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Dietary Zn deficiency, the current situation and potential solutions

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Abstract

Zinc (Zn) deficiency is a worldwide problem, and this review presents an overview of the magnitude of Zn deficiency with a particular emphasis on present global challenges, current recommendations for Zn intake, and factors that affect dietary requirements. The challenges of monitoring Zn status are clarified together with the discussion of relevant Zn bioaccessibility and bioavailability issues. Modern lifestyle factors that may exacerbate Zn deficiency and new strategies of reducing its effects are presented. Biofortification, as a potentially useful strategy for improving Zn status in sensitive populations, is discussed. The review proposes potential actions that could deliver promising results both in terms of monitoring dietary and physiological Zn status as well as in alleviating dietary Zn deficiency in affected populations.

Key words: **Zinc: Zn deficiency: Dietary Zn intake: Zn status monitoring: Biofortification**

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Overview of Zn deficiency: magnitude and clinical manifestations of Zn deficiency

In humans, Zn deficiency was first identified in the early 1960s[1], initially described in an adolescent Iranian male and 3 years later in an Egyptian boy[2,3]. In 1969 and 1972, Zn deficiency was reported in young children from the United States[4,5].

The historical significance of discovering Zn as an essential element for humans and its pronounced impact on health and disease is nicely summarised by Prasad *et al.* (2013)[6]. Similarly, the circumstances leading to the discovery of human Zn insufficiency in the Middle East are presented by Sandstead *et al.* (2013)[7]. Although, it was once disregarded as a 'global health problem', Zn deficiency is now acknowledged as an acute problem worldwide[8–10].

As stated by the World Health Organization (WHO), Zn deficiency ranks as the fifth most important health risk factor in developing countries and eleventh worldwide[8,11].

The magnitude of Zn deficiency is currently estimated based on the National Stunting Prevalence Data (acknowledged in the WHO Global Database on Child Growth and Malnutrition and UNICEF's Annual Report on the State of the World's Children (SWOC))[11,12], information on the amount of Zn in national food balance supplies is derived from the Food and Agricultural Organization of the United Nations' food balance sheets[13], and finally the prevalence of Zn inadequacy is based on the evaluation of plasma zinc concentrations from national surveys. Given, all these analyses, it is estimated that Zn deficiency affects approximately 17–20 % of the global human population[10].

The populations at the highest risk of Zn deficiency are concentrated in South and South East Asia, Sub-Saharan Africa, Central America, and the Andean region of South America where the diets are mostly plant based and the intake of animal sourced foods is low (Fig. 1). Additionally, in recent years, Zn deficiency has become progressively more apparent in developed countries[14,15].

Zn deficiency generally exists due to one or more reasons: insufficient Zn intake, interference of other dietary factors with the absorption and bioavailability of dietary Zn, enlarged losses of Zn, reduced utilisation, and increased requirements for Zn under physiological conditions such as periods of rapid growth, pregnancy and lactation[16–18]. Currently used cut-offs of plasma Zn concentrations for assessing the risk of Zn deficiency are presented elsewhere[19–21].

Clinical manifestations of Zn insufficiency are non-specific, differ extensively and depend on the severity of deficiency. The clinical features of severe Zn deficiency in humans are growth retardation, skin lesions, diarrhoea, dermatitis, alopecia, pneumonia, delayed sexual and bone maturation, impaired appetite, defects in the immune system, delayed wound healing, increased vulnerability to infections and the appearance of behavioural changes[22–24]. Zn deficiency has also been shown to be associated with sepsis[23,24] and may be a risk factor for developing asthma[25]. Impaired growth, child morbidity and mortality, and preterm births are manifestations most commonly seen in populations with inadequate Zn intake in low-income countries[18,26].

The variable estimates for Zn attributable mortality oscillate from 97·3 in the Global Burden of Disease Study 2010, to

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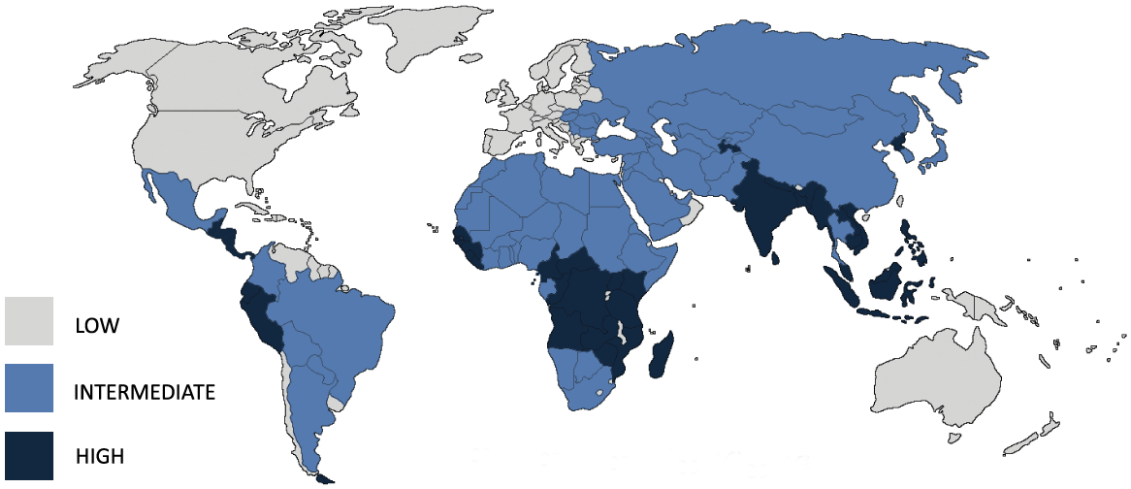


Fig. 1. Prevalence of Zn deficiency in developing countries. Based on data provided by Wessels and Brown (2012)[10] and Gupta *et al.* (2020)[88].

116·0 in the Lancet 2013 Maternal and Child Nutrition series, to 453·2 in a previous review[27,28]. An excessively high number of avertable childhood deaths is caused by Zn deficiency[28]. Poor Zn nutrition is associated with >50 % of diarrhoea deaths[29], 10 % of malaria and 7 % of pneumonia deaths[30]. The most recent Lancet Series data point out that 4–7 % of children are still simultaneously affected by both stunting and wasting, possibly leading to a four- to eight-fold increase in mortality[31]. Zn deficiency is present in close to half of all children worldwide[31]. Poor growth, subclinical inflammation and environmental intestinal dysfunction are commonly observed in children affected by Zn deficiency[31].

The consequences of marginal or mild Zn deficiency are less clear. The most vulnerable groups are prematurely born babies, infants and young children, particularly those 6–23 months of age. Mild Zn deficiency is frequently seen in healthy elderly subjects, contributing to impaired cell-mediated immune responses[32,33].

Moderate Zn deficiency can lead to hypogonadism, delayed puberty, reduced appetite, mental lethargy, hyperammonaemia, dermatitis, cell-mediated immune dysfunction, delayed wound healing and abnormal neurosensory changes[34,35].

Zn deficiency has been linked to certain health diseases in developed countries, including cancer, diabetes, depression, multiple sclerosis and coronary heart disease[36–38]. Childhood obesity, insulin resistance, metabolic syndrome and atherosclerosis are also associated with Zn inadequacy[39,40]. Numerous clinical risk factors and pathologies related to Zn deficiency have been described comprehensively by Mocchegiani *et al.* (2000)[41] and Roohani *et al.* (2013)[42].

Dietary Zn intake; food sources of Zn

Zinc is found in a wide range of foods; however, its bioavailability is variable[43,44]. Foods with a high protein content are rich in Zn, while foods and diets made of carbohydrates were found to be much lower in Zn content[45]. The richest sources of Zn include oysters (25–39 mg per 100 g), red meats (4·5–5·2 mg per 100 g), liver (4–7 mg per 100 g), and nuts and seeds (6·5–7·8 mg per 100 g). The highest content of Zn is found in animal source foods, which can range from 0·40 to 6·77 mg per 100 g[46]. Grains have 0·30–2·54 mg per 100 g, dairy products between 20·36 and 0·49 mg per 100 g, vegetables from 0·12 to 0·60 mg per 100 g, and fruits 0·02–0·26 mg per 100 g[47]. Moderate sources of Zn include cheese, whole-grain cereals and legumes. Zn in animal products is more easily absorbed when compared with Zn absorbed from plant foods.

Major food crops, that is, rice, wheat and maize, are relatively poor sources of Zn (16–25 mg/kg), yet they are basic food sources to millions of people in developing countries and, as such, the largest potential sources of Zn[47,48]. Cereal grains are rich in phytate, a compound known to bind Zn in the intestine, reducing its absorption[45,48]. The first recognised cases of Zn deficiency were linked with high-phytate-containing foods, unleavened bread from unrefined wheat flour and beans[49]. The intake of phytate around the world has been investigated to some degree, and omnivorous adult intakes of phytates range from 395 to 1293 mg/d in the United States and UK[50].

The molar ratio of phytate to Zn in the diet has been perceived as an indicator of Zn bioavailability, and ratios >15 have been related to suboptimal Zn status[48,51].

Currently, several useful resources provide data on Zn and phytate concentration in foods. The most complete source for low-income countries is accessible from the World Food System International Mini-list[13,52]. Industrialised countries mainly use the United States Department of Agriculture (USDA) database from the Nutritional Coordinating Center, University of Minnesota (USDA National Nutrient Database for Standard Reference) as well as the publications by Reddy and Sathe (2002)[50] and Wessells and Brown (2012)[10].

Dietary recommendations for Zn intake

A summary of the currently used dietary recommendations for Zn intake (mg/d) for various life stages and sex groups as set

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Table 1. Dietary recommendations for Zn intake (mg/d)

	WHO				IOM			IZiNCG			EFSA	
Age group/life stage	LBA	MBA	HBA	UL	EAR	RDA	UL	A	B	UL	EAR	UL
Infants and children												
0–6 months	6·6	2·8	1·1				4					
7–12 months	8·4	4·1	2·5	13	2·5	3	5	3	4	6	2·4	
1–3 years	8·3	4·1	2·4	23	2·5	3	7	2	2	8	3·6	3
4–6 years	9·6	4·8	2·9	23							4·6	
4–8 years					4	5	12	3	4	14		4
7–9 years	11·2	5·6	3·3	28							6·2	
9–13 years					7	8	23	5	7	26		7
Adolescents												
10–12, males	17·1	8·6	5·1	34								
12–15, males	17·1	8·6	5·1	40							8·9	
14–18, males					8·5	11	34	8	11	44		22
15–18, males	17·1	8·6	5·1	48							11·8	
10–12, females	14·4	7·2	4·3	32								
12–15, females	14·4	7·2	4·3	36							8·9	
14–18, females					7·3	9	34	7	9	39		22
15–18, females	14·4	7·2	4·3	38							9·9	
Adults												
18–60, males	14·0	7·0	4·2	45								
19–50, males					9·4	11	40	10	15	40	7·5	25
> 51, males	14·0	7·0	4·2	45	9·4	11	40	10	15	40		25
18–60, females	9·8	4·9	3·0	35								
19–50, females					6·8	8·0	40	6	7	40	6·2	25
> 51, females	9·8	4·9	3·0	35	6·8	8·0	40	6	7	40		25
Pregnancy												
14–18 years	11·0*	5·5*	3·4*		10·5	13	34	+2	+3		+1·3	
19–50 years	14·0†	70†	4·2†		9·5	11	40	+2	+3		+1·3	
	20·0‡	10·0‡	6·0‡									
Lactation												
14–18 years	19·0*	9·5*	5·8*		10·9	14	34	+1	+1		+2·4	
19–50 years	17·5†	8·8†	5·3†		10·4	12	40	+1	+1		+2·4	
	14·4‡	7·2‡	4·3‡									

WHO, World Health Organization; IOM, Institute of Medicine; IZiNCG, International Zn Nutrition Consultative Group; EFSA, European Food Safety Authority. A, IZiNCG P:Zn ≤ 18; B, IZiNCG P:Zn > 18. BA, bioavailability; LBA, low bioavailability (15 %); MBA, moderate bioavailability (30 %); HBA, high bioavailability (50 %); UL, upper limit of intake (the highest intake likely to cause no risks of adverse health effects); EAR, estimated average requirement (meets the needs of 50 % of individuals in the life stage group); RDI/RNI/RDA, Recommended Dietary Intake/Reference Nutrient Intake/Recommended Dietary Allowance (meets the needs of nearly all individuals in the life stage group).
There are insufficient data for upper level of Zn intake for children. Estimated additional average requirement for Zn needed during pregnancy and lactation (+).
Modified from WHO (1996)[11], IOM (2001)[53], IZiNCG (2004)[54], Lim *et al.* (2013)[27], King *et al.* (2016)[55] and Gibson *et al.* (2016)[26].
* First trimester.
† Second trimester.
‡ Third trimester.

by WHO, Institute of Medicine, International Zn Nutrition Consultative Group (IZiNCG) and European Food Safety Authority[11,53–55] is presented in Table 1.

Brief overview of dietary Zn intake assessment methods

Inadequate dietary Zn intake is usually the main cause of Zn deficiency. The assessment of dietary intake is accepted as the best scheme for estimating Zn exposure in individuals and populations and is the central element in evaluating the risk of Zn deficiency[55].

A comprehensive historical overview of the progress in the evaluation of dietary Zn intake as an indicator of Zn status was provided by Gibson in 2012[9].

The dietary Zn intake of individuals can be assessed by several methods (Table 2). The details of the methods presented here are described explicitly elsewhere[56–58].

It is important to highlight that the dietary Zn intake data provide only an approximation of Zn exposure or Zn insufficiency. The inadequacy cannot be identified with certainty as the actual Zn requirements of an individual are not always known. If dietary phytate can be assessed, the bioavailability of Zn can be defined.

Nevertheless, implications for adequacy of an individual's Zn intake can be made by comparing the variance among the reported intake and the estimated average requirements[59]; however, certain conditions need to be met before the method can be used[60], implying that the day-to-day variation in dietary Zn intake needs to be addressed appropriately. Nowadays, certain software programs are available for making necessary corrections. They include the Intake Modelling and Prediction Program (IMAPP) and the National Cancer Institute method[26,61].

The assessment of the dietary Zn intake of populations can be determined from the food balance sheet data provided by the Food and Agriculture Organization[62], which indicate the total amount of Zn accessible to populations within a country or region. Additional research is needed to evaluate the accurate proportion of populations affected by Zn deficiency or populations subgroups 'at risk' of Zn deficiency[10,55].

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Table 2. Assessment methods of dietary Zn intake for individuals

Method	Principle	Advantages	Disadvantages
Dietary record/food dairy	Quantities of food consumed per day are recorded and weighed	Detailed information about the food intake, provides quantitative information about food consumed	Motivation bias, high burden on participants, expensive, not suitable for people who frequently eat outside the home
24-h food intake recall	Food intake during the past 24 h; subjective measure using open-ended questionnaires	Provides detailed intake data, relatively small respondent burden, high literacy not required	Recall bias, requires skilled interviewers, depends strongly on the memory of participants, food intake may not be reported accurately, expensive, time consuming
Food frequency questionnaire (FFQ)	Questions on habitual consumption of certain foods (in the past several months or a year), data on frequency and size of food eaten	Easy and cheap to accomplish, cost effective, time saving, information of food intake over a longer period, no influence of eating behaviour, captures the intake of Zn rich foods that are consumed irregularly, useful in epidemiological studies	Closed-ended questions, recalling and measurement errors, not appropriate for clinical setting, cognitive effort required, no specific quantification of amounts, depends on memory, no correlations between consumption of foods, dietary phytate may not be included in the food composition tables, portion sizes need to be quantified cautiously
Diet history	Frequency of food intake and food preparation, estimation of usual consumption, use both open- and closed-ended questionnaires	No influence of eating behaviour, information of food consumption over a longer period of time, information on the whole diet is obtained	High cost, time consuming, consumption often misreported and cannot be precisely quantified

Based on data provided by Shim *et al.* (2014)[58] and King *et al.* (2016)[55].

Dietary Zn intake in developed and developing countries; challenges of monitoring Zn status

The adequacy of dietary Zn intake is assessed by comparing the usual dietary Zn intakes with the estimated average requirements[59]. As the assessment of Zn status by biomarkers is still challenging, evaluation of inadequacy is typically based on the assessment of dietary intakes, the prevalence of child stunting and the accessibility of Zn from the food supply[62]. The US, Canada, New Zealand and Australian government agencies have evaluated population dietary Zn intakes and documented the main dietary sources[63–66]. In Europe, the European Micronutrient Recommendations Aligned Network of Excellence (EURRECA; www.eurereca.org) is responsible for tracking the projected prevalence of inadequate Zn intakes and determining Zn requirements[67].

Results from the nutritional surveys conducted in the United States, Great Britain, Spain and Germany concluded that the recommended intake of Zn is not always achieved[68,69]. For example, 10 % of 1–3-year-old children from Poland had Zn intakes below the recommendations, and 39 % of girls aged 4–10 from Denmark had Zn intakes below the estimated average requirement (EAR)[69–71]. Similarly, the percentage of inadequate Zn intakes for adolescents in the UK was between 3 % and 48 %, while the percentage of inadequacy for adults was a bit lower, between 10 % and 21 %[65,69,72]. Inadequate Zn intake was reported in 15–25 % of apparently healthy Serbian adults[73]. Likewise, 39 % of Spanish men over 60 years of age had Zn intakes below the EAR[74], while people living in Ireland failed to meet the EAR for Zn (13 % of the elderly, 11 % of men and 29 % of women)[75]. Moreover, 83 % of the ANIBES study population did not meet the European recommendations for dietary Zn intake[76]. Comparable findings were provided for the United States, where data from the NHANES indicated that 11–17 % of people had Zn intakes below the EAR[77].

Similarly, the Canadian Community Health Survey from 2004 demonstrated that 10–35 % of Canadians consume Zn in inadequate amounts, with men over 70 years of age being most vulnerable, 41 % being Zn deficient[63]. People living in the UK also had Zn intakes below the EAR, and girls 11–18 years of age were at the highest risk of inadequate intakes[65]. The estimated prevalence of poor Zn intake among children in New Zealand ranged from 5·4 % to 9·2 % for 5–6-year-old males to 16·4 % for girls aged between 11 and 14 years[78]. An adult nutrition survey from New Zealand reported that 39 % of males and 11 % of female adults were at risk of dietary Zn deficiency[79].

In addition, 52 % of men and 9 % of women consumed below the Australia/New Zealand estimated average requirement for Zn, while 15 % of men and 7 % of women had low serum Zn levels[80]. Likewise, low serum Zn was measured in 18 % of men 50 years or older and 30 % of men 70 years or older[80]. Similarly, 19 % of premenopausal (age 18–50) Australian women were susceptible to inadequate Zn nutrition[81].

Finally, 7·8 % of people living in China and approximately 10 % of people from Central and Eastern Europe consume Zn in inadequate amounts[10]. The available micronutrient intake and status data in Europe[82], encompassing the majority of available research related to Zn intakes of people living in European

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countries, pointed out that data on Zn intakes in Europe are lacking for all life stages, while for certain countries, data on Zn intakes are either outdated or do not exist[82].

The nationally representative surveys that evaluated the adequacy of Zn intakes in low-income countries are very limited due to the high cost and logistical challenges. The global risk of Zn deficiency decreased from 22 % to 16 %, between 1992 and 2011, but it remains prevalent[83].

In 2011, 1·1 billion people were at risk of Zn deficiency[83]. Comparable findings were provided by Wessells and Brown in 2012[10], when the national food balance sheet data taken from the Food and Agriculture Organization of the United Nations were used to estimate a country- and region-specific risk of dietary Zn inadequacy in 188 countries. The global estimates on the prevalence of inadequate intakes were relatively stable over the 20 years, and there were no inter-regional variations in Zn intake among individual countries[10]. Of the world population, 17·3 % is at risk of inadequate Zn intakes, with more than 20 % inadequacy seen in people living in South and South East Asia and the Pacific (22 %), Sub-Saharan Africa (25 %) and South Asia (close to 30 %). The Zn deficiency risk for Africa in 2009 was estimated to be approximately 40 %[84]. A more recent systematic review documented that 34 % of women of reproductive age and 46–76 % of pregnant women in Kenya, Ethiopia, Nigeria and South Africa are Zn deficient[85]. Data on Zn status of certain population groups in the Australasian region are limited; however, the available evidence suggests that at-risk groups in Australasia are adolescents, toddlers (Pacific and Aboriginal ethnicities) and the elderly[86]. Pacific and M□ori children had a high prevalence of low serum Zn levels (21 % and 16 %, respectively) and were at a higher risk of developing Zn deficiency when compared with children from other ethnicities[87]. The risk of suboptimal Zn status was particularly high among younger boys in the Pacific region[87].

The prevalence data, based on the national plasma zinc concentration data, are currently available for only 25 low- and middle-income countries, of which in 23 the occurrence of Zn deficiency was above 20 % for at least one of the physiological groups examined[88,89], which indicates that there is an urgent need for additional assessment of Zn status in low- and middle-income populations.

The prevalence of inadequate Zn intakes is most common in low-income countries; however, it is also increasingly seen in developed country populations. In addition, there are no national data from a specific survey period that can show Zn intakes and their adequacy. No appropriate cross-country comparison could have been made as many countries have no regular nutritional monitoring programmes in place (this includes both developing and developed countries).

Comparison of data on Zn intake between countries is a challenging task because of the heterogeneity in methodologies and study purposes being used, various assessment methods of food intake, and no representative nutritional surveys at the national level for all countries. Additional, up-to-date and higher-quality studies are undoubtedly needed to address gaps in current knowledge.

Regular follow-ups are necessary to ensure that potential deficiencies of Zn get acknowledged and addressed on time, particularly in countries where their existence is less expected. More targeted measurements of population Zn status, including biochemical and dietary assessments, are needed for countries recognised as being at the highest risk of inadequate Zn intakes (i.e. South Asia, Sub-Saharan Africa and Central America).

Supporting evidence and additional research on dietary Zn intake globally would not only help in obtaining a more accurate estimate of Zn inadequacy but also in adequately directing nutritional interventions aimed at controlling Zn deficiency.

Zn bioavailability, inhibitors and enhancers

The bioavailability of Zn refers to the portion of dietary Zn intake that can be absorbed into the blood system and used for physiological functions within the body[42]. The main factors that define the bioavailability of Zn are the total Zn content of the diet, the individual's Zn status and the availability of soluble Zn from the diet's food components[90]. If the individual's Zn status is reduced, Zn absorption mainly depends on Zn solubility in the intestinal lumen, which is determined by the chemical form of Zn and the occurrence of certain inhibitors and enhancers of Zn absorption[42].

Long-term Zn intake (i.e. Zn status) affects the absorption of dietary Zn[91]. The long-term use of Zn supplements does not seem to down-regulate Zn absorption compared with normal, healthy subjects not taking any Zn supplements, yet low Zn intake and Zn status do affect Zn absorption[90]. As demonstrated in several studies, feeding low-Zn diets increases Zn absorption in all age groups, and homeostatic mechanisms up-regulate Zn absorption and retention[92,93]. Adults on diets with higher bioavailability are capable of regulating Zn absorption upwards or downwards to absorb 4–5 mg of Zn per day [94,95].

Concerning the time of adjustment, the variation of Zn absorption from the low-Zn diet took place within 4 weeks, and the degree of adjustment was no greater after 8 weeks[95].

In conclusion, the absorption efficacy of Zn is up-regulated as a consequence of extended low Zn intakes, and the major predictors of Zn absorption are daily Zn and phytate intakes.

Phytate, the main Zn inhibitor in plants

Phytate is the key dietary component known to limit Zn bioavailability, and it does this by strongly binding Zn in the gastrointestinal tract[96–98]. Phytate is the calcium, magnesium or potassium salt of phytic acid (myo-inositol hexakisphosphate, IP6) and is present in cereal grains, nuts, seeds and legumes[99].

In cereal grains, it is concentrated in the bran. The antinutritive effect of phytic acid is attributable to its molecular structure. At complete dissociation, the six phosphate groups of phytic acid transport twelve negative charges which, in weak acidic to neutral pH conditions, bind to various di- and trivalent cations (Ca, Mg, Fe, Zn, Cu, Mn) into a stable complex.

The phytate:Zn molar ratio of a diet is used to assess the quantity of Zn available for absorption. In general, unrefined cereal grains have very high phytate:Zn molar ratios (ranging from 22 to 88). Diets with a molar ratio >15 have poor Zn

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bioavailability and are linked to biochemical Zn insufficiency in human subjects[54,95,100]. The IZiNCG separates diets into high and low phytate:Zn molar ratios, with a cut-off of 18[54]. The inhibitory effect of phytate on Zn absorption was originally demonstrated by Lonnerdal *et al.* (1988)[101] via a radioactive isotope study in suckling rat pups and infant rhesus monkeys. The results indicated that the negative effect of phytate followed a dose-dependent response and that Zn absorption can be enhanced in humans by decreasing the phytate content of the diet. Similar findings were provided by others[102,103].

Dietary Zn intake that is needed to meet the Zn requirements of an adult doubles with every 1000 mg of phytate consumed[104]. With a phytate:Zn ratio of more than 15–20, any amount of Zn available for absorption is insufficient to up-regulate Zn absorption[95], so the goal for the phytate:Zn molar ratio should be less than 12 so that sufficient amounts of Zn are absorbed with unsupplemented diets[95,105]. Additionally, only the higher inositol phosphates (i.e. hexa- and penta-inositol phosphates) suppressed Zn absorption, whereas the lower inositol phosphates had no negative effect[101,106].

Humans have a negligible capacity to adaptively increase Zn absorption from diets high in phytic acid[95,107]. In humans, unlike in rats, hydrolysis of the higher inositol phosphates does not happen in the gastrointestinal tract because of the absence of phytase enzymes[108].

Lower inositol phosphates are formed during certain food preparation and processing procedures such as soaking, germination and fermentation that stimulate enzymatic hydrolysis of phytic acid in whole-grain cereals (i.e. wheat, rice, barley, oats) and legumes (i.e. soybean, cowpea, common beans) by increasing the activity of exogenous or endogenous phytate enzyme[100,109].

Similarly, non-enzymatic methods, such as milling, have also been shown to successfully lower the amount of phytic acid in plants as the aleurone layer of cereal grains, rich in phytates, is usually removed during the process[110].

Other dietary factors known to affect Zn bioavailability

In addition to phytate, some other dietary ingredients can also reduce Zn absorption (i.e. polyphenols, fibre, oxalate, tannin and lignin)[98,111]. Polyphenols, a class of antioxidant mainly found in berries, herbs, nuts, flaxseeds, vegetables, coffee and tea, reduce Zn bioavailability by forming complexes between the hydroxyl groups of the phenolic compounds and Zn[98]. Generally, the bioavailability is inversely related to the condensed polyphenol content[112].

Calcium (Ca) is a dietary ingredient for which there is no definite evidence regarding the effect it has on Zn absorption and Zn bioavailability. Some believe that Ca inhibits Zn absorption and that it additionally enhances the inhibition of Zn absorption by phytate[87], while others have shown no effect on Zn retention or balance. In animals, a high level of dietary Ca, present in dairy products, soy, beans, lentils and nuts, was shown to impair Zn absorption[113,114]; however, it is unclear if this also happens in humans, with inconsistent results being reported[98]. Increased Ca intake of post-menopausal women by 890 mg/d in the form of milk or Ca phosphate (total Ca intake; 1360 mg/d) diminished Zn absorption and Zn balance in post-menopausal women[115].

However, increasing the Ca intake in adolescent girls by 1000 mg/d (total Ca intake, 1667 mg/d) did not disturb Zn absorption or balance[116]. Similarly, a study with ten healthy women (21–47 years old) demonstrated that a high intake of dietary Ca (~1800 mg/d) did not further impair Zn absorption from a high-phytate diet[117]. The effect of Ca is not significant, and an enhancing effect is only observed in the diets with low phytate levels[116]. On the other hand, Sandström *et al.* (1989)[51] showed that the presence of Ca enhanced Zn absorption by the addition of dairy products to a high-phytate bread meal, but no change in absorption was evident in a low-phytate white bread meal[51].

The positive effect of Ca on Zn absorption was also shown when a soy formula was consumed with the addition of Ca[118]. The authors explained this effect by hypothesising that Ca is forming complexes with phytate in the gut, thus making phytate unavailable to bind Zn.

Miller *et al.* (2013)[119] based their mathematical model of Zn absorption on this hypothesis, stating that Ca has a positive effect on Zn absorption.

The inconsistent findings of the Ca studies on Zn absorption clearly show that complex nutrient interactions exist (beyond that with phytate) and that further research is needed to clarify the effect of Ca on Zn availability and absorption.

The effect of dietary protein on Zn absorption is also ambiguous. Dietary protein and protein digestion products (i.e. casein phosphopeptides) have been shown to increase Zn absorption[90,106], inhibit absorption[120] and have no effect[120,121]. The protein source, from animal or plant, can also have an effect[90]. Likewise with Ca, inconsistencies in research outcomes were explained through nutrient interactions where the phytate content has a central role.

Besides being affected by dietary components, Zn bioaccessibility and bioavailability depend on physiological luminal and basolateral factors[122]. The gastrointestinal mucus layer enhances luminal accessibility, leading to improved Zn bioavailability[101]. An important serosal factor is serum albumin, which operates as a basolateral Zn acceptor[20,123,124]. Finally, systematic factors also play a role. Hepcidin, a humoral factor, was shown to affect the export of Zn by intestinal cells, demonstrating the role of the liver in regulating intestinal Zn absorption[125]. These are all important aspects that require further investigation to enhance the current knowledge on intestinal Zn bioavailability and absorption of dietary Zn.

Methods for evaluating the intake of bioavailable Zn

There are currently three algorithms that can be employed for assessing the intake of bioavailable Zn. The initial algorithm, produced by Murphy *et al.* (1992)[126], was created on the semi-quantitative classification system of WHO for diets in low-income countries with a low content of animal protein, a moderate to low content of Ca, and a moderate to high content of phytate.

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The second model was developed in 2004 by the IZiNCG group and used a regression to calculate bioavailable dietary Zn[54]. Zn and the phytate:Zn molar ratios were involved in the final model, and both were very important predictors of the percentage of Zn absorption[19,127].

Hambidge *et al.* (2011)[105] calculated the effect of different levels of phytate on Zn absorption and intestinal excretion of endogenous Zn by using staple isotope studies[104,128] and developed a new physiologically based mathematical model of Zn absorption based on the amounts of dietary Zn and phytate. The new tri-variate model is the most commonly used nowadays and accounts for >80 % of the discrepancy in the amount of Zn absorbed[55,119].

Biofortification: a useful strategy for improving Zn status in sensitive populations

Since there is no efficient reserve or body store for Zn, appropriate consumption of dietary Zn is required on a regular basis[127]. Staple diets in low-income countries are primarily plant-based, and the consumption of animal products that contain a higher levels of Zn, for instance, red meat, fish and poultry, is often low for various reasons, including economic, cultural or religious restrictions[129]. Consequently, the amount of bioavailable Zn from such diets is low and often the main source of Zn insufficiency[87]. Severe Zn deficiency is seen in people whose diets are mainly based on cereal grains produced on Zn-deficient soils, for instance in India, Pakistan, China, Iran and Turkey[19,130].

This relationship of poverty with micronutrient malnutrition leads to the reality that it is not simple to accomplish adequate intake of Zn by dietary modifications (e.g., more fish, poultry and meat consumption), which would in theory provide an answer to undernutrition[131]. Furthermore, changes in dietary habits require primarily the availability of alternative foods at affordable prices, as well as individual and social acceptance. Alternatives to this approach are supplementation by an oral provision, such as Zn salts in the form of tablets, or the fortification of staple food such as flours through the addition of Zn[131]. Supplementation with pharmaceutical Zn preparations can be efficient in the alleviation of Zn deficiency on an individual basis; nevertheless, this strategy is often shown as unsuccessful at a population level in developing countries, due to the absence of suitable infrastructure and education[131–134]. Similarly, food fortification can be implemented promptly at a national level without personal contact and change of delivery habits by consumers, but its successful application into society involves the existence of safe delivery systems, steady policies, suitable social infrastructures and constant financial support[100]. All these strategies have limited success in developing countries as they were often difficult to sustain or were too expensive[135,136].

Taking all these aspects into account, biofortification, that is, the development of crop plants with greater levels of bioavailable Zn, is seen as the most manageable method for developing countries as it does not involve changes in customary diets and can reach rural families with very restricted access to infrastructure[21,131,137]. Besides, it may provide a significant increase in plant growth and the extra benefit of considerable yield increases on Zn-deprived soils[131,134,138].

Increased bioavailability of Zn in plant foods can be accomplished by plant breeding (conventional and agronomic) or genetic engineering approaches that either increase the concentration of Zn, reduce the content of inhibitors (primarily phytate) or increase the expression of compounds that augment Zn absorption (i.e. amino acids)[139–141]. Zn biofortification through Zn fertiliser application is also encouraged to increase grain Zn concentration[132,134,142] and Zn bioavailability[143]. This fertiliser route is identified as agronomic biofortification, and this is achieved by the application of minerals to the soil or by foliar application of fertilisers directly to the leaves of the plants. Foliar Zn application was shown to be effective in improving both Zn concentration and bioavailability of Zn in grains without changing the phytic acid concentrations[136,142,144].

Biofortification through Zn fertiliser application has been shown as a very effective method for improving the Zn content of major crop plants, while modern biotechnology tools are more and more often used for the development of Zn biofortified crops[142,145–150]. For example, through molecular breeding and genetic engineering, 'high zinc rice' varieties have been produced and are used to fight against hidden hunger[151].

The HarvestPlus Fertilizer and HarvestZinc project (www.harvestzink.org) established that foliar application of Zn fertilisers to crops can increase grain Zn concentration by 28–68 %[131,152,153]. Target Zn concentrations set by the HarvestPlus programme are 38 ppm of Zn in wheat[135]. The success of agronomic biofortification has been already reported for many crop varieties[138,154,155].

The agronomic biofortification strategy of breeding nutrient-rich staple food crops is anticipated to be of special benefit to poor rural populations affected by dietary Zn deficiency[156–159]. Several review papers highlighted the noteworthy increase in daily dietary Zn intake in people consuming Zn-biofortified crops, demonstrating the positive prospect of Zn biofortification interventions in lowering the risk of Zn deficiency in developing countries[160–164].

Economic analyses demonstrate that biofortification is the most concrete, cost-effective and durable strategy for increasing dietary Zn intake of vulnerable populations[127,137,165]. Certain economic analyses propose that genetic approaches towards biofortification are more cost-effective than dietary diversification, supplementation or food fortification programmes[131,166,167].

The likely influence of Zn biofortification has been calculated as the saving of disability-adjusted life years[21]. The annual burden of Zn deficiency in India was shown to be 2·8 million lost disability-adjusted life years, and it was predicted that Zn biofortification of rice and wheat could cut this burden by 20–51 %[21].

In conclusion, the improvement of Zn concentration in crop plants via conventional breeding and genetic engineering procedures represents the core biofortification strategy. Zn biofortification of grains by genetic and agronomic approaches is commonly suggested to resolve the Zn deficiency problem in people dependent on cereals as a main food

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source[131,135]. Relevant actions have been organised in the past 10 years, many of them initiated by the HarvestPlus programme.

The biofortification strategies, application of Zn fertilisers and modern biotechnologies have the potential to produce Zn-enriched crop varieties. Advanced technologies should be continuously employed to enhance genotypes used in biofortification programmes, to develop high-Zn biofortified crops and to further develop techniques for faster breeding, dissemination and implementation of Zn-enhanced cultivars. Additional research is certainly needed to assess various variables that will determine the ultimate success of biofortification of staple crops with Zn.

The effectiveness of Zn biofortification strategies, animal and human studies

As mentioned previously, methods to increase dietary diversification, mineral supplementation and food fortification have not always been effective in alleviating the problem of Zn deficiency in developing countries.

The biofortification of crops by either plant breeding or by using mineral fertilisers has been suggested as a potential strategy that could solve the Zn deficiency problem[131,168].

Biofortification strategies must focus on the staple food that dominates people diets, that is, wheat and rice, which are the staple food for almost half of the world's population[134,154].

In developing countries, a minimum of 60 % of Zn in human diets is derived from grain and legumes[169]. Wheat is one of the three major cereal crops worldwide[135,170] and is a major source of calorie and mineral intake in many developing countries[140,141]. Global wheat production goes beyond 720 million tonnes per year, most of it being used as food for humans[13,170]. The concentration of Zn in wheat plants is low (20–35 mg/kg of whole grain), due mainly to the low content of Zn in the soils where wheat is grown[132,142]. More than 40 % of wheat plants grow on low-Zn soils[130]. In addition, a significant amount of Zn in grain is lost during wheat processing (i.e. removal of aleurone layer and embryo during milling)[171].

The concentration of Zn in refined wheat flour is less than 15 mg/kg[172]. However, the percentage of Zn retained in flour after milling is anticipated to be 60 %[173]. Furthermore, besides being naturally low in Zn, wheat is rich in phytic acid, which is known to limit Zn bioavailability to a great extent[132,174]. The Zn concentration in wheat grain needed to prevent Zn deficiency in humans is estimated to be 45 mg/kg[167,174]. Daily net absorption of approximately 3 mg of Zn from 300 mg of wheat flour is necessary for human health[53,157].

Theoretical studies indicate that the agronomic biofortification strategy of staple crops would increase the delivery of Zn to human diets and improve the nutritional status of susceptible populations in developing countries[131]. The HarvestPlus group has recommended a target for added Zn in biofortified crops of 30 % of the estimated average dietary requirements for humans[19,154]. Wheat and rice varieties with enhanced Zn concentration have been produced, but before they can be introduced into the food supply, their efficiency in improving the Zn status of consumers requires confirmation.

Over the years, both *in vitro* and *in vivo* methods (animal and human studies) have been used to assess the efficacy of biofortified crops in improving the nutritional status of consumers. *In vitro* studies (mainly using Caco-2 cells) are suitable for preliminary screening of biofortified crops, but these studies do not provide data that are necessarily applicable to humans. Similarly, a protein that can be used as an indicator of Zn uptake and is specific for Zn only is still missing[175], making this approach unsuitable for merely assessing Zn absorption.

Human studies certainly provide the most valuable results, as they are capable of investigating host factors and physiological changes during digestion. However, they are time-consuming and very expensive[176].

An alternative approach to *in vitro* and human studies are animal models, which can provide a whole-body assessment of absorption as dissection of individual tissue parts is possible[176,177]. The faster output, the ability to assess an extensive range of physiological and molecular parameters thoroughly, and the cost-effectiveness are features that make the use of animal models appealing for testing dietary Zn bioavailability of staple crops. In recent years, the chicken (*Gallus gallus*) model has often been used for evaluating the effectiveness of biofortified crops[178–181].

The *Gallus gallus* model has been shown to be appropriate for Zn-related studies[182–184]. Lately, a good correlation between the results acquired through this animal model and via human efficacy trials was established, additionally confirming the suitability of the model in investigating mineral bioavailability[180].

The usefulness of biofortified wheat products to improve the Zn nutritional status of subjects has been tested. Welch *et al.* (2005)[156] were the first to show the favourable effect of Zn-biofortified wheat on the Zn status of rats. The wheat genotypes with increased grain Zn concentrations provided increased amounts of bioavailable Zn, thereby supporting the hypothesis that breeding for Zn-enhanced wheat grain may contribute to decreasing Zn deficiency in target human populations[156].

In a 2009 human trial, Zn absorption from biofortified versus conventional wheat (as 95 % and 80 % extraction flours) was compared[157]. Adult women were given 300 g of the high- or low-extraction flours (made into tortillas) for two consecutive days using either biofortified (41 mg Zn/g) or control (24 mg Zn/g) wheat. Zn intake from the biofortified wheat meals was 5·7 mg/d (72 %) higher at 95 % extraction ($P < 0·001$) and 2·7 mg/d (68 %) higher at 80 % extraction when compared with the corresponding control wheat ($P = 0·007$). The absorption of Zn from the Zn-biofortified wheat remained significantly higher than that of the control wheat.

Mean total Zn absorption from biofortified wheat was 2·1 ± 0·7 mg/d and 2·0 ± 0·4 mg/d for 95 % and 80 % extraction, respectively, 0·5 mg/d higher than for the control wheat ($P < 0·05$)[157]. The higher absorption was maintained with moderate extraction of the grain, even though substantial quantities of Zn were lost with extraction (80 %), which indicates that benefits of Zn-biofortified wheat are not lost with a moderate

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degree of milling. Zn absorption is greater from biofortified wheat than from typical wheat with lower Zn concentration, from the same quantities of each type of wheat flour consumed[157].

Carlson *et al.* (2012)[185] investigated the bioavailability of three wheat varieties using a pig as a model by collecting urine and faeces samples for 7 d.

The soluble Zn concentration of the three wheat samples was 9·9, 12·8 and 21·7 mg/kg. The Zn excretion in the urine of pigs was very low (below 1 mg/d) for all treatments.

The daily Zn intake fluctuated between all dietary groups ($P < 0·001$), with the highest intake in pigs fed the high-Zn diet (57·5 mg/d) and the lowest intake in pigs fed the low-Zn diet (7·3 mg/d). Accordingly, the net Zn absorption in milligrams per day differed ($P < 0·001$) among the dietary groups[185].

Furthermore, the efficacy of Zn-biofortified wheat in improving the Zn status of consumers was examined by using an *in vivo* (*Gallus gallus*) model of Zn absorption[186]. Two groups of birds ($n = 15$) were fed two different diets, a 'high-Zn' diet (46·5 ppm Zn) and a 'low-Zn' diet (32·8 ppm Zn), for 6 weeks. Dietary Zn intake, serum Zn, body weight and the erythrocyte fatty acid profile were evaluated. Concentrations of serum Zn were lower in the low-Zn group ($P < 0·05$). Correspondingly, the concentration of Zn in tissues (feather and nail) was lower in the low-Zn group of birds as opposed to the birds fed a high-Zn diet ($P < 0·05$). Duodenal mRNA expression of several Zn transporters (i.e. Zip4, Zip6, Zip9, ZnT1, ZnT5 and ZnT7) confirmed a lower mean value in the tissues collected from the birds fed a high-Zn diet ($n = 15$, $P < 0·05$). The higher amount of Zn in the biofortified wheat leads to a greater Zn uptake[186]. The wheat genotypes with enhanced grain Zn concentrations had increased amounts of bioavailable Zn, supporting the idea that breeding for Zn-enriched wheat grain may lead to reducing the Zn deficiency problem in target populations. This study demonstrates that the additional Zn present in the biofortified wheat is freely available for absorption; the higher amount of Zn in biofortified wheat contributed to a greater uptake of Zn by the intestinal enterocytes.

The efficacy of biofortification strategies of major staples in improving human health was lately confirmed by Sazawal *et al.* (2018)[187], Haas *et al.* (2016)[188] and Mehta *et al.* (2018)[189].

Recently, a systematic review (meta-analysis) examined the effect of the dose and duration of Zn interventions on the risk of developing type 2 diabetes and cardiovascular diseases, signifying that low-dose, long-duration Zn interventions (i.e. possible Zn biofortification strategies) can be very beneficial in reducing the risk of developing these diseases[190].

The measurement of Zn absorption from biofortified crop varieties is a crucial first step in demonstrating the efficacy of these products in improving Zn status of consumers. The available evidence shows that new biofortified varieties of staple crops may be useful in improving the Zn status of individuals. Nevertheless, it is still required to precisely determine the bioavailability of added Zn in plants and to explore if the additional Zn is at least equally absorbable as the native Zn content, and if Zn-enriched grain varieties can be used to effectively improve the Zn status of Zn-deficient people in developing countries dependent on cereals as a basic food source. It would be beneficial to investigate the potential beneficial role of Zn biofortification strategies in the prevention of certain non-communicable diseases.

Finally, an appropriate assessment of Zn bioavailability and absorption from Zn-biofortified crops is fundamental in estimating the efficacy of related breeding programmes.

Monitoring physiological Zn status in sensitive populations and dietary approaches for alleviating dietary Zn deficiency – prospective activities towards promising results

Information on Zn intake and status data for several countries is limited or outdated, or does not exist at all[80,82,83]. Similarly, the Zn intake and Zn status in healthy population subjects has not been regularly monitored. Monitoring of the prevalence and severity of Zn deficiency and development of Zn intervention strategies is not performed regularly besides the well-known consequences of Zn deficiency. Therefore, more current measurements of Zn intake and status data for a number of both developed and developing countries are needed. Regular monitoring of mineral intake and status for various population cohorts is necessary to make sure that deficiency of Zn is recognised and addressed promptly.

In many developed countries, micronutrient deficiencies are not linked to the quantity of food consumed, but rather to the quality of the diet[191,192]. Inadequate dietary intakes of Zn that failed to meet the high physiological demands were seen in different countries for various age groups (i.e. adolescent girls in Australia and New Zealand; non-pregnant premenopausal women from Seattle, Washington, United States)[56,127,191,193]. Dietary decisions made by individuals may lead to Zn deficiencies. Similarly, lifestyle changes taking place over the last few decades in many developed countries are characterised by increased dietary intake of low-cost but energy-dense foods and by reduced physical activity levels[59,194,195]. Finally, with the current global trend of people in developed countries eating less meat and increasing intake of grains[196,197], there is a tendency that more individuals may end up consuming inadequate intakes of many important nutrients. Therefore, regular monitoring of Zn intake and status data for various age groups is of crucial importance.

Lack of a reliable biomarker of Zn status is another major factor contributing to the paucity of international-level data on Zn deficiency. Plasma/serum Zn status and assessment of dietary Zn intake are currently the most employed methods, each with its well-acknowledged limitations[55,129]. New biomarkers have been suggested over the last few years, some of them being tested to a certain degree; however, additional work is necessary before any of these biomarkers can be employed as an accompanying biomarker of Zn status.

Several studies have demonstrated that the ratio of blood fatty acids (linoleic acid:dihomo-γ-linolenic acid ratio) could be a

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more appropriate biomarker of Zn status able to respond to dietary Zn manipulations and to differentiate between the various Zn deficiency/adequacy states[178,198].

The proposed biomarker has also been evaluated in humans[186,198–200]. Nevertheless, further work is needed to fully determine the efficacy of this newly proposed indicator.

Overweight and obesity are worldwide health problems present in both developed and developing countries[201,202]. The prevalence of obesity has tripled since 1975[202]. Currently, 650 million people are estimated to be overweight or obese worldwide[195]. However, even the excess dietary intake of energy and macronutrients does not ensure that people are taking the recommended intakes of micronutrients. Several national epidemiological surveys performed in some developed countries described the concurrence of obesity with inadequate intakes for certain vitamins and minerals, particularly Ca, Fe, Zn, and vitamins B_1, B_2, B_6, D and folate[203–205]. Lower Zn status was documented in overweight/obese individuals, in comparison with those who were normal weight[206–208]. Moreover, the double burden of malnutrition, that is, the coexistence of both over- and undernutrition, is nowadays seen not only in developed but also in middle- and low-income countries[190].

In addition to inappropriate intakes, impaired bioavailability and utilisation of micronutrients is frequently a major factor contributing to the inadequate micronutrient status in obesity. Zn is particularly important in this context as the low-level inflammation that accompanies obesity is contributing to decreased Zn absorption, which further on can result in impaired immune competence[46]. Furthermore, the links between Zn and the pathophysiology of non-communicable diseases have been noted over the years[190,209,210]. Zn affects insulin homeostasis and inflammatory response and plays a role in lipid metabolism[209,210]. All these factors should be taken into account when the adequacy of Zn intake in a population is evaluated.

Furthermore, other factors influence the daily intake of Zn: the amount of food consumed, Zn concentration in food, and consumption of dietary ingredients that delay Zn absorption, that is, polyphenols and phytate[90,100,105]. Food composition databases need to be updated, as many of them do not contain information on the content of phytate.

There is a need to update dietary Zn recommendations based on the recent advances in the determination of trace elements requirements. Bioavailability, dietary practices and mineral interactions should all be taken into account when setting and updating dietary recommendations. Inflammatory confounders should be considered when assessing and reporting the number of people affected by dietary Zn deficiency to ensure data accuracy and to support policy-making decisions. Adjustment methodologies for inflammation should be improved. The causal relationship between Zn status and inflammation and the potential of Zn to modulate and improve the immune system requires additional investigation.

Additional up-to-date and higher-quality studies are needed to address gaps in current knowledge. Regular follow-ups are necessary to ensure that potential deficiencies of Zn are acknowledged and addressed in a timely manner, predominantly in countries where their existence is less expected.

The introduction of Zn-biofortified crops is suggested as an approach for addressing nutritional Zn deficiency in people dependent on cereals as a basic food source[131,138]. As such, a comprehensive understanding of its effectiveness in improving the Zn status of consumers is needed. Additionally, an appropriate assessment of Zn bioavailability and absorption from Zn-biofortified crops is essential in estimating the efficacy of related breeding programmes and the health impacts of biofortified products. The effect of the consumption of Zn-biofortified crops on the risk of developing non-communicable diseases should also be examined.

The nutritional benefits of Zn-biofortified crops are known; however, people do not eat flour raw, and processing and baking are common practices worldwide. There is increasing evidence that food processing, including milling, fermentation and heat treatment during baking, can change the nutritional content and nutraceutical properties of cereal-based products[153,211–214]. The effect of bread-making processes on the concentration and availability of Zn and phytate in biofortified bread made of different wheat varieties should be evaluated and compared as variable responses with different wheat cultivars may be observed. The same applies to other Zn-biofortified crops.

Knowledge of the favourable effects of food processing techniques on Zn bioavailability from plant-based diets could be an effective food-based strategy to maximise their Zn content. Currently available evidence suggests that the levels of Zn do not change, while the concentration of phytate declines during fermentation and baking procedures[153,213,215], which in theory would mean that the bioavailability and absorption of Zn from baked products should be additionally augmented due to the lower phytate:Zn ratio. Zn absorption is considerably increased when the phytate:Zn molar ratio is below 15[100]. Therefore, the magnitude of this increase and its consequences on the availability of Zn and its absorption from processed Zn-biofortified products of various crop varieties need to be explored further.

In addition, it is still not entirely known how the rheological properties of flour are affected by Zn biofortification procedures. The effect of foliar agronomic biofortification of plants with Zn on the concentration of health-promoting compounds (i.e. phytate and polyphenols) and other minerals (i.e. Fe, Cu) has undergone limited testing and shown contrasting results. The estimated bioavailability of Fe, Mn and Cu was unaffected by Zn biofortification[174], and antagonism between Zn biofortification and content of other important minerals in wheat grains has been shown[216], so further research in this area is required to clarify these discrepancies.

The efficacy of Zn-biofortified wheat (and other staple crops) products in improving the Zn status of consumers should be tested for various biofortified wheat varieties available across the globe as each wheat variety may not necessarily produce an identical effect. Moreover, great variability in the number of traits and properties between the wheat/grain varieties has been observed[153,216–218].

Subsequently, different wheat cultivars need to be examined to reach biofortification targets aimed at enhancement of Zn content and its maximal possible bioavailability in grains.

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A large number of samples may be tested simultaneously using the high-throughput Caco-2 cell model. Duodenal enterocytes modulate their nutrient absorption in response to dietary intake, so the model can predict availability, uptake of Zn into the enterocytes, and sometimes even the absorption[219]. The cell model is less expensive, is quicker and allows several crop varieties to be compared simultaneously[220,221]. In addition, a well-developed cell model could diminish the need for isotopic labelling of the foods to measure Zn uptake[220,221].

However, there is mixed evidence of the efficacy of cell monolayers in predicting Zn absorption from food. When the formation of metallothionein, a cytoplasmatic protein that stores Zn, was tested as a proxy for Zn absorption, it was shown that the measurements of the cellular Zn and metallothionein concentrations are less reliable as their expression is often affected not only by Zn but also by other dietary components (i.e. phytate, casein, other metals)[220–222]. On the other hand, Caco-2 cells were successfully used for assessing Zn uptake into the cells[175,220,223].

The Caco-2 cell technique provided results comparable to those of certain *in vivo* methods and is suggested as a technique for preliminary screening of a large variety of Zn-biofortified cereal lines[176,220]. Nevertheless, a protein that can be used as an indicator of Zn uptake and is specific for Zn only is still missing[175,221], and the conditions for the *in vitro* digestion should be measured and selected cautiously, making this approach imperfect, so further improvement and optimisation of Caco-2 cells as a model for estimating Zn absorption from food is recommended.

Zn-biofortified crop varieties are developed primarily for resource-poor populations dependent on plants as a basic food source. They are meant to reach malnourished rural populations with restricted access to supplements and commercially fortified foods[224]. While these populations would certainly benefit the most from the consumption of Zn-biofortified plants, the use of the newly developed product could also be helpful to people in industrialised countries. Zn biofortification could be a suitable solution to the problem of inadequate Zn intakes in developed country populations where wheat bread is a staple. Short-term intervention with foliar application of Zn fertilisers and/or a long-term breeding programme to augment the Zn concentration of major bread wheat/maize cultivars should be considered to alleviate the problem of Zn inadequacy in these settings.

Recently, the gut microbial environment has been recognised as an important organ in the absorption and utilisation of Zn from the diet[165,209,210]. The gut microbial environment is fundamental to Zn homeostasis, and it is undesirably affected by suboptimal Zn status[225–227].

As the intake of Zn-biofortified staple food crops is anticipated to grow considerably due to the increasing implementation of population-wide biofortification strategies[131,181,228,229], characterisation of potential modifications in the gut microbiota following consumption of Zn biofortified cereal-based diets needs to be investigated.

Supplemental Zn alters the composition of the gut microbiome[227]. Zn deficiency deleteriously changes the composition of the gut microbiota through global reductions in taxonomic richness and diversity, a decrease in beneficial short-chain fatty acids, and changes in expression of bacterial micronutrient pathways[227].

Additionally, the protective effects of therapeutic Zn supplementation, including modifying intestinal permeability (via proliferation of the absorptive mucosa), influencing the immune response, reducing villous apoptosis, and reducing pathogenic infections and subsequent diarrheal episodes, are documented[181,227,230]. Further studies should evaluate how the consumption of Zn-biofortified plant-based diets modify the gut microbiota and whether a Zn-biofortified diet influences the metabolic and functional capacity of the host microbiome. The utilisation of some recently developed simulators of the human gut microbiome should be considered. Artificial gut models could be used to study dynamics of human-associated microbiota to dietary intake of Zn-biofortified foods[231].

Finally, with the current intention of stimulating the consumption of Zn-biofortified diets worldwide, exploring the role of gut microbiota in Zn biofortification remains important to further improve the nutritional outcomes provided by biofortification. The efficacy of biofortified staple food crops in improving the Zn status of humans should be measured directly. Thus, human studies are desirable to confirm the positive impact of Zn-biofortified plants on Zn status and support the further release of Zn-biofortified crops.

To conclude, the biofortification strategies based on plant breeding and application of Zn fertilisers have the potential to alleviate Zn malnutrition, both in developing and industrialised countries.

Additional research is certainly needed to assess various variables (i.e. health impacts of biofortified products, product acceptance by farmers, by consumers and decision-makers, recognition of the product by regulatory agencies, integration into development policies and private sector entities) that will determine the ultimate success of biofortification of relevant crops with Zn.

Conclusions

Zn deficiency remains an important malnutrition public health problem worldwide with numerous negative impacts on human health, lifespan and productivity. The number of people affected by dietary Zn deficiency has increased over recent years both in developed and developing countries. Regular updates with suitable assessments of the prevalence of dietary Zn deficiency at an international level are needed. A more accurate biomarker of Zn status is still missing, so further work towards identifying one is required. Biofortification is a strategy that can improve human Zn nutrition on a global scale. Agronomic Zn biofortification of staple plant foods is regarded as a highly effective strategy for increasing dietary Zn intake. Zn-biofortified crops are a feasible tool for addressing nutritional deficiencies, and understanding their efficiency in improving the Zn status of consumers is fundamental and needs to be appropriately verified. The food preparation and food processing techniques with a tendency to improve Zn bioaccessibility and bioavailability from plant-based

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diets should be effectively evaluated and promoted. Challenges associated with undernutrition, overnutrition and dietary practices are important considerations when setting dietary Zn recommendations and trying to reduce the risk of diet-related diseases of multiple aetiologies.

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M.K.: performed literature review, collected data, analysed and summarised results, wrote the manuscript. J.S.: supervised the project. All authors approved the final version of the manuscript.

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