MITOCHONDRIAL FUNCTION IN SEVERE CHILDHOOD MALNUTRITION

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Abstract

Severe malnutrition is a critical global health issue, affecting approximately 13.6 million children and accounting for 20% of deaths in children under 5 worldwide. Despite protocolized treatment with therapeutic food and antibiotics, mortality remains high, and survivors continue to endure long-term metabolic consequences. Mitochondria are indispensable for metabolism, producing the energy currency adenosine triphosphate, which powers all cellular functions. While mitochondria have been implicated in many diseases, the effect of undernutrition on mitochondrial biology in the context of severe malnutrition is not well understood. Through a comprehensive literature search, undernutrition has been found to exert profound structural and functional changes in mitochondria, including impairments in tricarboxylic acid cycle and oxidative phosphorylation, alongside increased oxidative stress. Furthermore, undernutrition affects mitochondrial abundance and processes such as mitochondrial biogenesis and mitophagy. Many low-risk, low-cost supplements are known modifiers of mitochondrial processes and could be used as co-therapies in the treatment of severely malnourished children, with the potential to improve mortality and long-term outcomes. This review serves as a critical step in this direction by highlighting the crucial role of mitochondria in severe malnutrition and identifying the most promising pharmacological targets and therapeutic interventions targeting mitochondria.

Keywords: adenosine triphosphate; electron transport chain; mitochondria; mitochondrial dysfunction; oxidative phosphorylation; severe acute malnutrition; tricarboxylic acid cycle; undernutrition

Abbreviations

ALA	alpha-lipoic acid
AMPK	adenosine monophosphate-activated protein kinase
ATP	adenosine triphosphate
CI	(respiratory) complex I
CII	(respiratory) complex II
CIII	(respiratory) complex III
CIV	(respiratory) complex IV
CoA	coenzyme A
ETC	electron transport chain
FA(s)	fatty acid(s)
FAD	flavin adenine dinucleotide
FCCP	carbonyl cyanide-p-trifluoromethoxyphenylhydrazone
GSH	glutathione
HSP	heat shock protein
LPD	low-protein diet
mPTP	mitochondrial permeability transition pore
mtDNA	mitochondrial DNA
mTOR	mammalian target of rapamycin
NAC	N-acetyl cysteine
NAD+	nicotinamide adenine dinucleotide
OCR	oxygen consumption rate(s)
PGC-1α	peroxisome proliferator-activated receptor gamma coactivator 1-alpha
PINK1	PTEN-induced kinase 1
ROS	reactive oxygen species
RUTF	ready-to-use therapeutic food
SIRT1	NAD-dependent deacetylase sirtuin-1
SM	severe malnutrition
TCA	tricarboxylic acid
TNF	tumor necrosis factor
TOM	translocase of the outer membrane

1. Introduction

Globally, 45 million children are estimated to be too thin for their height.[1] The most severe form of undernutrition, termed severe malnutrition (SM) or severe wasting, affects about 13.6 million children and accounts for about 20% of all deaths in children under 5 worldwide.[2] This excludes children with nutritional oedema, for which accurate epidemiological data is lacking. Despite protocolized treatment with ready-to-use therapeutic food (RUTF) and antibiotics, severely malnourished child suffers from a weakened immunity and faces an 11 times higher risk of death than a well-nourished child.[2] When contracted with an acute infectious insult, such as diarrhoea, pneumonia, or malaria, malnourishment increases poor clinical outcomes 2-10-fold.[3] Survivors also face a greater number of diseases in adulthood, a consequence of stunted growth and development.[4]

Mitochondria are cellular organelles found in almost every cell of the body.[5] As the site for cellular respiration processes such as the tricarboxylic acid (TCA) cycle and the electron transport chain (ETC), they are indispensable for metabolism, through the production of the energy currency adenosine triphosphate (ATP). Additionally, they are involved in many other processes including cell signaling, growth and adaptation, thermogenesis, calcium transport, regulation of inflammation, and production of reactive oxygen species (ROS). Mitochondrial homeostasis is primarily attained through mitochondrial biogenesis, in which new mitochondria are produced, and mitophagy, in which damaged mitochondria are degraded, in a tightly regulated fashion.

Undernutrition is known to have an impact on every function in the human body, but some mechanisms are more well-characterized than others.[6] Metabolic function is known to be altered in SM following disturbances in the homeostasis of carbohydrates, proteins, and fats. Several articles have also reported alterations in mitochondrial biology, such as changes in mitochondrial abundance and ultrastructure, increased oxidative stress, impaired ATP production, and impaired mitochondrial biogenesis and mitophagy. However, to date, no effective summary of the relationship between malnutrition and mitochondrial biology is available.

The goal of this review is to fill this research gap, to synthesize the available evidence on the relationship between undernutrition and mitochondrial function in children with SM. Secondly, the review summarizes the results of clinical trials with mitochondrial-function-modifying interventions carried out in SM so far and explores other potential interventions that could restore or improve mitochondrial homeostasis and function in malnourished conditions. Ultimately, I hope that this review will add to the growing knowledge on the pathophysiology of severe childhood malnutrition, contribute to the call for the development of new, innovative therapeutics, and serve to inform policy regarding the treatment of severely malnourished children.

2. Literature search

For this review, a literature search was conducted to identify all articles concerning mitochondrial biology and function in children suffering from undernutrition and SM.

The preliminary search strategy to retrieve results in PubMed and Google Scholar was as follows. The search terms ("mitochondrion" [tiab] OR "mitochondria" [tiab] OR "mitochondrial" [tiab] OR "sarcosome" [tiab] OR "sarcosomes" [tiab] OR "substrate oxidation" [tiab] OR "tricarboxylic acid cycle" [tiab] OR "TCA cycle" [tiab] OR "Krebs cycle" [tiab] OR "citric acid cycle" [tiab] OR "acetyl-coenzyme A" [tiab] OR "acetyl-CoA" [tiab] OR "oxidative phosphorylation" [tiab] OR "electron transport chain" [tiab] OR "ETC" [tiab] OR "cellular respiration" [tiab] OR "cellular metabolism" [tiab] OR "adenosine triphosphate" [tiab] OR "ATP" [tiab]) were used to retrieve results related to mitochondrial biology and function.

These search terms were used together with ("malnutrition" [tiab] OR "undernutrition" [tiab] OR "wasting" [tiab] OR "kwashiorkor" [tiab] OR "marasmus" [tiab] OR "malnourished" [tiab] OR "undernourished" [tiab] OR "underweight" [tiab] OR "low-protein" [tiab] OR "low protein" [tiab]) to restrict for information on the effect of undernutrition specifically. In past research on SM, there have been many different terms to describe the condition, including infantile malnutrition, protein-calorie malnutrition, protein-energy malnutrition, grade 3 malnutrition, kwashiorkor, marasmus, marasmic-kwashiorkor, (o)edematous malnutrition, (severe) wasting, undernutrition, severe acute malnutrition, and SM. In this study, I use the terms malnutrition and undernutrition interchangeably, and SM to denote specifically the severe form of the condition, which might or might not come with nutritional oedema (also referred to as the subtype (o)edematous malnutrition or kwashiorkor).

Finally, the search terms ("child" [tiab] OR "children" [tiab] OR "childhood" [tiab] OR "infant" [tiab] OR "infantile" [tiab] OR "newborn" [tiab] OR "young" [tiab] OR "weaning" [tiab] OR "weanling" [tiab] OR "weaned" [tiab]) were added to the query to restrict for articles concerning children (less than 5 years of age) and animal models of childhood undernutrition (e.g., less than 2 months old for rats). Studies that did not exclusively feature children or young animals were not included in this review.

Additionally, references, citations, and my own knowledge were used to identify other articles relevant to the study question. Due to the limited availability of SM-specific articles, multiple and varied aspects of mitochondrial biology and function to cover, and the heterogeneity in the results, i.e., in study types, study population, and definition of malnutrition, narrative review was chosen as the most suitable type of review for answering the study question.

3. Structural changes

Mitochondrial function, including the ability to synthesize ATP, is tightly linked with mitochondrial structure. Morphological and ultrastructural alterations in mitochondria have been documented in both human and animal studies investigating severe childhood undernutrition. More information on these studies can be found in **Table 1**.

Studies conducted by Brooks et al. (1992-1995) which looked at cellular structural changes in the liver, pancreas, and voluntary muscle in post-mortem samples of SM non-survivors all reported signs of mitochondrial swelling, a hallmark sign of mitochondrial dysfunction.[7–11] Mitochondrial swelling in children with SM was also noted by Shiner et al. (1973) in the jejunal mucosa, which improved upon treatment.[12] The study by Brooks et al. (1992) on hepatic ultrastructure further noted the loss of matrix granules, disorganization of cristae, the evidence of calcium influx, and depletion of peroxisomes.[9] Biopsies obtained from children during recovery showed restoration to normal mitochondrial morphology. Additionally, in a 1963 study from Theron and Liebenberg, parenchymal liver cells from children with kwashiorkor showed an association of mitochondria with lipid droplets and an increased density of the mitochondrial matrix.[13]

In a rat model of SM, from van Zutphen et al. (2016), in which rats were fed a low-protein diet (LPD), hepatic mitochondria exhibited pleomorphism, enlargement and elongation, abnormal cristae, formation of loops, presence of inclusion bodies, and elevated numbers of mitochondrial granules.[14] Svoboda and Higginson (1964) also reported structural alterations in hepatic mitochondria in rats, namely irregular large vacuoles, occasional disappearance of peripheral membranes, protuberances into adjacent mitochondria or presence of invaginations, presence of inclusions, and enlargement of 2-4% of mitochondria.[15] Interestingly, the study reports a reversal to normal ultrastructure after just 2 weeks of a standard higher-protein diet. Furthermore, Kvissberg et al. (2022) also noted enlargement, elongation, and presence of inclusion bodies in liver mitochondria in mice, while Patrick et al. (1973) reported no observations in liver mitochondria obtained from biopsies from malnourished baby baboons.[16,17] In the small intestine, Ling et al. (2023) noted enlarged and irregularly shaped mitochondria with severely disrupted cristae in mice, with similar findings reported by Nieto et al. (2000) in rats with chronic diarrhoea-induced malnutrition.[18,19] Lastly, Rossi & Zucoloto (1982) and Adebayo et al. (2016) observed mitochondrial swelling in their LPD-fed rat model, in cardiac muscle cells, and in the cortex and cerebellum respectively.[20,21]

Overall, the most common finding appears to be mitochondrial swelling. This is known to be a pathological response to stressors such as increased mitochondrial ROS and calcium overload. Mitochondrial swelling can impair mitochondrial and cellular function in multiple ways, including disruption to ATP production and initiation of cell death. I will discuss more on this topic in '4.5 Calcium homeostasis.' Elongation and enlargement might be related to adaptation to starvation by

increasing the efficiency of ATP production. Other observed changes might be either adaptations or pathological changes following nutrient deprivation and/or oxidative stress. The few studies that also investigated the responses to treatment, such as Brooks et al., van Zutphen et al., and Svoboda and Higginson, provide evidence that mitochondria can recover, which is a positive indication of the potential for therapeutic interventions targeting mitochondrial dysfunction in undernutrition.

There are many limitations to these studies. First, the small number of studies and small sample sizes limit the generalizability of the findings. Most studies focused on the liver, given its central role in metabolism and accessibility for biopsy samples. Furthermore, there is a great variation in terms of species, diet, and duration of undernutrition. Additionally, the observed changes might be attributable to terminal illness and other co-morbidities and not solely to undernutrition, especially when assessing post-mortem samples. Moreover, to an extent, the observed changes might be a result of delayed fixation and other pre-analytical errors. Lastly, most observations are cross-sectional, and overall, provide an incomplete picture of the effect of undernutrition on mitochondria. Future human and animal studies should assess how mitochondria change over time naturally and with treatment, while also collecting data on functional changes.

Table 1. Electron microscopy studies of structural changes in mitochondria in children with severe malnutrition and animal models of childhood undernutrition.

Study	Study type and population	Number of subjects	Location	Findings
Theron & Liebenberg (1963)[13]	clinical study, children with kwashiorkor	16	liver	attachment of mitochondria to lipid granules, increased density, and granulation of matrix in cells with more extensive FA infiltration
Svoboda & Higginson (1964)[15]	animal study, LPD-fed young rats	14 LPD, 5 control	liver	vacuolization, dissolution of peripheral membranes, protuberances and invaginations, enlargement (in 2-4%), presence of inclusions
Shiner et al. (1973)[12]	clinical study, children with SM	5	jejunal mucosa	mitochondrial swelling
Patrick et al. (1973)[17]	animal study, LPD-fed young baboons	6 LPD, 2 control	liver	no observed changes

Rossi & Zucoloto (1982)[20]	animal study, LPD-fed young rats	9 LPD, 9 control	heart	mitochondrial swelling
Brooks et al. (1992)[7]	clinical study, children with SM	7	exocrine pancreas	mitochondrial pallor and swelling
Brooks et al. (1992)[9]	clinical study, children with SM	8 post-mortem, 7 control	liver	mitochondrial swelling, loss of matrix granules, disorganization of cristae, evidence of calcium influx, depletion of peroxisomes
Brooks et al. (1993)[11]	clinical study, children with SM	7	islets of Langerhans	mitochondrial swelling
Brooks et al. (1994)[8]	clinical study, children with SM	8	liver	mitochondrial swelling
Brooks et al. (1995)[10]	clinical study, children with SM	8	voluntary muscle	mitochondrial swelling
Nieto et al. (2000)[19]	animal study, young rats fed lactose-enriched diet to induce chronic diarrhoea	20 (including controls)	ileum and colon	enlargement, low inner density, loss of cristae
Adebayo et al. (2016)[21]	animal study, LPD-fed young rats	not mentioned	cortex and cerebellum	mitochondrial swelling
van Zutphen et al. (2016)[14]	animal study, LPD-fed young rats	not mentioned	liver	pleomorphism, enlargement, elongation, abnormal cristae, formation of loops, presence of inclusion bodies, elevated numbers of mitochondrial granules
Kvissberg et al. (2022)[16]	animal study, LPD-fed young mice	not mentioned	liver	elongation, enlargement, presence of inclusion bodies
Ling et al. (2023)[18]	animal study, LPD-fed young mice	8 LPD, 8 control	small intestine	enlargement, irregular shape, disrupted cristae

4. Functional changes

4.1 Mitochondrial uptake and substrate oxidation

Before glucose, FAs, and amino acids can enter the TCA cycle inside the mitochondria, they undergo breakdown and conversion to acetyl coenzyme A (CoA).[5] This process is called substrate oxidation and predominantly occurs in the mitochondria. The uptake of substrates by mitochondrial transporters and the rate of oxidation both determine subsequent metabolism and ATP production.

Glucose undergoes glycolysis to produce pyruvate, which is then converted to acetyl-CoA. In a study on leucocytes in children with SM, Yoshida et al. (1967) reported significant decreases in pyruvate and lactate, suggesting a decrease in glycolysis.[22] Furthermore, in a rat model of SM, van Zutphen et al. (2016) have demonstrated reduced uptake of pyruvate, derived from glucose, into the mitochondria, where pyruvate oxidation occurs.[14] Both mitochondrial uptake and a decrease in glycolysis limit the amount of pyruvate available for subsequent conversion to acetyl-CoA, thus decreasing the amount of acetyl-CoA entering the TCA cycle. Furthermore, pyruvate oxidation may also be worsened by the deficiency in essential nutrients, such as thiamine, riboflavin, nicotinamide, pantothenic acid, and alpha-lipoic acid (ALA), which serve as co-factors for the enzyme pyruvate dehydrogenase complex responsible the conversion of pyruvate to acetyl-CoA.[23]

FAs undergo oxidation, called beta-oxidation, primarily within the mitochondria, but also within peroxisomes. Blood metabolomics profiles in Wen et al. (2022), obtained from children with SM, showed an impaired FA uptake and/or oxidation in SM with findings of increased even-numbered acyl-carnitines in SM, indicative of disruption in either the carnitine shuttle or the beta-oxidation machinery.[16,17] Van Zutphen et al. (2016) provides further evidence of impaired FA oxidation in SM with findings of a decrease in peroxisomes and enzymes involved in the beta-oxidation of FAs, and accumulation of long-chain acyl-carnitines, following a LPD.[14] Furthermore, the study showed an increase in acetyl-carnitine (C20) alongside an increase in ketone bodies, suggesting possible impaired entry into the TCA cycle and redirection towards ketogenesis. Low levels of the nutrient carnitine, essential for the carnitine shuttle which imports FAs into the mitochondria, may also contribute to impaired uptake.[24] Dysfunction in the FA oxidation, due to disrupted mitochondrial and peroxisomal function, and lack of nutrients such as carnitine, ultimately leads to ATP deficiency and fat accumulation within hepatocytes, promoting the development of hepatic steatosis, a prominent maladaptation in SM particularly associated with kwashiorkor.[14]

Overall, the limited evidence points to impairments in mitochondrial uptake and substrate oxidation, which could be at least partially attributed to deficiency in important nutrients such as B vitamins, ALA, and carnitine. Since, mitochondrial uptake and substrate oxidation are rate-limiting steps for cellular energy production, a decrease in these processes has a huge impact on energy production overall.

4.2 Tricarboxylic acid cycle

The TCA cycle, also known as the citric acid cycle or Krebs cycle, occurs in the mitochondria and functions to oxidize acetyl-CoA, produced during the breakdown of glucose, FAs, and amino acids, and to reduce nicotinamide adenine dinucleotide (NAD+) and flavin adenine dinucleotide (FAD), which then act as high-energy electron carriers for the ETC.[5]

Yoshida et al. (1967) reported significant decreases in TCA cycle intermediate oxaloacetate, alongside decreases in pyruvate and lactate, in leukocytes obtained from children with SM, suggesting an inhibition in glycolysis and the TCA cycle.[22] On the other hand, blood metabolomic profiles elucidated by Wen et al. (2022) showed that children who died from SM exhibited increased levels of TCA cycle intermediates, including pyruvate, fumarate, alpha-ketoglutarate, and succinate, compared to survivors.[25] While these are necessary for the TCA cycle function, their excessive accumulation can hinder the flux through the cycle. Hence these findings suggest a disruption of the TCA cycle among non-survivors of SM.[25] It's important to note, however, that blood metabolomics, although commonly used for inferring systemic metabolic alterations, has limitations in providing tissue-specific information. Additionally, as with pyruvate oxidation, the normal functioning of the TCA cycle might be disrupted by the deficiency of important nutrients, which serve as cofactors for the enzymes involved in the cycle.[23]

In animal models of malnutrition, van Zutphen et al. (2016), like Yoshida et al. observed a decreased hepatic content of TCA cycle intermediates, specifically fumarate, malate, and alpha-ketoglutarate.[14] The levels of TCA cycle enzymes were not found to be affected in the study. However, in Preidis et al. (2014), the levels of hepatic TCA cycle intermediates were found significantly elevated including acetyl-CoA, citrate, and succinyl-CoA in protein-calorie deprived young mice.[26] Lastly, in Kvissberg et al. (2022), the examination of the TCA cycle in the liver of LPD-fed young mice revealed lower levels of acetyl-CoA, 2-hydroxyglutaric acid, and several NAD factors, while the levels of acetyl-phosphatase, citrate, isocitrate, and glycolic acid were increased.[16]

The differences in the alterations in the TCA cycle metabolites might be attributed to several factors, such as differences in tissues, species, age, diet composition, time of measurement, comorbidities, and duration of exposure to undernutrition. The contradictory findings in the levels of TCA cycle metabolites might also reflect different stages of SM. Wen et al. (2022) specifically compares the metabolomic profiles between survivors and non-survivors providing insights into metabolic dysregulations associated with mortality in SM.[25] Overall, an impairment in the TCA cycle likely leads to decreased availability of reducing equivalents to donate electrons to the ETC. Further research is therefore necessary to clarify how undernutrition affects the TCA cycle and its relationship to overall metabolism and mortality in SM.

4.3 Electron transport chain and ATP synthesis

The ETC starts with the reduced equivalents NAD+ and FAD, donating electrons to respiratory complexes I (CI) and II (CII) respectively.[5] The electrons are then transferred through ubiquinone, respiratory complex III (CIII), cytochrome *c*, and respiratory complex IV (CIV), before joining oxygen in the matrix to form water. The flow of the electrons through the ETC enables proton translocation across the membrane, generating an electrical gradient. Protons then move back to the mitochondrial matrix through ATP synthase, which harnesses the gradient to generate ATP from adenosine diphosphate and inorganic phosphate. The ETC and ATP synthase together are commonly referred to as oxidative phosphorylation.

Waterlow (1961) showed in a study on oxidative phosphorylation using homogenates from the livers of malnourished children that oxidative phosphorylation was disrupted in SM and potentially associated with increased fat in the liver.[27] Yoshida et al. (1967) observed a reduced ATP content in leucocytes of malnourished children, which indirectly points to a decrease in ATP synthase function, ETC function, or glycolysis.[22] To the extent of my knowledge, no other clinical study has investigated the impact of undernutrition on ETC activity in children with SM, making this a significant gap in our understanding of the dysfunction in ATP synthesis associated with the condition.

In animal models of childhood malnutrition, several studies have examined biomarkers of ETC function across various tissues. An examination of liver ETC function in an LPD-fed rat model by Zutphen et al. (2016), revealed lower adenosine diphosphate-stimulated oxygen consumption rate (OCR), suggesting impaired electron flow, specifically at the CI level.[14] Significantly decreased OCR was also noted in a mice liver by Hu et al. (2022).[28] In Olorunsogo et al. (1989), brain mitochondria in LPD-fed rats exhibited diminished ADP-stimulated OCR and elevated OCR in the absence of ADP, suggesting impaired coupling of electron transfer to ATP synthesis.[29] The ratio of ADP to oxygen consumption was also reduced, indicating decreased ATP synthesis efficiency. The addition of carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP), an uncoupling agent which allows protons to bypass ATP synthase, increased the rate of mitochondrial respiration, suggesting partial preservation of ETC function despite malnutrition-induced changes. Furthermore, the study found altered proton dynamics, with reduced rates of proton efflux and increased rates of proton influx into the matrix in the malnourished mice and their progeny, which could be attributed to impaired structural integrity of the mitochondrial membrane.

Related to reduction in OCR, the expression and activity of ETC proteins was found reduced in several studies in animal models. Olorunsogo et al. (1989) observed reductions in the activities of CII and CIV in the brains of LPD-fed rats, by 24.1% and 65.3%, respectively, compared to rats fed a normal diet.[29] Furthermore, progeny of these malnourished rats fed a LPD during gestation and the postnatal period lead to reductions of 80.9% and 98.3%, respectively. The basal and FCCP-induced

activities of the ATP synthase were also reduced. ATP hydrolysis rates were reduced 82.1% in the malnourished rats and and 91.2% in their progeny, suggesting SM could lead to an almost complete loss of the activity of ATP synthase, and therefore a profound disruption in ATP synthesis. Similarly, Adebayo et al. (2016) showed significantly decreased activities of CI, CII, and CIV in both the cortex and cerebellum compared to rats fed normal diets, which was partially restored through supplementation with antioxidants zinc and selenium.[21] Furthermore, the study found a significant decrease in the activity of the ATP synthase in the cortex. Examining the ETC function in the liver, van Zutphen et al. (2016) found a significantly decreased hepatic CIV activity in their LPD-fed rat model, while Kvissberg et al. (2022) reported significantly decreased expression of CI and CIV.[14,16] Additionally, Hu et al. (2022) observed significantly decreased levels of hepatic CI, CIV, and ATP synthase.[28] Lastly, in the small intestine, Ling et al. (2023) observed a decrease in the expression of CI and CIV.[18]

In van Zutphen et al. (2016), the impairment in the ETC was corroborated by reduced hepatic ATP content with elevated adenosine monophosphate to ATP ratio, and increased phosphorylation of adenosine monophosphate-activated protein kinase (AMPK) a subunit, indicating cellular energy imbalance and insufficient compensatory mechanisms to restore ATP levels in the liver.[14] Decreased ATP content in the hepatocytes was also shown by Kvissberg et al. (2022) and Hu et al. (2022).[16,28] Finally, Ling et al. (2023) measured a significantly reduced ATP level in the small intestine.[18]

The variability in the impairment of specific ETC components observed across different studies could be attributed to several factors, including age, species, tissue, severity and duration of malnutrition, type of diet, nutritional deficiencies, and other variations. The ETC is a complex system involving multiple components, and dysfunction at any level can disrupt overall ATP synthesis. While some studies found significant impairments at certain complexes, others focused on different components or may not have detected significant differences due to methodological limitations or other factors. Additionally, the lack of reporting of nonsignificant findings in some studies could contribute to a biased perception of the overall impact.

However, the collective evidence across numerous studies indicates that malnutrition does indeed have a significant effect on ETC function and ATP synthesis. Impaired electron flow, particularly at the level of CI, diminishes the generation of the proton gradient, and impairs the function of ATP synthase, leading to ATP deficit. Moreover, impaired electron flow can lead to electron leakage and the generation of ROS, exacerbating oxidative stress and causing further disruptions to mitochondrial and cellular function. Therefore, while the specific manifestations of a dysfunction in oxidative phosphorylation in SM may vary across studies, the overall consensus supports the notion that malnutrition adversely affects ETC function and ATP synthesis.

4.4 Oxidative stress

Under physiological conditions, mitochondria produce many ROS which function as important signaling molecules. It is also important that the generation of ROS is balanced by an abundance of compounds that can neutralize them. This prevents oxidative damage to lipids, proteins, and nucleic acids including mitochondrial DNA (mtDNA), and disruptions in the TCA cycle, the ETC and ATP synthesis, calcium homeostasis, and other mitochondrial functions. Fortunately, normally mitochondria have several antioxidants, which can neutralize ROS directly or indirectly. A deficit in the levels of antioxidants compared to the amount of ROS is called oxidative stress.

Antioxidant levels are significantly decreased in SM, measured in the blood, including total antioxidant status, glutathione (GSH), GSH peroxidase, vitamin E, vitamin C, superoxide dismutase, ceruloplasmin, and albumin.[30–36] Oxidative stress in SM is also evidenced by increased lipid, protein, and DNA oxidation byproducts such as malondialdehyde, protein carbonyl, o,o'-dityrosine, ortho-tyrosine, and urinary 8-hydroxydeoxyguanosine.[33,35–38] Similarly, oxidative stress has been confirmed in animal models of childhood malnutrition, indicated by significantly decreased antioxidant levels in the liver, small and large intestine, and brain and significantly increased oxidation byproducts such as protein carbonyls, malondialdehyde, and thiobarbituric acid reactive substances.[14,19,21,39]

The free radical theory of kwashiorkor, proposed by Golden and Ramdath in 1987, posited that kwashiorkor, or edematous malnutrition, results from increased oxidative stress and should therefore be treated with antioxidants.[40] Since then further research has disproved the theory, albeit partially. Emerging evidence suggests that kwashiorkor has multifactorial etiology with increased oxidative stress, protein and micronutrient deficiencies, low plasma albumin, degradation of extracellular matrix, and mitochondrial dysfunction likely contributing to its development.[14,41–43] Treatment with antioxidants, which has been tried with varied results, will be discussed in section 7 titled 'Targeting mitochondria therapeutically.'

4.5 Calcium homeostasis

Mitochondria are central to maintaining cellular calcium homeostasis, serving as reservoirs for calcium ions that regulate vital processes including ATP production, cell signaling, and apoptosis.[5] Calcium ions stored within mitochondria exert their influence through various mechanisms, including the upregulation of rate-limiting enzymes in the TCA cycle and direct modulation of mitochondrial enzyme activity and dynamics.

Calcium homeostasis is likely to be disrupted by improper functioning of the ATP-powered calcium pumps following ATP deficit. Mg2+-dependent Ca2+-pumping ATPase, responsible for the active transport of calcium ions across cell membranes, was found less functional in erythrocytes of kwashiorkor children compared to healthy controls.[5] Impairment in calcium transport across cell

membranes has wide implications for muscle contraction, neurotransmitter release, and signal transduction. For example, growing evidence suggests that cardiac dysrhythmias in adulthood due to impaired calcium flux following post-natal undernutrition are related to impairment in oxidative phosphorylation and ATP synthesis.[44,45]

Dysregulated calcium homeostasis might also lead to calcium accumulation within mitochondria, which can trigger the opening of the mitochondrial permeability transition pore (mPTP). Induction of mPTP increases the permeability of the mitochondrial membrane to small molecules, leading to mitochondrial swelling (as discussed earlier), disruption of the mitochondrial gradient potential, loss of ATP production, and release of pro-apoptotic factors such as cytochrome *c* into the cytoplasm. In Olorunsogo et al. (1989), calcium uptake by brain mitochondria was found to be decreased in malnourished rats and their progeny, but mitochondrial calcium levels were not directly measured.[29] Similar results were obtained 3 years later by the same group, which the author suggested could be due to the malnutrition-induced changes in the permeability and structural integrity of the mitochondrial membrane.[46] No other studies I have found reported on this topic.

On the other hand, calcium deficiency would also be expected to disrupt mitochondrial function, but the potential effects of calcium deficit in SM on mitochondrial function remain unexplored. Despite the estimated prevalence of hypocalcemia (low calcium levels in the blood) in SM at 26%, there is currently no research on the potential impact of calcium deficiency on mitochondrial biology in this context.[23,47,48]

4.6 Nuclear gene expression

Some studies point to differential gene expression of certain proteins with functions in the mitochondria. The metaxin gene, which encodes for an outer mitochondrial membrane protein responsible for the import and assembly of precursor proteins into the mitochondria, including those involved in cellular metabolism, mitochondrial dynamics, and signaling pathways, was found downregulated in the lymphocytes of malnourished children in one study.[49] The finding might suggest a potential disruption of mitochondrial function and compromised immune response. However, as the authors hypothesize, this alteration might also be a protective mechanism against tumor necrosis factor (TNF)-induced apoptosis, a pathway implicated in immunosuppression.[49] This is important as the levels of TNF are known to be elevated in SM In addition.[6] Additionally, Hu et al. (2022) observed decreased expression of genes involved in the beta-oxidation pathways in the hepatocytes of LPD-fed mice, specifically *Acaa2* and *Hadha*, which persisted with nicotinamide treatment but improved with resveratrol treatment.[28] Further research is needed to elucidate how severe childhood malnutrition affects nuclear (and mitochondrial) gene expression and its implications for mitochondrial function and the overall health of malnourished children.

5. Mitochondrial abundance and quality control

5.1 Observed mitochondrial count

Studies examining the impact of undernutrition on the mitochondrial count so far produced varied results. In animal models, Svoboda and Higginson (1964) reported a profound decrease in the number of mitochondria in the hepatic cells of a rat model of childhood malnutrition.[15] Similarly, Kvissberg et al. (2022) and Hu et al. (2022) reported a decrease in the mitochondria count in the hepatocytes in LPD-fed mice together with a reduction in mitochondrial markers, heat shock protein (HSP) 60 and the outer membrane marker translocase of the outer membrane (TOM) 20.[16,28] Likewise, in Ling et al. (2023), in LPD-fed mice, the decreased abundance of mitochondria in the small intestine was suggested by a significantly decreased abundance of HSP60 and TOM20.[18] Conversely, Rossi and Zucoloto (1982) reported an apparent increase in the number of mitochondria in the cardiac muscle cells in their rat model of SM.[20] Lastly, a suggested increase was also observed in van Zutphen et al. (2016), who noted an increase in TOM20 and a more intense staining indicative of an increased mitochondrial volume or density in the hepatic cells in their rat model.[14]

5.2 Mitochondrial DNA

According to the endosymbiotic theory, mitochondria are believed to have originated from engulfed prokaryotic organisms, resulting in the acquisition of their own genetic material, referred to as mtDNA.[5] This mtDNA encodes 13 proteins crucial for oxidative phosphorylation (ETC and ATP synthesis). mtDNA is a marker of mitochondrial abundance and alterations in mtDNA content are hypothesized to be a sign of mitochondrial dysfunction. These alterations could be attributed to either impaired mitochondrial biogenesis or mitophagy, or both.

A recent cross-sectional study by Saha et al. (2022) identified a notable increase in the mtDNA content in blood samples obtained from children with SM.[30,50] This finding aligns with an observation from the rat model of SM in van Zutphen et al. (2016), which also exhibited elevated mtDNA content.[14] Other studies, such as Kvissberg et al. (2022) and Hu et al. (2022) reported a decrease in the mtDNA content.[16,28] Additionally, investigations into protein malnutrition during gestation and lactation in rats also reported alterations in the mtDNA content in the progeny, including both increases and decreases. Park et al. (2003), found decreased mtDNA content in the liver, muscle, and peripheral blood leucocytes, which partially increased in the resuscitation group.[51] In Park et al. (2004), mtDNA content measured in liver, skeletal muscle, and pancreas, was significantly decreased, except for liver mtDNA at 15 weeks of age.[52] Further studies in SM might look beyond mtDNA content and analyze its structural integrity.[30]

5.3 Mitochondrial biogenesis and mitophagy

Mitochondrial biogenesis and mitophagy are intertwined processes that regulate mitochondrial numbers and perform mitochondrial quality control.[5] Mitophagy plays an important role in removing damaged mitochondria, thereby safeguarding against excessive ROS production and cell death.[53] NAD-dependent deacetylase sirtuin-1 (SIRT1) regulates these processes directly by deacetylating proteins or indirectly through the activation of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α). SIRT1 itself can be activated by caloric restriction, as well as by compounds such as nicotinamide and resveratrol.[54]

In animal models, Kvissberg et al. (2022) reported an increase in PTEN-induced kinase 1 (PINK1), a marker of mitochondrial damage, suggesting greater levels of damaged mitochondria in mouse hepatocytes.[16] Under normal conditions, PINK1 is rapidly degraded in the mitochondria. However, in damaged mitochondria, PINK1 accumulation provides a signal to recruit Parkin, which ubiquitinates multiple proteins, marking the mitochondria for degradation by mitophagy. Despite the increase in PINK1 levels in the hepatocytes of LPD-fed young mice, the study observed a reduction in various examined autophagy markers, namely p62, LC3-II, and ULK1, suggesting an impairment in autophagy despite the presence of increased mitochondrial damage. A decrease in phosphorylated S6K suggests that the block in autophagy is not due to increased activation of the mammalian target of rapamycin (mTOR), which normally inhibits autophagy. Ling et al. (2023) noted a significant decrease in SIRT1 deacetylate enzymatic activity in LPD-fed mice, measured by a heightened ratio of acetylated p53 to total p53, despite no significant change in SIRT1 abundance.[18] Additionally, the decreased activity of SIRT1 was corroborated by the decreased levels of PGC-1α and decreased gene expression of TFAM and NRF-1, transcriptional factors regulated by PGC-1α. Similarly, to Kvissberg et al., Ling also reported a reduction in autophagy, observed through a decrease in LC3-II, despite elevated PINK-1 levels.

Overall, evidence from animal studies provides initial evidence that malnutrition causes a decrease in SIRT1 and PGC-1α expression and/or activity, leading to decreased mitochondrial biogenesis and mitophagy, which culminates in the accumulation of dysfunctional mitochondria. One potential explanation could be nutrient deprivation, such as a decrease in cofactors such as NAD+, which is required for SIRT1 function. Additionally, in conditions of reduced ATP availability, cells may prioritize essential cellular processes over autophagy. Lastly, it could be a result of altered cellular signaling in SM, increased oxidative stress, and inflammation. These studies also provide evidence that targeting these pathways in SM holds promise for ameliorating metabolic consequences associated with SM including hepatic steatosis.[16,18,28]

6. Short and long-term effects of mitochondrial dysfunction in severe malnutrition

The impact of mitochondrial dysfunction in SM extends across various tissues and organ systems, exerting profound effects on cellular function. Primarily, dysfunctional mitochondria disrupt cellular processes through three major mechanisms: energy depletion, increased ROS production, and altered signaling pathways.

ATP as a primary source of energy for all cellular processes such as ion transport, protein synthesis, and signal transduction. Consequently, ATP depletion can lead to compromised function of these processes. Ultimately, ATP depletion can lead to cell death, compromising tissue and organ function. Increased ROS due to depletion of antioxidants, ETC dysfunction, and increased inflammation, can exacerbate mitochondrial and cellular dysfunction, leading to oxidative damage to biomolecules. Additionally, molecules such as ATP, ROS, calcium, cytochrome *c*, and mtDNA, serve as signaling molecules, damage-associated molecular patterns, and apoptosis-inducing factors, thereby influencing cellular functions, and regulating immune response, inflammation, and cell death.

In the liver, the characteristic hepatic steatosis seen in kwashiorkor is attributed to disrupted FA oxidation due to mitochondrial and peroxisomal dysfunction.[14] This leads to intracellular accumulation of FA and exacerbation of oxidative stress. This cascade of events further impairs mitochondrial function, perpetuating the cycle of dysfunction.[55] Untreated liver steatosis can progress to liver cirrhosis and is associated with insulin resistance, dyslipidemia, and systemic inflammation, ultimately contributing to the development of metabolic syndrome.[56]

In the small intestine, mitochondrial dysfunction contributes to compromised barrier function, disrupting nutrient absorption, worsening nutrition deficiencies, and promoting the translocation of microorganisms and toxins into the systemic circulation.[18] This process fuels systemic inflammation and increases the risk of sepsis, a leading cause of mortality in malnourished children.[57] Furthermore, mitochondria and the microbiome are recognized to engage in cross-talk; however, no research to my knowledge explores the relationship between mitochondria and dysbiosis in SM.[58]

Moreover, mitochondrial dysfunction can compromise immune function, increasing susceptibility to infections and impairing immune response, which may contribute to the sepsis-like systemic inflammation and immunosuppression seen in SM, increasing mortality risk.[25,59] In a study in adults with sepsis, ATP synthase content in peripheral blood monocytes was decreased in septic shock compared to healthy controls, while a lower OCR was associated with organ failure and death.[60]

In skeletal muscle, disrupted mitochondrial function in muscle may result in muscle wasting, while in the heart, which contains the more mitochondria than any other tissue, it can lead to cardiomyopathies and organ failure.[61] In research on critical illness in adult patients, early activation of

mitochondrial biogenesis in skeletal muscle has been associated with increased survival, while patients with protracted critical illness following sepsis have been found to demonstrate impairments in skeletal muscle oxidative phosphorylation.[62,63]

In the brain, increased ROS and ATP deficits can lead to neurodevelopmental delays, cognitive impairments, and neurodegeneration.[64–66] Additionally, impaired mitochondrial function can result in renal impairment, endocrine disruption, and impaired wound healing, further increasing susceptibility to infections and sepsis.[6,67,68] Overall, mitochondrial dysfunction is known to have significant consequences in malnourished individuals, including children with SM, and should therefore not be disregarded in SM treatment.

Lastly, while this thesis focuses on the negative effects on mitochondrial biology and function following undernourishment in the postnatal period, research also clearly shows that mitochondrial dysfunction can arise earlier, i.e., following undernourishment during the gestational period, and persist even after optimal nutrition is started postnatally.[69,70] It is therefore also very important to not neglect pregnancy as a critical period where mitochondrial dysfunction can originate and in which optimal nutrition might help prevent the development of SM.

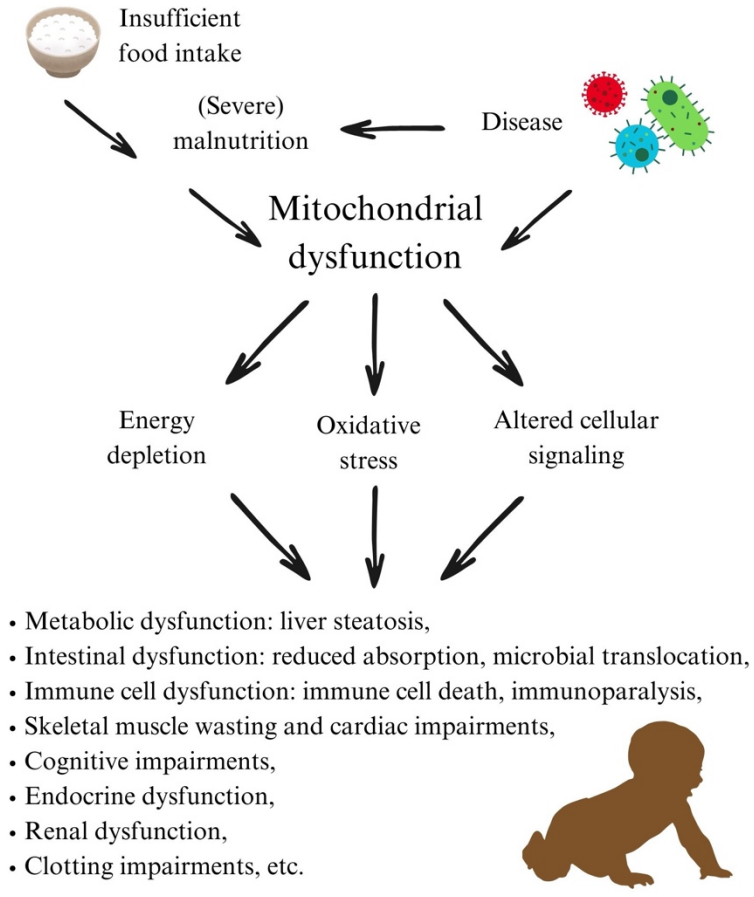

Figure 1. Consequences of mitochondrial dysfunction in severe malnutrition.

7. Targeting mitochondria therapeutically

7.1 Potential intervention

Numerous pharmacological and nutraceutical compounds have been identified as promising modulators of mitochondrial biology and function, offering potential avenues for therapeutic intervention in SM. These compounds have so far been understudied in the context of SM but hold significant promise in improving short- and long-term outcomes. They can be broadly classified into three categories based on their mechanisms of action: metabolic enhancers, antioxidant agents, and mitochondrial regulators. Advanced therapies, such as mitochondrial transplantation, are not discussed because of their lower cost-effectiveness, safety concerns, and lack of research on this topic in the context of severe childhood malnutrition.

This first category includes various amino acids, vitamins, and cofactors pivotal for driving the TCA cycle and the ETC, thereby facilitating ATP production and overall mitochondrial metabolism. For example, as mentioned before, carnitine is indispensable for the FAs import into the mitochondria, where they are oxidized before entering the TCA cycle.[24] Both the pyruvate dehydrogenase complex and oxoglutarate dehydrogenase complex, responsible for pyruvate oxidation and the TCA cycle respectively, require the cofactors thiamine pyrophosphate, FAD, NAD+, CoA, and ALA. Notably, studies suggest that the current thiamine content in therapeutic foods provides an insufficient amount of thiamine for children with SM.[23,71] Lastly, supplementation with ubiquinone/coenzyme Q, which is a necessary component of the ETC and an antioxidant, has shown promise in heart failure treatment.[72] In SM, therapies specifically targeting the TCA cycle, oxidative phosphorylation, ATP synthesis, and related pathways have not yet been tried in clinical trials, and thus represent a significant gap in undernutrition research so far.

The second category encompasses compounds such as GSH, vitamin C, vitamin E, and melatonin, which bolster antioxidative capacity within mitochondria, mitigating oxidative stress and preserving mitochondrial function. In a study by Becker et al. (2005), the efficacy of GSH, N-acetyl cysteine (NAC), and ALA supplementation in children with kwashiorkor was evaluated, comparing outcomes to standard treatment and healthy controls.[31] While both GSH and ALA decreased mortality, only GSH reached statistical significance after adjustments. Conversely, a larger study by Ciliberto et al. (2005) assessing NAC, riboflavin, vitamin E, and selenium in kwashiorkor prevention found no benefit.[73] The two studies suggest that GSH deficiency may be a consequence rather than a cause of kwashiorkor. The lack of data on mortality and oxidative stress leaves room for the possibility the dose might be insufficient and increasing GSH and antioxidant capacity could mitigate mortality from the condition by reducing oxidative stress.[36,74] Given this uncertainty, and the promising results with using antioxidants in other conditions, treatment with GSH or other antioxidant agents should be repeated, perhaps in conjunction with other mitochondrial modulators as a part of a comprehensive package.

Lastly, the third category comprises supplements aimed at promoting mitochondrial biogenesis and mitophagy, such as nicotinamide, resveratrol, rapamycin, and melatonin. These compounds facilitate the removal of dysfunctional mitochondria and promote the generation of new, functional ones. Experimental studies in animal models of malnutrition have shown promising results with nicotinamide, resveratrol, and rapamycin, in increasing mitochondrial biogenesis and mitophagy as well as improving mitochondrial dysfunction-induced hepatic steatosis through targeting SIRT1 and mTOR pathways, suggesting their potential therapeutic utility in SM.[16,18,28] Following up on the promising results in animal models, rigorous randomized controlled trials need to be conducted to assess their efficacy and safety in human subjects, paving the way for their clinical application in the management of SM.

7.2 Considerations and future directions

SM significantly impacts the pharmacokinetics of pharmaceutical and nutraceutical agents, necessitating careful consideration when selecting dosages for treating affected patients. Compromised gastrointestinal function, a known feature of SM, affects drug absorption and subsequent bioavailability.[57,75–77] Additionally, drug distribution is altered in SM, partially due to hypoalbuminaemia and nutritional oedema in children with kwashiorkor, and the lack of adiposity in malnourished children with the marasmic subtype.[75–77] The prevalence of hypoalbuminaemia, liver dysfunction, and alterations in glycoprotein levels and enzyme profiles in SM also influence drug metabolism and clearance.[75,77,78] Children with SM, aged 0.5-5 years, constitute a highly vulnerable population, at increased risk for organ dysfunction and mortality, which heightens their susceptibility to adverse drug reactions and warrants close monitoring. Ethical considerations often lead to the exclusion of the sickest children, including those with the kwashiorkor oedematous subtype, from clinical trials, potentially affecting treatment outcomes overall. Lastly, storage conditions and costs should also be carefully considered in resource-limited settings where SM typically occurs.

Nutraceutical agents, including vitamins, minerals, herbal medicine, and dietary supplements, are perceived as safer, with a wider therapeutic window and greater cost-effectiveness, but their adverse effects and efficacy remain less researched and understood. Single-compound trials with nutraceutical compounds conducted in SM so far yielded varied results, which could be related to the limitation of targeting only single pathways, suggesting the need for a more comprehensive approach.[24,31,79] Summary of these and other interventions targeting mitochondria in SM can be found in **Table 2**. Future clinical trials should consider testing a mitochondrial health package addressing all aspects of compromised mitochondrial function in children with SM highlighted in this review.

Table 2. Results of clinical trials with mitochondrial-function-modifying interventions in severe malnutrition.

Study	Number of subjects	Intervention	Relevance to mitochondria	Findings
Becker et al. (2005)[31]	11/12/12 treatment arms, 11 control	GSH / NAC / ALA, 1200 mg / 100 mg / 200 mg respectively, for 20 days	GSH is a crucial antioxidant, protecting the body from oxidative stress. NAC and ALA are cysteine donors for GSH. Additionally, ALA is a cofactor for enzymes in substrate oxidation and the TCA cycle.	GSH decreased mortality from SM after adjustments for height and initial blood GSH concentration. Mitochondrial function was not measured.
Ciliberto et al. (2005)[73]	1184 treatment, 1188 controls	antioxidant powder, 300 mg NAC, 55 µg selenium, 23 mg vitamin E, 1.8 mg riboflavin per day for 20 weeks	NAC, selenium, vitamin E, and riboflavin are antioxidants that protect the body from oxidative stress. Furthermore, riboflavin is necessary for the TCA cycle and for the regeneration of GSH.	Antioxidant powder had no significant effect in preventing kwashiorkor. Mitochondrial function was not measured.
Ghone et al. (2013)[36]	50 treatment, 50 control	antioxidant syrup, components not listed, 10 ml per day, for 30 days	Antioxidants protect the body from oxidative stress.	Antioxidant syrup significantly decreased levels of lipid peroxidation. Mitochondrial function was not measured.
Saleem et al. (2018)[79]	93 treatment, 92 control	high-dose vitamin D, 5 mg at week 2 and week 4 of starting RUTF	Vitamin D is a membrane antioxidant, which also upregulates other antioxidants including GSH. Additionally, It is hypothesized to regulate mitochondria transcription through the vitamin D receptor.[80] Low levels are thought to reduce mitochondrial function and increase oxidative stress.[81]	Treatment with high-dose vitamin D led to weight gain and improvement in development markers. Mitochondrial function was not measured.
Alam et al. (2024)[24]	49 treatment, 49 control	L-carnitine syrup, 100 mg/kg per day for 15 days	Carnitine is necessary for the carnitine shuttle, which transports FAs into the mitochondria for beta-oxidation.	Carnitine did not affect the rate of weight gain. Mitochondrial function was not measured.

8. Knowledge gaps and conclusion

Through an exploration of structural and functional changes within mitochondria, including alterations in energy production and redox balance, and regulation of mitochondrial numbers, it becomes evident that undernutrition exerts a significant impact on metabolism and overall mitochondrial function. These alterations can lead to serious consequences, including impaired growth and development, increased susceptibility to infections and non-communicable diseases, and organ dysfunction and failure. While there have been advancements in understanding the mechanisms underlying mitochondrial dysfunction in SM, significant knowledge gaps are yet to be addressed.

Firstly, further research is needed to elucidate the specific mechanisms of mitochondrial dysfunction at different stages of malnutrition and nutritional recovery, and of the interplay between mitochondrial dysfunction and other pathological processes in SM, such as inflammation.[25,59] Most knowledge we have comes from animal studies which mainly employ a LPD as a model and exclude the influence of infections and are thus less representative of the real world. Additionally, we need more information on the consequences of malnutrition-induced mitochondrial dysfunction on overall health across the lifespan. Moreover, the development of low-cost non-invasive methods to assess mitochondrial function in malnourished children would greatly facilitate the diagnosis of mitochondrial dysfunction and the monitoring of treatment responses. Lastly, while therapeutic strategies targeting mitochondria show potential, their efficacy and safety in the context of severe childhood malnutrition, in a population characterized by a high mortality rate, require rigorous evaluation in randomized controlled trials.

In summary, mitochondria are profoundly affected by undernutrition in childhood. Further exploration of mitochondrial dysfunction in this context is therefore necessary and holds promise for developing new therapeutics, improving the clinical outcomes and long-term consequences, and ultimately, improving the lives of the 13.6 million children worldwide currently suffering from the condition.

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