Chapter 30
The Impact of Micronutrients on Inflammation and Health in Low- and Middle-Income Countries

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Key Points

- Deficiencies of micronutrients (vitamins, minerals and trace elements) are common—up to a third of people in low- and middle-income countries are affected.
- Women and children, especially those living in poverty, are those most at risk often because of micronutrient-, protein- and energy-poor diets, increased metabolic demands of growth, pregnancy and lactation and repeated infections.
- A vicious cycle of undernutrition leads to reduced immunity that increases disease risk and then the disease itself causes further undernutrition and so on.
- Increasing challenges, in all countries, are overweight/obesity and non-communicable diseases—which have implications in management because they often are low-grade inflammatory diseases and frequently there are low micronutrient intakes of those affected.
- Immune systems are impacted by micronutrient deficiencies:
- Vitamin A deficiency impairs innate, cell-mediated and humoral antibody responses but probably not viral infection.
- Zinc deficiency affects both innate and cell-mediated immunity but effects of supplementation on antibody production in human less clear than in animals.
- Iron deficiency, and overload, impair both innate and cell-mediated immunity, with no effect on humoral antibody production.

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- Vitamin D deficiency impairs the regulation of innate immunity and other antimicrobial mechanisms and may be associated with cardiovascular disease risk.
- Vitamins B6, B12, folate and E deficiencies impair Th1 cytokine-mediated immune response through insufficient production of pro-inflammatory cytokines, shifting to an anti-inflammatory Th2 cell-mediated immune response thus increasing the risk of extracellular infections.
- Vitamin C deficiency in humans impairs leukocyte functions and decreases overall NK cell activity and lymphocyte proliferation.
- New avenues of immunomodulatory effect continue to be identified for these and other micronutrients.
- Supplementation with micronutrients generally reverses these impaired immune responses
- Micronutrient deficiencies can also be addressed by dietary improvement (if available and accessible), and by fortification.
- It is important to address other interventions such as control of infectious and chronic diseases, immunization, water and sanitation, breast-feeding and the reduction of social inequities.

Keywords Micronutrients • Immune system • Innate immunity • Cell-mediated immunity • Humoral immunity • Inflammatory mechanisms • Vitamin A • Zinc • Iron • Public health interventions • Public health nutrition • Women and children • Low- and middle-income countries (LMIC) • Chronic diseases

Introduction

The global under-five mortality rate has declined by nearly half (49 %) since 1990, dropping from 90 to 46 deaths per 1000 live births in 2013 (UNICEF 2014). Nevertheless, nearly 6.3 million children under 5 years of age continue to die unnecessarily in low- and middle-income countries (LMIC) [1, 2]. Undernutrition is the direct cause of almost half of these deaths [3] aggravated by infectious diseases and detrimental environments. Undernutrition contributes to over 3.5 million child deaths [3] and micronutrient deficiencies (vitamin A and zinc deficiencies in particular) have been estimated to account for one million of these deaths per year, or 9 % of global childhood burden of disease (under 5 years) [3]. At the same time, iron deficiency is a risk factor for maternal mortality, responsible for 115,000 deaths per year, or 20 % of global maternal deaths [3]. FAO estimates over 800 million people are hungry and at risk of food insecurity [4], with all the consequences on ill-health and impaired development and subsequent reduced productivity [5].

Malnutrition, both undernutrition and the more recent epidemic of overweight, obesity and related non-communicable diseases, impacts the development and function of the immune and inflammatory responses [6]. The relationship between poor nutritional status and impaired immunity and inflammatory responses is bidirectional [6]. Protein-energy malnutrition and micronutrient deficiencies, based on both animal and human studies, impact on immunity and inflammation through a variety of possible mechanisms: epithelial barrier function, innate physiological barriers, macrophage function; neutrophil function; NK cell function; APC function; T-cell function and B-cell function [6]. The global epidemic of overweight, obesity and non-communicable diseases in all countries, LMIC and more affluent [7], has also considerable implications due to the low-grade inflammatory nature of most of these conditions and the often accompanying micronutrient deficiencies [8]. The importance of micronutrient deficiencies on immunity, inflammation and infection is a critical piece in the global effort to address child and maternal mortality and global health. Vitamin A, zinc and iron will be discussed primarily, along with the other relevant vitamins and minerals such as folate and vitamins D and B12, selenium and others which are known to have an impact on immune function and status. These micronutrients will be discussed only in terms of their immune functions in humans and the

public health implications of deficiencies, as there are many other sources of information on structure, dietary sources, bioavailability, clinical manifestations, pathophysiology and the epidemiology of micronutrient deficiency.

Even just 40 or so years ago, it was generally assumed that, as far as the human was concerned, each vitamin served one particular main function, e.g. vitamin C preserved connective tissue; vitamin D, bone; vitamin B1, the nervous system; nicotinic acid, the skin; folic acid and vitamin B12, the blood and vitamin A, the eye [9]. This is now known to be clearly not the case and many vitamins, and minerals, are involved in many physiological actions, often, directly or indirectly, impacting on the immune system [6]. This is more apparent with vitamins A, D and folate and zinc, iron and selenium, and less so vitamins C, D and E although the specific mechanisms are not clear. In the seminal book from WHO [10] 40 years ago, it was noted that the formation of specific antibodies is inhibited by many severe nutrient deficiencies, including protein, tryptophan, vitamins A and D, ascorbic acid, thiamin, riboflavin, niacin, pyridoxine, pantothenic acid, folate and vitamin B12.

This chapter examines the cycle of deficiencies, and sometimes excesses of micronutrients, leading to impairment of the immune system and leading to infectious diseases and inflammation, as well as their relationship to chronic diseases. The public health implications of these interactions and public health interventions in the control and prevention of micronutrient deficiencies will be evaluated. Although there is a considerable literature on micronutrients and growth, that will not be considered here, although obviously of considerable public health importance. Although much of the investigative work on micronutrients and immunity has been carried out in animal studies, it has been found that the mechanisms in humans may differ and so the emphasis has been on the sparser literature on humans [10] and on public health aspects.

Dietary factors that impact on inflammation and immunity, and thus on health, operate throughout an individual's lifecourse, probably even before birth. It has been suggested that maternal supplementation might affect the newborn's immune development. A trial in SE Asia followed infants in the first 6 months of their life after maternal supplementation with beta-carotene and zinc (as well as iron and folic acid) [11]. There was no effect of beta-carotene on the infants' morbidity but maternal zinc did reduce infant morbidity and significantly reduced diarrhoea and a higher interleukin (IL)-6 production, and beta-carotene leading to lower interferon-γ (IFN-γ) production [11]. Early postnatal nutrition may lead to an inadequate gut microbiota composition and function in early life, which seems to partly account for the deviant programming of later immunity and overall health status [12]. There is increasing evidence that epigenetic mechanisms that regulate gene expression during immune differentiation are directly affected by dietary factors or indirectly through modifications in gut microbiota induced by different dietary habits [13]. With increasing age, the inflammatory response becomes dysregulated, with excess production of inflammatory cytokines, and these processes advance with age. The variation in immune protective function is one factor in differential risk of cancer in the elderly, and an individual's risk to autoimmune diseases also increases [14]. This is important because the world's population of those aged 60 years and over is predicted to almost double by 2025 (from the 672 million in 2005). Nearly two-thirds of older persons (64 %) already lived in LMIC in 2012 and this figure is projected to increase to 71 % by 2025 [15]. Nevertheless, the main emphasis here is on young children and women in countries with the developing and transitional economies of LMIC which have generally higher risks of micronutrient deficiencies, reduced immunity and increased incidence of infectious diseases. It will also briefly consider the specific impact of the inflammatory response on the selection, use and interpretation of nutrient biomarkers. The last is exhaustively and ably discussed in the record of a meeting ('INSPIRE' Project) coordinated by the USA National Institute of Child Health and Development (NICHD) [6] and the Biomarkers of Nutrition for Development (BOND) Initiative [16]. Lastly, public health implications of micronutrients and infectious diseases and inflammation are outlined, and methods of addressing micronutrient deficiencies are briefly addressed.

Inflammation and Immune Function

Inflammation is an integral part of the innate immune response to infection. The acute inflammatory response is initiated upon detection by cellular sensors and other pattern recognition receptors of inducers, mainly infections, but also reactive oxygen species (ROS) and tissue damage. In typical cases, the inflammatory response is localized to the site where the inflammatory trigger is present; however, an increasing number of inflammatory conditions have been described where the initiating factor is not infection, and inflammation appears to be chronic [17, 18], characterized by elevated levels of cytokines [19], such as in obesity.

Inflammation is usually associated with increased oxidative stress due to the production of ROS, which draws on available antioxidants such as vitamins C and E, and triggers cellular antioxidant responses. Antioxidative enzymes require the presence of nutrients such as zinc, iron and selenium at active sites or as structural components. This suggests that in chronic inflammation, the utilization and requirement of some vitamins and minerals is increased. The presence of low grade systemic inflammation in chronic diseases such as obesity and Type 2 diabetes mellitus (DM), has highlighted the challenges in managing the double burden of disease, with nutrition interventions required for not only the management of traditional risk factors for metabolic disease but also to decrease inflammation.

Optimal immune function is dependent on the availability and balance of nearly all macro- and micronutrients. For instance, diets that are high in saturated and *trans* fatty acids, and high glycaemic index carbohydrate, have been linked to elevated concentrations of C-reactive protein (CRP) [20]. Non-traditional diets are also typically high in *n*-6 relative to *n*-3 polyunsaturated fatty acids. *n*-6 Arachidonic acid and *n*-3 eicosapentaenoic acid are essential for the production of inflammatory modulating eicosanoids. *n*-6 Derived eicosanoids are powerful inflammatory agents compared to those derived from *n*-3 fatty acids [21, 22]. Nutrient deficiencies, such as protein-energy malnutrition, are a major cause of immunodeficiency because of high requirements for amino acids and energy for immune cell proliferation and synthesis of protein-mediators. Undernutrition, including of micronutrients, impairs immune function and increases susceptibility to disease. Understanding the mechanisms of action are complicated by the fact that stimulation of the immune system can also impair nutritional status. Mounting an immune response requires energy and amino acids, but also demands micronutrients [23]. The generation of energy itself requires vitamin B coenzymes, such as thiamin, riboflavin and niacin. Minerals such as iron and copper are essential at active sites of proteins involved in oxidative phosphorylation and the generation of ATP.

In the context of obesity, adipose tissue provides a source of circulating cytokines derived from adipocytes and resident immune cells. Cytokines and other mediators signal between immune cells to coordinate the inflammatory response in a manner specific to the particular inflammatory insult. Cytokines function synergistically, and are produced most commonly from T-lymphocytes and macrophages (Table 30.1). Many cytokines have both pro- and anti-inflammatory roles and the net inflammatory response depends on a range of factors, including the local environment in which they are released and the presence of synergistic or competing factors [24].

Adipose tissue recruits pro-inflammatory macrophages that contribute to chronic inflammation [25]. Studies in humans have demonstrated the ability of adipose-derived cytokines to inhibit insulin signalling and induce insulin resistance [19]. Furthermore, inflammatory status is improved following weight loss in obese patients and is associated with enhanced insulin sensitivity [26]. The morphogenic role of retinoic acid (RA) in regulating immunity shows regional concentrations of RA and commensurate RA signalling in CD8(+) cells within the tumour microenvironment. This intrinsic RA signalling is required for tumour associated antigen (TAA)-specific CD8(+) T-cell survival and hence for tumour surveillance [27].

T helper (Th)1/Th2 immune response has been linked to obesity-related immune disorders and retinoid-active derivatives improve immunity via regulating Th1/Th2 balance [28]. The decline seen in

Table 30.1 Selected cytokines and examples of their functions in inflammation

Cytokine	Primary sources	Key functions in inflammation
IL-1	Macrophages	Synthesis of acute phase proteins; local and systemic inflammatory effects
	Endothelial cells	
IL-2	Activated T-cells	Proliferation of T-cells, B-cells; proliferation and activation of NK cells
	Th1 cells	
IL-6	Macrophages	Synthesis of acute phase proteins; proliferation of B-cells; regulation of IL-1 and TNF production; activation of immune cells, osteoclasts, endothelial cells; hypothalamic pituitary axis—fever and hormone release
	Endothelial cells	
	Adipocytes	
	Myocytes	
IL-10	Macrophages	Resolution of inflammation; inhibition of inflammatory cytokine synthesis; inhibition of activated macrophages and dendritic cells
	Th2 cells	
IL-12	Macrophages	Promotion of Th1 differentiation; stimulation of IFN-γ production by T-cells, NK cells
	Dendritic cells	
CRP	Hepatocytes	Acute phase response; activation of innate immunity; macrophage phagocytosis; complement cascade; oxidative stress
	Adipocytes	
CAMs	Endothelial cells	Cell adhesion by interaction with other CAMs or extracellular matrix; cell binding and anchorage; transmembrane signal transduction
	Smooth muscle cells	
	Immune cells	
TNF-α	Macrophages	Synthesis of acute phase proteins by hepatocytes; recruitment and activation of neutrophils and monocytes at sites of infection; stimulation of CRP release from liver; activation of NF-kB pathway; induction of insulin resistance
	T-cells	
	NK cells	
	Lymphoid cells	
	Endothelial cells	
	Adipocytes	
	Neuronal cells	
TGF-β	Macrophages	Resolution of inflammation; inhibition of proliferation/activation of B-cells, T-cells, macrophages; limit production of IL-2, IFN-γ, and TNF
	T-cells	
IFN-γ	Th1 cells	Activation of macrophages; suppression of Th2 cell activity; promotion of leukocyte migration
	NK cells	

Adapted from [24]
CAM cell adhesion molecule, *CRP* C-reactive protein, *IFN* interferon, *IL* interleukin, *NK* natural killer, *NF-kB* nuclear factor-kappaB, *Th* T helper, *TGF* transforming growth factor, *TNF* tumour necrosis factor

serum concentrations of IL-1 beta and IL-1 beta/IL-4 ratio in obese women suggests that vitamin A is capable of regulating the immune system and possibly reducing the risk of autoimmune disease [28].

It is well documented that circulating levels of many micronutrients decrease rapidly due to inflammation [29]. The mechanisms by which this occurs are not clear, but likely are contributed to by the utilization of micronutrients for immune activities. Micronutrients such as zinc and iron may influence inflammatory signalling pathways at different levels and in a variety of ways, including via the modulation of cytokine production. In infection, serum vitamin A concentrations decline rapidly but are able to recover without vitamin A supplementation when the infection is resolved. This supports the notion that the coordinated response to inflammation includes the redistribution of micronutrients to tissues or cellular compartments. In contrast, the redistribution of micronutrients in non-resolving inflammation may contribute to the pathogenesis of a range of chronic conditions, including obesity, atherosclerosis and DM. It is known, for example, that impaired iron utilization in chronic disorders compromises many functions of iron, which further exacerbates the processes of disease.

Undernutrition and Disease: A Vicious Cycle

Interactions between malnutrition and infection contribute directly to the health of individuals and communities, and particularly so in lower socio-economic groups and less economically developed areas and countries (Fig. 30.1) [10, 30]. Infections and immunity can be synergistic in bidirectional ways: infections are likely to have more serious consequences among persons with clinical or subclinical undernutrition, including micronutrient deficiencies; and infectious diseases have the capacity to turn borderline nutritional deficiencies into severe clinical manifestations of undernutrition, e.g. marginal vitamin A deficiency into xerophthalmia [31]. One of the issues to be discussed, because of the public health aspects, is this concept of synergism, and antagonism (i.e. where aspects of undernutrition appear to limit infectious disease). In humans, the authors of an earlier seminal review concluded, however, that 'interactions between malnutrition and infection are regularly synergistic' [10]; a view more recently also concluded by Caulfield et al. [32] and Prentice [33].

Nutritionally induced determinants of synergism (between nutrition and infection) may include [10]:

1. Reduced capacity of the host to form antibodies
2. Decreased phagocytic activity of microphages and macrophages
3. Interference with production of non-specific protective substances
4. Reduced non-specific resistance to bacterial toxins
5. Alterations in tissue integrity
6. Diminished inflammatory response and alterations in wound healing and collagen formation
7. Effects originating in alterations of intestinal flora
8. Variations in endocrine activity

In response to infection, both innate and then acquired host defences are brought into play, and both processes involve activation and propagation of immune cells and synthesis of an array of molecules. These processes require DNA replication, RNA expression, and protein synthesis and

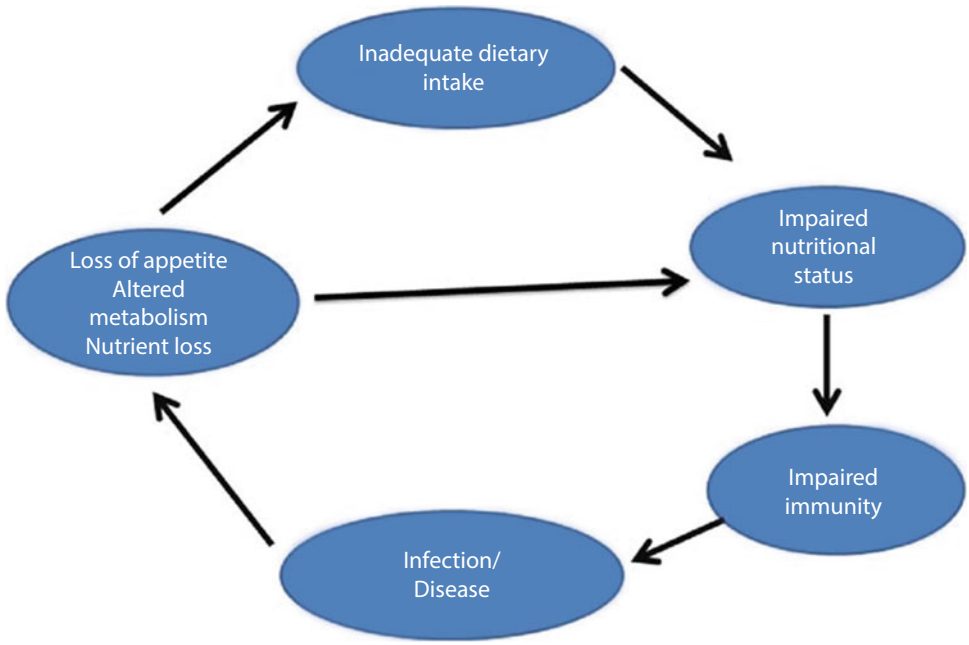

Fig. 30.1 Undernutrition/disease cycle

secretion, all consuming anabolic energy. Mediators of inflammation further increase the catabolic response [34]. The nutritional status of the host critically determines the outcome of infection and includes deficiencies in single nutrients such as micronutrients, fatty acids and amino acids, with general protein-energy mal(under)nutrition greatly increasing susceptibility to infection, particularly in LMIC, and particularly in children. Ultimately, productivity and well-being are affected at the community level which perpetuates what has beencalled the 'alarming spiral of malnutrition, infection, disease and poverty' [34].

As will be seen below, many of the micronutrients have the potential to have an association with impaired immune responses. Conversely, infectious disease adversely influences the nutritional state in several indirect ways, including loss of appetite and intolerance for food that result in metabolic effects, and an often-increased utilization of nutrients. Cultural factors can lead to substitution of less nutritious diets on the assumption of therapeutic effect and sometimes as purgatives, and antibiotics and some other drugs also can reduce appetite or digestion or absorption of specific nutrients [10]. An increased loss of body nitrogen is characteristic of all infectious disease. Among resource-poor societies the premature death of a mother and the lower income-generating capacity of iron-deficient and anaemic workers translate into greater rates of disease and overall undernutrition [35]. Women and girls are often discriminated against in terms of nutrition and health, including, in South Asia, the intrahousehold distribution of micronutrient-rich foods [36]. Disease can also affect the ability of populations to grow and harvest food if widespread enough, e.g. endemic malaria, onchocerciasis and more recently HIV/AIDS. Consequently the cycle can lead to poor nutrition leading to impaired immune systems leading to increased incidence of infectious diseases which in turn leads to further deterioration of nutritional status (Fig. 30.1).

Micronutrients, Immunity and Infectious Disease

Undernutrition, as noted above, can interfere with any body mechanism that interposes a barrier to the multiplication or progress of infectious agents [10] and that formation of specific antibodies is inhibited by many nutrient deficiencies. Severe protein depletion and folate deficiency are particularly important in reducing response and activity of phagocytes, both microphages and macrophages. The integrity of skin, mucous membranes and other tissues is important in preventing entrance of infection. Such changes associated with nutritional deficiencies include (1) alterations in intercellular substances; (2) reduction or absence of secretion of mucus; (3) increased permeability of intestinal and other mucosal surfaces; (4) accumulation of cellular debris and mucus to produce a favourable culture medium; (5) keratinization and metaplasia of epithelia surfaces; (6) loss of ciliated epithelium of the respiratory tract; (7) nutritional oedema, with increased fluid in the tissues; (8) reduced fibroplastic response and (9) interference with normal tissue replacement and repair (10).

Effect of Deficiencies on Immunological Status

> As elements of the antioxidant system, cofactors of enzymes, components of transcription factors, and epigenetic modulators, micronutrients influence various metabolic processes that are directly associated with immune functions [37].

All infectious diseases have direct adverse metabolic effects that, among other things, influence the amount and kind of food consumed and nutrients absorbed. Infectious disease nearly always makes co-existing undernutrition worse while the consequences of infection are more likely to be more serious in a malnourished host than a well-nourished one [10]. The possible importance of this

continues to be debated, especially in relation to iron supplementation in areas endemic for malaria [33] but the increasing body of evidence suggests that as long as malaria prophylaxis and treatment are available iron-deficiency status is better avoided [38].

The immune system can be broadly categorized into two groups: the innate immune system and the acquired immune system.

1. *The innate immune system*, the first line of defence, is naturally present and it is not influenced by previous contact with infectious agents. It includes epithelial barriers, the complement system, circulating phagocytes (neutrophils and macrophages) and other cytotoxic cells (natural killer (NK) cells). Innate immunity is regulated by two types of cytokines: pro-inflammatory cytokines such as IL-1, IL-6, IL-12 and tumour necrosis factor (TNF)-α, and anti-inflammatory cytokines such as IL-10, produced by neutrophils and macrophages.
2. On the other hand, *the acquired immune system* is antigen specific, where antibodies are produced by the B-lymphocytes, known as humoral-immunity, and cell-mediated immunity which depends on the T-lymphocytes system [39]. Acquired immunity involves the identification of an antigen by antibody or T-cell receptor on CD4+ T-helper (Th) cells or CD8+ effector T-cells. The antigen presenting cells carry the antigen to regional lymph nodes, where naïve Th cells are exposed to the antigen, and proliferate and mature to form memory T-cells. Memory T-cells then follow either of two pathways, Th1 or Th2 memory cells. In response to an intracellular pathogen, Th1 memory cells produce IFN-γ and IL-2, which in turn stimulate a response by cytotoxic T-lymphocytes (CTLs), activation of macrophages, response of delayed-type hypersensitivity (DTH) and provide limited help to stimulate B-cell development and antibody production. Th2 memory cells act in response to a pathogen produce IL-4, IL-5 and IL-10, which stimulate B-cells to produce antibodies, eosinophil and mast cell development and deactivation of macrophages.

It has long been accepted that malnutrition, in particular undernutrition, impairs immune function and increases the risk and severity of diseases. There are instances where the immunomodulatory effect is independent of any nutritional value, e.g. canthaxanthine, which does not have any provitamin A activity, has been shown in rodents to have the same ability to enhance immune responses as β-carotene [40].

Micronutrients and Their Immunological Roles in Disease and Inflammation

Vitamin A

Vitamin A deficiency was the contributing cause of over a million premature deaths each year in children globally in 2009 [41], as well as the commonest cause of childhood blindness, and remains a serious public health problem in 122 LMIC countries [42]. It is also likely to be a factor in the aetiology of several cancers [31]. Xerophthalmia was a recognized public health problem in much of Europe until early last century. The public health significance of vitamin A deficiency has been redefined beyond xerophthalmia in the last 35 years or so, to include its impact on deaths from infectious diseases in LMIC where vitamin A deficiency is frequently endemic. There has been tremendous progress in reducing the prevalence of the most severe manifestations of the disease (xerophthalmia and blindness), which has been on the decline in all regions of the world [43]. Subclinical vitamin A deficiency (serum retinol <0.07 μmol/L in children under 5 years), resulting from a chronic, dietary insufficiency of vitamin A, either preformed or from precursor carotenoids, and its impact on immunity and childhood infectious disease, however, is still a problem of considerable public health

significance [31, 42, 44, 45]. A deficiency state may arise with prolonged inadequate intake, often coupled with the high, normal demands imposed by rapid growth during childhood, pregnancy or lactation, or by excessive utilization and loss during infection [46]. The relative frequent occurrence in women during pregnancy in LMIC, and the possible consequences of that, have only relatively recently been widely recognized, which much increases the magnitude of the problem including possibly an impact on maternal mortality, at least in deficient populations such as Nepal, although apparently not in less severely deficient populations in Bangladesh [45, 47–49].

At least since the time of the Pharaohs were reigning in Egypt, vitamin A has been mostly known for its role in xerophthalmia and night blindness, and cases of xerophthalmia have been described since those times and especially throughout the eighteenth and nineteenth centuries [50]. A series of scientists, Hopkins, along with McCollum and Davies and Osborne and Mendel found that animals, fed only fats, protein, starch and inorganic fats not only failed to grow normally but became also more susceptible to infection and frequently died of overwhelming sepsis [50]. Bloch (cited in [50]), studying the growth and development of children in a Danish orphanage, found that when they were given butterfat and whole milk they were less susceptible to infections of the urinary and respiratory tracts and middle ear (and less likely to develop xerophthalmia). By 1928, Green and Mellanby had declared vitamin A as an 'anti-infective factor' [9, 46]. Ellison administered daily vitamin A and reduced by half the case-fatality rates due to measles. By 1930s, it was accepted that besides the ophthalmologic manifestations of vitamin A deficiency, there was also reduced resistance to some microbial infections [50].

With Wolbach and Howe's classic description in 1923 of widespread metaplasia and keratinization of epithelial linings of the respiratory and genito-urinary tracts and glandular ducts in vitamin A-depleted animals, loss of the 'barrier function' of epithelial linings became one plausible explanation for the associated decreased resistance to infection (cited in [31]). While animal experimentation continued, clinical studies in humans from the 1920s through the 1940s revealed associations between vitamin A deficiency or xerophthalmia and infectious diseases [51]. The inverse relationship between febrile illness and plasma vitamin A concentration, now understood as part of the acute phase response to infection, and the potential therapeutic efficacy of vitamin A in reducing childhood measles fatality, puerperal fever in women and other clinically relevant conditions were recognized [31, 51]. The regulatory roles of vitamin A in maintaining epithelial cell differentiation and function and immune competence have provided biologic plausibility to its importance in decreasing severity and mortality of infectious diseases [46, 52–54].

Vitamin A Deficiency and Immune Function

Vitamin A is one of the most extensively studied nutrients in relation to immune function. A review of the results from the supplementation of vitamin A in human studies was published in 2005 [55]. More recently, several lines of evidence have converged to show that retinoic acid (RA), a major oxidative metabolite of vitamin A, plays a key role in the differentiation of T-cell subsets, the migration of T-cells into tissues, and the development of T-cell-dependent antibody responses. Conversely, in a state of vitamin A deficiency, inflammatory T-cell reactions may be inadequately opposed and therefore become dominant. Although more data from human studies are still needed, the framework now developed from studies in rodent models suggests that adequate vitamin A status, whether derived from ingestion of preformed retinol or beta-carotene, is important for maintaining a balance of well-regulated T-cell functions and for preventing excessive or prolonged inflammatory reactions [56].

Vitamin A elicits a broad array of immune responses through its metabolite, RA and loss of RA leads to impaired immunity, whereas an excess of RA can potentially promote inflammatory disorders. Most of the effects of vitamin A on immune or inflammatory responses can be explained via binding of the vitamin A metabolite, all-*trans* retinoic acid, to one of three Zn-finger containing members of

the nuclear receptor superfamily (retinoic acid receptor (RAR)α, RARβ and RARγ). Recent estimates, in various cell lines, indicate that RAR are constitutively bound to around 500 genomic sites and all-*trans* retinoic acid treatment induces RAR binding to 500–600 DNA sites [6]. RA at basal levels is required for immune cell survival and activation. During immune responses, enzymes metabolizing vitamin A are induced in certain types of immune cells such as dendritic cells (DC) and tissue cells for induced production of RA. RA regulates gene expression, differentiation and function of diverse immune cells. The cells under the influence of RA in terms of differentiation include myeloid cells such as neutrophils, macrophages and DC. Also included are lymphoid cells such as effector T-cells, regulatory T-cells and B-cells [57]

Vitamin A maintains and restores the integrity and function of all mucosal surfaces, including a very sophisticated bidirectional mechanism that takes place in the digestive system and leads to immune tolerance across the entire gut lining. RA provides an intestine-specific environmental cue to differentiating immune cells. When T-cells and B-cells are activated in the intestine and associated lymphoid tissues, gut homing receptors are induced on the cells in a retinoic acid and antigen-dependent manner [58]. RA, produced by gut dendritic cells, is also an important signal that induces IgA-producing B-cells. The gut homing T-cells and B-cells play essential roles in protecting the digestive tract from pathogens.

The intestine is exposed continuously to complex environments created by numerous injurious and beneficial non-self antigens. The unique mucosal immune system in the intestine maintains the immunologic homeostasis between the host and the external environment. Crosstalk between immunocompetent cells and endogenous (e.g. cytokines and chemokines) as well as exogenous factors (e.g. commensal bacteria and dietary materials) achieves the vast diversity of intestinal immune functions in moderating the fine balance between physiologic and pathologic conditions of the intestine [59].

Innate Immunity

Vitamin A deficiency is associated with impaired innate immunity. Animal studies have shown that vitamin A deficiency is significantly associated with altered mucosal epithelial barriers in the conjunctiva of the eye [46, 60], respiratory [61], gastrointestinal [62] and genitourinary tract [63]. Vitamin A deficiency can result in a loss of microvilli, mucus-producing goblet cells and mucin in the small intestine [64–66]. Mucins are glycoproteins, secreted into the lumen, found on cell surfaces and serve as a first line of defense. Changes that occur due to vitamin A deficiency include squamous metaplasia of the conjunctiva and cornea, loss of goblet cells, and abnormal keratinization [46, 67] of the epithelium. In humans, using the lactose/mannitol urinary excretion test as an indicator of gut integrity in vitamin A supplementation trials in children suffering from severe infections, a rapid increase intestinal integrity was shown [68]. A few studies have failed to show a consistent effect on the mucosal anti-infective or inflammatory markers in milk, saliva or general fluid [55].

Animal studies suggest that vitamin A deficiency may lead to an increased total number of macrophages [69]. In addition, vitamin A deficiency leads to increased IL-12 produced by macrophages, with IL-12 promoting the development of Th1 cells, which produce IFN-γ. Increased IFN-γ leads to increased macrophage activation [70]. Although data from human studies are limited, clinical trials suggest that vitamin A supplementation may diminish the production of pro-inflammatory cytokines (TNF-α and IL-6) by macrophages, but only in response to infections [55]. Vitamin A supplementation was found to be associated with increased production of the anti-inflammatory cytokines IL-10 [71]. All these data suggest that vitamin A deficiency can lead to increased inflammation mediated by cytokines from macrophages, while impairing the ability of macrophages to ingest and kill bacteria.

NK cells are one of the components of innate immunity which work by killing virus-infected cells, as well as tumour cells. Studies in animals have shown that vitamin A deficiency impairs both the NK

cell number and its lytic activity [72, 73]. In a clinical trial among HIV-infected children in South Africa, vitamin A supplementation showed increased number of cells with the CD56 receptor expressed by the NK cells [74]. Vitamin A deficiency impairs normal neutrophil development, which can lower the capacity of phagocytosis to kill bacteria [74]. However, the evidence on the association of vitamin A and neutrophil function in humans is limited [55].

Acquired Immunity

Cell-mediated immunity can be affected also by vitamin A deficiency. Studies in animals have shown that vitamin A deficiency is associated with reduced weight of the thymus [66] and decreased lymphocyte proliferation in response to mitogens [69, 75]. In murine T-cells, all-*trans* RA has been shown to stimulate the expression of RA receptor-α and increased antigen-specific T-cell proliferation [76]. Vitamin A supplementation to infants has been shown to significantly increase total lymphocyte count [77], especially the CD4 subpopulation [78]. Similar findings have been observed in HIV-infected children [74], while when vitamin A was supplemented in HIV-infected women, no significant effect on CD4 T-cell counts was observed [79, 80]. Human study findings on Th1-mediated response are equivocal. One study showed increased DTH response in infants following high-dose vitamin A supplementation [81] whereas another study found no difference by treatment groups in the proportion of children with DTH response in a non-placebo-controlled trial of intramuscular vitamin A [82]. Further, in a study among children with measles, vitamin A supplementation apparently diminished the proportion of children with DTH response [83].

Human studies indicate that vitamin A can regulate the production of IL-10 from Th2 cells: vitamin A deficiency impairs secretion of IL-10 [84, 85], while supplementation of vitamin A increases the IL-10 secretion in vitamin A deficient subjects [71]. The cytokine IL-10 plays a role in the inhibition of the synthesis of pro-inflammatory Th-1 type cytokines, such as IFN-γ and IL-2, in both T and NK cells. In vitro lymphocyte stimulation to various mitogens was higher in vitamin A deficient rats, with higher IFN-γ and IL-2 production, indicating that vitamin A deficiency increased Th1 responses [86]. The results from animal studies suggest that modulation of the balance between Th1 and Th2 responses by retinoids may be influenced by the type of pathogens [87]. Results from human studies that examined the effect of vitamin A on either a Th1 or a Th2 responses also suggest that the immunological mechanisms through which vitamin A exert an effect are pathogen specific [55].

The growth and activation of B lymphocytes requires retinol [88]. The growth of B lymphocytes is also known to be mediated by the metabolites of retinol [89]. B lymphocytes are responsible for the production of immunoglobulins (antibodies). All-*trans* retinoic acid was found more active than retinyl acetate, retinaldehyde or retinol in restoring IgG responses in a murine model [90]. Vitamin A deficiency typically impairs antibody response to T-cell-dependent antigens [53, 69, 86] and in some T-cell-independent antigens [91]. Studies with a vitamin A deficient animal model have shown impaired serum IgG1 antibody response to purified protein antigens [54, 69], impaired serum IgG1 and IgE responses to the intestinal helminth *Trichinella spiralis* [92] as well as the intestinal IgA response to cholera toxin [86]. Most animal studies showed no impairment of serum antibody response to viral infection in vitamin A deficiency [73]. The evidence for an effect of vitamin A supplementation on T-cell-dependent antibody response in humans is equivocal. Administration of a large dose of vitamin A in children, aged 1–6 years, did not result in any significant effect on the antibody response against tetanus toxoid [82]. Another study compared the effect of different doses of vitamin A supplementation on the antibody responses against both tetanus and diphtheria toxoids in children, 1–6 years, and also found no effect [93]. In contrast, others have shown significantly higher antibody response against tetanus toxoid following vitamin A supplementation in tetanus-naïve 3–6 year old children [40, 94]. The effect of vitamin A in infants on the antibody response against diphtheria toxoid

was found positive, while there was no effect with tetanus toxoid [95]. Current consensus suggests vitamin A supplements can increase the antibody response to tetanus toxoid particularly in vitamin A deficient children who have not been exposed to tetanus. Effects of vitamin A supplements on antibody response against measles infection or measles immunization were found to be either positive [77, 96] or negative [97] or no change [60, 96]. The serum antibody response to polio vaccine showed no effect by vitamin A supplementation when given at routine immunization time [51, 98]. In a study when vitamin A was administered to both mother and children, a significantly higher proportion of children had protective titres against type 1 poliovirus than in the placebo group [99]. The differential effect of vitamin A supplementation observed could to be attributed to doses of vaccines, time of supplementation or baseline vitamin A status of the population studied [55, 94].

Public Health Implications

It is now well accepted that all-cause mortality among children 6 months to 5 years of age is reduced by about a quarter when supplementation with vitamin A capsules takes place as recommended by WHO [46]. A national cross-sectional survey, a large, population-based, prospective study, and several hospital-based clinical studies of xerophthalmia among Indonesian children by Sommer and colleagues in the late 1970s built on earlier work and demonstrated aspects of causation, progression, risk factors and health consequences of childhood xerophthalmia and vitamin A deficiency in LMIC [46]. Reports from this work, in the early 1980s, showed that non-blinding, mild xerophthalmia (night blindness and Bitot's spots) was associated with markedly increased risks of preschool child mortality [100]. Presumably vitamin A supplementation increased resistance to the severity of infection (measles and diarrhoeal diseases) by reducing the functional degree of vitamin A deficiency. In contrast to evidence relating vitamin A deficiency to respiratory tract compromise and infection [46], vitamin A supplementation has not had a consistent effect in reducing the incidence, severity or mortality of acute lower respiratory infection in children, and vitamin A supplementation of infants under 6 months of age has generally not shown a survival benefit in early infancy.

This considerable public health effect (the reduction of childhood mortality by an average of 23 %) can be partly explained by an ability of vitamin A to lower case fatality from measles by almost half, as observed in field trials and hospital-based measles trials [46], mortality from severe diarrhoea and dysentery, by approximately 40 % [50] and, based on morbidity findings from a recent supplementation trial, possibly falciparum malaria [32]. Vitamin A deficiency and infection interact within a 'vicious cycle' [10], whereby one exacerbates and increases vulnerability to the other. The bidirectional relationship complicates frequent cross-sectional evidence of depressed plasma retinol levels with diarrhoea, acute respiratory infections, measles, malaria, HIV/AIDS and other infectious illnesses [31]. Combining mortality effects with data on the prevalence of vitamin A deficiency, it has been estimated that 1.3–2.5 million early childhood deaths each year can be attributed to underlying vitamin A deficiency [46].

Zinc

Low zinc intakes, through an effect on immune function, reduce resistance to infection. Conversely, zinc supplementation reduces the morbidity and mortality of common childhood diseases, including diarrhoea, lower respiratory tract infection, and probably malaria [101]. Zinc was used topically as calamine lotion as far back as 1500 BC by the Egyptians. Its current name probably originates from an early German word meaning 'tooth-like, pointed or jagged' (presumably referring to the

needle-like metallic zinc crystals). Zinc mines near Udaipur in the Indian State of Rajasthan were active during 400 BC and there are references to medicinal uses in *Charaka Samhita* (300 BC). Pure zinc was not isolated in China until the seventeenth century although the smelting and extraction of impure forms was being undertaken around 1200 AD in India. There is a record however of the metallurgist Andreas Libavius receiving in 1597 from Asia a quantity of pure zinc metal, unknown in the West before then, although several different Englishmen and Germans probably isolated zinc independently in the late first half of eighteenth century.

Cellular zinc concentrations are maintained by two classes of zinc transporter families (ZnT and Zip). ZnT transporters promote cellular zinc efflux or its sequestration into intracellular organelles, whereas Zip transporters facilitate extracellular or organellar zinc influx into the cytoplasm. Metallothionein plays a central role in the maintenance of zinc homeostasis. Inflammatory cytokines have been reported to both up- and down-regulate the expression of specific transporters—with the net effect thought to increase the intracellular zinc in response to an increased demand for zinc in inflammatory conditions [24]. Although advances have been made towards understanding cellular zinc metabolism, the identification and quantification of zinc deficiency is hindered by the lack of a suitable diagnostic test. Under the EURopean micronutrient RECommendations Aligned (EURRECA) consortium, Lowe et al. [102] used meta-analysis to examine the usefulness of biomarkers of zinc status in humans. They showed that plasma zinc concentration responded in a dose-dependent manner to dietary manipulation in a range of population groups. Data on urinary zinc excretion, though limited, appeared to respond in the same manner. Further analysis by Lowe's group [103] revealed that for every doubling in zinc intake, the difference in serum or plasma zinc concentration is 6 %. The small magnitude of this relationship places further emphasis on the technical aspects of sample collection and analytical processing.

The effects of zinc on immune function have been demonstrated by intervention trials showing an impact on infectious diseases, more frequently diarrhoea, and to a lesser extent respiratory tract infection. There is a consensus now that zinc deficiency is a problem in many countries with high child mortality rates [104–106]. The impact on growth and maturation has been long recognized where the deficiency is more florid and has a clinical effect on growth and failure to thrive, immune effects and delayed sexual maturation are clinical manifestations of zinc deficiency. It has also been observed as an inborn error of zinc metabolism, acrodermatitis enteropathica, in patients fed incomplete parenteral solutions, in patients with Crohn's disease and occasionally in infants. Zinc-responsive night blindness has been observed in alcoholism and Crohn's disease [107].

Zinc Deficiency and Immunity

An initial consequence of zinc deficiency is an impairment of immunological functions. Zinc is crucial for the normal development and function of cells mediating both innate and acquired immunity. Several reviews in the available scientific literature have summarized the effect of zinc deficiency and immune function and the possible mechanisms [24, 108, 109] which appear to be multifaceted, from the physical barrier of the skin to gene regulation within lymphocytes. Even with mild zinc deficiency, multiple aspects of the immune system are impaired [110–112].

Innate Immunity

Zinc deficiency may impair epithelial linings of the gastrointestinal and pulmonary tracts [112, 113], and also damage epidermal cells, resulting, e.g. in the skin lesions of acrodermatitis enteropathica [114]. In both human and animal studies, NK cell activity has been found to be depressed [110, 115],

and treatment of human peripheral blood NK cells with exogenous zinc has been found to stimulate production of IFN-γ [116]. Rajagopalan et al. [117] have suggested that zinc is required for killer cell inhibitory receptor on NK cells and so zinc deficiency results in the inhibition of the killing activity.

Zinc deficiency impairs chemotactic responses of neutrophils, while absolute numbers of neutrophils are not affected [114, 118, 119]. The chemotactic response of monocytes is impaired and can be rapidly restored by the in vitro addition of zinc [114, 118]. Macrophage phagocytosis in zinc deficient animals (both mice and rats) has been found to be reduced [120], enhanced [121] or to remain unchanged [122]. High concentrations of zinc in vitro inhibit macrophage activity [123]. Information regarding the effects of zinc on macrophage function in humans is limited and more studies are needed to confirm the role of zinc on macrophage phagocytosis.

Acquired Immunity

Zinc plays an important role in cell-mediated immunity. In their review article, Shankar and Prasad [124] summarized the available data from both animal and human studies of subjects showing thymic atrophy, a reduction in the size of thymus, due to zinc deficiency. Thymus is the main organ for T-cell development, and the reported atrophy would confirm the role that zinc plays in the early stages of T-cell maturation. Zinc deficiency causes depleted numbers of T-cells in the spleen, lymph nodes and peripheral blood in animals [110, 125], and in the blood and peripheral lymphoid tissues in humans [124]. Studies demonstrate that zinc supplementation reverses these conditions [115, 125, 126].

Delayed hypersensitivity response and cytotoxic activity of T-lymphocytes are impaired in zinc deficiency and are reversed by zinc supplementation [127]. Additionally, zinc supplementation in malnourished children restores their delayed hypersensitivity responses [128]. Besides maintaining the proliferation, there is a role of zinc in lymphocyte homeostasis by suppression of apoptosis [129]. Several studies have reported that apoptosis of T-lymphocytes induced by in vitro treatment of toxins and other agents can be prevented by adding high doses of zinc [130, 131]. The thymic atropy seen in zinc deficiency, mentioned above, is accompanied by apoptosis of lymphocytes [132].

Thymulin, a thymus-specific hormone, binds to the high affinity receptor on T-cells and promotes T-cell functions, such as allogenic cytotoxicity, suppressor functions, and IL-2 production [133–135]. Thymulin regulates the cytokine release by peripheral mature T-cells [136] and induces the proliferation of CD8+ T-cells that function as cytotoxic cells able to recognize and kill pathogens [137]. Thymulin requires zinc for its biological activity to be expressed [133, 134]. Experimental zinc deficiency decreases the activity of serum thymulin, which is required for the maturation of T-helper cells [109], leads to an imbalance of T-helper 1 (Th1) and T-helper 2 (Th2) functions, decreases the recruitment of T-naive cells [138], and reduces NK cell lytic activity [139]. Zinc modulates the oxidative burst that is generated by polymorphonuclear leucocytes as part of their microbiocidal activity [140]. Although the activity of thymulin in serum has been found to be significantly impaired in zinc deficiency, this was able to be corrected by both in vivo and in vitro zinc supplementation [141, 142].

A T-cell subpopulation study showed a significant decrease in the ratio of CD4+ to CD8+ during zinc deficiency that was corrected by zinc supplementation [138]. It has been suggested that zinc is required for regeneration of new CD4+ T-cells [109]. Studies on experimental human models have shown a decreased proportion of CD73+ in the CD8+ subset of T-lymphocytes in zinc deficiency [143]. The CD73 molecule on CTLs is required for antigen recognition, proliferation and cytolysis [143]. Zinc deficiency is known to affect the production of a variety of cytokines, such as IL-1, IL-2, IL-4 and IFN-γ [24, 144], by influencing the functions of T-lymphocytes and macrophages [145]. IL-1β production is higher in zinc-deficient adults [146, 147] and, compared to zinc-sufficient individuals, production of IL-2 is lower [148]. Inconsistent results are reported for IL-6. Zinc deficiency in Indonesian infants was accompanied by lower production of IL-6 [85], while no significant

differences were observed in IL-6 between zinc-sufficient and zinc-deficient adults [146]. The addition of zinc to human peripheral blood mononuclear cells was found to induce the release of IL-1, IL-6, TNF-α and IFN-γ [149]. Studies in the experimental human model and in patients with sickle cell disease suggest that the impaired cell-mediated immunity of zinc deficiency is caused by the imbalance between Th1 and Th2 cell functions [138, 143, 146]. While there was a decrease in the production of IFN-γ, and IL-2 (Th1 response), the production of IL-4, IL-6 and IL-10 (Th2 response) were not affected during zinc deficiency [138, 143, 146].

Human intervention studies measuring the effects of zinc on plasma cytokine concentrations or cytokine production in primary human blood cells have been reviewed previously [24]. Increased cytokine concentrations have been shown in stimulated mononuclear cells isolated from populations supplemented with ≤20 mg zinc/day [150], suggesting a zinc dose–response. Measurements of plasma cytokine concentrations in response to zinc supplementation support a difference in effect depending on zinc dose; plasma concentrations of IL-6 have been shown to decrease with zinc supplementation of 45 mg/day [151] but to increase with 10 mg zinc/day [24]. The significance of these changes is unclear but the ability of zinc supplementation to influence cytokine concentrations in humans is consistently reported.

Effects of Zinc Supplements on Infection and Inflammation

Otitis media is inflammation of the middle ear, which is common in young children in LMIC, and may lead to hearing loss. Gulani and Sachdev [152] evaluated the evidence on whether zinc supplementation can reduce the incidence of otitis media in healthy children living in LMIC and found the outcomes to be inconsistent. The authors identified limited signs of benefit in children being treated for marasmus.

Singh and Dass [153] assessed RCTs to determine the efficacy of zinc supplementation in reducing the incidence and symptoms of the common cold. Zinc supplements were associated with a significant reduction in the duration, by approximately one day, but not the severity of symptoms. Very high heterogeneity was observed in the included trials, suggesting that the estimates must be viewed with caution.

The effect of zinc supplementation on the prevention of pneumonia in children aged 2–59 months of age was investigated by Lassi et al. [154]. Their analysis showed that supplementation reduced the incidence and prevalence of pneumonia by 13 % and 41 %, respectively. Brown et al. [155] conducted a meta-analysis of zinc supplementation in infants, preschool, and older prepubertal children, and showed that supplementation reduced the incidence of acute lower respiratory tract infections by approximately 15 %. The authors reported significant heterogeneity among the studies, with the magnitude of reduction in infection being greater in children who were stunted.

Zinc supplementation for treating children with diarrhoea was evaluated by Lazzerini and Ronfano [156]. In their Cochrane review they included 24 eligible trials, with the majority of the data obtained from Asia, and from individuals at high risk of zinc deficiency. In children aged greater than 6 months with acute diarrhoea, zinc supplementation may shorten the duration of diarrhoea, and as reported previously [155], the improvement was greater in malnourished children. For children with persistent diarrhoea, zinc supplementation shortened the duration of diarrhoea by around 16 h although the trials were considered of moderate quality evidence. As reported by Brown et al. [155], age is an important contributor to the outcome: zinc supplementation reduced the incidence of diarrhoea by approximately 20 %, but the impact was limited to studies that enrolled children age greater than 12 months. In children with initial age >12 months, the relative risk of diarrhoea was reduced by 27 %.

One study investigated the effects of zinc supplementation for 6 months in HIV-infected children [157]. Children supplemented with zinc had fewer clinic visits in which watery diarrhoea was observed, and there was more weight gain in the zinc group, but the difference was no longer significant 3 months after supplement cessation.

Public Health Implications

Supplementation under experimental conditions has shown reduced poor growth rates and possibly an association with an increase in energy and protein intake. More recent field trials in Bangladesh, India, Pakistan and sites in Africa have shown conflicting results in growth, reduction in disease, severity of disease, and varied results depending on the disease. Nevertheless, results of two recent meta-analyses of such studies seem to indicate a definite role for zinc supplementation (probably with other micronutrients), especially in growth and diarrhoeal disease [104]. Consequently, zinc deficiency and its impact on reducing child mortality has come much more to the fore, with sufficient evidence that the accepted treatment of diarrhoea is now oral rehydration therapy with a 2 week course of zinc supplementation [158] and is a recommendation by WHO [159]. Effectively, the guidance recommends that mothers, other caregivers and health workers should provide children with 20 mg per day of zinc supplementation for 10–14 days (10 mg per day for infants under the age of 6 months) [159]. There is considerable debate now on whether this also applies to respiratory disease and the public health prevention of zinc deficiency, as opposed to therapeutic use.

Zinc and the Double Burden of Disease

In Type 2 DM, patients exhibit an impaired immune function as part of their pathogenesis that ultimately results in a decreased functional pancreatic β-cell mass; while the failure of β-cells in Type 2 DM occurs over a prolonged period and involves the chronic activation of the innate immune system [160]. The aberrant expression in DM of a number of important immune mediators that are zinc-responsive, including the proinflammatory cytokines, suggests a potential interaction between zinc status, impaired immunity, and DM. A systematic review and meta-analysis of randomized placebo-controlled trials was conducted to determine the effect of zinc supplementation on markers of glycaemic control, with the trials carried out mainly in LMIC. A reduction in fasting glucose concentrations was observed following zinc supplementation and in those with underlying chronic metabolic disease zinc supplementation produced a greater reduction in glucose concentrations compared to the effect in healthy participants [161]. The results suggest that zinc could play a role in the management of hyperglycaemia and may serve as co-adjuvant therapy for DM, particularly in LMIC [162].

Part of the need for low-dose zinc supplementation is the difficulty of increasing intakes of zinc through dietary methods, especially poor diets low in animal-source foods [163]. Large variations in zinc content can be found between otherwise nutritionally similar food sources but tend to be high in meat, cheese, lentils and cereals. These tend to be components of more expensive diets with cereals being the major source of energy and zinc in large parts of the world. As the zinc is mainly located in the outer layer of the grain, a low extraction rate means that the majority of the content of zinc, as well as other minerals are removed, although this should also reduce the phytates that affect bioavailability. Use of zinc-rich galvanized cooking pots and canning may contribute zinc in the diet. Unrefined cereal-based diets present the largest risk for low zinc absorption [164]. Contributing factors to poor zinc intakes may be geophagia and large zinc losses due to intestinal parasitic infections [164]. Lower zinc intakes than in western type diets have been described in Brazil around the Amazon where the diet is fish based, and where signs of zinc deficiency were also observed [163]. Similarly low intakes have been described in other parts of the developing world including Papua New Guinea and South Asia. Nevertheless, Gibson and others have demonstrated the theoretical possibility and the feasibility in West Africa of increasing the bioavailability of micronutrients in plant-based diets [165].

Iron Deficiency, Iron Deficiency Anaemia and Other Nutritional Anaemias

Conservative estimates indicate that 1500 million people are anaemic worldwide, with perhaps over 90 % of these in LMIC, mainly South Asia and Africa [166]. All the figures suggest that over half of all women in LMIC are anaemic. Iron deficiency, the main cause of anaemia, is a major contributor to low birth weight, prematurity and maternal mortality [167, 168]. Iron deficiency anaemia (IDA) is even more prevalent in infants and young preschoolers, and while there are only very recently global data on prevalence of IDA in infants and children, in some sample populations prevalence reaches 70 % or more [169]. Nutritional anaemia, largely because of iron deficiency, remains the major nutritional problem facing the poorer nations, although even in more affluent countries, it remains a significant problem in certain, usually disadvantaged, groups. Earlier WHO estimates give prevalence data for preschool-age children, non-pregnant and pregnant women, according to information available and that were included according to pre-specified criteria [169]. For infants and young children, the range is from 3.4 % in North America to nearly two-thirds (65.4 %) in Africa. In women, the range is 7.6 % in North America to 44.7 % in Africa (non-pregnant), and for pregnant women, 4.7–55 %. However, individual studies have identified far higher prevalences for infants and women, especially in South Asia that show, e.g. 84.9 % of pregnant women anaemic (Hb < 110 g/L) with 13.1 % having severe anaemia (Hb < 70 g/L). In India, adolescent girls had levels of 90.1 % with 7.1 % having severe anaemia, in the 16 Districts of India surveyed [170].

Iron Deficiency and Immunity

In both humans and animals, the effects of iron deficiency on immune response have been studied extensively. Overall, there is little evidence that shows any effects of iron deficiency, especially in humans, on B-cell-mediated immunity and antibody production. On the other hand, specific defects in several components of both innate immunity and cell-mediated immunity have been well documented [171]. Overall though, from a public health perspective there has been limited appreciation of a role. An earlier review in the Lancet, besides a mention of increased susceptibility to upper respiratory infections associated with iron deficiency anaemia [172], the only mention was that 'the effect of iron status on immune function and cognition in infants and children needs to be clarified' [173].

Much progress has been made in the understanding of iron metabolism. Hepcidin, a peptide that prevents the efflux of iron across the intestinal mucosa and from macrophages [174], has attracted much attention in the last few years, partly because infection and inflammation increase hepcidin synthesis and this discovery has enhanced understanding of the association between iron and innate immunity [175]. Hepcidin appears to be regulated by body iron status, and is synthesized by hepatocytes, macrophages, neutrophils and adipocytes [175].

Innate Immunity

In both human and animal studies, several components of nonspecific immunity have been found to be impaired by iron deficiency. NK cell activity was found to be depressed in iron deficiency [176, 177], presumably because the NK cell needs iron for its differentiation and proliferation. Macrophage phagocytosis in general appears to be unaffected by iron deficiency, while bacteriocidal activity of these macrophages has been reported to be impaired [176, 177]. In iron deficiency, neutrophils have reduced activity of myeloperoxidase, which is involved in the killing process of pathogens [176].

Acquired Immunity

Iron plays an important role in cell-mediated immunity [178, 179]. In most but not all studies, the number of T-cells was found to be reduced with thymic atrophy during iron deficiency [178, 179]. In addition to a reduced number of T-cells, more reports than not on iron deficiency show an impairment of lymphocyte blastogenesis and mitogenesis in response to a number of different mitogens [181, 182]. This change is largely correctable with iron repletion [180]. Further, studies on iron deficient patients have reported either an absent or diminished DTH response, compared with control subjects, to a variety of antigens such as Candida, mumps, diphtheria, trichophyton and streptokinase-streptodornase. Following iron supplementation, the impairment of the DTH responses, including tuberculin reactivity, were found to be reversed [182].

Studies have shown a reduced in vitro production of IFN-γ by spleen cells taken from iron deficient mice [183]. In a study in hospitalized children with iron deficiency, they were found to have a lower percentage of lymphocytes producing IFN-γ in vivo (spontaneously), while they had a higher percentage of lymphocytes producing IFN-γ following in vitro stimulation [184]. The IFN-γ is a potent macrophage activating lymphokine and an important mediator of the DTH response and cellular cytotoxicity [185]. Cellular iron availability modulates the differentiation and proliferation of Th cells subsets, with Th1 cells being more sensitive than Th2 cells to iron deficiency [186]. Further, the ratio of CD+ to CD8+ T-lymphocytes in blood was found to be reduced in iron deficiency, whereas the number of cells remained unchanged [187].

Humoral immunity on the other hand, appears to be normal in iron deficient individuals. In iron deficient patients, the serum IgG, IgA and IgM concentrations were either normal or elevated [188]. Antibody production in response to specific immunization with most antigens was found to be well preserved in iron deficient humans [189].

Infection and Iron Supplementation

It is well established that serum iron concentrations decrease markedly in response to systemic inflammation or infection [190]. In patients with tuberculosis (TB), supplementation with iron increases mycobacterial growth [190] and is associated with increased morbidity and mortality [190]. Similarly, the deleterious impact of iron supplementation in parasitic disease such as malaria is well documented [33]. The provision of iron supplements in endemic malaria regions could increase morbidity and mortality with the most likely explanation being the appearance of non-transferrin-bound iron (NTBI) in the plasma. NTBI forms when the rate of iron influx into the plasma exceeds the rate of iron binding to transferrin. Malaria decreases iron absorption in single-meal studies, but there is no evidence of decreased efficacy of iron-fortified foods, and no significant increase is observed in NTBI on consumption of iron-fortified food. Therefore strategies such as fortification of staple foods and condiments, or the use of micronutrient powders for home fortification, could be effective for improving iron status in susceptible groups such as women and children [191, 192].

Thus, based on the existing literature, it can be concluded that iron deficiency impairs both innate (reduce bactericidal macrophage activity and NK cell activity) and cell-mediated immunity (reduce T-cell proliferation, DTH response, decrease in the ratio of CD4+ to CD8+ cells). It also impairs a variety of cytokines (IFN-γ, TNF-α, IL-2, IL-10), and suppresses Th-1 cells response with a small decrease in Th2 response.

On the other hand, iron overload affects various components of the immune system. Several relatively recent reviews have summarized the effects of iron overload on the immune system [193, 194]. Iron overload, as seen in hereditary haemochromatosis patients, enhances suppressor T-cell (CD8) numbers and activity, decreases the proliferative capacity, numbers, and activity of helper T-cells (CD4)

with increases in CD8/CD4 ratios, impairs the generation of cytotoxic T-cells, and alters immunoglobulin secretion and increased levels of IL-4, IL-6 and IL-10 [193, 194]. Thus iron overload may result in increased susceptibility to infection by impairing Th1 cytokine-mediated response through diminished activity of regulatory cytokines (IFN-γ, IL-2 and IL-12), and by increasing Th2 response, by impairing the killing of intracellular pathogens by macrophages.

Public Health Implications

The overt physical manifestations of iron deficiency include the generic symptoms of anaemia, which are tiredness, lassitude and general feelings of lack of energy. While neuromaturational delays and reduced productivity, physical activity and work performance are the most important clinical features, reduced immunocompetence, thermoregulatory function, and energy metabolism are also consequences [195, 196].

Iron and the Double Burden of Disease

Increased inflammation and iron stores have been correlated with established risk factors of DM, obesity and the metabolic syndrome [190]. A link between obesity and iron deficiency was first made over 40 years ago following the publication of a study reporting significant associations between obesity, CRP and iron deficiency [190]. With the increasing incidence of overweight and obesity in virtually all parts of the world, there has been renewed interest in the area of iron status in the obese. More recently a number of studies have reported an association between obesity and iron deficiency or 'hypoferremia' in adults [190]. A recent systematic review reported a tendency for lower transferrin saturation and higher ferritin concentrations in obese populations consistent with the inflammation hypothesis in which hepcidin plays a central role [197].

As obesity is known to be a state of chronic low-grade inflammation, a mechanistic link between inflammation in obesity and hypoferremia is plausible. In obese populations circulating hepcidin concentrations are reported to be higher than in non-obese subjects [198]. The underpinning mechanisms that lead to higher hepcidin levels in obesity are likely to be driven by higher concentrations of IL-6 [199]. One in vitro study involving leptin, which is markedly increased in obesity, suggests that leptin also may stimulate hepcidin expression [200]. Therefore iron deficiency adds further to the burden of obesity and complicate weight management. Iron deficiency has been associated with fatigue, depression and reduced exercise capacity and this may impact negatively on the efficacy of behavioural weight management programmes aimed at increasing physical activity and improving motivation and psychological well-being [201]. Restricted energy diets used for weight management may be low in iron, particularly for women during reproductive years where requirements are high.

In a cross-sectional survey in Moroccan adults, where iron deficiency is prevalent, biomarkers of inflammation were linked significantly with serum ferritin concentrations and with body mass index (BMI). The prevalence of iron deficiency was underestimated by not adjusting for serum ferritin concentrations, and the difference increased with increasing adiposity. This suggests that in LMIC where the double burden of disease is increasing, markers of inflammation should be used to correct biomarkers of iron status even if infectious or parasitic diseases are no longer widespread [202]. Aderibigbe et al. [203] undertook a review of the literature with the aim of elucidating the link between iron status and adiposity in women in LMIC. They showed that the studies had inconsistent outcomes, and factors such as infection, alcohol consumption, dietary intake and genetics were significant confounding factors.

In obese young women in Australia, where anaemia (haemoglobin <120 g/L) and iron deficiency (serum ferritin <15.0 μg/L) are less prevalent than those in LMIC, BMI was shown to be a significant predictor of serum iron, transferrin saturation and CRP. When the study participants were investigated based on their BMI, those with BMI ≥ 35 had significantly higher CRP than those in lower BMI categories but with no apparent effect on hepcidin. The authors concluded that obesity per se was not sufficient to induce clinically significant disturbances to iron metabolism, possibly due to the lack of co-morbidity in this cohort [204].

A relatively recently published review volume on nutritional anaemia gives considerably expanded information from a largely public health perspective [167]. Iron deficiency anaemia has been re-recognized as an important cause of cognitive deficit in this age group [205], including in the very recent and potentially influential *Lancet* series on early child development [206]. Iron deficiency also has a profound effect on productivity and hence has economic implications for countries in which it is a significant public health problem [207, 208] with physical work capacity being reduced even in moderate anaemia [209, 210].

The greater understanding of factors in the control and prevention of nutritional anaemias for public health interventions are still being evaluated but are likely to be important. For example, if populations have high levels of infection, then they will also have high levels of hepcidin—this may then block the uptake of iron that is in the diet from fortification and supplementation [211]. However, hepcidin has not been linked to any effect on dietary haem uptake, thus lending support for promotion of animal-source foods in poor diets [212]. Therefore it is becoming more apparent that treatment strategies encompass all the health concerns of a population—nutritional anaemia can only be completely addressed if other diseases are concurrently treated.

Selenium

Selenium plays a pivotal role in maintaining the functions of the immune and antioxidant systems as well as affecting the networks of genes that are central to anti- and pro-inflammatory mediators [213]. Low selenium status is associated with increased risk of mortality and poor immune function, with demonstrated adverse effects on immune cells during activation, differentiation and proliferation [214]. This is related to increased oxidative stress, but additional functions such as protein folding and calcium flux may be impaired also in immune cells under deficiency conditions [214].

Animal models and human studies with supplementation have been shown to enhance immune competence and resistance to viral infections. However, while the influence of selenium on immune responses is generally to enhance them, it may not always be beneficial, e.g. on antiparasitic responses or allergic asthma suggest the levels of selenium may affect different types of immunity [215]. While micronutrients can influence the ability of the host to respond to a viral infection [216], the virus itself may respond to the nutritional status of the host [217]. For example, a deficiency of selenium influences the expression of mRNA for the chemokine monocyte chemo-attractant protein-1, which may contribute to the development of myo-carditis in the selenium-deficient host [217]. In selenium deficiency, benign strains of Coxsackie and influenza viruses can mutate to highly pathogenic strains, and increasing selenium intake or supplementation alone or in combination with other micronutrients, may improve outcomes in patients infected with HIV and/or TB. Selenium promotes the acute cellular immune response [218]. Several trials assessed the effects of multi-micronutrient supplements, which also contained selenium in small doses but there was only low-level evidence in support of a beneficial effect on mortality in patients with tuberculosis, but little or no effect on mortality in those with TB and HIV. Plasma levels of selenium are improved by supplementation during the early stages of tuberculosis treatment, but a consistent benefit on tuberculosis outcomes has not been demonstrated [219]. Supplementation with Selenium beyond the upper tolerable limit can impinge on immune cell

function, with some types of inflammation and immunity particularly affected [214]. A crucial factor that needs to be emphasized is the U-shaped link with status in that selenium supplementation may benefit people with low status, those with high status might be affected adversely [220].

Iodine

The public health importance of iodine deficiency is that it is the most common cause of preventable intellectual impairment in the world. It is important in terms of women's reproductive outcomes and probably infant mortality. The fact that infants born to mothers who are iodine deficient are likely to suffer impaired intellectual impairment, even when there may be no clinical manifestations of cretinism- the most extreme manifestation, makes this extremely important both in community terms but also for national economic development. An estimate of iodine deficiency, or those suffering from iodine deficiency disorders as assessed by goitre prevalence, was estimated globally at around 740 million in 1998 [43]. More reliably (with urinary iodine the global prevalence is around 35.2 % (or 1,988,700,000 people) [221]. It is unlikely to have an impact on immune status and so will not be further considered in this chapter.

Other Micronutrients of Less Current Public Health Significance

Deficiencies of vitamins A, C and D, the B group of vitamins, especially B6, B12, riboflavin and folate, have all been associated with poorer health outcomes, although the pathways are not all clearly established and likely do not all work through impaired immunity [10, 205]. Besides vitamin A, iron and zinc, in public health terms, there are other micronutrients that are important through having either low or deficient status in certain population groups, such as pregnant and/or lactating women, and the elderly. In this section, the roles of these micronutrients on immune response are briefly discussed. Allen [222] has identified, in addition, riboflavin, vitamins B6 and B12, calcium, and depending on local variations in inadequate diets of poorer populations, β-carotene, folate and vitamin C. Similarly, Zimmermann [223] notes that single micronutrient deficiencies do not occur in isolation and that overlapping deficiencies affect more than 50 % of children and women in many LMIC.

The framework used here of micronutrients only of current public health interest is used to justify, in an already very brief background, why micronutrients such as niacin, thiamin and calcium are not addressed more explicitly. Historically and even now in certain geographic areas, these are, or have been of important public health interest. Niacin was widespread in many maize-consuming areas, including the south of the USA in the early nineteenth century but the deficiency disease *pellagra*, is considerably less often seen because of widespread fortification of flour with niacin amongst other B vitamins and iron [224, 225]. Thiamin deficiency, expressed as the disease *beriberi* was widespread throughout rice-eating populations [226] and is again now less often seen in rice-consuming populations due to generally improved diets (so that there are other sources of thiamin in the diet) or by fortification, as in Japan. However the contribution of thiamin deficiency to Wernicke–Korsakoff's syndrome in alcoholics means it is being addressed in a public health manner (in this case fortification of flour) in Australia. The report of the FAO/WHO meeting in Bangkok in the late 1990s gives useful information on other micronutrients [226], especially those now of somewhat more historical interest. Many of the B vitamins continue to be of interest as fortificant pre-mixes being added to flour (both wheat and maize) along with iron, and sometimes other fortificants such as zinc and vitamin A [227, 228]. Fortification is discussed in more detail below.

Vitamin D

Vitamin D is known to be essential to immune function [229, 230]. Relatively little is known about vitamin D status in equatorial populations but a recent study in Tanzania showed that hypovitaminosis D is common among pulmonary tuberculosis patients (and is not explained by the acute phase response) [230]. At the turn of the last century, ultraviolet light was successfully used to treat TB of the skin but it has only been in the last decade or so, that the understanding of vitamin D beyond its role as a determinant of mineral metabolism and rachitic bone disease, skeletal homeostasis and prevalent bone disorders such as osteoporosis, has emerged [231]. It is now clear that vitamin D has an important anti-infective role, involved in the production of defensins and cathelicidin (antimicrobial peptides) [232] and the induction of antimicrobial peptides and autophagy in cells of the monotype/macrophage lineage [233].

Calcitriol, or 1,25-dihydroxyvitamin D3, is well known as an endocrine regulator of calcium homeostasis. It is now known that local calcitriol production by immune cells also exerts autocrine or paracrine immunomodulating effects. Immune cells that produce calcitriol express the vitamin D receptor (VDR) and the enzymes needed to metabolize vitamin D3 (1 alpha-, 25- and 24-hydroxylases). These immunomodulatory effects may explain the reported epidemiological associations between vitamin D status and a large number of autoimmune and inflammatory diseases such as rheumatoid arthritis, lupus, inflammatory bowel disease, and Type 1 DM, as well as infections, malignancies, transplant rejection and cardiovascular disease [234]. Induction of the vitamin D-activating enzyme CYP27B1 in monocytes via pathogen recognizing receptors has highlighted an entirely new function for vitamin D as a potent inducer of antibacterial innate immune responses [235]. Vitamin D deficiency may affect Th17 responses and microvascular function, which may protect against IL-17-mediated inflammation and vascular dysfunction [236].

Vitamin D levels are independently and inversely associated with IL-6 in older populations suggesting a potential anti-inflammatory role for the vitamin [237]. Treatment with high dose vitamin D3 reduces CD4+ T-cell activation, a clear human example of influence of cell-mediated immunity [238], confirming the potential role of vitamin D in chronic inflammation. However, different inflammatory biomarkers have been shown to be differently associated with vitamin D with beneficial effects of increasing 25(OH)D for fibrinogen and WBC. In contrast, the U-shaped association between vitamin D and CRP indicates that increased vitamin concentrations may also be related to pro-inflammatory states [239].

Impaired vitamin D status is common to many populations across the globe with associations with chronic health problems including autoimmune and cardiovascular diseases, hypertension and common cancers [231]. Adequate vitamin D status now appears to be protective against a variety of conditions: musculoskeletal disorders (muscle weakness, falls, fractures), infectious diseases, autoimmune diseases, cardiovascular disease, Type 1 and Type 2 DM, several types of cancer, neurocognitive dysfunction and mental illness, and other diseases, as well as infertility and adverse pregnancy and birth outcomes. Vitamin D deficiency/insufficiency is associated with all-cause mortality [240]. The long-term effects of low vitamin D status remain somewhat unclear but increasingly of the opinion that optimization of vitamin D status in otherwise healthy individuals may potentially have lasting beneficial impacts on the immune system [241]. Optimal vitamin D levels and appropriate dosing schedules have yet to be determined [232] and guidelines for supplementation are urgently needed.

Folate

Folate (or its most common supplemental form of folic acid) has come to prominence recently as the flour in the USA, and now other countries, is being fortified with folic acid. Folate is required for DNA synthesis and so its deficiency is clinically expressed in tissues with high rates of cell turnover.

The principal sign is megaloblastic anaemia. However, its current public health importance is as a cause of anaemia, a cause of neurological tube defects (NTDs) and a possible role in cardiovascular disease. The recent fortification with folic acid in an increasing number of countries [228] is to prevent NTD. The public health importance of folic acid in fortified cereals has increased and has had a dramatic effect on the incidence of NTDs. At current fortificant levels, there is emerging some concern around unintended effects, e.g. some cancers. There is increasing awareness of the public health importance of folate deficiency in immune function, including its association with impaired cell-mediated immunity. Blood folate status and the expression of over 60 proteins that are involved in immune function, inflammation, and coagulation are both affected in deficiency. In response to long-term synthetic folic acid supplementation the protein response can be categorized into metabolic pathways related to complement fixation (e.g. C1, C3, C4, Factor H, Factor 1, Factor B, clusterin), coagulation (e.g. antithrombin, alpha-1-antitrypsin, kininogen) and mineral transport (e.g. transthyretin, haptoglobin, ceruloplasmin) [242]. Low folate status is associated with lower levels of proteins involved in activation and regulation of immune function and coagulation [242].

Folate deficiency has been associated with reduced cell-mediated immunity by reducing the proportion of circulating T-lymphocytes and their proliferation in response to mitogen activation [243]. Folate deficiency has been demonstrated to be associated with increased ratio of CD4+ to CD8+ T-lymphocytes due to decreased CD8+ T-lymphocytes proliferation, and which was reversible by in vitro addition of folate [244]. It has been suggested that the reduction in CD8+ cell replication in folate deficiency may be related to the finding of an increased carcinogenesis due to reduced cytotoxic activity [244]. Studies among post-menopausal women aged 50–70 years with diets low in folate showed an increased NK cell activity following folate supplementation [245]. All these findings indicate that folate deficiency is associated with impaired Th1 response.

Vitamin B12 and Other B Vitamins

Studies on vitamin B12 deficiency and immune response are limited. In patients with vitamin B12 deficiency (with pernicious anaemia or post-gastrectomy megaloblastic anaemia) a significant decrease was found in the number of lymphocytes and CD+ T-cells and a reduction in the proportion of CD4+ T-cells. Further, there was an abnormally high CD4+/CD8+ ratio and reduced NK cell activity [246]. Following treatment with methylcobalamin, CD8+ T-cells were restored and NK cells activity improved [246]. In an elderly population with low serum vitamin B12 concentrations, a reduction in antibody response to pneumococcal polysaccharide vaccine was observed suggesting an impaired synthesis of specific immunoglobulins [247].

Possible mechanisms that link obesity/visceral fat to DM and cardiovascular complications include inflammation and increased oxidative stress. Measures of plasma antioxidant vitamins status, markers of oxidative damage (malondialdehyde (MDA) and protein carbonyls), and inflammation (CRP, IL6 and TNF alpha) are part of an increased effort to find appropriate biomarkers, including of vitamin B12 itself, and its physiological activity [16]. Using these measures, antioxidants supplementation with B-group vitamins enhances antioxidant capacity, and may have an anti-inflammatory effect on obese diabetic patients [248].

A variety of inflammatory disease conditions have been found to be associated with low levels of plasma pyridoxal 5′-phosphate (PLP), the active form of *vitamin B6*. The inverse association between plasma PLP and inflammation may be the result of mobilization of this coenzyme to the site of inflammation, for use by the PLP-dependent enzymes of the kynurenine pathway of tryptophan degradation, metabolism of the immunomodulatory sphingolipids, ceramide and sphingosine 1-phosphate, and for serine hydroxymethylase for immune cell proliferation [249]. Vitamin B6 (pyridoxine) deficiency in humans has been found to be associated with reduced lymphocyte maturation, growth and proliferation, impaired NK activity, decrease in pro-inflammatory cytokines IL-1-β, IL-2, IL-2

receptors, and a decreased antibody response of DTH [250]. Thus vitamin B6 is associated with suppressed Th1 response and increased Th2 response, which is reversed following repletion of the vitamin [251].

Other Vitamins

In animal models, *vitamin C* (ascorbic acid) deficiency has been associated with decreased neutrophil function and impaired delayed cutaneous hypersensitivity [252, 253], decreased T-cell proliferation and abnormal complement concentrations [254]. In humans, vitamin C deficiency was associated with decreased DTH response to several antigens, which could be reversed by high dose supplementation [255]. Administration of vitamin C in humans has been described as resulting in improvement of anti-microbiocidal and NK cell activities [256]. Supplementation of vitamin C has been found to enhance neutrophil chemotaxis in adult healthy volunteers [257], and an increase in the proliferative response of T-lymphocytes to PHA and concanavalin A in the elderly [258]. Thus vitamin C deficiency in humans can impair leukocyte functions, and decrease overall NK cell activity and lymphocyte proliferation. A study looking at associations between circulating ascorbic acid, alpha-tocopherol, 25-hydroxyvitamin D and plasma cytokine concentrations in young adults concluded that alpha-tocopherol (vitamin E), but not ascorbic acid or 25(OH)D, is inversely associated with inflammation in healthy young adults [259]. One review, however, found that one of the consequences of vitamin C deficiency is impaired resistance to various pathogens, whereas an enhanced supply increases antibody activity and infection resistance [260].

Vitamin E is a fat-soluble vitamin important for normal function of the immune system. In the few rare cases of vitamin E deficiency in humans, impaired T-cell function and DTH response were observed [261, 262]. Dietary vitamin E may play a protective role a protective role in the development of allergic sensitization [263]. Supplementation of vitamin E in healthy adults showed a significantly increased T-cell proliferation in response to PHA, an improved CD4+/CD8+ ratio and decreased parameters of oxidative stress [264]. In general, the elderly are at a greater risk for lower vitamin E intake. A review by Meydani et al. [265] presented a comprehensive coverage of the role of vitamin E and immunity in humans, especially in the elderly. Vitamin E supplementation above currently recommended levels has been shown to improve immune functions in the aged including DTH skin response, increased mitogen-stimulated lymphocyte proliferation and increased production of IL-2, enhanced NK cell cytotoxic activity, and increased phagocytic activity by macrophages [265, 266]. Antibody production in response to vaccination was shown to be significantly associated with the nutritional status of vitamin E, which was mediated through increased production of IL-2, leading to enhanced proliferation of T-cells [267]. Thus higher vitamin E intake is associated with enhanced Th1 response and decreased Th2 response. Besides its protective role as an antioxidant, the possible mechanism for the improved immune function due to vitamin E supplementation is because of the reduced production of the T-cells suppressive factors, such as PGE_2 by macrophages [265]. Low vitamin E concentration and vitamin E have both been associated with obesity, and in a further link to non-communicable diseases, low concentrations of zinc, vitamins A and E in children who were overweight and obese were associated with lipids, inflammation and insulin resistance [268].

Vitamin K derivatives attenuate T-cell-mediated immunity by inhibiting the proliferative response and inducing apoptosis in activated cells [269]. Data from the Framingham study show that vitamin K status is inversely associated with concentrations of inflammatory markers, including CRP, suggesting a possible protective role for vitamin K [270].

To briefly summarize, the above section shows how little some understandings have changed; however, it is the mechanisms and the understanding of the incredible complexity of micronutrients, immunity and inflammation that has expanded so dramatically in the last decade or so. But in 1968, Scrimshaw et al. [10] concluded that vitamin A is regularly synergistic with infection; vitamin D deficiency commonly fails to show evidence of an interaction but synergism has been demonstrated;

deficiencies of the vitamin B-complex and some individual B vitamins behave variably, sometimes showing synergism and at other times antagonism, depending on species, the agent and host; vitamin C deficiencies are usually synergistic, but antagonism has been demonstrated; and finally, lack of minerals may result in either synergism or antagonism, depending on agent, host and species [10].

Impact on Infectious Diseases

The evidence for the impact of protein-energy undernutrition on immune status, in humans, has been stronger than in micronutrients but the evidence, and complexity, continues to expand. The causal line between undernutrition, including micronutrient deficiencies, to impaired immunity and then to increased incidence and/or severity of diseases leading further to a cycle of poor intakes leading to poor nutrition and so on, can continue until death or resolution. However, the direct evidence of the actual mechanisms linking micronutrient deficiencies to increased disease is sometimes less clear, e.g. the inadequacy of vitamin A and subsequent diseases, especially with respiratory tract infection [31]. Some of the stronger evidence comes from the role of micronutrient deficiencies in ageing and infectious disease. Of course, ageing is itself associated with impaired regulation of the immune system contributing to a higher incidence of morbidity and mortality from infectious, inflammatory, autoimmune and neoplastic diseases [229]. Subtle subclinical deficiencies of micronutrients such as zinc, selenium and vitamin E and inadequate macronutrient intake contribute to the decline in immune functions in the elderly [229]. Nevertheless, there is considerable more evidence in the last 10 years on the role of the many and complex roles of micronutrients in disease, inflammation and immunity. There is, for example, the much-increased understanding around vitamin D [6] and the associations of micronutrient deficiencies and metabolic syndrome signs and symptoms, even in (overweight) children [268].

What are the public health impacts of addressing compromised immune function through improving micronutrient status? The first section showed that the public health impact of micronutrient deficiencies extends far beyond their impact on infectious diseases, therefore it is important, when addressing micronutrient deficiencies, to go beyond a medical model. Micronutrient deficiencies affect both intellectual development and potential, and as has been graphically demonstrated in the recent *Lancet* nutrition series in early 2008 [2] and 2013 [3] and elsewhere, including also impacts on individual earning capacity and the economic development of whole countries [208]. Similarly the correction of the deficiencies needs to be far more than supplementation, especially in terms of sustainability.

The challenges of getting expensive foods, the ones that are usually higher in iron and zinc and preformed vitamin A, into the diets of the poor are discussed below. Both the problem and the solutions need to be perceived broadly. The challenge is bigger than just impaired immunity and inadequate diets but is one of wider development and reduction of inequities. This must include broad solutions, not least the improvement of women's status and education and other opportunities for female children, adolescents and women [271]. It is encouraging, that child deaths under 5 years of age each year in poorer communities and countries has been reduced from over 12 million children in 1990 to 6.3 million in 2013, a drop of 49 % [272]. The average annual reduction has accelerated—in some countries it has tripled—but overall progress remained short of meeting the MDG global target of a two-thirds decrease in under-five mortality by 2015. Nevertheless, most of the nearly 17,000 child deaths a day are entirely preventable and existing limited programmes need to be scaled-up nationally [273] along with considerably greater efforts on improved water and sanitation measures [274].

Consequently the following section is about the prevention, control and treatment of micronutrient deficiencies rather than about treating diseases or infectious diseases control, and improving hygiene and sanitation measures that would be expected to have an impact on immune status. Another whole area that is not addressed is the increased immunity over time of children, and adults, continuously

exposed to disease and the likely compromising of this defence in affluent populations where exposure is usually delayed and reduced. In this context, a study with pregnant women in Indonesia showed that when they were supplemented with zinc or β-carotene (along with the routine iron and folic acid), the mothers having zinc in pregnancy had a better ability to produce IL-6, and those receiving β-carotene, produced less IFN-γ, independently of nutritional status or birthweight [275]. So the authors suggest that giving mothers improved antenatal nutrition might even have the unintended consequence of an increase in the incidence of allergy and atopy in their offspring. A further aspect is the possibility that vaccinations and immunization outcomes may be compromised if the child is inadequately nourished, e.g. vitamin A and triple antigen, as briefly discussed earlier.

Infectious disease and the inflammatory response also present a number of challenges to an individual's nutritional status. Not only can infection result in poor intake, but for many nutrients it affects the body's natural homeostatic processes. This latter effect can have implications both in terms of nutrient physiology and function as well as the ability to assess nutrient status [6]. These challenges are perhaps best exemplified by iron particularly in the context of malaria. Although a need exists for a clear determination of the relative risks versus benefits of the most prominent iron interventions strategies (i.e. supplements vs. multiple micronutrient powders vs. fortification), the increasing body of evidence suggests that at least in the context of malaria, interventions to improve iron nutrition appear to be safe and effective in conjunction with malaria prophylaxis [191, 192].

Prevention, Control and Treatment Interventions to Improve Micronutrient Status

The recognition of the magnitude of the prevalence and impact of micronutrient deficiencies, and the knowledge of the possibility of doing something about them on a large scale, has resulted in a series of international goals. A meeting in Ottawa in 1991 reviewed and recommended ways to reach these goals [276]. These built on experience gained over previous decades (since the early 1960s in the case of iron-fortified cereal flour and iodized salt). As more experience has been gained, and funding increased, these have been continuously refined, and expanded. However, there were no goals or targets for micronutrients in the MDGs (to be achieved by end 2015) and there is unlikely to be in the Sustainable Development Goals that will replace them. The prevention and control of micronutrient deficiencies has become a higher global priority over the last couple of decades, but the extent of the programmes and the level of funding remain vastly under-resourced. This section briefly examines the currently most commonly used interventions.

A suggested categorization of such interventions is seen in Table 30.2 and is broadly:

1. Food-based approaches, including dietary diversification, nutrition education and fortification of staple and value-added foods.
2. Supplementation with vitamin A capsules, iron-folic acid tablets and iodized oil with increasing interest in a multi-micronutrient supplements and weekly low-dose supplements.
3. Public health interventions such as immunization, adding vitamin A supplementation to other programmes such as national immunization days and child health days, promotion of breast-feeding, and treatment of infectious diseases.
4. Change in the possibilities that are available to people by modification of the political, socio-economic and physical environment. As with so much of public health, those most vulnerable are those who are poorest.

The important point about these different approaches is that they are complementary, and should be started in concert, as they may have different time-frames, and differing feasibility, depending on local circumstances. Behaviour change to improve the intake of micronutrients is an essential part of whatever method is being used; through communications, social and political facilitation, social

Table 30.2 Public health approaches to modifying micronutrient intake used in the prevention and control of micronutrient malnutrition

Food based
Dietary diversification
Home gardening
Nutrition education
Development of high micronutrient content varieties of staple foods ('bio-fortification')
Fortification
Staples, e.g. flour, noodles
Fats and oils, e.g. margarine, edible oils
Condiments, e.g. salt, sugar, soy sauce, fish sauce
Complementary foods for infants 6 months and older
Home-based fortification, e.g. 'sprinkles'
Beverages, e.g. fortified juices, condensed milk and other dairy products
Supplementation
National distribution to all preschool children
National immunization days
Through health system centres, including maternal and child health programmes, and routine treatment
Outreach, e.g. with E.P.I. and other programmes
Post-partum supplementation
'Life cycle' distribution to adolescents and young women through schools and factories
Home-based supplementation, e.g. 'foodlets'
Public health measures
Improved antenatal and obstetric care
Immunization
Appropriate prevention and control of diseases such as diarrhoea, respiratory tract infections and malaria
Promotion of exclusive breast-feeding
Appropriate complementary feeding
Water and sanitation measures
Appropriate birth spacing
Global equity corrections, poverty reduction and socio-political change
Increased availability and accessibility of micronutrient-rich foods
Improved health systems
Improved status and education of women

marketing, and nutrition education. The overall strategy is to reduce the size of the most vulnerable group (to the left of the curve in Fig. 30.2) by improving the coverage of the middle group by fortification, dietary diversification and reduction of the disease burden [277]. The most at-risk group is likely to continue to need supplementation for many years to come. The factors listed in Table 30.2 have all been shown, to a greater or lesser degree, to have an evidence-based impact on micronutrient deficiencies prevention and control programmes [278].

In the following section, the prevention, control and treatment of micronutrient deficiencies are described. Improving immune response is not directly addressed as it is presumed to be a function of improved micronutrient status where that is the cause of the impaired immune function and increased risk of infectious disease. Ways in which improving nutrition may reduce the negative impact of infections on growth by the following actions can be seen in Table 30.3 [279].

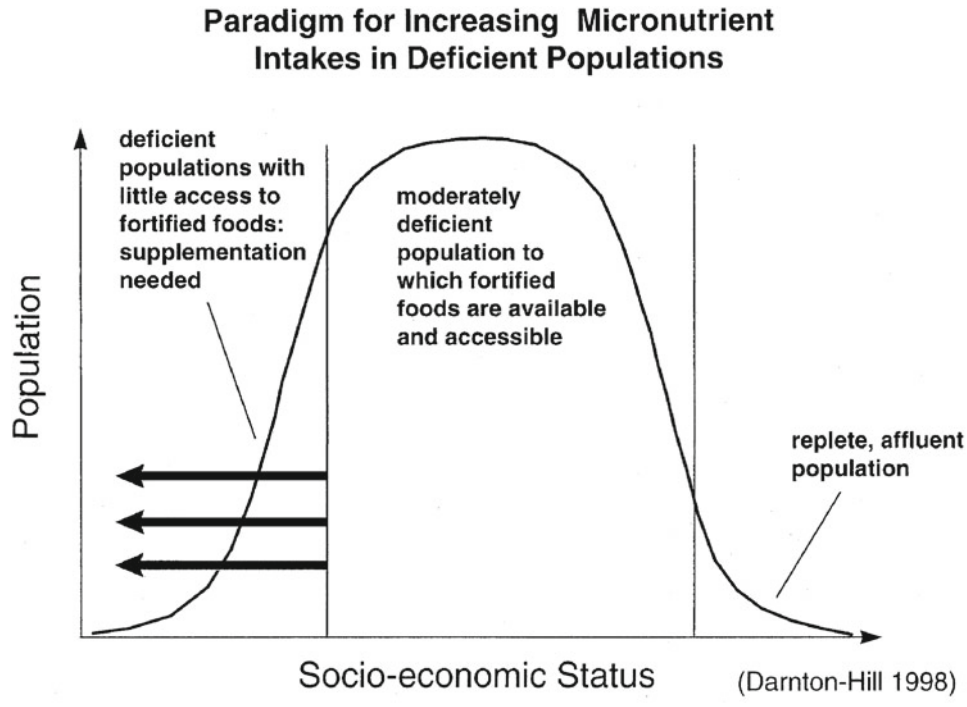

Fig. 30.2 Paradigm for increasing micronutrient intakes in populations by socio-economic status

Table 30.3 Improved nutrition may reduce the negative impact of infections on growth by the following actions

	Interventions
1	Strengthening the child's immune system, thereby reducing the severity and duration of infections and their impact on growth
2	Providing extra amounts of nutrients to compensate for those that are not well absorbed during infection, lost during diarrhoea, reallocated elsewhere in the body due to immune system activation or consumed in lower amounts than usual because of reduced appetite during infection
3	Providing the required amounts of nutrients for catch-up growth following infection, particularly the nutrients that are needed to build lean body tissue such as protein, potassium, magnesium, phosphorus, zinc and sodium
4	Preventing poor appetite caused by micronutrient deficiencies, thereby facilitating catch-up growth
5	Favouring the growth of beneficial bacteria in the gut that enhance gut function and immune defences

Adapted from [279]

Food-Based Approaches and Fortification

Dietary and Horticultural Interventions

With the exception of iodine in certain ecological settings, micronutrients are found abundantly in many plant foods and animal products. However, many families in resource-poor settings simply do not have enough to eat—over 800 million people according to FAO [4]. In the Indian sub-continent, nearly half of all women are categorized as underweight, e.g. Bangladesh [280]. But it is even more the quality of the diet, as diets characterized by poverty are less likely to include many micronutrient-rich foods

which are in any case generally more expensive and often less accessible, and so diets are likely to be low in vitamins and minerals, as well as energy [281]. This low accessibility to food sources is aggravated by the usually low bio-availability of micronutrients in the diets eaten by poor families, and it is poor dietary quality, rather than quantity, that is considered to be the key determinant of impaired micronutrient status [282]. In the current environment of high food price cycles, the accessibility of the poor to all foods is critically affected. The resulting shift to increased cereal staples such as rice, as other more micronutrient-rich animal-source foods becomes priced out of poor households' ability to purchase, the changes in household food expenditure patterns have a negative impact on the clinical vitamin A status of women of child-bearing age [283] amongst other micronutrients [284].

Food-based approaches have been categorized as (1) increasing small-scale production of micronutrient-rich foods, by community fruit and vegetable gardening, school gardening and/or small animal, poultry or fish production; (2) increasing community production of micronutrient-rich foods, such as horticultural products, oil seeds, palm oil, beverages and natural nutrient supplements; (3) maintaining micronutrient levels in commonly eaten foods with food storage and preservation techniques, improving food safety, and better food preparation; (4) plant breeding to increase micronutrient levels, including through genetic engineering and (5) community strategies to increase consumption of micronutrient-rich foods [212, 285].

Improving dietary diversification through increasing variety and frequency of micronutrient-rich food sources through nutrition education and horticultural approaches has been shown to be effective in many settings. Measuring effectiveness should use indicators of outcomes that go beyond increased serum levels of micronutrients, to clinical outcomes (reduction in night blindness) to social outcomes such as women's empowerment [286–288]. Food preparation interventions to achieve dietary diversification can include nutrition education concerning available foods and their more effective utilization; horticultural approaches such as home gardens; and improved methods of food preparation, preservation and cooking that better conserve the micronutrient content. There is increased interest in the genetic manipulation and breeding of staples and other foods to increase micronutrient content ('biofortification') [289–291].

While home gardening is a traditional family food production system widely practised in many LMIC [285, 292], anecdotal experience suggests home gardening (as an intervention method for improving nutrition) has been generally successful at the pilot or local phase, but often not been scaled up successfully. Recent experience in Bangladesh has demonstrated a successful example where it has, now reaching 800,000 families [292], and some of the lessons learned are being tried, with apparent good acceptance in Cambodia, Nepal [280], and parts of Africa such as Ethiopia [293]. An evaluation has shown that food gardening programmes also strengthened the capacity of local non-government organizations as a contribution towards sustainability of improvements in the community [294]. They have been found to increase income and empowerment of women and that can result in increased intake of micronutrient-rich foods such as eggs and meat as well as other foods such as oil, and improved caring practices [280, 287, 295]. Where home gardening is traditionally practised, using such an approach to increase micronutrient intake is more likely to be successful. In Indonesia, ownership of a home garden appears to indicate long-term vitamin A intake from plant foods, which explains its relationship with vitamin A status [296]. In the Bangladesh national survey, young children who had not received a vitamin A supplement were half as likely to be night blind if the family had a home garden [295].

Biofortification, also a food-based approach, uses traditional plant-breeding methods such as identifying plants that have cereal seeds naturally high in zinc or iron, or low in phytates, and then breeding for these, and more recently transgenic methods [289, 297]. Effectiveness of the resultant grains to raise micronutrient status in humans has been shown in one study to date of a successful feeding trial in the Philippines (using Catholic nuns to ensure adequate control conditions) [297]. The use of genetic engineering is expanding the possibilities, and a relatively recent alliance among the International Rice Research Institute (IRRI) and the International Maize and Wheat Improvement

Center (CIMMYT) has increased both efforts and coordination of research efforts on rice, wheat and maize aimed at 'improving the lives of poor farmers' [289, 290, 298]. Poor farmers are a group that has not much benefited from transgenic food research up to this point, which has mainly benefited horticulture for western markets, despite much of the rhetoric [291]. Probably the best known micronutrient example of this research approach, at least in terms of micronutrients, is the 'golden rice' where four different genes from the daffodil (*Narcissus pseudonarcissus*) and two from a bacterium (*Erwinia uredovora*) have been introduced to allow a non-biologically active precursor of beta-carotene, to proceed to the next three biological steps to become beta-carotene [290, 299]. However, it is not anticipated nutrigenetics will be a significant source of micronutrients in population terms within the next decade [291, 300].

Fortification

Probably the most cost-effective food-based approach to improving micronutrient availability and accessibility is fortification, with the proviso that the fortified foods must reach those who most need them. Not infrequently, those most at risk are outside established market systems that provide many of the 'value-added foods' most likely to be fortified. It has also not been shown that the fortification of staples will be able to provide adequate micronutrient content in most of the complementary foods given to young children (due to the small volumes involved), and so commercially processed and fortified foods will generally be necessary where available and accessible. There does appear to be increasing evidence that some animal sources in the diet are necessary for adequate micronutrient status [212, 294, 301]. Nevertheless, for the majority of many populations, fortification of foods with micronutrients has been shown to be a technologically, programmatically and economically effective method of increasing micronutrient intakes in populations [277]. Food fortification is likely to have played a significant role in current nutritional health and well-being of populations in industrialized countries [225]. Starting in the twentieth century, fortification was used to target specific health conditions: goitre with iodized salt; rickets with vitamin D fortified milk; beriberi, pellagra, and anaemia with B vitamins and iron enriched cereals; and more recently in the USA and other western countries, but also lately Pacific Island Nations and South Africa, risk of pregnancy affected by NTD by adding folic acid to fortified flour and cereals.

A relative lack of appropriate centrally processed food vehicles, less developed commercial markets, and relatively low consumer awareness and demand has meant that nearly 50 years have passed since its recognized successful impact in industrialized countries [225]. However, fortification is now increasingly seen as a viable option for the less developed and industrializing countries to increase micronutrient intakes [41, 302], including more recently in Africa [303]. As many of the previous constraints to widespread accessibility are minimized and with an increasingly global market, there is a great deal of current investment in fortification as an approach to the prevention and control of micronutrient malnutrition in LMIC [227, 302]. Fortification is but one arm of a micronutrient deficiency prevention and control strategy, but by becoming commercially viable, can reduce the size of the at risk population needing other measures such as supplementation (Fig. 30.1). Where the costs are passed onto the consumer, and the food industry routinely fortifies, sustainability is potentially high [304].

Globally 82 countries currently have legislation to mandate fortification at least one industrially milled cereal grain: 81 countries plus the Punjab province in Pakistan have legislation to fortify wheat flour; 12 countries have legislation to fortify maize products; and six countries have legislation to fortify rice [305].

A single micronutrient addition to an appropriate food vehicle is increasingly an uncommon approach in food fortification programmes, except iodine in salt and vitamin A in sugar. Even with iodine there is now considerable work in double fortification of salt with iodine and iron [41] and even triple fortification with vitamin A as well [306]. As Huffman et al. [307] and others have described,

women in LMIC often are consuming diets of poor bio-availability and limited micronutrient content, leading to concurrent deficiencies of iron, vitamin A, zinc, folic acid, B6, B12 and occasionally other vitamins and minerals [308–310]. Such deficiencies have important consequences for women's own health, pregnancy outcomes and their breast-fed children's health and nutritional status [307], and increasingly it seems on the birthweights of their children [310, 311]. Mason et al. [43] have estimated that nearly a quarter of children have multiple deficiencies. Consequently, it is now generally recommended that fortification be with a mixture of micronutrients, often in a pre-prepared fortificant mix of iron, folic acid and other B vitamins [227].

Supplement-type home fortification, e.g. 'Sprinkles', are microencapsulated micronutrients, including usually ferrous fumarate, which are available in a single dose sachet, and can be sprinkled onto complementary and weaning foods and other foods. In a randomized, controlled trial in Ghana, they were found to be as efficacious as iron drops in the treatment of anaemia [312], and have extensive efficacy experience such as those carried out in Bangladesh, Benin, Bolivia, Canadian First Nations and Inuit areas, China, Haiti, India, Nicaragua, Pakistan, Sri Lanka and Vietnam [313]. Although there was initially some concern about the levels of iron being given, these have now been reduced and there seems no doubt about their efficacy. Cure rates from anaemia have ranged from 55 to 90 % in children in the studies conducted [312, 313]. While the effectiveness applications need further demonstration, their use is already gaining considerable experience in the post-Tsunami disaster areas in South Asia [314] and non-emergency settings such as Mongolia [312].

In the more affluent industrialized countries, micronutrient deficiencies have been, and continue to be, addressed by food fortification, as well as by overall economic growth and general improvements in health, sanitation and nutrition that have contributed to the prevention and control of these deficiencies. These same aspects must be addressed in any prevention and control programmes in non-industrialized countries. Fortification, supplementation, other food-based approaches, and complementary public health measures are all necessary. This will only be done by partnerships with government, industry, and the consumer. There is a need to assess more widely the impact of interventions, not least for advocacy. Ultimately the success, impact, and sustainability of food fortification, like other interventions, rest with educating the consumer, developing consumer demand and demonstrating impact.

Supplementation

Supplementation has often been characterized as a short-term approach, criticized as an example of medicalization of a public health intervention, and presumed to have difficulty with likely sustainability, especially when supplements are supplied by foreign donors. Nevertheless, iron supplementation with folic acid, has been the method of choice to address anaemia in pregnant women despite little evidence of its effectiveness and likely limited impact [43, 210, 315], although efficacy has been repeatedly shown [205, 316]. Vitamin A supplementation has now been in place for over 40 years in countries such as Bangladesh and so hardly merits being seen as short-term, and many would argue that the need will be there for many years yet [31, 46, 317] although others are increasingly questioning this as the infectious diseases situation globally is so different now to what it was 35 years ago [43]. Consequently the effectiveness of vitamin A supplementation to preschool children to continue to reduce the risks of mortality and morbidity from some forms of diarrhoea, measles and malaria is thought to be decreasing, especially with the decline in measles rates. Nevertheless the observed effects in the earlier studies should be continued where vitamin A deficiency remains a serious public health issue. It is presumed these positive effects are the result of the actions of vitamin A on immunity [31]. Some of the immunomodulatory mechanisms of vitamin A have been described in clinical trials and can be correlated with clinical outcomes of supplementation, despite serum levels staying elevated for only a couple of months at most. The effects on morbidity from measles are related to

enhanced antibody production and lymphocyte proliferation. Benefits for severe diarrhoea could be attributable to the functions of vitamin A in sustaining the integrity of mucosal epithelia in the gut, whereas positive effects among HIV-infected children could be related to increased T-cell lymphopoiesis. The colostrum of women supplemented with retinyl palmitate has higher levels of SIgA, which suggests that the production of antibodies is modulated by vitamin A [318].

Zinc supplementation is now the recommended treatment for diarrhoea in children in LMIC [156], and while not used, at least as yet, in prevention, does reduce the risk of recurrent attacks of diarrhoea for some months after treatment. Since the release of the UNICEF-WHO Joint Statement on Clinical Management of Acute Diarrhea with new oral rehydration solutions and zinc, at least 54 countries have changed national child health policies to include zinc for treatment of diarrhoea. The experience of zinc treatment for diarrhoea has been instructive in terms of demand being generated before supply was assured. However the actual roll-out of zinc at country level has been slow for a number of reasons, including the need for changes to national policy and treatment guidelines, as well as adequate supply of zinc supplements.

Iron and folic acid supplementation has been the traditional approach for preventing and treating iron deficiency, particularly during pregnancy [168, 319] but logistics continue to be an issue, it is relatively expensive (for better quality iron/folic acid tablets that have far better compliance) and coverage is often poor [210]. Compliance is usually blamed but it is likely that distribution and logistical problems are every bit as important [320]. The efficacy of intermittent dosages, once or twice a week, has been demonstrated, suggesting that this may be a possibility for prevention, although not to treat anaemia in pregnancy [321]. However it does appear appropriate to recommend a dosage regimen of one or two times per week before pregnancy, e.g. to adolescents and young women in schools and factories [322]. It is presumed this approach would encourage compliance and reduce side effects and would certainly reduce costs [315, 316, 323]. Logistic constraints in many settings would still be a potential problem, although work in four Asian countries has shown promise with a social marketing approach [323]. With iron supplementation, gains in productivity and take-home pay have been shown to increase 10–30 % [318]. Consequently there are important reasons, in addition to the already compelling health, cognitive development and reproduction consequences, to accelerate programmes to prevent and control iron deficiency anaemia.

Nevertheless, there is increasing consensus that new approaches to scaling-up supplementation coverage are required [205, 210]. Anthelmintics treatment improved the haemoglobin and serum ferritin concentrations of Tanzanian schoolchildren [324], growth, appetite and anaemia [325] with similar positive synergies in other settings [326]. As the strategy for improving micronutrient status moves more towards integrated approaches as a way of helping to improving child survival [327, 328], reducing micronutrient deficiencies will be increasingly seen as an approach to increasing child survival and development in general.

Interactions are also a potential issue in other multiple micronutrient intervention settings. Most of the research to date has focused on the effect of single nutrient deficiencies on immune response and few studies have examined the simultaneous association of multiple nutrients, or their status, with immune function [6]. However, it is known that four micronutrients at least (vitamin A, vitamin D, zinc and folic acid) have specific points of convergence on the regulation of two major regulators of inflammation, NF-κB activity and the induction, and maintenance of Treg cells [6]. Up until now, much of the work on multimicronutrient supplementation has been in the relatively affluent elderly in western societies, with a considerable amount of self-medication as immunologic function, particularly cell-mediated immunity declines with age, this probably contributes to the increased incidence of infectious diseases in the elderly [14, 329]. High [329] has concluded that ‘multivitamin/mineral supplements or specific micronutrients such as zinc and vitamin E maybe of value … oversupplementation may be harmful’. Nevertheless women in North America, with generally micronutrient replete diets, are recommended to take multiple micronutrient supplements during pregnancy. On the hand, many women in less affluent economies survive on diets of poor quality and micronutrient

deficiencies are common in LMIC [309]. As these authors note, the ability of the newborn to maintain health, withstand disease, grow and develop normally is influenced by the gestational nutritional experience, and replacing likely deficient micronutrients would presumably correct these deficiencies. However it is not known exactly how such micronutrients interact in depleted/infected populations, or against habitually compromised diets [309] but positive evidence is accruing [278, 330].

An independent systematic review and meta-analysis of 12 randomized, controlled trials in LMIC, comparing multiple micronutrient supplementation with iron-folic acid supplementation found that both supplements were equally effective in reducing anaemia (even though iron content was often lower in the multimicronutrient supplement) and resulted in a small, significant increase in mean birthweight [330]. Following these findings, it was suggested that replacing iron-folic acid supplements with multiple micronutrients in the package of health care, including improved obstetric care of health and nutrition interventions, would improve the impact of supplementation on birthweight, small-for-gestational age neonates, and perhaps child growth and development [330]. Despite some initial concern in some settings of (non-significant) risk of increased neonatal mortality (not found in other reviews), the conclusion to recommend antenatal multiple micronutrients was subsequently endorsed by the second *Lancet Series on Maternal and Child Nutrition* following further evidence supporting the approach [3]. Trials are underway in a number of countries at present. A meeting to review nutrition as a preventive strategy against adverse pregnancy outcomes concluded that effective interventions with micronutrients are likely to be required at an earlier stage than happens in public health programmes at the present time, certainly before mid-pregnancy, and for some interventions, probably during the pre-conceptual period [331]. There is already an existing WHO/WFP/UNICEF joint statement on preventing and controlling micronutrient deficiencies in populations affected by emergencies [332], which includes both women and young children, but not for prevention in non-emergency settings.

Control, Prevention and Treatment of Infectious Diseases by Strengthening Immunity

Once an infection is established, a host can do one of three things to minimize the agent's impact on its health. The immune system of the host can directly attack the growing pathogen population to contain or eliminate it (*resistance*); or it can attempt to minimize the harm caused by a given number of pathogens by increasing tissue repair or by detoxifying pathogen by-products (*tolerance*); or some combination of both [333]. Traditionally, it is suggested that immunologists, microbiologists and parasitologists have focussed on the ability to limit parasite numbers or on the overall ability to maintain health irrespective of parasite burden (resistance plus tolerance), with less emphasis on just tolerance although there are good examples of that such as the presence of α-thalassaemia and the resulting reduced life-threatening episodes of malaria [333].

With micronutrient deficiencies, the line between prevention, treatment and control is often blurred except in serious deficiency, e.g. xerophthalmia and serious anaemia (Hb < 7 g/dL). Children with any stage of xerophthalmia should be treated with vitamin A according to WHO treatment guidelines, as should pregnant women with such life-threatening levels of anaemia. But, in a population with say 50 % prevalence of a particular deficiency and the resultant clinical outcomes, such as may occur with anaemia in pregnant women in resource-poor settings, is giving iron a treatment or prevention [334], especially in the case of the relatively recent recommendation by WHO of weekly preventive iron and folic acid supplementation [335]. In the majority of the programmes, it is clear that to address impaired immunity, integrated programmes that also address food security, care, the health services, community measures and the water, sanitation and hygiene environment will all be necessary [273].

As there are standard guidances for treatment of the clinical outcomes of micronutrient deficiencies and these are usually well tried and efficacious, they will not be discussed further. WHO is the technical agency of the United Nations system that takes a normative role in developing these and coordinating available research information on an evidence-base and the consensus of experts and are available through their electronic library of recommendations. The BOND Initiative (Biomarkers of Nutrition and Development) is assisting WHO and others in updating six of the micronutrients of public health concern [16]. The prevention of micronutrient deficiencies, although undoubtedly efficacious, remains challenging in terms of effectiveness, especially in hard-to-reach populations.

Improving the Immunological Status and Resistance to Disease Through Related Public Health Interventions

Despite the recognized bidirectional interactions between nutrition and immunity [336], it is clear that while important, improving the nutritional status, and micronutrient adequacy, will alone not be enough to improve immunological status of an individual. Most children at risk of increased risk of undernutrition and early child death from infectious diseases live in unhealthy and deprived environments. The World Bank increased the definition of people living in poverty as those with under US$1.25 a day, and so in 2005, 1.4 billion people were defined as living in poverty (a quarter of LMIC)—although an improvement on the 1.9 billion in 1981. It is hard for most people not living in such conditions to have any idea of what this means in terms of inadequate food security, impossibly unhygienic conditions and increased risk of maternal and child death. Five years ago, over 80 % of all children stunted lived in just 20 countries [337] and 90 % of the global burden of under 5 mortality is borne by 36 countries [2, 3]. Consequently there is a need to address other issues as well, quite apart from the need to reduce inequities both within and between countries. Also there will need to be a scaling-up of water and sanitation measures, infectious disease prevention and treatment, improved measures to improve household food security and nutrition security and a reduction of parasite infections, as well as social measures. *The Lancet* has recently reported the estimate that a tenth of the global disease burden could be addressed by properly tackling water and sanitation issues [338], almost certainly now considered a conservative figure [274].

For maximum impact other public health interventions are essential. These integrated interventions include, amongst other locally appropriate actions, control of infectious diseases, expansion of measles and other childhood immunization interventions, deworming for intestinal parasites (hookworms), malaria control, promotion of breast-feeding, and proper health care such as oral rehydration therapy, all of which have an impact on micronutrient status [326, 339, 340], and hence, in many cases, immune status. Multiple factors are associated with immune response to vaccines administered during childhood including the timing of antigen exposure, age, concurrent infections, undernutrition, particularly micronutrient deficiencies of vitamin A, iron and zinc [341]. Breast-feeding is identified in the Bellagio child survival reports as the most important intervention providing 13 % of the total impact on potential young lives saved [2, 3]. An earlier study from rural Ghana showed epidemiological evidence of a causal association between early breast-feeding and reduced infection-specific neonatal mortality [342]. Vitamin A and iron supplementation of pregnant Indonesian women benefited the vitamin A status of their infants, but still, the authors concluded in that study, the infants may need vitamin A supplementation or increased dietary intake after 6 months [343]. Human milk ascorbic acid levels can be doubled or tripled by increased intake of ascorbic acid in women with low human milk ascorbic acid content with the impact far more evident in African women compared with European women [344].

The International Food and Nutrition Policy Research Institute (IFPRI) has identified the four main factors contributing to infant and child undernutrition: food accessibility and availability; mother's

education; women's status relative to men in the society; and the health and sanitation environment [271]. Bendich [345] has demonstrated the roles of nutrients in optimizing women's health and immune function, as well as other roles of micronutrients in women's health [216]. While critical, the actual interventions are outside the scope of this chapter but there is increased recognition that parallel, vertical programmes are no longer enough (although the funding community finds them easier to manage); but as it is the same families and communities that need investment in all these areas, and usually the same inadequate health systems trying to support them, and a lack of nutrition capacity, there are increasing attempts to recognize and implement these realities on the ground.

Among the major remaining constraints, as the recent re-analysis of the Child Survival approach has reminded the international health community, are amongst other things, poor health systems and inadequate resources [2, 3, 327]. They demonstrate convincingly that it is not that cost-effective interventions for both child survival and development and young child undernutrition are not known about, but that they are not being implemented on a sufficient scale [327, 337, 340]. Largely based on this series, but drawing on years of often poorly documented experience, international agencies, with national governments, are directing their efforts more towards an integrated approach: reduction of the diseases of childhood, reduction of the vaccine-preventable diseases, neonatal causes of death, undernutrition, water and sanitation-related disease and national policy making to strengthen health systems and coordinate funding through the various political and policy mechanisms.

Micronutrient deficiencies prevention and control, largely through mechanisms discussed in this chapter, will need to be scaled-up to be a more important part of overall public health approaches in resource-poor settings [346]. Noting that potential investments appear under-resourced, Behrman and colleagues have also noted the high rates of benefit-to-cost ratios and that the 'gains appear to be particularly large for reducing micronutrient deficiencies in populations in which prevalences are high' [347].

Conclusion

Micronutrient deficiencies are a recognized problem in as many as a third of people living in LMIC and in disadvantaged sub-populations in more affluent countries. The impact of such deficiencies, and more uncommonly excesses, on immune status and disease incidence, growth, development and survival is now well appreciated although some of the underlying mechanisms are still not clear. What is often less appreciated is the impact of micronutrient deficiencies on immunological status. As has been described, there is good evidence that vitamin A, vitamin D, other vitamins, and iron and zinc and other mineral and trace element deficiencies all impact on the incidence and prevalence of infectious diseases through an impairment of the immune system resulting from these same deficiencies.

At the same time, there are many environmental, societal and cultural reasons, all aggravated by poverty, that are increasing the risk of these same populations of a heightened risk of contracting diseases. As in undernutrition in general, there is a vicious cycle that is set-up with micronutrient deficiencies further reducing resistance to infection with increased disease and further reduced appetite and absorption which then re-enforces the underlying micronutrient deficiency. Because of the broader environmental determinants of increased disease through undernutrition and impaired immunity, the interventions need to be broad ranging across health, nutrition, environment, water and sanitation and reduction of social inequities. The relatively recent recognition of the role of inflammation in overweight and obesity and associations between many non-communicable diseases and impairment of immunity, adds another dimension of complexity to addressing these issues.

The efficacy of micronutrient prevention and control, and treatment, is well established. The big challenge is effective national or sub-national intervention programmes being adequately scaled-up. Transition from vertical to more integrated programmes, and getting donor support for these, are a

current major challenges. As is frequently quoted, the cost-effectiveness of most micronutrient interventions continues to need advocacy to policy makers; overall, it has been estimated by the World Bank that for 'less than 0.3 % of their GDP, nutrient deficient countries could rid themselves of these entirely preventable diseases, which now cost them more than 5 % of the GDP in lost lives, disability and productivity'. Given the comparative success of many of the micronutrient deficiency prevention and control programmes in many parts of the world, because of the known interventions, the challenge is now to scale-up such programmes to a more comprehensive national level. This would achieve results in improving the survival and development of children and women, through improving immunity and reducing micronutrient deficiencies in integrated, community-based programmes supported by adequate resources at district, national and international levels.

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