



Collaborating to improve the management of  
acute malnutrition worldwide

## Kwashiorkor: still an enigma – the search must go on

André Briend, MD, PhD

Department of Nutrition, Exercise and Sports,  
Faculty of Science,  
University of Copenhagen,  
DK-1958 Frederiksberg,  
Denmark

and

Department for International Health,  
University of Tampere School of Medicine,  
FIN-33014, Tampere,  
Finland

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## Abbreviations

<b>ADH</b>	Antidiuretic Hormone
<b>CMAM</b>	Community-based Management of Acute Malnutrition
<b>CoA</b>	Coenzyme A
<b>EFA</b>	Essential Fatty Acids
<b>GAG</b>	Glycosaminoglycans
<b>G6PD</b>	Glucose 6-Phosphate Dehydrogenase
<b>HSPG</b>	Heparin Sulphate Proteoglycan
<b>NADPH</b>	Nicotinamide Adenine Dinucleotide Phosphate (reduced form)
<b>LPS</b>	Lipopolysaccharide
<b>RUTF</b>	Ready-to-Use Therapeutic Food
<b>TNF</b>	Tumor Necrosing Factor
<b>VLDL</b>	Very Low Density Lipoprotein
<b>WHO</b>	World Health Organisation

## Table of Contents

Acknowledgments .....	1
Abbreviations .....	1
Introduction .....	3
Public health importance.....	3
Diagnosis and management.....	4
The kwashiorkor enigma.....	7
Kwashiorkor as a consequence of insufficient protein intake .....	8
Limitations of the protein hypothesis .....	8
The possible role of insufficient intake of some amino acids .....	12
A possible role of kidney dysfunction .....	13
Kwashiorkor resulting from a dysadaptation to a low protein high carbohydrate diet .....	14
Kwashiorkor and aflatoxins.....	16
The role of oxidative stress – the free radical hypothesis .....	17
Disruption of sulphated glycosaminoglycans (GAGs).....	22
Possible role of the gut microbiota .....	23
Conclusions: kwashiorkor remains an enigma .....	25
The way forward – priorities for research.....	25
Epidemiological studies.....	25
Pathophysiological studies .....	26
Clinical trials.....	27
References .....	29

## Tables

Table 1: Estimation of protein requirements by different committees over the last few decades .....	8
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## Figures

Figure 1: Basic disturbances in homeostasis during the development of kwashiorkor.....	15
Figure 2: Initial interpretation of the causal association between increased oxidative stress and clinical features of kwashiorkor.....	17
Figure 3: Alternative interpretation of the association between increased oxidative stress and clinical features of kwashiorkor. ....	20
Figure 4: Percentage of CD4+ cells of lymphocytes in malnourished children with and without oedema. ....	21
Figure 5: Number of publications with “kwashiorkor” as keyword since 1945 .....	25

## Photos

Photo 1: Pitting oedema on the feet .....	4
Photo 2: Pitting oedema on the legs.....	5
Photo 3: Kwashiorkor with oedema of the face (oedema +++)......	5
Photo 4: Skin lesions of kwashiorkor - scaly skin. ....	6
Photo 5: Skin lesions of kwashiorkor - peeling skin.....	6

## Introduction

The term kwashiorkor was introduced in the medical literature by Cicely Williams, a Jamaican physician working in what is now Ghana in an article published in the *Lancet* in 1935<sup>1</sup> (republished in 2003<sup>2</sup>). It is derived from a word of the Krobo language from Ghana and refers to a child displaced from the breast by the birth of a younger sibling.<sup>3</sup> The paper was not the first to describe this form of oedematous malnutrition. Arguably it had been known under different names in different languages back to biblical times.<sup>4</sup> It was not even the first description of the condition in English, as Cecily Williams herself had already given a full picture of the disease in 1933<sup>5</sup> (republished in 1983<sup>6</sup>).

In its original description, kwashiorkor was presented as a full clinical syndrome, seen mainly in children under the age of 2 years fed a monotonous diet and associating oedema, skin lesions, hair changes and affect dominated both by irritability and apathy. Its reported case fatality was 90% and at *post mortem*, fatty infiltration of the liver was a constant finding. In the following years, some authors used the term kwashiorkor to describe conditions which included only some of the clinical signs of the original description. This resulted in some confusion and following a meeting of experts at the invitation of the Wellcome Trust in 1970, it was recommended to use the terms kwashiorkor and marasmic kwashiorkor only for children having nutritional oedema, independently of other associated symptoms.<sup>7</sup> To be more specific, some authors went on to abandon the term “kwashiorkor” and just use the term “oedematous malnutrition.”

In the original Wellcome classification, marasmus was defined by a weight-for-age less than 60% of the US reference used at that time. Currently, it is defined by a weight-for-age less than -3 z-score of the World Health Organisation (WHO) growth standard, or a mid-upper arm circumference less than 115 mm in 6-60 month old children and absence of oedema.<sup>8</sup> This changing definition complicates the interpretation of studies based on the comparison of the pathophysiology of marasmus and kwashiorkor as they refer to different comparison groups.

## Public health importance

Currently, there is no reliable estimate of the number of children suffering from kwashiorkor around the world. This condition is usually transient, i.e. children usually recover or die within a few weeks of onset, and kwashiorkor is poorly captured by cross-sectional surveys which are commonly used to assess the importance of malnutrition.<sup>9</sup> As a result, it is not mentioned in the recent *Lancet* series on nutrition, although it is the most common form of severe acute malnutrition in many parts of Africa.<sup>10,11,12,13</sup>

Kwashiorkor incidence can be high in some areas. During an attempt to prevent kwashiorkor by an antioxidant mix in rural Malawi, 2.6% of children developed oedema during the 20 weeks follow-up.<sup>14</sup> In a study in Malawi following up twins, in about half of the pairs of twins, at least one developed kwashiorkor.<sup>15</sup> Extrapolated at the southern Africa regional level, these studies suggest that hundreds of thousands of children are affected in this area only, every year. The number of kwashiorkor cases worldwide would be even larger.

The number of children who die worldwide from kwashiorkor every year is difficult to establish. Several studies suggest that presence of oedema is an aggravating factor for severe acute malnutrition and is associated with a high risk of death.<sup>16,17,18,19</sup> In contrast, a community study from Malawi suggests a lower mortality among kwashiorkor children compared with children with non oedematous malnutrition.<sup>20</sup>

There is indirect evidence that the number of children suffering from kwashiorkor has declined over the last 30 years or more, presumably in parallel with a reduction of infectious diseases, especially with the increased coverage of measles immunisation.<sup>21,22</sup> Despite this encouraging trend, the problem remains important in terms of public health and available data suggest it should not be neglected.

## Diagnosis and management

To determine the presence of oedema, normal thumb pressure should be applied to the dorsum of both feet for at least 3 seconds. If a shallow print persists, then the child has oedema.<sup>23</sup> The severity of oedema is often graded as mild (+) when it is present in feet only, as moderate (++) when it is present in legs and feet and lower arms, and severe (+++) when it is visible on the face and / or arms (**Photos 1 to 3**). Predominance of oedema in the legs is more frequent than in nephritic and nephrotic syndromes, the most common causes of non nutritional oedema. There are no skin lesions in the oedema of renal origin and apathy is more pronounced in kwashiorkor. If in doubt, a simple urine test strip shows the presence of albumin in the urine of children with a renal disease in contrast to kwashiorkor where it is absent or present in very small amounts.<sup>24</sup> Blood, present in urine during nephritis, is always absent in kwashiorkor.

**Photo 1: Pitting oedema on the feet**



*Credit: Nicky Dent (Nutritionist)*

**Photo 2: Pitting oedema on the legs**



*Credit: Nicky Dent (Nutritionist)*

**Photo 3: Kwashiorkor with oedema of the face (oedema +++)**



*Credit: Kerstin Hanson (MSF)*

Skin lesions are associated with a higher risk of death.<sup>25</sup> Skin may be scaly, or peeling (**Photos 4 and 5**). Skin lesions are associated with a higher risk of hypothermia and also predisposes to infections.<sup>23</sup> The liver is usually enlarged, but there is no associated jaundice. Slight elevation of bilirubin can be observed and is associated with an increased risk of dying.<sup>26</sup> The presence of significant elevation of liver enzymes is also a sign of profound illness and high risk of death.<sup>27</sup>

**Photo 4: Skin lesions of kwashiorkor - scaly skin**



*Credit: Nicky Dent (Nutritionist)*

**Photo 5: Skin lesions of kwashiorkor - peeling skin**



*Credit: Nicky Dent (Nutritionist)*

Compared to children with marasmus, children with kwashiorkor are more apathic. They often suffer from anorexia, but in the absence of a quantitative measure, it is not clear whether this is more pronounced than in marasmus.

Children with kwashiorkor seem susceptible to an excessive sodium load leading to heart failure.<sup>28</sup> They seem especially at risk at the time of starting intensive feeding.<sup>29</sup> This sodium load can result from inappropriate use of oral rehydration solutions (including ReSoMal) or from intravenous infusion.<sup>23</sup>

The management of kwashiorkor improved considerably after decades of research and mortality decreased dramatically first in a few pilot centres and then more generally after the publication of a standardised protocol by WHO in 1999.<sup>30,31</sup> Still, mortality remains high in referral treatment centres, especially in the context of high HIV prevalence.<sup>32,33</sup>

The WHO treatment protocol has been recently updated.<sup>34</sup> With the endorsement of the Community-based management of severe acute malnutrition in 2007 by WHO and UNICEF<sup>35</sup> kwashiorkor cases in the mild and moderate categories, in the absence of complications - the vast majority of cases - are usually treated entirely in the community using ready-to-use therapeutic food (RUTF) and antibiotics.<sup>34</sup> RUTF provides all essential nutrients and is designed to replace deficits, promote rapid catch-up growth of lean as well as adipose tissue, and repair physiological function. Early detection of oedema by community health workers facilitates the treatment of less severe forms of kwashiorkor and contributes to the reduction of its mortality.

When complications are present, children should be treated as inpatients and receive a broad spectrum antibiotic treatment and are fed F-75, a low protein milk-based diet with added vitamins and minerals, until they have a major decrease in their oedema, recover their appetite and show improvement in their affect. They receive an intake of 100 kcal/kg/day, which is designed just to maintain their body weight. Once associated infections are under control and oedema is resolving, children are fed RUTF in the community until full recovery.

High doses of vitamin A (60 mg) have been reported to be associated with higher risk of death in kwashiorkor.<sup>36</sup> As F-75 and RUTF are already fortified with sufficient levels of vitamin A, children with kwashiorkor should not be given pharmacological doses of vitamin A on admission.<sup>34,37</sup>

## The kwashiorkor enigma

The first paper from Cicely Williams, originally published in 1933, remained understandably circumspect regarding the origin of the disease.<sup>6</sup> It noted simply that it is frequently associated with a monotonous maize-based diet and concludes with the following statement:

*“Breast milk is probably deficient in some factors, which are at present uncertain. As maize is the only source of supplementary food, some amino acid or protein deficiency cannot be excluded as a cause. As regards vitamin deficiency, there is no evidence pointing to lack of vitamin A, C, D or E. That there is a deficiency of some part of vitamin B complex cannot be excluded, although the disease described here does not resemble either pellagra or beri-beri.”*

In those days, vitamins were in fashion and some authors argued that the newly described syndrome was just a variation of pellagra. In her Lancet 1935 paper, Cecily Williams insisted that this was a different condition but again remained circumspect about the aetiology.<sup>2</sup>

Despite the spectacular improvements in its management, 80 years later, the aetiology of kwashiorkor remains an enigma. There is no doubt that it usually occurs at the time of weaning, in a context of poverty, in children having a monotonous diet with low nutrient density. Kwashiorkor responds to dietary treatment, in which a milk-based diet seems important, suggesting a key role of some form of macro or micro nutrient deficiency in its aetiology. But beyond this, there is no general agreement on what is its original cause and there are still many uncertainties regarding its management and particularly its prevention. This technical brief will review different explanations which have been proposed as a cause of kwashiorkor, describe their shortcomings and highlight areas which deserve attention.

## Kwashiorkor as a consequence of insufficient protein intake

Following failed attempts to treat kwashiorkor with niacin, the specific treatment for pellagra, it became generally accepted that protein deficiency was the cause of kwashiorkor. This hypothesis was supported by the apparent consumption of a low protein diet and also by the frequent observation of low plasma albumin concentration in oedematous children. A simple mechanism was postulated: low protein intake resulted in insufficient albumin synthesis which in turn was the cause of oedema as a result of low plasma oncotic pressure.<sup>38</sup> The association of fatty liver with oedema could further be explained by a depression of the synthesis of apo-lipoprotein. As this protein is needed for the release of triglycerides from the liver into the plasma, an insufficient synthesis was supposed to explain the accumulation of fat.<sup>39,40</sup> This hypothesis however, was later challenged and this simple mechanism seems now unlikely to explain the clinical picture of kwashiorkor.

### Limitations of the protein hypothesis

#### *Lack of supporting epidemiological evidence*

First, the protein deficiency hypothesis was put forward at a time when different committees thought that child protein requirements were quite high in comparison with currently accepted values (**Table 1**). With the successive readjustments which took place over the years, it became less clear that children in areas where kwashiorkor was prevalent had an insufficient protein intake, unless their overall food (and energy) intake was itself insufficient.<sup>41,42</sup> Of note, the protein requirements of children has been recently challenged based on stable isotope studies and may be higher than currently estimated.<sup>43</sup>

Table 1: Estimation of protein requirements by different committees over the last few decades

Year	Protein (g/day)	Source
1948	3.3	NRC (USA)
1957	2.0	FAO
1964	2.5	NRC (USA)
1965	1.1	FAO/WHO
1968	1.8	NRC (USA)
1969	1.3	DHSS (UK)
1973	1.27	FAO/WHO
1974	1.35	NRC (USA)
1985	1.57	FAO/WHO
2007	1.14	FAO/WHO

Source: adapted from Waterlow and Payne,<sup>42</sup> FAO-WHO-UNU,<sup>198</sup> WHO<sup>74</sup>

With this background of falling estimates of protein requirements, the protein deficiency hypothesis, which was predominant until the 1980s, was also challenged on different grounds. First, a study from

<sup>i</sup> Oncotic pressure is the fraction of the osmotic pressure which is due to protein. In contrast to proteins, electrolytes and small molecules which readily cross capillaries membranes have no effect on oncotic pressure. The effect of oncotic pressure is to maintain fluids within the capillaries.

India examined the food and nutrient intake of about 1800 children among which 23 developed kwashiorkor.<sup>44</sup> According to the authors:

*“...the most careful examination failed to show that the dietary pattern of the children who developed kwashiorkor or marasmus was qualitatively different from those of other children in the community.”*

This simple sentence, published in a narrative book chapter, not in a formal scientific journal, with little information on the dietary intake measure, and which was not supported by quantitative data nor statistical analysis, would not meet present criteria to be considered as solid evidence. Yet, it was sufficient at the time to question the relevance of the protein deficiency hypothesis. A hospital based study from Nigeria, published in 1976, also mentioned an absence of detectable difference in the diet of children with marasmus or kwashiorkor without giving a quantitative description of their respective diets.<sup>45</sup> These findings were confirmed years later by more rigorous studies. A first retrospective study failed to find marked differences of intake of protein rich foods, except for a slightly lower consumption of eggs in children with kwashiorkor compared to those with marasmus.<sup>46</sup> A later retrospective case-control study compared the food diversity of children from households where kwashiorkor or marasmus cases came from and found a slightly lower consumption of eggs in households of children with kwashiorkor. Frequency of consumption of other protein rich foods, such as milk, beans or fish was not significantly different.<sup>46</sup>

Longitudinal studies provide more rigorous information but the quantity of data that can be collected is always limited. A first longitudinal study from Malawi used food frequency questionnaires that were used afterwards to calculate the nutrient content of the diet of children in the community using standard portion sizes obtained from a previous survey. This study found that children who later developed kwashiorkor had on average a higher protein intake than those who did not during follow-up.<sup>47</sup> A second study examined the food frequency of children every three months and compared the food frequency among children who later developed kwashiorkor and others who did not. This study failed to show a lower frequency of consumption of high protein foods in children who later developed kwashiorkor.<sup>48</sup>

Taken all together, studies on the relationship between diet and the occurrence of kwashiorkor are not in favour of the hypothesis of a lower protein intake in children who develop kwashiorkor. It should be acknowledged, however, that these studies rely on a very rough estimate of nutrient intake based on food frequency questionnaires, and consumption of lower amounts of high quality protein rich foods or of specific amino acids in children who developed kwashiorkor cannot be ruled out.

#### ***Link between low protein intake and low plasma albumin concentration***

The link between low protein intake and low plasma albumin concentration was first suggested by early experimental animal studies, going back to the 1940s.<sup>38</sup> Albumin concentrations, however, fell only with diets which are very low in proteins, levels unlikely to be consumed by children in real life.<sup>49</sup>

A link between protein intake and albumin synthesis was also suggested by a study in malnourished children estimating *de novo* albumin synthesis indirectly by the shape of the curves of distribution over time of albumin labelled with [<sup>131</sup>I] in the intra and extra vascular compartments.<sup>50</sup> This early study compared estimated albumin synthesis in children on a low (0.7-1g/kg/day) and a high (2.0-4.8 g/kg/day) protein diet and found it to be higher in the latter. This finding was later supported by a comparison of albumin concentrations in Ugandan children receiving a diet providing either 2.3 or 4.4 g/kg/day of protein suggesting a faster increase of serum albumin to normal concentrations in children in the high protein diet.<sup>51</sup> A later study, however, using a direct method of estimation of albumin synthesis based on measurement of the incorporation of [<sup>2</sup>H<sub>3</sub>] labelled leucine, failed to show a difference in albumin

synthesis at the beginning of treatment in children with oedematous and non oedematous forms of malnutrition.<sup>52</sup>

Albumin concentrations are also influenced by concurrent infections,<sup>49,53</sup> and more generally by the level of metabolic stress, and cannot be regarded as a specific consequence of a low protein intake.<sup>54</sup> The effect of protein-losing enteropathy on plasma albumin concentration is uncertain: it has been reported to influence albumin concentrations in children with marasmus, but not in those with kwashiorkor.<sup>55</sup>

### ***Link between low plasma albumin concentrations and presence of oedema***

Water represents about 60% of the total body weight in adults, more in children, depending on age. About two thirds of this water is within the cells and represents the intracellular compartment. Water outside the cells (about 33 % of total body water) represents the extracellular compartment. About 25% of this water is in blood vessels, in the intravascular compartment. Extracellular water which is not in blood vessels (about 75% of the extracellular water) represents the interstitial compartment. Pitting oedema, as seen in kwashiorkor, is the clinical manifestation of increased water in the interstitial compartment.<sup>56</sup>

Water is continuously flowing at the capillary level from the intravascular to the interstitial sector. Excess water accumulation is prevented by the lymphatic system which actively returns the water from the intravascular compartment into the venous system. Normally, the capacity of the lymphatic system to move the water from the interstitial compartment back into the venous system can increase 10 to 50-fold and represents an important safety factor to prevent the occurrence of oedema. Oedema occurs only when there is a massive increase of the filtration of water into the interstitial space.<sup>56</sup>

The passage of fluid from the intravascular to the interstitial sector is ruled by the Starling's equation:

$$\text{Filtration} = K_f (P_c - P_{if} - \Pi_p + \Pi_{if})$$

where  $K_f$  is the capillary filtration coefficient which depends on the membrane permeability,  $P_c$  and  $P_{if}$  are the hydrostatic pressures within the capillaries and the interstitial fluid respectively, and  $\Pi_p$  and  $\Pi_{if}$  are the respective oncotic pressure of plasma and interstitial fluid.<sup>56</sup>

Albumin is a major determinant of the plasma oncotic pressure ( $\Pi_p$ ) and the low plasma albumin concentrations frequently observed in kwashiorkor were proposed to explain the oedema.<sup>57</sup> The observed association, however, does not necessarily imply direct causality. One can argue that, to some extent, a correlation between a reduction of serum albumin and the extent of oedema is almost inevitable as a result of a dilution effect if the intravascular volume increases and the total albumin pool remains constant.<sup>58</sup> This dilution mechanism, however, implies an increased plasma volume, which does not seem to be present in kwashiorkor.<sup>38</sup>

Nephrotic syndrome has some similarities with kwashiorkor as it associates low plasma albumin concentration and oedema, but the causal link between these two events is debated.<sup>59</sup> Oedema in the nephrotic syndrome seems related to change in the endothelial capillary barrier, i.e. an increase in its hydraulic conductivity and permeability to proteins, rather than to an imbalance of Starling's forces. These changes may be indirectly related to hypoalbuminaemia via an increase in intracellular calcium and possibly to an increased TNF $\alpha$  (Tumor Necrosing Factor) plasma concentration.<sup>60</sup>

A primary role of low albumin concentrations as a cause of oedema has been questioned in view of the large overlap of albumin concentrations between children with oedematous and non oedematous malnutrition which has been noted for some time.<sup>61,62</sup> Also, oedema can disappear without any major change in plasma albumin concentration.<sup>58,63</sup> The frequent absence of oedema, and, if present, its minor

importance in patients who have analbuminaemia,<sup>64</sup> a rare genetic disease preventing the synthesis of albumin, also suggests that low albumin concentrations do not necessarily lead to oedema.

These observations however, do not exclude a low albumin from being a possible contributing cause of oedema. They suggest that other factors are also involved, as described by Starling's equation, and that these other factors may play a more important role than albumin. Among the other terms in the equation, a change in albumin concentration in the interstitial sector has an effect on its oncotic pressure ( $\Pi_{if}$ ) but experimental studies suggest it decreases during malnutrition, opposing the development of oedema. This decrease in albumin concentration in the interstitial sector possibly results from an increased fluid flow across the capillary membrane<sup>57</sup> and a redistribution of albumin in favour of the intravascular compartment.<sup>50,65</sup>

The hydrostatic pressure of the interstitial fluid ( $P_{if}$ ) decreases in response to some bacterial endotoxins, thereby increasing the pressure gradient which drives filtration across the capillary wall,<sup>66</sup> with different toxins having different effects.<sup>67</sup>

Studies on the relationship between low plasma albumin concentrations and oedema are difficult to interpret. The effect of the difference:

$$(P_c - P_{if} - \Pi_p + \Pi_{if})$$

(also called the net filtration pressure) on interstitial space volume is non linear and increases sharply about 3 mmHg above the normal range.<sup>56</sup> This suggests that hardly detectable changes in albumin and on oncotic pressure can have a dramatic effect on appearance of oedema. This can mask the effect of albumin especially if other factors influencing the appearance of oedema, notably sodium and potassium intake, change at the same time.<sup>68</sup>

### ***Fatty liver and export proteins***

Fat accumulation in the liver is unlikely to be due to a lack of dietary protein leading to an insufficient production of lipoproteins needed to export fat from the liver. The evidence in favour of this mechanism is indirect and weak, based mainly on the low concentration of beta-lipoprotein in the plasma of children with kwashiorkor,<sup>39,69</sup> which represents a poor indicator of its synthesis and seems an inconsistent finding.<sup>70</sup> Direct measure of very low density lipoprotein (VLDL) apolipoprotein B100 synthesis by a stable isotope method disproved this interpretation by showing that children who had the highest proportion of fat in their liver had the fastest rate of synthesis.<sup>70</sup>

### ***Response to treatment***

Response to treatment has been central in the discussion on the cause of kwashiorkor. Initially, the hypothesis that protein deficiency was a possible cause was supported by response to treatment of children receiving milk described in early reports.<sup>2</sup> However, as was pointed out at a meeting in Uganda in the 1950s, curing headache with aspirin does not mean that headache is due to aspirin deficiency.<sup>38</sup> Milk-based diets provide many more nutrients than proteins, and have diverse effects on metabolism, and it is not correct to conclude that the effect of milk is necessarily due to its protein content.

In an early metabolic study on kwashiorkor, oedema was shown to disappear in 5 children while receiving for 4 to 7 days a nitrogen-free but potassium rich electrolyte solution, pointing to the possible role of potassium deficiency as contributing to the development of oedema.<sup>71</sup> Disappearance of oedema was later found to be unrelated to the level of protein intake in Jamaica also suggesting this is not the main causal factor.<sup>72</sup> Since 1999, WHO has recommended the low protein milk-based formula F-75 diet for children with oedema<sup>31</sup> and daily experience shows that it is effective in treating kwashiorkor in the initial phase.

These observations of response to low protein diets are hardly compatible with a central role of protein deficiency as a cause of oedema, and here the logic seems absolute, in contrast to the link between protein intake associated with milk-based diets and the disappearance of oedema.<sup>4</sup> To continue the previous comparison, one cannot argue that headache is due to aspirin deficiency if it can be cured without aspirin.

Interestingly, in one experimental study of induced kwashiorkor in children, the oedema was cured by the addition of egg yolk to the diet, but not by the addition of egg white, which is rich in protein,<sup>73</sup> suggesting that the effect of protein rich diets may be due to the other nutrients they provide beyond proteins.

Arguably, the quantity of protein provided by F-75 is slightly above the maintenance level. F-75 contains 9 g/L of protein. When 135 ml/kg/day of F75 is consumed, the protein intake is equivalent to 1.2 g/kg/day of proteins which is clearly higher than the currently estimated daily requirement for body maintenance, which is 0.66 g/kg/day in children.<sup>74</sup> Also, the lack of effect of protein intake on the disappearance of oedema was established based on comparisons of diets with different protein contents given over different time periods, and not by a direct comparative trial. So some doubt theoretically persists about the relation between protein intake, protein synthesis and disappearance of oedema.<sup>38</sup> The protein content in F-75 is low, however, representing about 5% of its energy content, a proportion which is in the lower range of observed intake in the poorest countries.<sup>42</sup> Thus, the consistent response to treatment by low protein diets makes the hypothesis of a protein deficiency as the primary cause of kwashiorkor unlikely.

## The possible role of insufficient intake of some amino acids

In her early papers, Cicely Williams mentioned an insufficient intake of individual amino acids as a possible cause of kwashiorkor. This hypothesis was tested in the 1960s by a multicentric study examining the plasma amino acid profile of 64 children suffering from kwashiorkor in 9 different countries.<sup>75</sup> The authors observed that the aminogram of these children was remarkably similar across countries, despite great variation in the type of protein consumed. In all countries, plasma concentrations of branched chain amino acids (valine, leucine and isoleucine) were markedly depressed. Among aromatic amino acids, tyrosine plasma concentration was depressed but phenyl-alanine was maintained. The concentration of 3 non essential amino acids (tyrosine, arginine, citrulline) was decreased whereas that of the others was increased. These aminograms were quite different from those observed in experimental amino acid deficiencies and the authors concluded that overall protein deficiency, and not the lack of a specific amino acid, was the cause of the observed abnormal plasma amino acid profile. The decrease of some amino acid concentrations was attributed to insufficient protein intake. The decrease of tyrosine plasma concentrations when its essential precursor phenylalanine was maintained was explained by an insufficient conversion.<sup>75</sup> The increase of some of the non essential amino acids was attributed to altered enzymatic pathways involved in their metabolism, suggested by the similarities of the excretion in the urine of products of amino acid metabolism with what is seen in inborn errors of amino acid metabolism.<sup>76</sup>

The evidence ruling out the role of individual amino acids was however indirect, and was challenged by Roediger who observed that many characteristics of kwashiorkor could be explained by an insufficient intake or metabolism of sulphur amino acids.<sup>77</sup> Among his arguments was the marked decrease of the plasma concentration of methionine, the only essential sulphur amino acid, before starting nutritional therapy (mean reduction in 5 different studies,  $\pm$ SE: 60.3%  $\pm$ 11.8). Also, he noted the marked reduced urine excretion of sulphur in children with kwashiorkor, with hardly any overlap with concentrations observed in marasmic patients.<sup>78</sup> Kwashiorkor is also associated with a decreased plasma concentration of glutathione,<sup>79</sup> which could also result from an insufficient sulphur amino acid intake.

Formation of coenzyme A (CoA) requires methionine derived cysteine. Methionine is important for maintenance of liver CoA. CoA is central to the control of lipid synthesis and breakdown and methionine deficiency leads to production of fatty liver in experimental animals.<sup>77</sup> Methionine insufficient intake or availability could play a role in the fatty liver associated with kwashiorkor.

Skin is rich in sulphur, especially in the young,<sup>80</sup> and some of the skin lesions could also be explained by sulphur amino acid deficiency. In addition, kwashiorkor is commonly seen in population consuming cassava,<sup>81</sup> a staple food often contaminated with cyanogens which require sulphur amino acid for detoxification.

A clinical trial giving cysteine supplement (as N-acetyl-cysteine) showed a faster disappearance of oedema in children who were supplemented, compared to children who received alanine supporting the hypothesis of a role of sulphur amino acids in the occurrence of oedema.<sup>82</sup> A similar effect was not observed with methionine supplementation, although cysteine can be synthesised from methionine which is considered to be the only indispensable sulphur amino acid in healthy children.<sup>83</sup> The ability to convert methionine to cysteine, however, is limited and in a number of cases where cysteine demand is high, cysteine must be supplied directly through the diet - thus cysteine can be considered as "conditionally essential."<sup>83</sup>

All together, available evidence suggests that insufficient intake or availability of sulphur amino acid may be involved in the development of kwashiorkor. The absence of efficacy of a supplement providing 300 mg of N-Acetyl-cysteine (equivalent to 222 mg of cysteine) to prevent kwashiorkor in Malawi<sup>14</sup> - when the estimated requirement is of 22 mg/kg/day of sulphur amino acid in children aged 1 to 2 years - is intriguing in this context. Methionine, however, has specific functions and cannot be synthesised from cysteine. A deficiency of the two sulphur amino acids as a cause of kwashiorkor cannot be ruled out by this finding.

A possible role of other isolated amino acid deficiency is also suggested by the kwashiorkor-like clinical presentation of Hartnup disease when associated with malnutrition.<sup>84,85</sup> Hartnup disease is caused by a genetic metabolic disorder affecting the transport of neutral amino acids, and in particular the intestinal uptake of tryptophan.<sup>86</sup>

## A possible role of kidney dysfunction

An involvement of kidney dysfunction has also been postulated to explain the presence of oedema in some children. With the protein deficiency hypothesis in mind, it has been proposed that this kidney dysfunction was a consequence of the low plasma albumin concentration which would result in low plasma volume, low cardiac output, low blood pressure, decreased peritubular hydrostatic pressure and increased reabsorption of salt and water, possibly associated with increased renin and angiotensin and aldosterone concentrations, as a result of a decreased glomerular filtration rate.<sup>87</sup> This possible mechanism has been little explored but the rare observations of variations of urinary aldosterone excretion and oedema during treatment do not support this mechanism.<sup>88</sup> Arguably, a hyperactivity of the renin-angiotensin system in oedematous malnutrition has been reported,<sup>89</sup> but it is difficult to state whether this is a cause of oedema or the consequence of cardiac dysfunction.<sup>90</sup>

An excessive production of antidiuretic hormone (ADH) has also been proposed as contributing to the formation of oedema. The proposed mechanism is that liver damage leads to the release of ferritin into the plasma and that ferritin has a stimulating effect on ADH secretion by the posterior pituitary.<sup>91</sup> This hypothesis was supported by a study showing by a biological assay an ADH effect of plasma from

children with kwashiorkor that decreased during treatment.<sup>92</sup> Using the same assay, an increased ADH effect was observed in children with kwashiorkor compared to those with marasmus.<sup>93</sup> This observation is not consistent, however, with a decreased ADH activity in malnutrition reported by other authors.<sup>87</sup> An increased ADH activity should be associated with an increase in urine osmolarity in kwashiorkor, which is inconsistent with the decreased urine osmolarity observed in malnutrition.<sup>87</sup>

A direct dysfunction at the kidney level has been proposed in view of the increased leakiness of leucocyte cell membranes with a cellular response of increasing sodium efflux by the sodium pump. If an increased permeability existed as well at the renal tubule level this could explain an excessive sodium retention.<sup>58</sup> A study in dogs suggested that cell membranes permeability is influenced by some trace elements such as zinc, copper and cobalt.<sup>94</sup> The nature of the membrane lipids may also affect cell membrane permeability. The sodium pump activity is also regulated by vanadium<sup>58</sup> which may play a role as well in the development of oedema.<sup>95</sup> This hypothesis, however, is difficult to explore as vanadium has different oxidation states of which only the one with the highest oxidation level, vanadate, apparently has an effect on the sodium pump.<sup>95,96</sup>

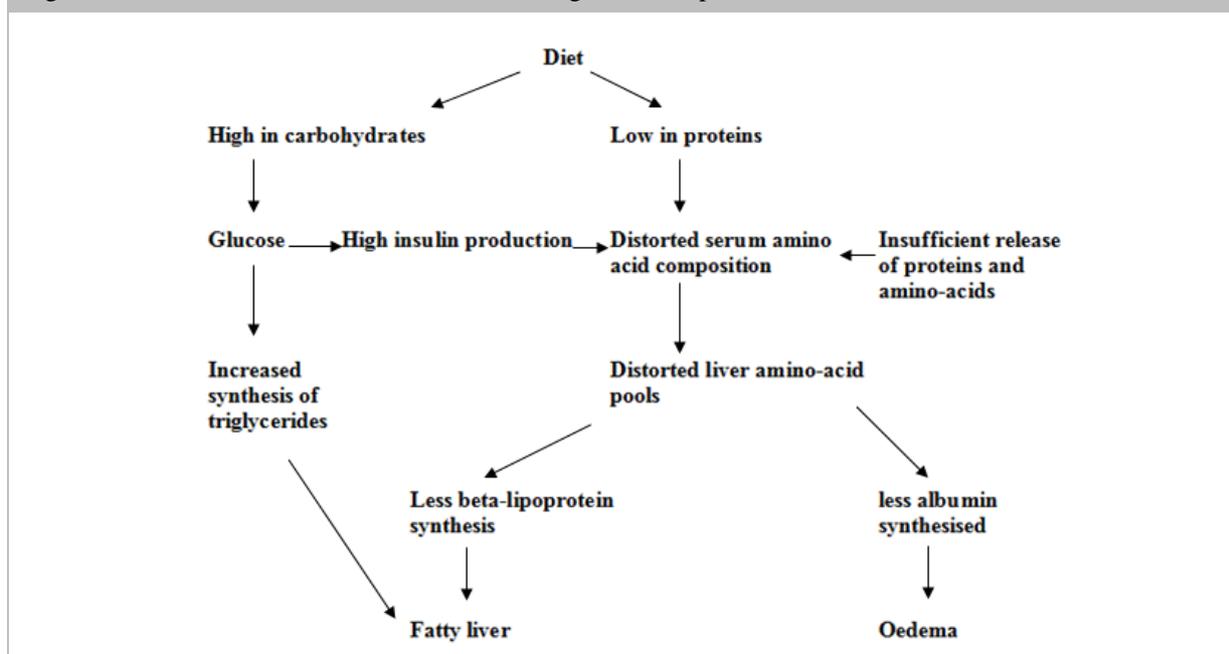
Presence or absence of oedema in malnourished subjects seemed to correlate well with the dietary history of salt intake.<sup>87</sup> Sodium is mainly distributed in the extracellular sector and in case of excessive intake it may lead to expansion of the interstitial space and cause oedema. Animal studies suggest that this sodium retention is aggravated in cases of low potassium intake.<sup>97</sup> A combination of excessive sodium intake and a low potassium intake may also explain the development of oedema in malnourished children.

## Kwashiorkor resulting from a dysadaptation to a low protein high carbohydrate diet

Gopalan in 1968 was the first to raise the possibility that kwashiorkor was not the consequence of a low protein diet itself but the result of a failure of the organism to adapt to a low protein diet, which would explain why some children would develop kwashiorkor, whereas others would not, while consuming similar low protein diets.<sup>44</sup> There was no mechanism leading to this dysadaptation described in this initial paper.

A detailed mechanism leading to kwashiorkor by a failure to adapt to a low protein diet was proposed a few years later.<sup>98,99</sup> In brief, these authors suggested that this dysadaptation was the result of a high carbohydrate intake, leading to an increased insulin secretion inhibiting amino acid release from muscle, which in turn may lead to reduced albumin synthesis and oedema (**Figure 1**). The high carbohydrate intake would also inhibit the synthesis of beta lipoprotein which would limit the release of triglycerides from fat stores leading to hepatic steatosis. These changes in insulin were associated with low cortisol concentrations which also inhibited the use of muscle amino acids which usually takes place in children with marasmus. Growth hormone concentration is increased in kwashiorkor, with an inverse correlation with albumin, possibly as a result of low somatomedin concentration (IGF1 and 2 with the current nomenclature).<sup>100</sup>

Figure 1 – Basic disturbances in homeostasis during the development of kwashiorkor



Adapted from Whitehead and Alleyne<sup>98</sup>

This hypothesis of an involvement of high insulin and low cortisol plasma concentration in the mechanism leading to kwashiorkor was supported by comparison of apparently healthy children in Uganda, where kwashiorkor was the commonest form of malnutrition, with children from the Gambia where marasmus was predominant: after 6 months of age, children in Uganda had high insulin and low cortisol plasma concentrations compared to children in the Gambia.<sup>100</sup> This comparison between Uganda and the Gambia should be interpreted with caution, as it was cross-sectional, based on community surveys carried out years apart, and relied on comparison between countries (ecological studies), both aspects of which represent a very low level of evidence in favour of causality.

This interpretation ascribing many of the clinical features of kwashiorkor to an insufficient mobilisation of protein stores has received some confirmation from metabolic studies using stable isotopes.<sup>101</sup> In brief, these studies showed a slower amino acid flux, indicating a slower protein breakdown, in children with oedematous malnutrition compared to the non oedematous form.<sup>102,103,104,105</sup> In the same way, a study of fat metabolism with stable isotopes showed that fat release from adipocytes and fatty acids oxidation is lower in kwashiorkor compared to marasmus, again suggesting a failure to use fat as an energy source, as suggested by this dysadaptation hypothesis.<sup>106</sup>

The role of increased insulin production in the process of inadequate protein and fat mobilisation associated once kwashiorkor is established is questionable. In Uganda, plasma concentrations of insulin were lowest in children with the lowest plasma concentration of albumin.<sup>107</sup> Children with kwashiorkor and those with marasmus both have an impaired insulin production and there is no indication that they differ in this respect once malnutrition is established.<sup>108,109</sup> An increased insulin secretion may only play a role as an underlying factor at an early stage of the disease.

Observations made in malnourished baboons suggest that addition of sugar to a nutrient poor diet lead to clinical deterioration and precipitates the onset of kwashiorkor.<sup>110</sup> This intriguing observation may be related to an inappropriate insulin secretion leading to hypophosphatemia and hypokalemia as seen in refeeding syndrome.<sup>111</sup> Serum phosphate is more depressed in children with kwashiorkor than in marasmus.<sup>112</sup>

Another mechanism than hormonal imbalance may be involved in the insufficient protein and fat mobilisation observed in kwashiorkor. A study from Jamaica found that children with kwashiorkor had a higher birth weight than those with marasmus.<sup>113</sup> They speculated that children with marasmus adapted to an inadequate nutrient intake right from foetal life. In support of this, they refer to the higher protein turnover in children with kwashiorkor after recovery compared to those with marasmus.<sup>101</sup> In absence of a healthy control, however, this study could be interpreted as evidence that a low birth weight is a risk factor with marasmus, with less effect on the risk of kwashiorkor. In a prospective study from Malawi, children who later developed kwashiorkor were found to be more wasted and stunted than those who did not.<sup>47</sup>

Experimental studies suggest that a low protein, high energy diet represses the transcription of albumin mRNA in rat liver and this is the main mechanism behind the low albumin plasma concentration observed in this model of malnutrition.<sup>114,115</sup>

## Kwashiorkor and aflatoxins

Aflatoxins represent a family of toxins produced by *Aspergillus flavus*, a fungus which grows worldwide but produces its toxins mainly in tropical climates. In view of the difficulties with explaining the epidemiology of kwashiorkor by a protein deficiency, it was proposed in the 1980s that aflatoxins may play a role in its pathogenesis.<sup>116</sup> This hypothesis was suggested by a similar geographical distribution of kwashiorkor and of aflatoxin presence in food, and by the similarities of the metabolic disturbances induced by aflatoxin in animals and those observed in kwashiorkor. Aflatoxins have an effect on several organs but especially on the liver where they induce a depression of protein synthesis. Hence, it seemed plausible that aflatoxin contamination could explain the low plasma albumin concentrations in kwashiorkor, and then oedema and an altered lipid metabolism. The association with a background of malnutrition could be explained by a higher toxicity of aflatoxins in young and malnourished children as suggested by animal studies.<sup>117</sup>

This hypothesis was supported by studies from Sudan showing that aflatoxin was more commonly found at higher concentrations in plasma of children with kwashiorkor compared to those with marasmus.<sup>118</sup> A similar observation was found in Kenya.<sup>119</sup> This also was supported by pathological studies showing a higher concentration of aflatoxin in the liver of children with kwashiorkor compared to control children.<sup>120,121</sup> When put on an aflatoxin-free diet, it was found that children with kwashiorkor excreted aflatoxin for a longer period compared to children with marasmus.<sup>122</sup>

These observations, however, should be interpreted with caution. First, in all these clinical observations, there was a considerable overlap between the aflatoxin concentration of children with kwashiorkor and marasmus. Second, the difference in aflatoxin concentrations in the liver of children with marasmic kwashiorkor was not shown in a later series which found that children dying from marasmus had higher hepatic aflatoxin concentrations than those dying from kwashiorkor.<sup>4</sup> Also, aflatoxin was found in post mortem analysis in children who died from causes other than kwashiorkor.<sup>123</sup> An alternative interpretation of these findings is that of a reverse causality as aflatoxins are detoxified in the liver and the impaired liver function in kwashiorkor could cause aflatoxin accumulation in the tissues. In this interpretation, all children are exposed to aflatoxin in some countries, but those with kwashiorkor or who are severely ill for another reason lose the capacity to rapidly detoxify aflatoxin which then accumulates in the liver or other organs.

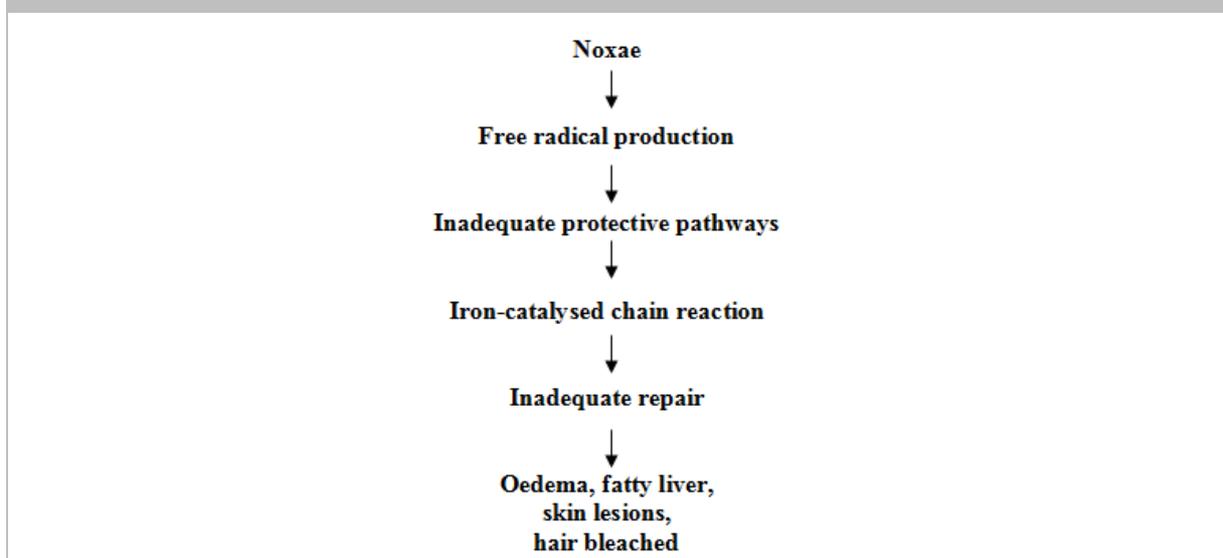
A primary role of aflatoxin as a cause of kwashiorkor is not consistent either with observations made during recent outbreaks of acute aflatoxin poisoning. During these outbreaks there are consistent

observations of leg oedema, but they are associated with abdominal pain, vomiting, fever, jaundice and ascites<sup>124</sup> which are not part of the clinical picture of kwashiorkor. There was no mention in the observations made during these outbreaks of an increase of the association of oedema as seen in kwashiorkor, with hepatic steatosis and skin lesions but without fever and without jaundice. Nevertheless, aflatoxins can stimulate free radicals production and may aggravate kwashiorkor as discussed in the next section.

## The role of oxidative stress – the free radical hypothesis

In 1985 and 1987, in two landmark papers, Golden and Ramdath proposed an alternative interpretation of the pathophysiology of kwashiorkor, denying any role of protein deficiency and presenting oxidative stress as the initial cause of kwashiorkor.<sup>125,126</sup> Free radicals are atoms or molecules which have an unpaired electron which makes them chemically hyper-reactive. They are produced in small quantities in healthy subjects in the mitochondria during respiration but their production is greatly increased in leukocytes in response to infection as they are involved in mechanisms killing potential pathogens. The body uses multiple mechanisms to deactivate these free radicals which have a strong oxidative action and may damage other molecules. According to the free radical hypothesis, the production of free radicals is increased in kwashiorkor as a result of infections or toxic aggression (both grouped under the general term of *noxae*) but crucially the defence mechanisms needed to remove these free radicals are inadequate (**Figure 2**). Protecting the organism against oxidative stress involves multiple detoxification mechanisms requiring the presence of many essential nutrients, including sulphur amino acids, several vitamins (E, riboflavin, nicotinic acid), carotene, selenium, copper, zinc and manganese, many of which are usually lacking in the monotonous diet typically consumed in regions where kwashiorkor is prevalent.<sup>126</sup> This results in an oxidative stress which would explain the clinical features of kwashiorkor.

Figure 2 . Initial interpretation of the causal association between increased oxidative stress and clinical features of kwashiorkor



*Adapted from Golden and Ramdath<sup>126</sup>*

The hypothesis was initially proposed following the observation that plasma concentration of glutathione, a tripeptide involved in the detoxification of free radicals, was lower in children with kwashiorkor compared to marasmus with minimal overlap between the two forms of malnutrition.<sup>79</sup> This suggested an increased oxidative stress in kwashiorkor,<sup>127</sup> which was confirmed in several other settings.<sup>128,129,130</sup> Other studies found an increase in free iron in the plasma of children having kwashiorkor compared to

marasmus, which is also in favour of a role for the oxidative stress as free iron is a powerful pro-oxidant.<sup>131,132,133</sup>

The free radical hypothesis is attractive as it offers a unique explanation to many apparently unrelated clinical features of kwashiorkor. Oedema could be related to an increased cell membrane permeability due to lipid oxidation or to alteration of membrane pore permeability which is speculated to be due to oxidation of a sulphhydryl group on band-3 pore protein.<sup>134</sup> The consequent increase in intracellular sodium could explain the increased activity of the sodium pump seen in children with kwashiorkor.<sup>135,136,137</sup>

Fatty liver could be related to an impairment of fatty acid oxidation by peroxisomes in the liver.<sup>138</sup> Peroxisomes are small subcellular bodies which are present in large quantities in the liver and are involved in the initial stage of the beta-oxidation of long chain fatty acids, producing hydrogen peroxide, a strong and potentially damaging oxidant. In the case of inadequate protection against oxidation, this could lead to destruction of peroxisomes and inadequate fatty acid oxidation, leading to fatty liver.<sup>138</sup> This mechanism is consistent with the observation of lower fatty acid oxidation in children with kwashiorkor compared to marasmus.<sup>106</sup> It could be tested by examining the lipid profile of fatty liver, an excess of long chain fatty acids being in favour of a dysfunction of peroxisomes. Peroxisomes, however, oxidise only a small part of lipids in the liver and are mainly involved in the oxidation of very long chain fatty acids such as hexacosanoic acid (C26:0) or branched chain fatty acids which are mainly found in dairy products or meat, rarely consumed by children with kwashiorkor.

Skin lesions could be related to an insufficient availability of reduced nicotinamide adenine dinucleotide phosphate (NADPH) consumed in large quantities to reduce glutathione as part of the defence against free radicals. The similarity of skin lesions of kwashiorkor to those of pellagra, due to an insufficient intake of niacin, could be explained by this mechanism as niacin is an essential component of NADPH which is likely to be reduced as well in pellagra.<sup>4</sup> In contrast to pellagra where the total NADP<sup>+</sup>/NADPH is decreased, only the reduced form NADPH is decreased in kwashiorkor.<sup>127</sup>

### ***Limitations of the free radical hypothesis***

In its initial version, the free radical hypothesis postulated that kwashiorkor was not related at all with protein and /or amino acid deficiency. The low concentrations of reduced glutathione observed in kwashiorkor could be in theory due either to an insufficient intake or availability of amino acids needed for its synthesis or to an excessive oxidation, but the emphasis was put on the latter mechanism based on the observation that glutathione concentration in whole blood of malnourished children increased *in vitro* when oxidation was prevented.<sup>126</sup> The role of an insufficient intake of sulphur amino acids is suggested, however, by a clinical study showing that supplementing children with kwashiorkor with cysteine increased their glutathione concentrations.<sup>82</sup> Cysteine is one of the three amino acids (glutamic acid, cysteine and glycine) needed for glutathione synthesis and can be obtained from the diet or from mobilisation of body proteins, or synthesised from methionine, which is an essential amino acid. This effect of cysteine suggests either an insufficient dietary intake or an inadequate release of cysteine from the body protein pool, as part of an overall dysfunction of protein metabolism.<sup>139</sup> An abnormal protein metabolism could explain the low concentration of glutathione in addition to the increased level of oxidative stress. Another possible link complicating the interpretation of these results is that serum albumin, the concentration of which is markedly decreased in kwashiorkor, is also a major antioxidant due to the presence of cysteine residues.<sup>140</sup> So it is not clear which is the first causal mechanism in the association between altered protein metabolism and increased oxidative stress. The difficulty of determining whether oxidative stress is the real cause of kwashiorkor or one of its many consequences has been highlighted previously.<sup>128</sup>

### ***Link between free radicals and oedema***

The mechanism which could explain how excessive free radical production can lead to oedema is not clear. A major difficulty for accepting a causal link is that the association of oxidative stress with oedema is inconstant. Oedema has been described in premature infants receiving a diet high in polyunsaturated fatty acids favouring the production of free radicals and which was to be corrected by the addition of the antioxidant vitamin E to the diet.<sup>141</sup> Consumption of edible oil adulterated with argemone oil results in oxidative stress and epidemics of oedema (epidemic dropsy).<sup>142</sup> But exposure to ionizing radiation, as occurs during a nuclear accident, exposes the organism to an intense attack by free radicals and is not associated with oedema.<sup>143</sup> Exercise at high altitude generates a significant oxidative stress which can lead to cerebral or pulmonary oedema,<sup>144</sup> but not generalised oedema as observed in kwashiorkor. Preeclampsia is associated with an increased oxidative stress associated with oedema, but antioxidants do not reduce the risk,<sup>145</sup> which suggests a non causal association. AIDS, which is known to be associated with increased oxidative stress<sup>146</sup> is not associated with oedema. Among children treated for severe acute malnutrition, those infected with HIV are less likely to have kwashiorkor than marasmus compared to uninfected children.<sup>147,148,149,150,160</sup> Although these differences may partly be explained by a bias in treatment seeking behaviour, they are also at odds with the hypothesis that oxidative stress leads to oedema.

### ***Lack of association between genetic variants of enzymes involved in the protection against oxidative stress and kwashiorkor***

When deactivating free radicals, reduced glutathione is oxidised and the oxidised form has to be reduced by NADPH to be used again. The NADPH used in this reaction is mainly produced by the pentose phosphate pathway which oxidises glucose into ribose 5-phosphate by a series of reactions of which the first one is catalysed by glucose 6-phosphate dehydrogenase (G6PD). This enzyme has several genetic variants, some of which are less effective and lead to a reduced capacity to produce NADPH. Clinical manifestation of G6PD deficiency results mainly from the effect of oxidative stress on red cells. As red cells have no mitochondria, they rely only on the pentose phosphate pathway to produce NADPH and are especially vulnerable in case of G6PD deficiency.<sup>151</sup> G6PD, however, is present in all cells, and one would expect kwashiorkor to occur more frequently in patients with less effective G6PD variants. This was examined in a study in Nigeria which failed to find an association between G6PD activity and kwashiorkor.<sup>152</sup> Arguably, the level of G6PD deficiency may have been insufficient in these patients to lead to kwashiorkor, especially considering the other existing pathways to produce NADPH in all cells except red cells.

The same approach of examining the effect of genetic variants of enzymes involved in the protection against oxidative stress was used in a later study from Jamaica. Genes coding for different enzymes involved in a wide range of protection against free radicals were compared in children with marasmus and kwashiorkor.<sup>153</sup> Variations of tested genes could not explain the occurrence of kwashiorkor or marasmus in some children. The authors acknowledged that this does not rule out the free radical hypothesis, as differences in important untested genes may have remained unnoticed, but again this finding is not in favour of failure to respond to an oxidative stress as the primary cause of kwashiorkor.

### ***Absence of effect of supplementation with antioxidants on kwashiorkor incidence***

A double blind randomized controlled trial failed to show a preventive effect of a supplementation with an antioxidant cocktail (1.8 mg riboflavin, 23 mg Vitamin E as d- $\alpha$  tocopheryl acetate, 55 mcg selenium as sodium selenate, and 300 mg *N*-acetylcysteine) on the incidence of kwashiorkor.<sup>14</sup> The *N*-Acetylcysteine dose was equivalent to 222 mg of cysteine, to be compared with an estimated requirement of 22 mg/kg/day of sulphur amino acid in healthy children aged 1 to 2 years. The incidence of kwashiorkor was

higher in the intervention group (3.3%) than in the placebo group (1.9%) with a relative risk of 1.7 almost reaching statistical significance (95% CI: 0.98 to 2.42).

Another trial carried out also in Malawi in moderately wasted children receiving three different food supplements, two being more effective than the last one to prevent wasting, failed to show a differential effect on the incidence of kwashiorkor, which was 8% for the three groups over the study period despite the presence of a vitamin and mineral antioxidant mix in all the supplements.<sup>154</sup>

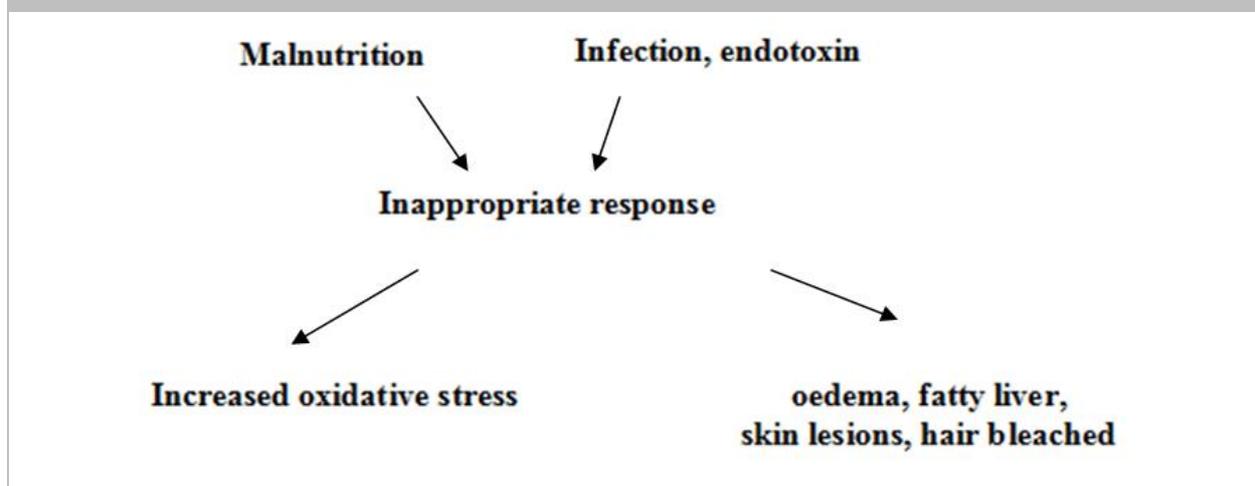
These results are not in favour of inadequate protection against an oxidative stress as being the primary cause of kwashiorkor. This negative finding, however, does not formally eliminate the free radical hypothesis. In all these studies, neither antioxidant status nor oxidative stress were measured so it can be argued that the antioxidant mix was not optimal, or not sufficient in relation to an important oxidative stress.<sup>155</sup>

A large dietary survey found an association between the intake of high carotene foods, likely to have antioxidant properties, with a reduced incidence of kwashiorkor.<sup>48</sup> This association, in favour of a role of carotene in protecting against kwashiorkor, however, should be confirmed by intervention studies. Also, carotene-rich foods may contain other nutrients which are needed for kwashiorkor prevention and the effect of carotene is uncertain.

#### ***Alternative interpretation of the high level of oxidative stress observed in kwashiorkor***

The association between kwashiorkor and oxidative stress is now well established and appears to be part of the pathogenesis of kwashiorkor. Its role as a primary cause of kwashiorkor, however, seems uncertain. A possible interpretation of these findings is that oxidative stress may come at a late stage of the causal pathway leading to kwashiorkor or constitute a side event (**Figure 3**). In this interpretation, malnutrition and an external stressor, when present together, may lead by independent pathways to an increased oxidative stress and to oedema. For example, malnutrition is often associated with the presence of the bacterial endotoxin lipopolysaccharide (LPS) in the systemic circulation.<sup>156</sup> LPS can trigger an inflammatory response inducing a cascade of events, including an increased vascular permeability to albumin<sup>157</sup> and a decrease in the hydrostatic pressure of the interstitial fluid,<sup>67</sup> both of which can lead to oedema. LPS also increases the level of oxidative stress by making polymorphonuclear neutrophils more responsive to pro-oxidant agents.<sup>158</sup> As a direct role of aflatoxin as a cause of kwashiorkor seems unlikely nowadays, the general term “noxae” was replaced in **Figure 3** by infection or endotoxins.

Figure 3. Alternative interpretation of the association between increased oxidative stress and clinical features of kwashiorkor



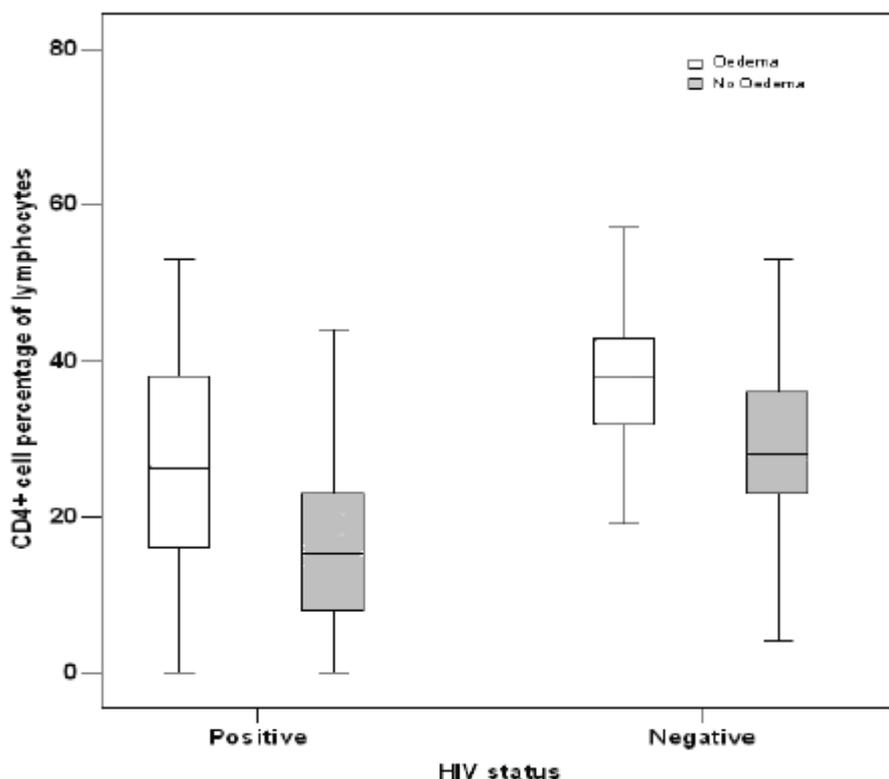
*In this interpretation, an inappropriate response to some stressor as a result of malnutrition is the cause of both the oxidative stress and the clinical features of kwashiorkor which are not causally related*

This interpretation, not involving oxidative stress as the primary cause of kwashiorkor, is compatible with an inadequate protection against oxidative stress being an aggravating factor of kwashiorkor and is compatible with a possible role of antioxidants in the treatment of kwashiorkor. In this regard, a first pilot study showed that supplementing children with kwashiorkor with cysteine (as *N*-acetyl-cysteine) increased glutathione synthesis and plasma concentrations and was associated with a more rapid resolution of oedema compared to controls ( $9 \pm 1$ , vs.  $14 \pm 2$  days).<sup>82</sup> Another pilot study showed that supplementation with glutathione or the antioxidant alpha-lipoic acid had a favourable effect on survival.<sup>159</sup> Similar studies to test the potential of different antioxidant supplementations on the clinical outcome of kwashiorkor seem warranted.

### Immunity and inflammation

The activation of the immune system seems to operate early in the causal pathway leading to kwashiorkor. This is suggested by the already mentioned lower prevalence of HIV infection among children admitted to hospital with kwashiorkor compared to marasmus. This is also suggested by the finding of a study from Uganda showing that among HIV negative children with severe malnutrition, the presence of oedema was associated with a higher CD4 count<sup>160</sup> (**Figure 4**).

Figure 4: Percentage of CD4+ cells of lymphocytes in malnourished children with and without oedema



Box and whisker plot showing the median and the interquartile range of the percentages of CD<sup>+</sup> cells in severely malnourished children who were grouped based on their HIV status and type of malnutrition

Source: Bachou et al<sup>160</sup>

These observations are difficult to explain with the hypothesis of an insufficient protein intake, or to a dysadaptation to a low protein, high carbohydrate diet. It is also not easily explained by the free radical hypothesis, at least in its initial form assuming a direct link between an increased oxidative stress and kwashiorkor.

Among HIV infected children with severe acute malnutrition, treatment with antiretroviral therapy to restore immunity has been shown to be frequently associated with development of oedema.<sup>161</sup> Whether this is due to the restoration of immunity or to a refeeding syndrome with hypophosphataemia and hypokalaemia<sup>162</sup> is unclear, however.

An inappropriate inflammatory response to an external stimulus is suggested by an elevated concentration of inflammatory mediators in children with kwashiorkor compared to marasmus. Among these mediators Interleukin-6 and soluble receptors of tumor necrosis factor alpha sTNFR-p55 and sTNFR-p75 seem elevated even in the absence of clinical infection.<sup>163</sup>

The production and excretion of leukotrienes in kwashiorkor also suggests an inappropriate inflammatory response as a contributing cause.<sup>195</sup> Particularly intriguing is the increased urinary excretion of interleukin E4, as its synthesis requires glutathione which is in short supply as it is needed to respond to the oxidative stress. Quantities of glutathione needed for interleukin E4 synthesis are small, however, compared to its concentration in plasma.

The dysregulation of the inflammatory response, and in particular the increased leukotrienes production, suggests a possible contributing role of essential fatty acids (EFA) in the pathway leading to kwashiorkor, as these inflammation mediators are produced from fatty acids of the omega-6 family (n-6). Also, the type of predominant EFA in the diet has a modulating effect on the inflammatory response.<sup>164</sup> A role for an excessive (n-6) EFA in kwashiorkor has been previously suggested, but with the hypothesis that cellular immune response, especially CD4, is decreased in kwashiorkor,<sup>165</sup> which is not consistent with available evidence suggesting that the CD4 response is actually increased. A possible role of EFA is also suggested by the observation that children with cystic fibrosis, which is associated with severe fat malabsorption, may have a kwashiorkor-like clinical aspect.<sup>166,167</sup> Of note, the early observation of Cicely Williams suggested that cod liver oil, which has a high content of eicosapentaenoic acid (n-3) had a favourable effect on kwashiorkor.<sup>2</sup> An early description of kwashiorkor in Vietnam noted that it rarely occurred in fishing communities.<sup>168</sup> And whereas in Malawi as a whole, kwashiorkor is the predominant form of severe acute malnutrition, it is not mentioned in a longitudinal study examining the growth and nutrition of children in the community of Lungwena, near the lake Malawi, with fish being part of the common foods.<sup>169</sup> At the individual level, however, the fish intake is not significantly lower in children who later develop kwashiorkor compared to other children of the same community.<sup>47,48</sup>

## Disruption of sulphated glycosaminoglycans (GAGs)

Water in the interstitial space is mainly entrapped in a gel formed by very long molecules of sulphated glycosaminoglycans (GAGs) which are long chains of polysaccharides with attached sulphate molecules. Free water represents a small proportion of interstitial tissues (usually less than 1%) and is normally contained in small non-communicating pockets. The lack of communication between these vesicles explains why water does not flow down to the lower parts of the body in healthy individuals. During oedema, there is an excess of free water and these vesicles grow, come into contact with each other and channels appear between them. Water can then flow from one vesicle to the other and accumulates in the lower parts of the body.<sup>56</sup>

An abnormal GAG structure could favour the development of these micropockets of free water and could be a cause of oedema. In the early paper presenting the free radical hypothesis, disruption of sulphated GAGs was presented as a possible consequence of oxidative stress and as a mechanism to explain occurrence of oedema in kwashiorkor.<sup>126</sup> The constitutive antioxidant function of superoxide dismutase, a key enzyme for neutralising free radicals, is dependent on binding to heparan sulphate proteoglycan

(HSPG), another form of sulphated GAG. Also, complex carbohydrates are a target for oxidative damage.<sup>170</sup>

In the kidney, epithelial cells which line the outer surface of the glomerulus have long foot-like processes (podocytes) that encircle the outer surface of the capillaries.<sup>56</sup> These podocytes have an ultrastructure rich in sulphated GAGs which were examined in a post mortem study of the kidneys of 6 children who died from kwashiorkor. The histological analysis showed an effacement of glomerular foot processes, similar to that of minimal change nephritic syndrome, suggesting a disruption of the structure of sulphated GAGs.<sup>171</sup> This abnormality can be reproduced experimentally in animals by infusion of polycationic substances neutralising the negative ionic charge of GAGs. This suggests that children with kwashiorkor could have sulphated GAGs with a decreased ionic charge, altering the physical properties of the interstitial space leading to oedema. A more recent study confirmed that there is a decreased production of sulphated GAGs, in particular of HSPG in kwashiorkor, but not in marasmus, also suggesting a possible role of GAGs disruption in kwashiorkor.<sup>170</sup> This hypothesis is also suggested by a low urinary sulphate and sulphated GAG excretion in kwashiorkor.<sup>172,78</sup> Sulphated GAG disruption could also explain a greater resistance to different infections, in particular to cholera observed in kwashiorkor: *Vibrio cholerae* binds to GAGs present in the gut and their disruption during kwashiorkor may prevent infection.<sup>4</sup>

Amadi *et al.* ascribed this disruption of sulphated GAGs to an interaction of malnutrition and enteric infection with a genetic predisposition associated with a decreased capacity for GAG synthesis. A genetic variation leading to anomalies of sulphated GAGs could also explain a lower risk of acquiring HIV prenatally or during lactation, as HIV entry across endothelial barriers is mediated by interaction with HSPG.<sup>170</sup> In this interpretation, this protection against HIV may explain the lower prevalence of infection in kwashiorkor compared to marasmic children, an observation which is not consistent with the original free radical hypothesis.

The acceptance of the hypothesis of disrupted sulphated GAGs as a cause of kwashiorkor should be based on its capacity to resist testing and to explain all manifestations of kwashiorkor. Children with congenital defects of the metabolism of heparin sulphate, a sulphated GAG, suffer from a non oedematous form of malnutrition.<sup>173,174</sup>

A possible test would be to examine the effect of stimulating sulphated GAGs synthesis either by N-acetyl glucosamine (GlcNAc) or by heparin analogs on the evolution of kwashiorkor. Examining the effect of the capacity to synthesise HPSG on the risk of kwashiorkor possibly by examining the polymorphism of genes involved in sulphated GAGs synthesis also seems a promising option.<sup>170</sup>

## Possible role of the gut microbiota

In recent years, the gut microbiota<sup>ii</sup> has been shown in experimental models to have an effect on the overall metabolism and in particular to have influence on the energy harvested from the gut.<sup>175,176</sup> While this has important implications for the control of obesity, a possible role of the gut microbiota as a contributing factor leading to malnutrition has been also suggested by studies showing a delay in its maturation in children suffering from severe acute malnutrition.<sup>177</sup>

The role of the microbiota in the development of kwashiorkor has been suggested by a major longitudinal study done in Malawi on 317 twin pairs during the first 3 years of their life.<sup>15</sup> Among these twin pairs,

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<sup>ii</sup> This term is preferable to the old term “gut flora” which implies that all microbes in the gut are bacteria whereas there are also archae, viruses and eukaryotes, which belong to different kingdoms.

half remained well-nourished, whereas in 43%, one child developed kwashiorkor, and the other one did not. Interestingly, among these discordant pairs, there was no significant difference in the incidence of kwashiorkor among the identical and the fraternal pairs of twins of a case, suggesting that genetic factors do not have a major role in the origin of kwashiorkor. Analysis of the microbiota of children who developed kwashiorkor showed they had a delayed maturation compared to the healthy twin.

To disentangle the cause from the effect of this association between abnormal microbiota and kwashiorkor, the microbiota of 3 discordant pairs of twins were inoculated to germ-free mice, some of which were fed a standard laboratory diet, while others received the typical food consumed by rural children in Malawi. Germ-free mice who received the Malawi diet and were also inoculated with the microbiota of kwashiorkor children suffered a major weight loss, suggesting that the association between a nutritionally poor diet and an abnormal microbiota was leading to malnutrition. The metabolism of germ-free mice who were inoculated showed many differences of their metabolism compared to controls, in particular in carbohydrate and sulphur amino acid metabolism, which suggests a possible link with the “dysadaptation” hypothesis and with the hypothesis of an insufficient sulphur amino acid availability.

The Malawi twin study attracted wide attention from the scientific community as it definitely offers new perspectives for the understanding of kwashiorkor.<sup>178,179,180,181</sup> A plausible scenario would be that the effect of microbiota on metabolism and translocation of intestinal bacteria or of bacterial endotoxins could trigger the development of kwashiorkor. The abnormal metabolism of sulphur amino acids of mice who received the microbiota of kwashiorkor children deserves attention, as it is also considered as a key event in other explanations of kwashiorkor, such as the free radical or the disrupted sulphated GAGs hypotheses. An abnormal microbiota associated with gut dysfunction could explain the presence of endotoxin in the blood of these children with overstimulation of the immune system and of the inflammatory response, and increased oxidative stress. Endotoxins produced by intestinal bacteria, and in particular LPS, are suspected to be involved in the pathophysiology of non alcoholic fatty liver and associated stimulation of pro-inflammation cytokines.<sup>182,183</sup> A similar mechanism could be involved as well in the steatosis of kwashiorkor. Or an effect of microbiota on sulphur amino acid metabolism leading to an insufficient availability of methionine could also explain the hepatic steatosis.

The results of the twin Malawi study, however, should be regarded as preliminary. The hypothesis that an abnormal gut microbiota may cause kwashiorkor, suggested by these early studies, needs further testing. In this first study, microbiota of kwashiorkor children was compared with that of well nourished controls, not that of children with marasmus. Hence it is not possible to say if the observed change are specific to kwashiorkor or are related to malnutrition. The mouse model used in this study is interesting, but in the absence of observed oedema in experimental mice, this should not be regarded as a kwashiorkor model. Reproducing kwashiorkor in an animal model if this hypothesis is true might be difficult as humans have a very different gut physiology compared to other mammals and even to other primates.<sup>184</sup> In particular, their gut microbiota is different, even compared to closely related primates.<sup>185</sup> Kwashiorkor, however, has been reproduced to a large extent in baboons<sup>110</sup> and this model could be explored to test the microbiota hypothesis. Oedema disease, observed in piglets at the time of weaning, and in which the role of a bacterial toxin is well established,<sup>186</sup> could also be considered as a possible experimental model which could give a clue on the pathophysiology of oedema.

Ultimately, trials having a direct effect on the gut microbiota, such as giving an antibiotic or a specific pre or probiotic, are needed to confirm a possible primary role of an abnormal gut microbiota as a cause of kwashiorkor.

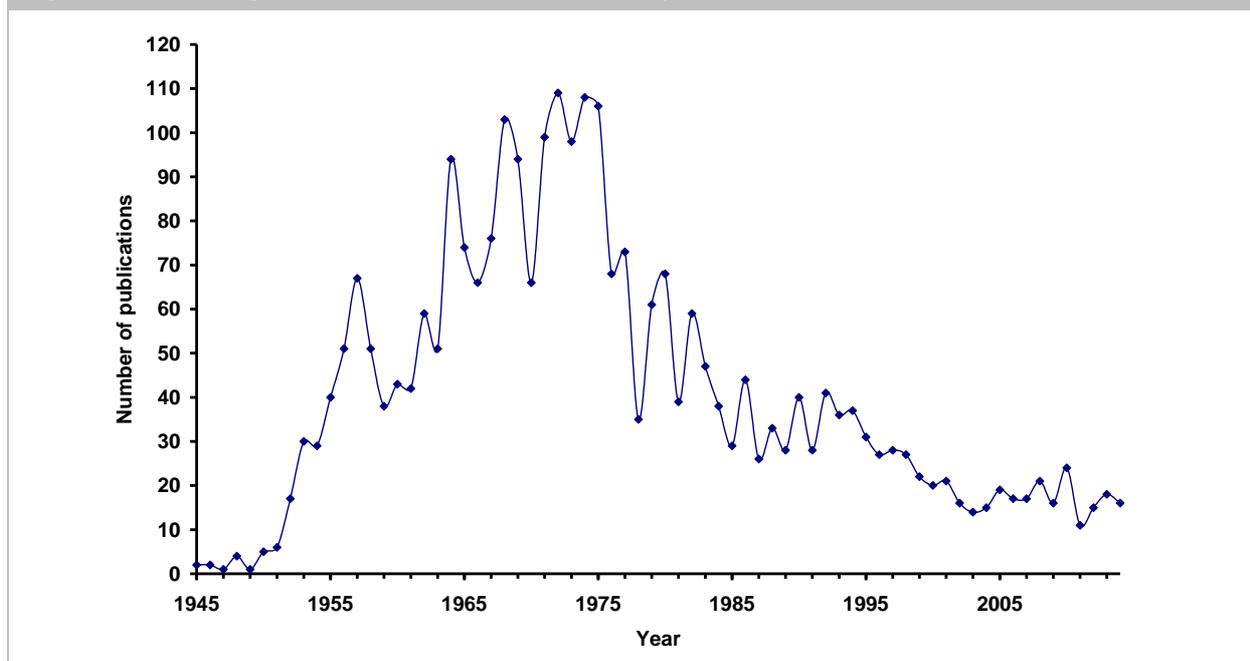
## Conclusions: kwashiorkor remains an enigma

None of the many hypotheses about the origin of kwashiorkor have succeeded in explaining correctly all of its clinical and pathophysiological manifestations. The low prevalence of kwashiorkor in HIV infected children and the possible early activation of the CD4 immune systems are especially intriguing, as they are not easily explained by any of the hypotheses proposed over the last 50 years. The hypothesis of an abnormal microbiota is attractive but this hypothesis, however, is in its early stages, and is based on very limited evidence and testing it in clinical and animal models is needed before going further in its acceptance.

## The way forward – priorities for research

Many studies quoted in this review go back to 30 or 40 years ago, sometimes even more. Kwashiorkor was the topic of high quality research and intense debate in the 1950-1970s. Surprisingly, it later became an “orphan disease”, with very few groups actively involved in disentangling its mechanism and decreasing number of publications over the years (**Figure 5**). In contrast to most other understudied diseases, kwashiorkor affects hundreds of thousands if not millions of children every year, killing many of them, and is a serious public health problem in many parts of the world. The lack of a coherent explanation to its aetiology is a major obstacle to further optimise treatment and to design effective prevention strategies.

Figure 5: Number of publications with “kwashiorkor” as keyword since 1945



(Source: PubMed)

Several areas deserve special attention. They are suggested below without ranking their level of priority beyond the scope of this review.

### Epidemiological studies

#### *Assessing the extent of the problem*

Currently, there is no reliable estimate on the number of children developing kwashiorkor each year. As mentioned in a previous section, there was no mention of the problem in the recent Lancet series on child nutrition. Importance of kwashiorkor, an acute condition, is poorly evaluated by prevalence surveys, which are often used to assess nutritional problems. These prevalence data could be used for mapping,

and identifying areas where it is a problem, but should be complemented by studies of the ratio of kwashiorkor to marasmic patients admitted for treatment.

Detection of children with oedema is currently done in community-based programmes of management of malnutrition and this can generate incidence data in different regions of the world if screening is repeated in the same area sufficiently frequently. These data should be systematically collected and compared at the international level.

### ***Getting a clue about aetiology***

Kwashiorkor is known to occur only in some areas, but currently, there is no systematic attempt to link these differences with dietary patterns or other environmental variables. Although this kind of evidence is very weak to show causality, it may give hints about possible mechanisms involved. A special case is Malawi where large variations of kwashiorkor prevalence have been reported within small geographical areas without a clear explanation.<sup>187</sup> Anecdotal reports suggest the same pattern occurs in other settings but has not been documented.

These epidemiological investigations should go beyond simple comparisons on protein or antioxidant intakes. Comparison could be made on the intake of sulphur amino acids, ideally studying separately cysteine and methionine intake, as these amino acids have different effects. The pattern of essential fatty acids intake could also be examined. Sodium and potassium intake should be compared in populations with high and low prevalence of kwashiorkor. Beyond these pure dietary aspects, the different insulin and cortisol profiles observed in Uganda and in the Gambia is intriguing, and this pattern should be examined in other settings with different forms of malnutrition. The nature of the soil could provide a clue as well. Wet soil with a lot of organic matter which represent a reducing environment can make selenium unavailable, and lead to selenium deficiency.<sup>188</sup> As selenium is needed to protect the organism against free radicals, natural variations of selenium availability could provide a clue to test the role of a deficiency of this particular trace element as a contributing cause of kwashiorkor.

Microbiome studies (studies of the collective genome of the microbiota) can be carried out in large scale human studies as they do not require blood nor tissues and could be used to explore potential differences in areas of high and low kwashiorkor prevalence.

Although the Malawi twin study, which reported a similar incidence of kwashiorkor in identical and fraternal twins of a confirmed case, is not in favour of a genetic factor,<sup>15</sup> case-control studies examining genetic variants of genes coding for enzymes involved in the defence against oxidative stress or in the synthesis of sulphated GAGs could also be considered. Properly designed and well powered genome wide association studies could give very important mechanistic insights.

### **Pathophysiological studies**

Major aspects of the pathophysiology of kwashiorkor are still poorly understood and deserve exploration with modern techniques. A few areas deserve urgent attention.

#### ***Immunology***

The possible role of an active CD4 response suggested in one study<sup>160</sup> is intriguing and needs confirmation. If confirmed, this should be further explored and factors leading to this active role of the immune system should be explored. Our knowledge of the immune system in malnutrition, and in particular in kwashiorkor, is incomplete and relies on studies done years ago with techniques which are currently often regarded as unreliable.<sup>189</sup> All possible factors including a continuous stimulation of the immune system by infections or by specific organisms proliferating in the gut possibly releasing LPS and other bacterial metabolites in the blood should be examined. Factors possibly regulating the immune

response, and in particular essential fatty acids, should be examined. The role of non-specific immunity in kwashiorkor should also be explored, as its role was largely ignored at the time most studies on the immune system in malnutrition were carried out.

### ***Pathophysiology of oedema***

The pathophysiology of oedema is still poorly understood. The role of different terms of the Starling's equation, and in particular the role of albumin and hydrostatic pressure in the interstitial fluid should be explored. The possibility of an increased hydraulic conductivity and permeability to proteins, as observed in the nephrotic syndrome, should also be examined. The possible role of endotoxins, and in particular of LPS in generating oedema has been largely ignored. Antibodies to different LPS could be looked at, and compared with those favouring protein leakage into the interstitial sector in animal studies. More generally, animal models, and ideally primate models, could be used to disentangle factors leading to oedema. The few studies using non-human primates to reproduce kwashiorkor were carried out more than 40 years ago,<sup>110</sup> and go back to a time when the understanding of kwashiorkor was fragmentary and relied mainly on the protein deficiency hypothesis. Studies exploring more complex aetiology and pathogenic pathways using the exciting advances in technology and exploring more recent hypothesis are urgently needed.

### ***Mechanisms leading to fatty liver***

The origin of this major characteristic of kwashiorkor is still poorly understood. Hepatic steatosis occurs in marasmus as well as kwashiorkor<sup>190</sup> and is associated with a poor prognosis.<sup>38</sup> Fat is very slowly mobilised from the liver as assessed by repeated biopsies<sup>191</sup> or by ultrasound assessment.<sup>190</sup> This contrasts with the successful treatment of children with high fat diets, showing that dietary fat is efficiently utilised. The reasons for these contrasting observations are not clear. The hypothesis of an insufficient export of protein synthesis seem hardly tenable<sup>70</sup> but no adequate alternative explanation has been validated. The hypothesis of an insufficient oxidation of fatty acids, proposed more than 20 years ago<sup>138</sup> has not been adequately tested so far. This could be done by examining the fatty acid composition of fatty liver. Here again, animal studies, preferably in non-human primates, checking the nature of accumulated fatty acids, would help. This question could be also examined in post mortem liver samples taken from children who died from kwashiorkor.

### ***Effect of the microbiota on overall metabolism***

Results of the pilot Malawi study suggesting that microbiota associated with kwashiorkor can have general metabolic effects on mouse metabolism with some similarities with those observed in kwashiorkor should be further explored, ideally in non-human primate models.

### **Clinical trials**

In recent years, WHO's emphasis when generating guidelines has been to use an evidence-based approach;<sup>192</sup> this has led to giving a special weight to data generated by randomised clinical trials. Although this approach has its limitations, especially in nutrition,<sup>193</sup> randomised trials have an important role to play in improving current protocols and also in understanding causal links. This approach has been underused in the context of malnutrition. Most studies describe associations which are not sufficient to have any conclusion on causality. As a result, many critical aspects of clinical management of severe acute malnutrition are still based on clinical impression and experience of experts which represents a low level of evidence.<sup>194,34</sup> Several questions should be addressed urgently.

### ***Testing the effect of different antioxidants - minimising the level of oxidative stress***

The pilot study suggesting that supplementation with reduced glutathione or with alpha-lipoic acid had an effect on mortality<sup>159</sup> has never been confirmed. There is an urgent need to test the potential of different antioxidants to improve clinical recovery of children with kwashiorkor.

The level of oxidative stress can be influenced by the nature of the fatty acids included in the diet.<sup>141</sup> Leukotrienes which are derived from unsaturated fatty acids are elevated in kwashiorkor compared to children with marasmus.<sup>195</sup> Diets with different fatty acid composition should be compared for their efficacy for the treatment of children with oedema.

### ***Optimising amino acid intake***

Clinical data suggesting that cysteine supplementation can accelerate cure from oedema<sup>82</sup> should be confirmed by large clinical trials in different settings. The use of different protein sources with a high content of sulphur amino acids, in particular cysteine (egg proteins, or specific milk fractions) should also be tested. A preliminary study suggested that a diet with egg white as a protein source might have a favourable effect on acute phase protein synthesis.<sup>196</sup> Egg white, however, has a low phosphorus content and its use was associated with lower phosphate plasma concentration and egg yolk might be a better choice as suggested by early studies.<sup>73</sup> In all these clinical trials skin lesions and other organ systems dependent upon sulphur metabolism, including sulphated GAGs,<sup>77</sup> should be included as secondary outcomes.

### ***Optimising the fat intake***

Little attention has been given to optimising the fat intake of children with kwashiorkor. The nature of lipids in the diet used for treatment is likely to have an effect on the level of oxidative stress and the production of leukotrienes and other mediators of inflammation. Medium chain fatty acids are likely to be better absorbed. When the protein intake is kept constant, increasing the fat intake will decrease the carbohydrate intake, with possibly a favourable effect on plasma phosphorus concentrations.<sup>111</sup> Different options to optimise fat intake should be tested in formal clinical trials.

### ***Treating skin lesions***

The origin of skin lesions of kwashiorkor still remains an open question. Some clue about the mechanisms involved can be obtained by clinical trials testing supplementation with sulphur amino acids or changing essential fatty intake during treatment. Skin lesions can also respond to topical application of some nutrients, especially zinc and fatty acids. This can lead to clinical trials, on a small sample of children, each child being his own control, by leaving some lesions untreated.<sup>197</sup>

### ***Modifying the gut microbiota***

One of the best ways to confirm or disprove the effect of the gut microbiota on the clinical picture of kwashiorkor would be to test the efficacy of different interventions which could affect it by using randomised clinical trials. The microbiota can be modified by antibiotics, prebiotics, and probiotics. Testing any of these options should be considered. A randomised trial in Malawi showed that treating children with kwashiorkor with antibiotics has a favourable effect on survival,<sup>20</sup> consistent with a possible role of infection in the pathogenesis of this form of malnutrition.

Despite the high quality of the work led by the few groups, many avenues of research have been left untouched as is shown by this list. This contrasts with the unprecedented development of techniques to explore metabolism over the last 20 years. One reason for low research output is the scarcity of human capacity and poor access to the innovative technologies in areas with the disease burden. The situation may be improving with increasing academic connectivity and resurgence of interest in nutrition related health internationally. It is hoped in this context that the challenge of identifying the mechanism of kwashiorkor will attract the attention of donors and encourage scientists with access to modern exploration techniques to study its mechanisms in depth.

**Questions and comments may be submitted through the CMAM Forum:**

**[cmamforum@gmail.com](mailto:cmamforum@gmail.com) [www.cmamforum.org](http://www.cmamforum.org)**

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