogenesis during fasting. PGC-1\(\alpha\) is a transcriptional co-activator that is involved in multiple metabolic processes, but mainly in the upregulation of gluconeogenesis, fatty acid oxidation, and mitochondrial biogenesis under fasting conditions\(^3\). During fasting glucagon activates both CREB and PGC-1\(\alpha\)\(^1\). In addition to PGC-1\(\alpha\), the main regulator of hepatic \(\beta\)-oxidation is the nuclear receptor peroxisome proliferator-activated receptor alpha (PPAR\(\alpha\)) which is activated by fatty acids and glucagon\(^4\), and in turn upregulates FGF21 and PGC-1\(\alpha\), thereby further stimulating \(\beta\)-oxidation\(^5,6\). PPAR-\(\alpha\) also induces ketogenesis enzymes 3-hydroxy-3-methylglutaryl-CoA lyase (HMGCL), acetyl-CoA acetyltransferase 1 (ACAT1), and 3-hydroxy-3-methylglutaryl-CoA synthase 2 (HMGCS2) promoting ketogenesis, which is also stimulated by CREB activity\(^1\).

1.7 The role of FXR in liver metabolism
Nuclear receptors are a family of 48 intracellular proteins that typically function as ligand-regulated transcription factors, with their individual activation leading to a distinct gene expression profile\textsuperscript{37}. One of the most well studied nuclear receptors is farnesoid X receptor (FXR), which is activated by bile acids and belongs to the sub-group called metabolic nuclear receptors that also includes peroxisome proliferator-activated receptor (PPAR), liver X receptor (LXR), pregnane X receptor (PXR) and liver receptor homolog-1 (LHR-1)\textsuperscript{37}. In addition to working as transcription factors, nuclear receptors also affect cellular metabolism through activating coregulatory proteins and through interacting with key energy sensory complexes, such as, adenosine monophosphate-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR)\textsuperscript{38}. It is a complex regulatory network and for this section we will only focus on one nuclear receptor, FXR, and its role in cellular energy metabolism as it is the focus of chapter 6.

FXR is mainly expressed in the liver, intestine, kidney and adrenal cortex, and is activated by bile acids\textsuperscript{37,39}. Bile acids are synthesized in the liver from cholesterol through either the classical (neutral) or the alternative (acidic) pathway that produce cholic acid (CA) and chenodeoxycholic acid (CDCA), respectively. The classical pathway account for about 75% of BA production and has the rate limiting enzyme cholesterol 7 alpha-hydroxylase (CYP7A1), while the acidic pathway has sterol 27-hydroxylase (CYP27A1)\textsuperscript{40}. Bile acids are then conjugated with taurine or glycine and secreted from the hepatocytes into the bile canaliculus via the bile acid export pump (BSEP) and stored in the gallbladder\textsuperscript{40}. Bile is secreted into the intestine after ingestion of food, for the uptake of lipid soluble fats and fat-soluble vitamins, and the majority of bile acids are reabsorbed in the terminal ileum by apical sodium-dependent bile acid transporter (ASBT)\textsuperscript{40}. After reabsorption, bile acids are effluxed from the enterocyte into the portal blood through organic solute transporter α and β (OSTα/β), and then reabsorbed into the hepatocytes through Na\textsuperscript{+}-dependent taurocholate cotransport peptide (NTCP)\textsuperscript{39}. Most of bile acids are reabsorbed with a small proportion being lost in feces, which is compensated for by de novo synthesis in the liver. This excretion and reabsorption of bile acids is known as the enterohepatic circulation of bile acids, in which FXR has a key regulatory role. Hepatic FXR activation reduces bile acid synthesis and promotes hepatocyte bile acid outflow by increasing expression of BSEP\textsuperscript{39}. Intestinal activation of FXR induces fibroblast growth factor 15/19 (FGF15/19) signaling, which in the liver inhibits CYP7A1 mediated bile acid synthesis\textsuperscript{39}. Hepatic FXR activation induces SHP expression that decreases bile acid synthesis via downregulation of expression of CYP7A1 and CYP8B1\textsuperscript{39}.

The role of FXR in liver lipid metabolism has mostly been studied in non-alcoholic fatty liver disease (NAFLD) models\textsuperscript{41}. In these models FXR activation has shown to reduce hepatic steatosis by decreasing fatty acid uptake through decreased expression of fatty acid transporter CD36\textsuperscript{42} and by reducing lipogenesis through direct and indirect SREBP-1c suppression\textsuperscript{43,44}. It has also been shown to increase fatty acid oxidation in hepatocytes through PPAR\textsubscript{α} activation\textsuperscript{45} which increases the expression of CPT1 and fibroblast growth factor 21 (FGF21)\textsuperscript{46}. FXR has also been shown to decrease serum
triglyceride levels by inducing Apo-C2, which in turn activates LPL activity and increases VLDL hydrolysis and by lowering hepatic VLDL secretion. FXR whole body knock out mice have been shown to have increased VLDL secretion. FXR activation has, through these pathways, been shown to be beneficial in treating hepatic steatosis in NAFLD. The role of FXR in hepatic glucose and amino acid metabolism has also mostly been studied in NAFLD models. In these models FXR activation has been shown to directly and indirectly lower plasma glucose levels, through suppression of PEPCK and reduced gluconeogenesis by SHP and FGF15/19, respectively; however, other studies have shown that FXR can also induce gluconeogenesis through PEPCK upregulation. This discrepancy is thought to be due to differential functioning of FXR in a fed vs fasted state, with FXR and FGF15/19 working as a metabolic switch. Data on glycogen is more consistent and its synthesis is induced by hepatic FXR activation and FGF15/19 induction. A study by Kir et al. reported that FXR through FGF15/19 promotes protein synthesis through rat sarcoma (RAS) and extracellular signal-regulated kinase (ERK) signaling, while a study by Massafra et al. found that FXR promotes protein catabolism. Again, this difference in effect is thought to be due to FXR being able to switch function in between the fed to fasted state.

1.8 The role of mTORC1 and autophagy in liver metabolism

In chapter 4 and 5 we look at the role of mTORC1 inhibition in malnutrition induced enterohepatic dysfunction. The mTOR pathway has been shown to affect many cellular processes. mTOR contains two complexes composed of several proteins, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) and which are important in transitioning between an anabolic and catabolic state. mTORC1 has been better characterized than mTORC2, and has a wide range of different upstream signals, such as stress, growth factors, oxygen, amino acids, and energy levels (Figure 5). It has a key role in promoting protein and lipid synthesis, as well as increasing cellular metabolism and ATP production, and inhibits catabolic processes such as autophagy (discussed below). During fasting the activity of mTORC1 is reduced, allowing for increased ketone production by the liver, as well as, higher PPAR-α and PGC1-α activation to stimulate oxidative metabolism. In the fed state mTORC1 promotes lipogenesis via SREBP1C. Inhibition of mTORC1 with rapamycin has been shown to stimulate gluconeogenesis, as well as, autophagy. Autophagy is a cellular recycling process that breaks down intracellular components in response to different stressors, such as nutrient deprivation. mTORC1 is also inhibited by AMP-activated protein kinase (AMPK), which in turn upregulates autophagy. AMPK is an important regulator of cellular metabolism and is activated by decreased levels of ATP and relative increased levels of AMP and ADP, which stimulates catabolic processes to restore ATP levels. However, AMPK also stimulates autophagy independent of mTORC1 through
phosphorylation of unc-51-like kinase 1 (ULK1). AMPK is activated in response to a variety of conditions that deplete cellular energy levels, such as nutrient starvation and it inhibits gluconeogenesis and lipid synthesis, while promoting fatty acid oxidation, glucose uptake and autophagy to restore energy balance.

Autophagy, or more specifically macro-autophagy, recycles intracellular components by encapsulating them in autophagosomes that are then fused with lysosomes and degraded, liberating amino acids, carbohydrates and lipids. These intracellular components are marked for degradation by autophagy receptors. Autophagy receptors then promote the recruitment of autophagy related proteins (ATGs) that cause the formation of a membrane surrounding the component that fuses with a lysosome for degradation. Figure 6 provides a schematic overview of autophagy. Protein restriction is one of the best studied activators of autophagy mediated through mTORC1 inhibition. Increased autophagy liberates amino acids necessary for ATP and glucose production from the TCA cycle to help maintain cellular energy levels. Beyond supplying substrates for the TCA cycle, autophagy also has an important role in the maintenance of mitochondrial homeostasis. This is achieved by the breakdown of dysfunctional mitochondria through a specific form of autophagy, also known as mitophagy. Mitophagy is activated by the accumulation of PINK1 in the membrane of damaged mitochondria, which then recruits Parkin and marks the mitochondria for autophagic degradation. Mitophagy and mitochondrial biogenesis are opposing pathways that are crucial in maintaining cellular health and energy function. AMPK activation promotes mitochondrial biogenesis through PGC-1α upregulation.
which is also stimulated by mTORC1. AMPK’s ability to both stimulate mitophagy and mitochondrial biogenesis is important for the renewal of mitochondria and maintain cellular energy levels.

**Figure 6. Schematic overview of autophagy**

1. The formation of an autophagosome around the intracellular component that is marked for degradation.
2. An autophagosome and lysosome.
3. The fusing of the autophagosome and lysosome.
4. The degradation of the autophagosome.

### 1.9 The metabolic changes seen in severe malnutrition

Severe malnutrition affects basal energy expenditure compared to well nourished children, as in severely wasted children visceral organs and the brain contribute relatively more to the total energy expenditure and visceral organs have a higher metabolic rate than muscle. Severely malnourished children have reduced thermogenesis possibly due to adaptive hypothyroidism, related to low leptin levels. Children with severe malnutrition have shown to have decreased plasma leptin, insulin, and insulin growth factor-1 (IGF-I) levels, with increased cortisol and growth hormone (GH) levels. IGF-I levels are regulated by nutrient intake and low IGF-I is thought to be at least partially responsible for the stunting seen in malnourished children. Low IGF-I levels cause increased secretion of GH. The decreased insulin and IGF-I levels with the increased cortisol and GH promote lipolysis and gluconeogenesis, and this contributes to maintaining energy homeostasis. This promotion of gluconeogenesis and glycogenolysis, however, is not sufficient to meet the children’s glucose requirements, as it has been shown that severely malnourished children have a decreased endogenous glucose production and are prone to develop hypoglycemia. Hepatic biopsies of severely malnourished children have shown both decreased or increased glycogen stores, potentially indicating that glycogenolysis is impaired in some children. Hypoglycemia in malnourished children is common and associated with mortality. Severely malnourished children also show a reduced ability for glucose clearance, which is likely not related to insulin resistance but to an impaired insulin secretion response indicating an impairment in beta-cell function.

The lipid metabolism in severe malnutrition has also been shown to reflect a catabolic state with increased lipolysis seen in one study, which is further supported by increased plasma fatty acid, ketone and acylcarnitine concentrations. However, results have not been consistent with another
study that didn’t find increased lipolysis when comparing children in a malnourished state to a recovered state. Other studies did not find a difference in total plasma fatty acid levels between malnourished children and healthy controls, but a clear difference in fatty acid composition with malnourished children having higher saturated fatty acids and lower essential and polyunsaturated fatty acids. The lower levels of these fatty acids indicate insufficient intake as the body cannot produce essential and polyunsaturated fatty acids, which have important anti-inflammatory properties and are important in cardiovascular and brain function and development. Another condition reflecting a disturbed lipid metabolism seen in severely malnourished children is hepatic steatosis. The pathophysiology behind malnutrition induced hepatic steatosis is unknown. It was long speculated that low protein intake in malnourished children reduced lipoprotein synthesis and production of VLDL particles thereby causing the hepatic triglyceride accumulation; however, Badaloo et al. reported that there was no decrease in VLDL ApoB100 synthesis. Another hypothesis to explain the hepatic steatosis is impaired hepatic β-oxidation due to mitochondrial dysfunction, which is supported by lower oxidation rates observed in kwashiorkor children and mitochondria and peroxisome dysfunction in severely malnourished children. This hypothesis will be further elaborated on in the section of animal models of severe malnutrition. Lipogenesis has not been assessed in severely malnourished children.

Other signs of hepatic dysfunction in severely malnourished children include hypoalbuminemia, which is thought to be due to reduced albumin synthesis because of low availability of amino acids and/or micronutrient deficiencies, as well as, increased breakdown through proinflammatory state. Hypoalbuminemia is highly associated with increased mortality in patients admitted to hospital, but this has not been seen in severely malnourished children. Albumin is important in maintaining colloidal osmotic pressure, plasma antioxidant activity and binding compounds, such as long chain fatty acids, cations and bilirubin. In addition to hypoalbuminemia, these children also present with a significant decrease in plasma non-essential and essential amino acids, with decreased rates of proteolysis indicating a state of trying to preserve amino acids. Jahoor et al. observed that protein breakdown is especially low in edematous malnutrition compared to the wasting phenotype of severe malnutrition, which had a faster return to normal protein synthesis during recovery. The study also saw in increase in oxidative stress in the edematous children with decreased glutathione synthesis. Di Giovanni et al. observed a lower availability of essential amino acids especially the kynurenine-tryptophan pathway in edematous children, which were shown to be significantly increased in children who survived from severe malnutrition compared to those who died. Low levels of amino acids is one of the factors that inhibit mTORC1 leading to activation of autophagy; in a low energy setting AMPK also upregulates autophagy through direct stimulation and through mTORC1 suppression. As previously mentioned FXR also has an important role in nutrient sensing by working as a transcriptional factor, as well as activating cofactors and other important pathways, such as inhibiting autophagy. Furthermore, it regulates bile acid
homeostasis, which has shown to be disturbed in malnourished children\textsuperscript{113}. These important nutrient and energy pathways have not been studied in malnourished children.

1.10 Animal models of severe malnutrition

There is no well-established pre-clinical model of severe malnutrition. In the 60’s and 70’s different degrees of protein deprivation and caloric restrictions were used in different mammals to induce severe malnutrition. A special emphasis was put on trying to reproduce edematous malnutrition, to uncover why some children develop the severe wasting (marasmus) phenotype and others the edematous (kwashiorkor) phenotype. Appendix 1 gives an overview of pre-clinical mammalian models of malnutrition by protein or caloric restriction. The most common finding in these models has been wasting, stunting, hepatic steatosis and hypoalbuminemia. Edema has been reproduced in monkeys and rats with lethargy, loss of fur, skin lesion, changes in posture, and in some cases death; mostly by protein or caloric restrictions diets\textsuperscript{94,114–116}. Interestingly in other models’ similar protein restrictions and phenotypic changes were not observed to lead to edema\textsuperscript{117}, while some studies they documented edema after increasing sucrose content in the diet\textsuperscript{118}.

In animal models of severe malnutrition, the main observed finding is weight loss\textsuperscript{94,115–137} or a lack of weight gain\textsuperscript{118–157} compared to controls during a protein or calorie restrictive diet. Some studies also reported reduced longitudinal growth\textsuperscript{121,129,131,133,134,144}. Malnourished animal models have consistently shown changes in metabolism, similar to those found in malnourished children. While some studies using pre-clinical malnutrition models have reported lower insulin plasma levels\textsuperscript{122,141,146}, other studies observed no difference in insulin levels\textsuperscript{151,157}. Bandsma et al.\textsuperscript{139}, looked at both in vivo and ex vivo insulin secretion in malnourished rats, showing a lower steady state insulin concentration with a lower islet insulin secretion, and increased peripheral insulin sensitivity with decreased hepatic insulin sensitivity. Fasted glucose plasma levels have generally been reported to be decreased in animal malnutrition models\textsuperscript{134,135,139}. Hepatic gluconeogenesis has been shown to be decreased\textsuperscript{139,146}, with low hepatic glycogen stores. Plasma triglycerides\textsuperscript{123,129,136,138,141,158} and cholesterol\textsuperscript{123,136} have also been shown to be decreased in animal models of malnutrition, while non-esterified fatty acids (NEFAs) are reportedly increased\textsuperscript{123,129,141,158}. Together, this indicates that low protein diet or calorie restriction over prolonged time periods generally induces a catabolic state with increased lipolysis in animal models of malnutrition. This has further been supported by Predis et al.\textsuperscript{129} who used metabolomics to demonstrate a state of amino acid and lipid catabolism, with increased hepatic oxidative stress and a buildup of TCA intermediates\textsuperscript{129}. Another metabolomic malnutrition study in piglets also reported an increase in markers of hepatic oxidative stress\textsuperscript{127}, which also has been seen in rat models\textsuperscript{134,155,159}. Van Zutphen et al.\textsuperscript{134} showed that the increased oxidative stress was related to loss of peroxisomes and mitochondrial dysfunction with decreased mitochondrial oxidative phosphorylation capacity, and that this may explain the malnutrition induced hepatic metabolic defects. These findings are further supported by another
article from our group that observed a loss mitochondrial number and mass in mice on a low protein diet, with a reduced oxidative capacity, as well as, a reduced β-oxidation gene expression. Hypoalbuminemia is commonly reported in both calorie restrictive and protein deficient studies of malnutrition, but has not always been reported with some studies requiring an additional stressor to protein restriction to induce hypoalbuminemia. Srikantia et al. saw a loss in serum albumin which was compensated by an increase in serum globulin maintaining total protein levels. While, Wu et al. saw a decrease in albumin, globulin and total plasma proteins in a calorie restriction model of severe malnutrition. Another commonly reported finding is increased concentrations of liver dysfunction markers alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT), and hepatic steatosis. Some studies, however, using similar protein deficient diets or calorie restriction did not find hepatic triglyceride accumulation, and just as with edema increased sucrose content reportedly induced this. Other malnutrition models also displayed an increase in unconjugated plasma bile acids, which further suggests hepatic dysfunction. Brown et al. showed a decrease in intestinal tauro-conjugated bile acids and an increase in unconjugated bile acids, while Preidis et al. showed an altered hepatic bile acid profile with decreased primary bile acids cholate and beta muricholate.

Low protein and calorie restriction diets also have a negative effect on intestinal architecture and function, as seen in animal models by villous blunting, increased intestinal permeability, loss of intestinal microbiota diversity, and loss of goblet cells. The increased intestinal permeability is likely partially due to a loss of tight junction proteins. The loss of villous height and flattening of microvilli could affect the ability of optimal nutrient absorption, further contributing to the cycle of malnutrition.

1.1 Aim of the thesis

With this thesis I aimed to better understand the underlying metabolic and specifically intestinal and hepatic pathophysiological changes seen in severe malnutrition, by using clinical studies and applying a murine model of severe malnutrition that mimics the clinical hallmarks of severe malnutrition. While rats have previously been more extensively used in malnutrition models, I aim to implement a mouse model of severe malnutrition to be able to not only test therapeutic interventions but also allow for genetic manipulation. Mice are further more cost effective with shorter gestation periods and can be co-housed in greater numbers. With this murine model I aim to better understand the enterohepatic cellular adaptations and mal-adaptations that takes place in severe malnutrition, with a specific focus on the liver and the accumulation of triglycerides. Improving our understanding of the pathophysiology in severe malnutrition can lead to the identification of novel pathways that can be modulated to improve acute and long-term outcome in this population, including reducing the 3.1
millions of malnutrition-related deaths in children under the age of five. I have organized my thesis into a clinical-research and a pre-clinical malnutrition model section.

The clinical research section is composed of two chapters. In Chapter two, I synthesized the knowledge on carbohydrate malabsorption in severely malnourished children using a systematic review. This chapter highlights the lack of good quality evidence on the prevalence and severity of carbohydrate malabsorption in malnourished children which has relevance for nutritional approaches to help children recover from severe malnutrition. In Chapter three, the aim was to gain more understanding on the metabolic changes in children with severe malnutrition applying a metabolomic approach using urine samples. These chapters better defined the metabolic derangements seen in malnourished children. To improve our understanding of the pathophysiological changes an established animal model of severe malnutrition can be transformative, and the following three chapters are based on such a model.

I have implemented a novel pre-clinical model of severe malnutrition using a low protein diet in mice. Chapter four first characterizes the model by showing that feeding a low protein diet induces stunting and wasting, and leads to metabolic disturbances such as hepatic steatosis, hypoalbuminemia, with increase hepatic dysfunction marker and cholestasis. Chapter five focuses on the intestinal changes seen in malnutrition, including villous blunting, impaired barrier function and increased inflammation. Both these chapters study assess the role of mTORC1 and the importance of mitochondrial health in both hepatic and intestinal function in severe malnutrition. Chapter six aimed to further understand the malnutrition induced hepatic steatosis and the role of the non-canonical response of FXR.

Together this thesis provides novel insight in the whole body and intestinal and hepatic metabolic changes in severe malnutrition and the central role of mitochondrial health herein. My work suggests, promoting mitochondrial health could be a target for novel treatments for severely malnourished children.

Images created using Bio Render.
REFERENCES


