



# Nutrition, infection and stunting: the roles of deficiencies of individual nutrients and foods, and of inflammation, as determinants of reduced linear growth of children

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## Abstract

The regulation of linear growth by nutritional and inflammatory influences is examined in terms of growth-plate endochondral ossification, in order to better understand stunted growth in children. Linear growth is controlled by complex genetic, physiological, and nutrient-sensitive endocrine/paracrine/autocrine mediated molecular signalling mechanisms, possibly including sleep adequacy through its influence on growth hormone secretion. Inflammation, which accompanies most infections and environmental enteric dysfunction, inhibits endochondral ossification through the action of mediators including proinflammatory cytokines, the activin A-follistatin system, glucocorticoids and fibroblast growth factor 21 (FGF21). In animal models linear growth is particularly sensitive to dietary protein as well as Zn intake, which act through insulin, insulin-like growth factor-1 (IGF-1) and its binding proteins, triiodothyronine, amino acids and  $Zn^{2+}$  to stimulate growth-plate protein and proteoglycan synthesis and cell cycle progression, actions which are blocked by corticosteroids and inflammatory cytokines. Observational human studies indicate stunting to be associated with nutritionally poor, mainly plant-based diets. Intervention studies provide some support for deficiencies of energy, protein, Zn and iodine and for multiple micronutrient deficiencies, at least during pregnancy. Of the animal-source foods, only milk has been specifically and repeatedly shown to exert an important influence on linear growth in both undernourished and well-nourished children. However, inflammation, caused by infections, environmental enteric dysfunction, which may be widespread in the absence of clean water, adequate sanitation and hygiene (WASH), and endogenous inflammation associated with excess adiposity, in each case contributes to stunting, and may explain why nutritional interventions are often unsuccessful. Current interventions to reduce stunting are targeting WASH as well as nutrition.

**Key words:** Endochondral ossification: Protein: Micronutrients: Zinc: Iodine: Milk: Environmental enteric dysfunction

## Introduction

The stature of human adults reflects individual genotype and those environmental factors which influence child linear growth and limit the phenotypic expression of the genotype. Tanner identified growth as a 'mirror of the conditions of society', especially the 'nutritional and hygienic status' of the population<sup>(1)</sup>. In Victorian Britain, the industrial revolution and urbanisation of the population had detrimental effects on child growth, reducing adult height to the extent that a 1904 government report<sup>(2)</sup> identified 'urban poverty leading to insufficient food and malnutrition' as a main cause of 'ill health, poor physical and mental performance and a general deterioration of the race'. In fact, this report is credited with the subsequent introduction of free school meals and other benefits for poor children and their families. The result was an increase in adult height in British men

born between 1900 and 1946 of about 1.25 cm/decade, a secular trend in height which continued in those born between 1946 and 1960 at 0.6 cm/decade<sup>(1)</sup>. The most recent study of adult height changes in the century between the 1896 and 1996 birth cohorts indicate increases ranging from 20.2 cm in South Korean women to little or no change in adult height in some sub-Saharan African countries and in South Asia, and a current gap of 22–23 cm in men and 20 cm in women between the tallest and shortest adult populations<sup>(3)</sup>. This is an indication that throughout the developing world today the 'nutritional and hygienic status' is preventing normal growth.

Poor growth in children is currently defined as inadequate height, weight and weight for height, in relation to growth standards, currently those defined by the WHO<sup>(4)</sup>. Stunting, underweight and wasting describe a height-for-age (HA), weight-for-age (WA) and weight-for-height (WH)  $\geq 2$  SD below

**Abbreviations:** 1,25(OH)2D, 1,25-dihydroxyvitamin D; ASF, animal-source food; CRP, C-reactive protein; FGF, fibroblast growth factor; GH, growth hormone; HA, height-for-age; IGF, insulin-like growth factor; IGF1BP, insulin-like growth factor binding protein; LNS, lipid-based nutrient supplement; mTORC1, mammalian target of rapamycin complex 1; PTH, parathyroid hormone; PTHrP, parathyroid-hormone-related protein; QPM, quality protein maize; T3, triiodothyronine; T4, thyroxine; TGF, transforming growth factor; TSH, thyroid-stimulating hormone; WA, weight-for-age; WASH, clean water, adequate sanitation and hygiene; WH, weight-for-height; ZD, Zn deficient.

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the median of the relevant standard with severe stunting or wasting at  $\geq 3$  SD below the standards. In practice, HA Z score or WH Z score are calculated: the differences between the observed values and the growth standards as a fraction or multiple of the SD of the mean values of the standards. Because this SD increases with age, the absolute HA difference (cm) has been suggested to be more appropriate in terms of identifying the time course of stunting and appropriate periods for intervention<sup>(5)</sup>. Thus the levelling off of the HA Z score deficit seen after 24 months in multiregional analyses of stunting up to 5 years is not seen if HA difference deficits are examined<sup>(5)</sup>. The argument has been made that in reality there are not distinct populations of normal stunted or severely stunted children but rather a gradation of growth faltering. Thus in India the entire length-for-age Z score/HA Z score distribution is shifted to the left (compared with the WHO standards), indicating that all children, and not only those falling below a specific cut-off, are affected by some degree of growth faltering<sup>(6)</sup>.

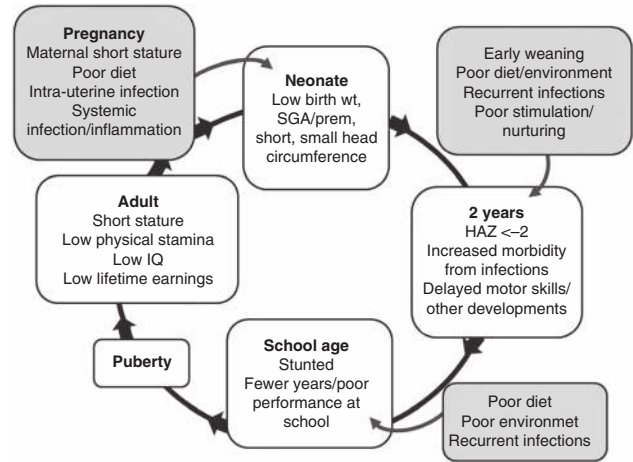
### A stunting syndrome

Globally more children are stunted than wasted, with prevalence rates and burdens in 2013 of 25% or 161 million stunted preschool children compared with 8% or 51 million wasted<sup>(6-8)</sup>. Prevalence rates are particularly high in sub-Saharan Africa and South Asia but some 7% or 5 million of children in the developed world are also stunted.

Stunting can begin *in utero* and intra-uterine growth faltering is a greater problem than previously believed; for example, in India stunting rates are about 20% at birth, and this may account for about half of growth faltering in Indian children under 5 years of age. After birth average HA Z score among infants in deprived populations continues to decline to a greater or lesser extent in all regions until about 24 months of age, and given the well-documented rapid brain growth in the first 2 years, this early period is also critical for long-term neurodevelopment<sup>(6)</sup>. Thus, emphasis on the first 1000d as a crucial period for intervention is based not only on the magnitude of faltering but also on its long-term impact on adult human capital. After 24 months of age on the basis of HA differences, growth continues to falter in poor environments with no indication of a levelling off<sup>(5)</sup>, so that global stunting at 5 years comprises 11.2% *in utero*, 60.6% between birth and 2 years and 28% between 2 and 5 years. Substantial height catch-up can occur between 24 months and mid-childhood and again between mid-childhood and adulthood, even in the absence of any interventions in some populations as a result of an extended pubertal growth phase especially in adolescent girls<sup>(9)</sup>, indicating that adolescence represents an additional window of opportunity during which substantial life-cycle and intergenerational effects can be accrued.

The complexity of the interactions between environmental adverse influences which impair linear growth has been recently reviewed in terms of a 'stunting syndrome'<sup>(10)</sup> and a somewhat simplified version of this is shown in Fig. 1<sup>(5,6,10-15)</sup>.

As for the details of the nutritional deficiencies and of the infectious and inflammatory insults which inhibit height growth, the pathogenesis underlying linear growth failure is said to be surprisingly poorly understood<sup>(10)</sup>. In the present review the



**Fig. 1.** The stunting syndrome. Modified and simplified from Prendergast & Humphrey<sup>(10)</sup>. The shaded boxes indicate adverse influences while the unshaded boxes indicate outcomes. Stunting is identified as a cyclical process, often starting *in utero*, connecting maternal nutrition to an intergenerational cycle of growth failure transmitted across generations through the mother<sup>(11)</sup>. High rates are apparent for at least the first 2 years of postnatal life, hence the identification of the critical window of the first 1000d<sup>(6)</sup> but growth continues to falter in poor environments with no indication of a levelling off<sup>(5)</sup>, resulting in school children and adults of short stature. Mothers with short stature and especially teenagers are more likely to have low-birth-weight babies who are subsequently more likely to have growth failure during childhood<sup>(12)</sup>. Potential mechanisms explaining intergenerational effects on linear growth are shared genetic characteristics, epigenetic effects, programming of metabolic changes, the mechanics of a reduced space for fetal growth<sup>(6)</sup> and sociocultural factors such as the intergenerational transmission of poverty and deprivation<sup>(13)</sup>. Multiple environmental influences contribute to impaired growth including poor maternal and child nutrition throughout the cycle, inadequate breast-feeding and inappropriate complementary feeding<sup>(14)</sup> together with infectious and inflammatory insults<sup>(15)</sup>. These interactions are mutually reinforcing through infection exacerbating any malnutrition, because of appetite suppression and reduced food intake, and any malabsorption reducing nutrient intake, while malnutrition reduces immune defence systems, thereby worsening the adverse influence of infections. The multiple pathological changes marked by linear growth retardation in early life are associated with increased morbidity and mortality, reduced physical, neurodevelopmental and economic capacity and an elevated risk of metabolic disease into adulthood<sup>(10)</sup>. IQ, intelligence quotient; SGA, small for gestational age; prem, prematurity; HAZ, height-for-age Z score.

intention is to attempt to examine the importance of both nutritional deficiencies, especially energy, protein, Zn and iodine, and infectious-inflammatory insults in the context of what might be described as a first-principles model of linear growth regulation.

### Physiology, cellular biology and endocrinology of linear bone-growth regulation

The growth potential of an individual in height and overall shape, mainly a function of bone growth, is genetically determined and each individual will follow a growth curve canalised in terms of both extent and time course if conditions are favourable<sup>(16)</sup>. In the context of the present review favourable conditions include a diet which can exert an appropriate regulatory anabolic drive on growth<sup>(17,18)</sup> and provide necessary substrates, in an environment which presents minimal inflammatory challenges. While mechanical loading can influence bone density, length growth is relatively insensitive to physiological dynamic loading, although direct compressive stress on the growth plate inhibits growth<sup>(19)</sup>.

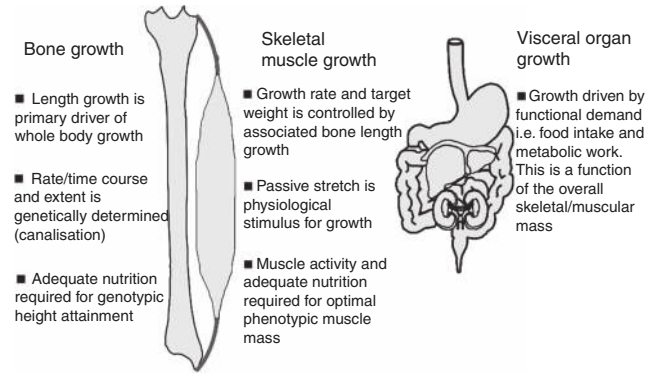
One widely quoted model<sup>(20)</sup> involves three additive and partly superimposed phases of postnatal linear-growth from birth to maturity, i.e. infancy, childhood and puberty (the ICP model). When interrupted by malnutrition or infection there is usually some period of catch-up growth in WH<sup>(21–23)</sup> and in HA<sup>(24)</sup>, i.e. a self-correcting response returning the growth pattern to the individual growth channel.

The nature of the genetic programming is quite complex. Genetic factors are often estimated to account for 80% of the variation in height and genome-wide association studies in adults of recent European origin indicate hundreds of SNP variants clustered in genomic loci and biological pathways that affect human height<sup>(25)</sup>. Nevertheless, these identified variants explain only about 10% of the phenotypic variance, with unidentified common variants of similar effect sizes possibly increasing the overall influence to about 20% of heritable variation. The genetic programming of the time courses of linear-growth, especially in relation to events in the growth plate which mediate the slowing down of the initial very rapid fetal, early infancy growth phase with eventual cessation of linear growth after puberty, is particularly complex<sup>(26)</sup>, as are the mechanisms which link linear growth to the growth of other organs and tissues. The progressive decline in cellular proliferation throughout the organism may result from a programmed down-regulation of a large set of growth-promoting genes<sup>(26)</sup>.

However, non-genetic mechanisms also exist to coordinate growth throughout the organism. A protein-stat model of growth control accounts for the accumulation of protein-containing structures, in which dietary protein provides key regulatory and permissive (substrate) roles<sup>(27)</sup>. Within this model, the overall metabolic demand for amino acids for lean tissue growth is linked to dietary intake through an aminostatic appetite mechanism which enables protein intake to meet the demand. Regulation of the demand involves a mechanism in which amino acid intake exerts a largely endocrine-mediated anabolic drive on the growth plate of the long bones which in turn, through passive stretching, activates growth of associated muscles at the level of muscle connective tissue synthesis and myofibrillar protein deposition. This ensures that skeletal muscle growth occurs at a rate and time course which ensures sufficient muscle mass and strength to allow development of body function with increasing bone length and associated height, and the protein deposition in muscle signals increases in appetite. Thus the genetically programmed postnatal height-growth pattern is enabled by sufficient and appropriate dietary protein and other key nutrients, which initiate the endocrine-mediated anabolic drive on the growth plate of the long bones and permit linked muscle growth. This hierarchical scheme is shown in Fig. 2<sup>(27)</sup>.

### Endochondral ossification

The target of the anabolic drive for linear growth of bones, which directly influence height (limb bones, ribs and vertebrae), is endochondral ossification within the growth plate<sup>(28–32)</sup>. This starts with the differentiation cascade initiated by stem cell clonal expansion as proliferative chondrocytes, followed by hypertrophy, cartilage matrix secretion and apoptosis which



**Fig. 2.** Physiological coordination of whole-body growth. The hierarchy of height-growth control involves bone length growth, regulating skeletal muscle growth, which influences whole-body energy expenditure, consequent food intake and the size of the visceral organ mass. See Millward<sup>(27)</sup> for details, including the molecular basis of the muscle growth response to passive stretch by bone.

releases angiogenic factors that stimulate vascular invasion and migration of osteoblasts and osteoclasts, leading to remodelling of calcified cartilage and formation of trabecular bone. Synchronously, the diameter of the long bone diaphysis increases by osteoblastic deposition of cortical bone beneath the periosteum, and the marrow cavity expands as a consequence of osteoclastic bone resorption at the endosteal surface. This ordered process mediates linear growth until adulthood. Progression of endochondral ossification and linear growth is regulated by the combined influence of those systemic endocrine and local paracrine/autocrine anabolic influences<sup>(32)</sup>, acting together with small molecules which include specific amino acids such as leucine<sup>(33)</sup>, and Zn<sup>2+</sup>, which mediate a signalling cascade via a variety of pathways. Linear growth continues until the growth plates fuse during puberty, but bone mineralisation and consolidation of bone mass continues until peak bone mass is achieved during the third to fourth decade. Considerable progress has been made in unravelling the marked complexity of the regulation of the growth plate<sup>(29)</sup>. Here the main features are briefly summarised.

### Endocrine regulation

The endocrine signalling of the dietary anabolic drive which acts on the growth plate includes insulin and the growth hormone (GH)–insulin-like growth factor (IGF)-1 axis, a mixed endocrine paracrine–autocrine system, the thyroxine/triiodothyronine (T<sub>4</sub>/T<sub>3</sub>) axis, androgens, oestrogens, vitamin D, glucocorticoids, and possibly leptin<sup>(34,35)</sup>, making for an extremely complex system which is by no means understood and will only be briefly reviewed here. As far as the insulin–GH–IGF-1 axis is concerned, the Karlberg ICP (infancy, childhood and puberty) model of an insulin/IGF-1-driven fetal infancy phase and a GH/IGF-1-driven childhood phase<sup>(20)</sup> may need reassessment, with several authors arguing that GH has an important role in fetal growth<sup>(36,37)</sup>. It would appear that: (1) GH can be produced within the fetus both from the pituitary and from other fetal tissues<sup>(36,37)</sup> and derives to a limited extent from the placenta<sup>(38)</sup>, the source of most maternal GH during pregnancy<sup>(38,39)</sup>; (2) there is a positive correlation

between placental GH levels in pregnant women and the birth weight of their offspring<sup>(39–41)</sup>; (3) a functional GH receptor is widespread in the human fetus at all stages of development<sup>(36)</sup>, including on growth plate chondrocytes<sup>(42,43)</sup>; and (4) GH increases IGF-1 gene expression in human fetal epiphyseal chondrocytes when conditioned by 1,25-dihydroxyvitamin D<sub>3</sub> (1,25(OH)<sub>2</sub>D<sub>3</sub>)<sup>(43)</sup>.

In the postnatal growth plate, insulin, GH and IGF may have overlapping functions. There can be cross-talk between IGF-1 and insulin at the receptor level<sup>(44)</sup> and post-receptor signalling is identical<sup>(45,46)</sup>, involving the Ras/mitogen-activated protein kinase pathway which signals cell proliferation and the PKB/Akt kinase and mammalian target of rapamycin complex 1 (mTORC1) pathway which stimulates cell growth. Also, although the GH receptor is distinctive, some of the GH Janus activated kinase-2 (JAK2)-initiated signalling is similar to insulin/IGF-1 post-receptor signalling<sup>(47,48)</sup>. Because the GH receptor is found on chondrocytes of all zones of the growth plate<sup>(49)</sup>, GH action is more complex than simply activating expression of IGF-1, bone morphogenetic protein and other genes in resting-zone chondrocytes through the JAK/STAT pathway<sup>(50)</sup>. Also IGF-1, like GH, can stimulate proliferation of resting-zone chondrocytes and chondrocyte hypertrophy<sup>(34)</sup>. For IGF-1, both its endocrine action through its mainly hepatic-derived circulating form and its paracrine–autocrine action contributes to postnatal growth<sup>(51,52)</sup>. However, the distinctive feature of IGF-1 action is its regulation through the IGF-binding proteins (IGFBP) 1–6<sup>(53)</sup>. These have a variety of functions, with IGFBP-1, -2, -4 and -6 mainly inhibitory by preventing receptor binding. Also IGFBP-1, which is increased in plasma of protein-deficient rats<sup>(54)</sup>, increases IGF-1 clearance from the plasma and subsequent catabolism<sup>(55)</sup>. IGFBP-3 can protect IGF-1 by maintaining a circulating ternary complex with IGF-1 and the acid-labile subunit (ALS), a circulating hepatic-derived glycoprotein. ALS functions to prolong the half-life of the IGF-1, IGFBP-3 (or IGFBP-5) binary complexes<sup>(56)</sup>. IGFBP-5 can enhance IGF-1 activity, including induction of chondrocyte proliferation<sup>(57)</sup>. Both IGFBP-3 and -5 can be made by chondrocytes under IGF-1 control<sup>(57)</sup>. Interestingly, in the context of a role for Zn in the growth plate, IGFBP-3 possesses a metal-binding domain<sup>(58)</sup>, and Zn<sup>2+</sup> influences binding of IGF to cell surface-associated IGFBP-3 and -5, possibly affecting IGF bioavailability<sup>(59)</sup>.

As for thyroid hormones, the thyroid-stimulating hormone (TSH) receptor is expressed in growth plate cartilage and cultured chondrocytes, consistent with evidence of its action to inhibit chondrocyte proliferation<sup>(31)</sup>. T<sub>3</sub> is widely involved<sup>(31)</sup>. Its indirect effects involve enhancing the direct effects of IGF-1 on cartilage and influencing the levels of GH-binding protein, IGF-1, IGF-2, IGFBP-3 and IGF bioactivity<sup>(60)</sup>. It also acts through thyroid hormone receptor (TR) $\alpha$ 1, one of three T<sub>3</sub> nuclear receptors (the other two TR $\alpha$ 2 and TR $\alpha$ 3 acting as antagonists) to inhibit proliferation and stimulate pre-hypertrophic and hypertrophic chondrocyte differentiation. In addition, it has a number of non-genomic actions involving various signal transduction pathways<sup>(31)</sup>. Thus, overall thyroid hormone is essential for coordinated progression of endochondral ossification, acting to stimulate genes that control

chondrocyte maturation and cartilage matrix synthesis, mineralisation and degradation<sup>(31)</sup>.

Both androgens and oestrogen contribute to the pubertal growth spurt<sup>(34)</sup>. Androgens can stimulate longitudinal bone growth acting directly on growth plate chondrocytes and indirectly by increased local IGF-1 expression<sup>(34)</sup>. Androgens also act in boys after aromatisation to oestrogens which signal through the ER- $\alpha$  receptor to mediate the growth spurt directly and, like androgens, through stimulation of the GH–IGF-1 axis. Eventually with skeletal maturation and epiphyseal fusion, the proliferative capacity of the growth plate chondrocytes is exhausted<sup>(34,35)</sup>.

Finally, vitamin D is involved in endochondral ossification, although this is poorly understood in terms of its direct as opposed to indirect influence, and its influence may be subtle. Although chondrocytes contain both the vitamin D receptor (at least in hypertrophic but not proliferating chondrocytes<sup>(61)</sup>) and can produce 1,25(OH)<sub>2</sub>D, the major skeletal manifestations of vitamin D deficiency, rickets and osteomalacia, can be corrected by increasing the intestinal absorption of Ca and phosphate, indicating the importance of indirect effects<sup>(62)</sup>. However, direct effects are observed in the human fetal epiphyseal growth plate with 1,25(OH)<sub>2</sub>D, like T<sub>3</sub>, a potent inhibitor of chondrocyte proliferation but enhancing chondrocyte differentiation in terms of stimulating expression of several GH–IGF axis genes such as IGF-1, IGFBP-3 and GHR<sup>(43)</sup>. Vitamin D also participates with parathyroid hormone (PTH) in a functional paracrine feedback loop in the growth plate between 1,25(OH)<sub>2</sub>D and PTH-related protein (PTHrP). Thus 1,25(OH)<sub>2</sub>D decreases PTHrP production, while PTHrP increases chondrocyte sensitivity to 1,25(OH)<sub>2</sub>D by increasing vitamin D receptor production<sup>(63)</sup>.

The main inhibitory endocrine influence is cortisol, which is increased in energy deficiency and inflammation and its action is discussed below (see the Inflammation and endochondral ossification section).

### Paracrine signalling within the growth plate

The linkage between nutritionally mediated endocrine changes in insulin, GH/IGF-1, T<sub>3</sub> androgens and oestrogens, as described above, and endochondral ossification is complex and not entirely understood. A brief summary of those key factors involved is reported here<sup>(28,29,31,64)</sup>. Chondrogenesis is initiated by one of the bone morphogenetic protein (BMP) family of signalling molecules, together with the SOX series of transcription factors. Chondrocyte proliferation is directly stimulated by Indian hedgehog, which ensures that sufficient chondrocyte proliferation occurs by increasing PTHrP signalling, which in turn inhibits chondrocyte hypertrophic differentiation. Wntless/integrated protein signalling promotes hypertrophic differentiation, whereas fibroblast growth factors (FGF) FGF18 and 19, acting through FGFR3, inhibit chondrocyte proliferation and differentiation and stimulate their apoptosis. Epidermal growth factor, vascular endothelial growth factor and transforming growth factor (TGF)- $\beta$  also play a part, most probably with additive effects as with the additive effects of IGF-1 and TGF- $\beta$ 1 observed on human chondrocytes in culture<sup>(65)</sup>.



One of the principal apparent functions of the endocrine system is to allow rapid growth only when the organism is able to consume abundant and appropriate nutrients. Thus any GH-driven linear growth can only occur when GH can induce IGF-1 and the various other paracrine growth factors as a result of the appropriate dietary signalling from key nutrients, such as amino acids and Zn, to increase growth factor levels. This anabolic drive is apparent in the growing rat in which growth and protein synthesis rates in muscle and bone reflect circulating levels of insulin, IGF-1 and free  $T_3$ , in turn a function of dietary protein intakes<sup>(66–71)</sup>, and especially leucine which enhances insulin secretion from islet  $\beta$ -cells as well as having direct tissue effects (see Millward<sup>(33)</sup>). However, in children, length growth is very much slower and saltatory (discontinuous) with 90–95% of infant development growth-free<sup>(72)</sup>, and the physiology and endocrinology of this saltatory growth is by no means understood. What is known is that GH secretion, which is pulsatile during the day and night and extremely variable on a day-to-day basis, does appear to relate to height growth velocity, in that synchrony between successive changes in height and GH output is associated with increased stature in healthy children<sup>(73)</sup>. Furthermore, in infants each saltation appears to follow periods of increased sleep<sup>(74)</sup>, and because from early childhood the onset of sleep is a robust stimulus for GH secretion<sup>(75)</sup>, this may link sleep quality to length growth. In this case low-quality sleep of children in a poor environment could be part of the aetiology of stunting.

#### *Direct anabolic influences of amino acids and zinc*

The extent and nature of direct anabolic influences of amino acids or Zn on endochondral ossification are poorly understood. What is known is that chondrocyte development in cell culture is sensitive to amino acid levels<sup>(76)</sup> and that in a wide variety of cell types, the major pathway through which amino acids influence cell growth and protein synthesis is as obligatory upstream regulators of the mTORC1 signalling pathway, with leucine as the main effector<sup>(33,77,78)</sup>. Given that mTORC1 signalling has been reported to promote osteoblast differentiation from pre-osteoblasts in mouse primary calvarial cells<sup>(79)</sup>, it can be expected that similar signal-transduction pathways operate in chondrocytes. Dietary leucine is not likely to be limited by poor protein quality since it occurs in high concentrations in most dietary proteins including cereals<sup>(33)</sup> and its signalling role may simply indicate intake of protein in general. In this context it is the case, as discussed below, that plasma leucine levels are particularly low in stunted children in Malawi<sup>(80)</sup>.

A role for Zn within the growth plate would be expected given that up to 10% of human genes code for proteins with Zn-binding domains<sup>(81)</sup>, with >100  $Zn^{2+}$ -dependent enzymes and >2000  $Zn^{2+}$ -dependent known transcription factors<sup>(82)</sup>. Labile  $Zn^{2+}$  in extra- and intracellular compartments exerts a wide range of influences on cellular functions with an imbalance in  $Zn^{2+}$  homeostasis linked to dysfunction<sup>(81,83)</sup>, and a role in growth-related signalling is emerging involving those transporters which mediate intracellular Zn trafficking.

Of the two families of Zn transporters which either reduce (ZnT 1–10) or increase (ZIP 1–14) intracellular Zn, transporter-mediated Zn signalling roles appear to involve the ZIP family<sup>(84–87)</sup>. Thus an up-regulation of ZIP8 in human osteoarthritis cartilage increases chondrocyte intracellular  $Zn^{2+}$  levels, activating the metal-regulatory transcription factor 1 (MTF1) which increases matrix-degrading enzymes<sup>(85)</sup>. ZIP13 may be important for bone BMP and TGF- $\beta$  signalling during chondrocyte maturation on the basis of ZIP13 knockout mice studies and a ZIP13 loss of function mutation in human Ehlers–Danlos syndrome which is associated with short stature<sup>(86)</sup>. ZIP14 controls G-protein-coupled receptor (GPCR)-mediated signalling and ZIP14 knock-out mice exhibit growth retardation, attributable to disrupted GPCR signalling in the growth plate and elsewhere<sup>(87)</sup>. As indicated above, the metal-binding domain of IGFBP-3<sup>(58)</sup> allows  $Zn^{2+}$  to influence binding of IGF to cell surface-associated IGFBP-3 and consequent IGF bioavailability<sup>(59)</sup>. Taken together, these examples show that the availability of  $Zn^{2+}$  could influence the regulation of growth in diverse ways.

#### *Inflammation and endochondral ossification*

Inflammation, which occurs as part of many chronic disease conditions<sup>(88)</sup>, and to a greater or lesser extent in response to infective insults by bacterial pathogens or immunogenic macromolecules either ingested or translocated across a compromised gut mucosa, is associated with increased and sustained production of pro-inflammatory cytokines, chemokines, adhesion molecules, eicosanoids, NO, oxygen radicals, FGF21<sup>(89,90)</sup> as well as the activin A–follistatin system<sup>(91,92)</sup>. This is usually coupled to both GH and insulin resistance and a reduction in circulating IGF-1 and IGFBP-3<sup>(88,93)</sup>. Clinically, low-grade inflammation often results in increased circulating hepatic acute-phase reactants, C-reactive protein (CRP) and  $\alpha$ -1 acid glycoprotein, elevations in inflammatory markers in stool such as myeloperoxidase,  $\alpha$ -1 antitrypsin, and neopterin, and reduced circulating IGF-1 and IGFBP-3 (for example, Prendergast *et al.*<sup>(94)</sup> and DeBoer *et al.*<sup>(95)</sup>). In rat models these responses associated with insults such as endotoxaemia<sup>(96,97)</sup>, a parasitic infection<sup>(98)</sup> or elevated levels of glucocorticoid<sup>(99)</sup> induce a profound insulin resistance<sup>(96)</sup>, and markedly inhibit tissue protein synthesis and growth. Children suffering from specific inflammatory diseases such as inflammatory bowel disease, Crohn's disease, ulcerative colitis, and juvenile idiopathic arthritis, who exhibit physiologically elevated levels of glucocorticoids and proinflammatory cytokines, usually display abnormal growth patterns as well as delayed puberty<sup>(88,93)</sup>. Furthermore, linear growth can be increased in these patients by the inhibition of these cytokines<sup>(88,93)</sup>.

Key pro-inflammatory cytokines known to inhibit endochondral ossification include TNF $\alpha$ , IL-1 (particularly IL-1 $\beta$ ) and IL-6 (in some<sup>(100)</sup> but not all studies<sup>(88)</sup>). Each of these signals via its specific type I cytokine receptor which are structurally divergent from other cytokine receptor types. High concentrations of these cytokines suppress growth in a dose-dependent manner by decreasing chondrocyte proliferation and hypertrophy while increasing apoptosis<sup>(93)</sup>. TNF $\alpha$ , a potent mediator of the inflammatory action of the innate immune system,

is secreted mainly from activated macrophages, and by many other cell types in response to endotoxins<sup>(101)</sup>. It induces cytokine production, activation and/or expression of adhesion molecules, with additional functions linked with lipid metabolism, coagulation, insulin resistance and endothelial function, and is one of the most important and pleiotropic cytokines, mediating inflammatory and immune responses. TNF $\alpha$  is produced endogenously throughout the growth plate and can inhibit chondrocyte proliferation, especially in combination with IL-1 $\beta$ <sup>(102)</sup>. IL-1 $\beta$  expression is induced mainly in response to microbial molecules and it signals via both mitogen-activated protein kinases and the transcription factor NF- $\kappa$ B, thereby resulting in further pro-inflammatory cytokine expression. IL-1 $\beta$  induces rapid dedifferentiation of chondrocytes in culture<sup>(65)</sup> and acts in synergy with TNF $\alpha$  to inhibit rat longitudinal bone growth in culture<sup>(102)</sup>. IL-6 is a pleiotropic cytokine with a wide range of biological activities<sup>(103)</sup> involving haematopoiesis, B-cell maturation and T cell activation, differentiation and regulation, the secretion of acute-phase proteins by the liver, which it does in cooperation with IL-1 while inhibiting hepatic IGF-1 production. Most importantly, IL-6 has direct inhibitory effects on growth-plate chondrocytes<sup>(100,104)</sup>.

Activin A is released rapidly into the circulation during inflammation, in advance of TNF $\alpha$  or IL-6<sup>(91)</sup>. Activin A and its antagonist follistatin are members of the TGF- $\beta$  superfamily, deriving from a wide range of resting and activated immune and other cell types including bone cells, and modulating several aspects of the inflammatory response, including release of pro-inflammatory cytokines, NO production and immune cell activity<sup>(91)</sup>. Increased circulating levels of activin A occur in inflammatory conditions such as inflammatory bowel disease and rheumatoid arthritis and during bacterial septicaemia, hepatitis C infection, and trauma<sup>(92)</sup>. Less is known about the role of this system in the growth plate, but both activin A and follistatin are produced by human osteoblasts, and are involved in controlling the extent of mineralisation and the prevention of over-mineralisation of bone tissue<sup>(105,106)</sup>.

FGF21, one of the endocrine-like FGF which is also expressed by chondrocytes, is increased by inflammatory stimuli<sup>(107)</sup> and by undernutrition in mice<sup>(108)</sup>, and in humans in late-stage fasting<sup>(109)</sup> and sepsis<sup>(110)</sup>, and directly inhibits chondrocyte proliferation and differentiation as well as preventing GH-mediated stimulation of chondrocyte proliferation and differentiation<sup>(88,108,111)</sup>.

Inflammation is also accompanied by elevated levels of cortisol, which inhibits length growth directly in terms of chondrocyte proliferation, hypertrophy and cartilage matrix production<sup>(88,112)</sup> and by the blockade of anabolic growth factor signalling<sup>(34)</sup>. Children treated with corticosteroids for various reasons exhibit impaired growth<sup>(113)</sup>. In the rat, exogenous corticosterone at physiologically elevated plasma levels inhibits longitudinal bone growth within 24 h with total arrest after 4 d, at which time epiphyseal cartilage width had shrunk, proteoglycan synthesis was markedly suppressed but overall protein synthesis was unchanged<sup>(70,71)</sup>. One target appears to be apoptosis of proliferative growth-plate chondrocytes<sup>(114)</sup>, particularly activation of the caspase cascade triggering Bax-mediated mitochondrial apoptosis<sup>(115)</sup>, as well as suppression of the PKB/Akt kinase IGF-1 signalling pathway<sup>(114)</sup>.

Given that some or all of the proinflammatory cytokines and activin A–follistatin are produced within the growth plate<sup>(93,105,106)</sup>, the implication is that individually they may be involved in normal bone growth regulation with their inhibitory effect resulting from their combined effects and/or at high concentrations. The key question which remains is the profile and thresholds in the changes from normal to increased levels of these various mediators induced by low-grade inflammation, which does inhibit linear growth.

## Nutrition and linear growth

Nutrients can be classified according to their influence on linear-growth<sup>(116,117)</sup>. Type 1 nutrients, the majority group, have specific functions with easily identifiable deficiency symptoms, of which growth failure may or may not be included. Type 2, the smaller group, have ubiquitous functions and it is more difficult to detect deficiency symptoms apart from growth failure.

### Type 1 nutrients

Of the type 1 nutrients, iodine deficiency does influence linear growth, as discussed below. For Fe and vitamin A, intervention studies suggest that linear growth is not affected by their individual deficiency<sup>(118)</sup>. For the bone minerals, it is usually assumed that low Ca intakes are not responsible for stunting. In animal models, while Ca deficiency results in low bone mineralisation and reduced bone strength, it does not result in reduced bone length<sup>(119)</sup>. It has been suggested that slow growth rates of children in many developing countries may represent an adaptation to limited Ca supply and poor bioavailability even after adaptation of losses to match low intakes. However, where these low intakes occur, case reports indicate biochemical signs of hyperparathyroidism, low bone mineral contents, and rickets even in the presence of adequate vitamin D status<sup>(120)</sup>. Ca supplementation studies in developing countries mostly found no significant effects on linear growth retardation and studies of bone mineral acquisition in younger children reported no height effects<sup>(121)</sup>. Interestingly, Ca supplementation for 13 months in adolescents which did increase bone mineral content, increased height in boys but not girls<sup>(122)</sup>, possibly through an interaction between the sex hormone systems and GH/IGF<sup>(121)</sup>.

### Type 2 nutrients

Type 2 nutrients, of which linear growth inhibition is an immediate response to their deficiency, include protein (specifically N and indispensable amino acids), Zn, P and the main electrolytes K, Na and Mg. Of these, there is evidence that deficiencies of protein and Zn can occur in the human diet, especially for populations consuming diets based on starchy roots with little or no animal-source foods (ASF); their potential role in the nutritional aetiology of stunting is discussed below. There is some limited evidence for phosphate deficiency occurring through a dietary lack<sup>(23)</sup>, although phosphate deficiency is likely to be very rare. Indeed, average intakes of P and Mg from most diets are usually substantially greater than their biological requirements and, if not, increased absorption

and conservation of their endogenous losses may prevent an inadequate dietary supply contributing to the poor linear growth of Third World children<sup>(120)</sup>.

### Multiple nutrient deficiencies and growth

Limitations in the type 1–type 2 classification need to be recognised. First, some type 1 nutrients can influence growth in situations where the symptoms of their deficiency may be missed, one example being mild iodine deficiency<sup>(123–128)</sup>. Second, endochondral ossification is a complex system involving multiple hormonal and small molecule signalling systems responding to multiple dietary factors. This means that multiple type 1 and type 2 nutrient deficiencies may induce growth effects through interactions of their deficiencies which would only be identifiable by multiple nutrient supplementation studies. Finally the type 1/type 2 concept was developed in relation to postnatal growth and is probably less relevant to fetal growth where maternal deficiencies of many nutrients including those involved in cell division such as folate and vitamin B<sub>12</sub> can influence fetal development. In this context it is not surprising, as discussed below, that multiple micronutrient supplementations of pregnant women have been shown to be beneficial for birth outcomes including birth weight.

### Linear growth regulation as observed in animal models

Unlike the human diet in which multiple nutrient deficiencies occur, animal studies allow the role of individual nutrient deficiencies to be examined.

#### Protein and energy deficiency

Because animal growth is usually rapid, it is very sensitive to dietary protein intake and quality, reflecting a metabolic demand for protein almost entirely driven by deposition in lean tissue. The protein efficiency ratio (PER) rat growth assay became the industry-standard method of assessing dietary protein quality<sup>(129)</sup>, until it was recognised that the assay markedly overestimated the importance of protein quality in the human diet<sup>(130)</sup>. This means that while rat growth studies enable the mechanisms of the dietary activation of growth to be evaluated, they are much less likely to indicate how variation in dietary protein quality influences linear growth in children with a quite different metabolic demand<sup>(131)</sup>.

The concept of the anabolic drive<sup>(18)</sup> and the protein-stat model of dietary protein-mediated growth control<sup>(27)</sup> was developed from a detailed study of the nutritional and endocrinological regulation of muscle and bone length growth in the rat<sup>(66,67,69–71)</sup>. In response to protein deficiency, slowed tibial length growth was apparent by 3 d<sup>(69)</sup>, and this was graded according to the extent of dietary protein deficiency, ceasing with a near-protein-free diet. Tibial length growth was restored in a graded way with increasing dietary protein concentration over a wide range of protein intakes.

As for energy deficiency, while food restriction has long been known to inhibit linear growth<sup>(132)</sup>, the specific effects of energy

deficiency, independently from protein deficiency, are more difficult to evaluate. Energy restriction in young rats to 25 % of normal intakes or total starvation induced immediate weight loss but maintained tibial length growth over 4 d, albeit at reduced rates<sup>(70)</sup>, although corticosterone treatment inhibited tibial growth immediately. Progressive food restriction (75, 50 and 25 % *ad libitum*) of diets containing increasing concentrations of protein, to maintain constant protein intakes, arrested body-weight growth in all groups, with weight loss in the severely restricted rats, but allowed some tibial length growth to continue at all levels of energy restriction with only a slow development of inhibition. Taken together, these experiments strongly support a dietary control mechanism for linear growth in which protein intake is the most powerful macronutrient influence, acting to promote high circulating levels of insulin, IGF-1 and free T<sub>3</sub>, which together with amino acids act on the growth plate to optimise endochondral ossification at the level of protein and proteoglycan synthesis maintaining both ribosomal capacity (RNA:protein ratio) and activity (protein synthesis:RNA)<sup>(66,67,69,71)</sup>. The inhibition of length growth by energy deficiency or corticosteroids was dissociated from and preceded inhibition of protein synthesis and <sup>35</sup>S uptake, possibly reflecting an elevation of rates of proteolysis and proteoglycan degradation associated with elevated corticosterone levels.

#### Zinc deficiency

Notwithstanding the importance of Zn for pre- and postnatal development<sup>(133)</sup>, evaluating the mechanism of its actions in growth control has proved remarkably difficult. Weight and length growth inhibition is the immediate response to Zn deficiency in the rat and its influence on catch-up growth in infants on a high-energy soya-based formula, which slowed unless additional Zn was added, resulted in the analogy of Zn with an essential amino acid<sup>(134)</sup>, given the large number of Zn<sup>2+</sup>-dependent enzymes and transcription factors in tissues. Although some very limited initial muscle growth occurs in the severely Zn-deficient (ZD) rat, a normal Zn concentration is maintained, expanding the muscle Zn pool, by drawing on Zn mobilised from bone<sup>(135,136)</sup>. However, the key problem of animal studies of ZD is to allow for the characteristic inhibition of appetite, i.e. cyclic changes in food intake and associated body weight<sup>(135,137)</sup>. Careful pair-feeding studies indicated that ZD specifically inhibited body weight and muscle growth<sup>(135)</sup>, through both a corticosteroid-induced blockade due to anorexia and reduced food intake, and an impaired insulin response to food intake when appetite returns, with insufficient insulin to fully activate the translational phase of protein synthesis<sup>(138)</sup>. This indicates a Zn deficiency-induced impairment of the anabolic drive at a key initial step of insulin production. However, in terms of linear growth, while 28 d of a very-low-Zn (ZD) diet<sup>(136)</sup> induced marked differences between ZD and pair-fed animals in bone Zn and bone histology, the reductions in femur weight and length, in circulating IGF-1, and in thicknesses of both the overall growth plate and of the hypertrophic cartilage, differed little between ZD and pair-fed animals. When food intake and serum IGF-1 levels were maintained in ZD rats

given megestrol acetate, a synthetic progestin used clinically to correct anorexia, growth inhibition persisted, a relatively unambiguous demonstration of the inhibition of the anabolic signalling by IGF-1 with Zn deficiency<sup>(139)</sup>. Subsequent studies with ZD rats<sup>(140)</sup> demonstrated the need for Zn in the IGF-1 mediation of cell division in cultured 3T3 cells, possibly at the level of a specific step in Ca-dependent mitogenic signal transduction through the IGF-1 receptor protein kinase C (PKC) pathway<sup>(141)</sup>, PKC being a Zn metalloenzyme. It remains to be seen whether similar Zn effects explain Zn's role in regulating endochondral ossification, and/or whether low growth-plate Zn<sup>2+</sup> levels decrease chondrocyte IGF bioavailability through impaired surface-associated IGFBP-3 function given its metal-binding domain<sup>(58,59)</sup>.

### Iodine deficiency

Although dietary iodine is an essential nutrient for maintenance of the T<sub>4</sub>/T<sub>3</sub> system, animal models of iodine deficiency are problematic. This is because of a well-documented conservation of iodine at the level of T<sub>3</sub>. Thus rats fed an iodine-deficient diet for long periods<sup>(142)</sup>, or for two generations<sup>(143,144)</sup>, or pigs fed a goitrogenic diet<sup>(145)</sup>, maintain plasma T<sub>3</sub> and some growth although symptoms of hypothyroidism can be observed<sup>(142)</sup>. T<sub>3</sub> conservation is thought to involve increased iodine uptake into the thyroid, increased T<sub>3</sub> secretion from the thyroid and continued extrathyroidal T<sub>4</sub> to T<sub>3</sub> conversion<sup>(124)</sup>. Because of this, early rat studies involved thyroidectomy to lower T<sub>3</sub> levels and such studies showed the slow growth was associated with a reduction in DNA-dependent RNA polymerase activity<sup>(146)</sup>, and a loss of the capacity for tissue protein synthesis in terms of ribosomal RNA<sup>(147)</sup>. T<sub>3</sub> administration by osmotic minipump revealed a dose-response of growth, muscle protein synthesis and ribosomal RNA to circulating T<sub>3</sub> levels<sup>(148)</sup>, similar to the relationship between plasma T<sub>3</sub> levels and muscle ribosomal RNA observed when growth was manipulated by protein deficiency<sup>(66)</sup>. Since these early animal studies, studies in GM mice have been the main source of information on the complex physiological relationship between centrally regulated thyroid status and the peripheral anabolic actions of T<sub>3</sub> on the growth plate outlined above<sup>(31)</sup>.

### Human studies of nutrition and linear growth

Plant-based diets are consumed by many communities with a high prevalence of stunting in developing societies. These are often limited to staples which for most starchy root crops are nutritionally poor and of low protein quality, and with few vegetables or pulses and often little or no ASF. However, for children raised in sanitary environments by economically independent parents, plant-based diets can be nutritionally adequate. Thus, near-normal linear growth of children is observed in developed countries within vegan communities who adopt the principles of protein complementation and supplementation with limiting nutrients such as vitamins A, D and B<sub>12</sub> (for example, O'Connell *et al.*<sup>(149)</sup>). With non-supplemented, relatively restricted vegan diets such as the

macrobiotic diet, retarded linear growth is observed<sup>(150)</sup>, with faltering after 4 months to a rate which is 3-5 cm/year less than normal<sup>(151,152)</sup>.

Most studies of linear-growth failure in the developing world are more difficult to interpret in terms of an uncomplicated nutritionally poor diet because of an associated impoverished environment. In fact, the overall number of interventions aimed in improving height which have been successful is low. In 2001, of the trials of complementary foods since 1988 in fourteen countries<sup>(153)</sup>, only three improved height<sup>(154-156)</sup>. Trials conducted since then, examining both individual nutrients and specific foods, are referred to below.

### Energy intake

The introduction of the term 'protein-calorie malnutrition' in the 1960s resulted in a debate into the extent of a protein, as opposed to a food or energy gap<sup>(157)</sup>, and this prompted intervention studies. Undernourished Indian children, consuming a cereal-based diet with some buffalo milk, judged to provide sufficient protein but inadequate energy were given an energy-rich low-protein supplement (310 kcal (1300 kJ) and 3 g protein/d)<sup>(158)</sup>. The intervention increased both height and weight gain over 14 months, enabling growth on the 50th percentile of American children. Furthermore, supplementation protected the linear growth of the children from an outbreak of measles during the study, whereas the measles outbreak depressed height gain and induced some weight loss of the non-supplemented children.

A similar conclusion that energy intake was the limiting factor for linear growth was reached following the INCAP longitudinal intervention study of Guatemalan mothers, infants and severely stunted children in 1969-1977<sup>(159)</sup>. The provision of either a good-quality high-protein (protein:energy ratio 28%) supplement (Atole), or a protein-free low-energy supplement (Fresco) as control, each with some micronutrients, resulted in a greater increase in height with Atole than Fresco at 3 years. However, analysis of variation in the actual intakes of the two supplements consumed by both pregnant women and children showed that energy, not protein, intake predicted both birth weight and height at 3 years of age<sup>(159)</sup>. This was consistent with the background diets of this community (mainly maize and beans, with tomatoes, sorghum and cassava)<sup>(160)</sup> which provided sufficient protein but inadequate energy intakes. Allen has argued<sup>(161)</sup> that their very low energy intake explains why Guatemalan children were more stunted than those from Egypt, Mexico and Kenya studied in the Nutrition Collaborative Research Support Program (CRSP). Overall, the Atole-supplemented children showed only small benefits of supplementation and remained severely stunted even though many of them consumed more energy and much more protein than their requirements<sup>(154,159)</sup>. Given their impoverished environment, it is possible that enteric dysfunction as discussed below was an additional important factor limiting growth.

Krebs *et al.*<sup>(162)</sup> suggested the possibility of energy insufficiency in their meat intervention study in stunted infants and toddlers in which stunting rates increased during the trial (see below under Animal-source foods and linear growth).



They gave 293–439 kJ/d (70–105 kcal/d), a very modest amount compared with their energy requirements of up to 3350 kJ/d (800 kcal/d).

Malcolm's studies of the stunted Bundi children in a mission school in Papua New Guinea<sup>(163–165)</sup> included the testing of energy deficiency. These children and the adults were very severely stunted consuming a nutrient-poor, low-protein-quality diet of mainly sweet potato and taro. Given the bulk and low energy density of this low-fat diet, an energy insufficiency was possible, especially for children in the mission school, who had a restricted three meals/d schedule. They were more stunted than village children who had an unrestricted access to food throughout the day. There was a small but significant increase in height growth with 60% more energy and protein when the children ate five rather than three meals per d, but no stimulation of height growth with extra energy as margarine, although there was a marked increase in skinfold thickness. This suggests that energy intake was not the limiting factor for height growth in these children. As discussed below (Animal-source foods and linear growth section), a skimmed milk supplement had the greatest effect on height. Malcolm argued that there were few signs of overt deficiency or morbidity other than stunting, with malaria rates low at 3–7%. However, diarrhoea and dysentery did occur, accounting for a quarter of the admissions to the mission hospital, and given this evidence of infection, Malcolm's assertion that 'It is unlikely that disease is a significant factor in the slow linear-growth rates in Bundi' has been challenged<sup>(166)</sup>.

Taken together, these studies do indicate that inadequate energy intakes can occur and that in some circumstances the energy deficiency is associated with stunting.

### *Protein and amino acid intake*

Contrary to popular belief, the dietary protein requirement of young children is low in terms of the protein:energy ratio of the requirement: the safe level is about 5% energy for healthy preschool children<sup>(167,168)</sup>. This means that with the exception of some very low-protein starchy-root staples like plantain, cassava, taro and sweet potato, most diets and especially cereal-based diets provide more than adequate amounts of protein if sufficient is consumed to meet the energy requirement. The challenge is to define the lower limits of the range of protein:energy ratios of intakes which ensures that the genotype in relation to stature can be expressed.

During exclusive breast feeding, length growth is generally considered to be optimal with protein intakes much lower (about 6–7% protein:energy) than after weaning and at later stages of childhood. Furthermore, the linear growth of healthy, breast-fed or formula-fed infants in the USA during the first year of life does not differ and is independent of protein intake for both groups<sup>(169)</sup>. No difference in length growth in infants fed either breast milk or experimental formulae was observed with protein intakes either higher than or similar to breast milk<sup>(170)</sup>. Whether this means that during infancy linear growth is insensitive to dietary signals or that the threshold for dietary proteins' influence is below the lowest level of intake observed in healthy breast- or formula-fed infants is not known. Current regulatory advice for infant formula in Europe<sup>(171)</sup> is for a minimum protein content

of 1.8 g/100 kcal (4.184 kJ) (protein:energy ratio=0.072) for cows' and goats' milk-based formulae (of which protein quality and digestibility are assumed to match breast milk (which is the case)<sup>(172)</sup>), and 2.25 g/100 kcal (4.184 kJ) (protein:energy ratio=0.09) for formula based on isolated soya protein because of a slightly lower quality (lower sulfur amino acid content) and digestibility.

In developed societies the change to a diet based on family foods at weaning involves a dramatic increase in protein intake to levels considerably in excess of requirements, for example, 13% protein energy in Danish infants at 9 months<sup>(173)</sup> with even higher values maintained in many Nordic countries up to puberty<sup>(174)</sup>. The consequences of this increase in protein intakes for linear growth and whether any influence is desirable is a complicated issue. A systematic review of protein intakes and growth of children in the Nordic countries up to puberty<sup>(174)</sup> concluded that a higher protein intake in infancy and early childhood is convincingly associated with increased growth and higher BMI in childhood and possibly related to an undesirable earlier puberty. However, some of the studies quoted in support of this indicated no relationship between protein intake at early childhood and body fatness at 10 years expressed as body fat percentage or BMI<sup>(173)</sup>. Also, in healthy Danish preschool children, height was positively associated with protein intake from milk but not from meat<sup>(175)</sup>, a possible example of the 'milk effect' on growth as discussed below. Thus the issue of excess protein intakes in early childhood and obesity in late childhood and adult life requires further clarification.

As far as protein quality is concerned, it is not clear how well linear growth can occur in the best circumstances for cereal-based diets with minimal ASF, given the likely dietary limitations of micronutrients and minerals, as well as the suboptimal protein quality. The high protein:energy ratio of cereals, especially wheat, means that their lysine limitation is to some extent offset by the higher protein supply, although digestibility may limit the protein quality of cereals like millet<sup>(168)</sup>. In fact, the largely cereal-based diets reported for the rural, urban, slum and tribal communities in India, which exhibit high prevalence rates of stunting, appear adequate in terms of protein and lysine intakes<sup>(176)</sup>. Although countries with the lowest protein and lysine intakes on the basis of FAO food balance sheets have the highest prevalence rates of stunting<sup>(177)</sup>, they also have the lowest gross domestic product per capita and highest infant mortality rates, indicating a poor environment. This means that it is unwise to suggest, as these authors did, a causal relationship between stunting and protein adequacy and quality of the adult diets.

The older literature does include some lysine supplementation studies, in one case examining child growth and bone density<sup>(178)</sup>. Lysine supplementation of the diets of sub-adolescent orphanage children for 5 months increased growth and bone density compared with supplemented controls. However, total lysine intakes were >5 times their requirement. Thus the changes were most probably pharmacological effects of lysine which is known to increase Ca absorption<sup>(179)</sup>.

There is evidence that improving the protein quality of maize can increase height growth. Zein, the main storage protein of maize, is low in lysine and tryptophan, hence its association with pellagra (niacin deficiency). Maize varieties with good agronomic

properties have been developed by selective breeding with much higher levels of lysine and tryptophan. One variety is quality protein maize (QPM) in which the protein content is unchanged but the concentrations of lysine, tryptophan, the sulfur amino acids and threonine are increased by 50, 75, 90 and 40%, respectively. The adequacy of QPM protein content and quality for child linear growth was demonstrated in rapidly growing infants recovering from malnutrition<sup>(180)</sup>. When fed as the sole source of dietary protein, fat and energy (but with micronutrient and mineral supplementation), it enabled height growth comparable with that observed with a cows' milk formula<sup>(180)</sup>. Although the introduction of QPM has been relatively slow, a recent meta-analysis of community-based studies in maize-eating populations<sup>(181)</sup> showed modest positive effects of QPM on both weight gain (12% increase) and height gain (9% increase) compared with conventional maize in infants and young children with mild to moderate undernutrition.

Serum concentrations of most (sixteen out of nineteen) amino acids were low in stunted preschool children from rural Malawi with no evidence of severe acute malnutrition, compared with non-stunted children<sup>(80)</sup>. Leucine and the other essential amino acids were particularly low<sup>(80)</sup>. While this suggests that dietary protein intake was low, without any indication of the timing of the blood sampling in relation to their last meal it would be unwise to over-interpret the findings.

Taken together, these studies provide some suggestion that height growth in children is sensitive to dietary protein and specifically dietary indispensable amino acids intakes as in the animal models, but with the exception of studies with milk protein (discussed below) any influences are small and some of the evidence is indirect. On the other hand, sufficient or even excess dietary protein intake may not be able to prevent the stunting observed in poor communities. Thus the Nutrition Collaborative Research Support Programme (CRSP) found that linear growth faltering was prevalent in preschool children in Egypt, Kenya and Mexico, even though their intakes of protein and essential amino acids were adequate<sup>(182)</sup>.

### Zinc intake

The prevalence of Zn deficiency is widely believed to be widespread in developing countries because of low intakes of Zn-rich animal products, diets high in phytates, which inhibit Zn absorption, and Zn losses due to diarrhoea. However, few nationally representative surveys of Zn status<sup>(183)</sup> or intakes have been conducted in low-income countries, and clear evidence of its role in the aetiology of stunting has been difficult to demonstrate. Recently, Zn deficiency prevalence has been modelled from both country-specific absorbable Zn content of the national food supply (from national food balance sheet data), and estimates of physiological requirements for absorbed Zn<sup>(184)</sup>, finding 17.3% of the world's population to be at risk of inadequate Zn intake, with estimates ranging from 7.5% in high-income regions to 30% in South Asia. Overall, the prevalence of inadequate Zn intake correlated with the prevalence of stunting in children under 5 years of age, explaining almost a quarter of the between-country variation in stunting.

As for Zn supplementation trials, evidence for their efficacy in terms of pregnancy and birth weight is very mixed, with the majority of studies identifying no influence<sup>(153)</sup>, and Zn supplementation during pregnancy is not included in the most recent *Lancet* series on Maternal and Child Undernutrition<sup>(185)</sup>. For children, benefits of Zn supplementation include reductions in the duration and severity of both diarrhoea and dysentery and acute lower respiratory infections<sup>(153,186)</sup>, all of which may indirectly influence height growth. As for linear growth, results of randomised controlled trials of Zn supplementation for children have been mixed, as might be expected given the limitations of Zn supplementation trials: i.e. difficulty of discerning baseline status in supplemented populations, low bioavailability of supplements, low adherence and side effects, etc. Thus although a 1997 meta-analysis indicated a small, but highly significant, impact of Zn supplements in stunted children<sup>(187)</sup>, more recent meta-analyses have found no influence<sup>(118)</sup>, a significant positive effect in children under 5 years of age<sup>(188)</sup>, and, in the case of a Cochrane review<sup>(189)</sup>, moderate-quality evidence of a very small improvement in height in children aged 6 months to 12 years of age which was unlikely to be clinically important.

The small-quantity lipid-based nutrient supplements (discussed below) contain Zn and these have not been shown to be effective in Malawi<sup>(190-192)</sup> or in Ghana except as part of a Nutributter supplement<sup>(193)</sup>. Overall, it seems that for Zn, the initial indications that this could easily be remedied with Zn supplements<sup>(187)</sup> have yet to be achieved. Nevertheless, it is probably appropriate that in the most recent *Lancet* Series on Maternal and Child Undernutrition<sup>(185)</sup>, preventative Zn supplementation in children is included as an intervention in the Lives Saved Tool modelling, acting both on diarrhoea incidence and stunting to reduce mortality.

### Iodine intake

Iodine deficiency is seldom included in discussions of the nutritional aetiology of stunting. Yet even after the widespread introduction of salt-iodination programmes, 2 billion individuals globally may have an insufficient iodine intake, especially in South Asia and sub-Saharan Africa, and 241 million (30%) of school-age children globally are estimated to have insufficient iodine intakes<sup>(123,125)</sup>.

The health consequence of severe iodine deficiency for human growth and development is clear in terms of delayed physical development through growth arrest and delayed bone maturation, and impaired mental function<sup>(124,126)</sup>. An insufficient iodine supply can be aggravated by goitrogens (glucosinolates, cyanogenic glucosides and some flavonoids) in many plant foods and unclean drinking water, which interfere with thyroid metabolism<sup>(123,126)</sup>. Deficiencies of Se, Fe and vitamin A exacerbate the effects of iodine deficiency. Given the importance of the T<sub>4</sub>/T<sub>3</sub> axis for growth and development, with hypothyroidism decreasing circulating IGF-1 and IGFBP-3 levels, and thyroid hormone replacement increasing them<sup>(194,195)</sup> and the fact that severe iodine deficiency results in severe linear-growth retardation, it might be expected that growth faltering would occur in otherwise asymptomatic iodine deficiency. In fact, the evidence for this is scarce.



Adaptive changes in iodine and thyroid hormone metabolism occur with iodine deficiency, initially conserving iodine within thyroid tissue in association with increased TSH, and maintaining T<sub>4</sub> and T<sub>3</sub> levels (subclinical hypothyroidism), eventually followed by reductions in T<sub>4</sub> (overt hypothyroidism). Thus, in both severely iodine-deficient and moderately iodine-deficient children, as indicated by their urinary iodine concentration, median T<sub>4</sub> concentrations can be in the low-normal range, with only a minority identifiable as hypothyroxinaemic<sup>(128)</sup>. Serum total and free T<sub>3</sub> concentrations may not decrease until disease is far advanced, because increased TSH concentrations stimulate T<sub>3</sub> release from the thyroid<sup>(126)</sup>. Thus, the enlargement of the thyroid may precede any fall in T<sub>3</sub> and any impairment of linear growth. In fact, most randomised controlled trials of iodine supplementation have involved pregnancy, aimed to prevent irreversible brain damage associated with cretinism<sup>(127)</sup>, and, of these, a recent systematic review identified only two studies which reported on child linear growth, observing no improvements<sup>(196)</sup>. However, hypothyroid iodine-deficient Columbian children with goitre, short stature and retarded bone age increased in stature when given T<sub>4</sub><sup>(197)</sup>. Iodine repletion in school-age children who were severely iodine deficient (7- to 10-year-old Moroccan children), moderately iodine deficient (10- to 12-year-old Albanian children) or mildly iodine deficient (5- to 14-year-old South African children) increased IGF-1 levels in each case, and also increased total T<sub>4</sub>, IGF-1, IGFBP-3 and both WA and HA Z scores in the Moroccan and Albanian children<sup>(128)</sup>. This suggests that in communities of low iodine availability where use of iodised salt is limited, if stunting is prevalent, iodine deficiency should be included as a potential part of any nutritional aetiology.

### Multiple micronutrient intakes

Given that multiple deficiencies of both type 1 and type 2 nutrients are likely to be common in the diets of many mothers and stunted children, poor growth may reflect interactions of their deficiencies. The recognition of this has resulted in the introduction of multiple micronutrient supplements.

These programmes have developed from an initial focus on Fe, vitamin A, iodine, folate and Zn<sup>(198)</sup> to multiple-micronutrient supplementations (MMS) of pregnant women in developing countries<sup>(199)</sup>, recommending a single daily tablet containing the ADA (or higher for folic acid) of fifteen micronutrients (vitamins A, B<sub>1</sub>, B<sub>2</sub>, B<sub>6</sub>, B<sub>12</sub>, C, D, E, niacin, folic acid, Fe, Zn, Cu, I and Se). It has often been given as a powder. A Cochrane review of MMS during pregnancy indicated that it was efficacious for maternal, fetal and infant health outcomes<sup>(200)</sup>: i.e. a reduced frequency of low birth weight, small for gestational age and stillbirths compared with supplements of only Fe, with or without folic acid. However, for postnatal linear growth, while an early MMS 12-month trial in Mexican infants aged 8–14 months showed benefit for the younger children (<12 months)<sup>(201)</sup>, a 2009 meta-analysis of multiple micronutrient supplementations of at least three micronutrients identified only a very small significant change in height<sup>(118)</sup>. Reviews of trials of micronutrient powders given to children under 2 years of age<sup>(202)</sup> and in children mainly aged 6 months to 6 years of age<sup>(203)</sup> concluded that micronutrient powders did not increase linear growth. Since these reviews,

as discussed below<sup>(162)</sup>, a multi-micronutrient-fortified cereal supplement given as a control for a multi-country meat intervention trial in stunted infants and toddlers was unable to prevent the worsening of stunting during the trial, even though both Zn and especially Fe status benefitted from the intervention.

The most recent approach is to provide MMS as part of a small-quantity lipid-based nutrient supplements (LNS), for home fortification during complimentary feeding<sup>(204)</sup>. This provides about 20 g/120 kcal (502 kJ) of fat (73% energy of which essential fatty acids are about 50%), protein at 9% energy, and aims to be nutritionally complete in terms of both type 1 and type 2 nutrients. Whilst this International Lipid-Based Nutrient Supplements project is ongoing<sup>(205)</sup>, to date they have achieved only very limited success, with a modest linear growth increase in Ghana, but no growth effects in three trials in Malawi. Thus, LNS appear to promote linear growth in some but not all infant populations. The project team suggests that lack of an intervention effect could reflect asymptomatic infections, environmental enteropathy and an unfavourable composition of intestinal bacterial microbiota, restricting linear growth through a multitude of inflammation-related or other pathways in children with adequate dietary intakes<sup>(192)</sup>. As a result, trials of LNS are now underway combined with interventions aimed at water quality, sanitation and hygiene (WASH)<sup>(206,207)</sup> in which the nutrition intervention will use a LNS comprising what is described as a next-generation version of Nutributter<sup>(206)</sup>.

### Animal-source foods and linear growth

ASF are nutrient dense in terms of many highly bioavailable micronutrients and are often more energy dense than plant-based foods through their fat content; thus, they are a good source of fat-soluble vitamins and essential fatty acids. They are also the only source of vitamin B<sub>12</sub>. It is the case that for many population groups of children, ASF often comprise only a very small part of their diet, if any at all. As discussed above, while diets free of animal products can support near-normal growth, they need to contain a wide variety of plant food sources and to be supplemented with key micronutrients. However, in poor communities in much of the developing world, diets are much less varied and often contain few ASF or energy-dense foods, with ASF culturally unacceptable in some communities.

Several observational studies, for example The Nutrition Collaborative Research Support Program (N/CRSP) in Egypt, Kenya and Mexico, show associations between intakes of ASF and better growth which persisted even after controlling covariates and confounders such as socio-economic status, morbidity, parental literacy and nutritional status<sup>(208)</sup>. In these studies the greatest deficits in linear growth were found in those with little or no ASF in their diet. On the basis of these studies, Allen & Dror<sup>(209)</sup> have widely promoted the strategy of increasing consumption of ASF, i.e. meat, fish, eggs and milk, for improving the amount and bioavailability of micronutrients available to children in the developing world.

As far as meat *per se* is concerned, few of the interventions associated with better growth identified by Allen & Gillespie<sup>(153)</sup> involved meat as such and these generally failed to increase



height growth or reduce stunting. In a Kenyan trial that supplied school children daily snacks of a local dish (maize, beans and greens) for 2 years with additions of meat, milk or equivalent energy, while all children increased weight compared with no snacks, there was little effect on height, although meat did improve muscle mass and cognitive function more than milk<sup>(210,211)</sup>. A more recent multicentre 12-month intervention at 6 months of age compared the effect of lyophilised beef with an equi-energetic multiple micronutrient-fortified cereal. This involved infants with an estimated prevalence of stunting of  $\geq 20\%$ , in rural communities in the Democratic Republic of Congo and Zambia, in semirural communities in the Western Highlands of Guatemala, and in urban communities in Karachi, Pakistan<sup>(162)</sup>. The micronutrient content of the rice-soya cereal supplement was based on guidelines for multiple micronutrients in complementary foods. Consequently, apart from vitamin B<sub>12</sub>, dietary levels of minerals and vitamins were higher in the cereal compared with beef supplement, four times higher for Fe, and Fe status at 18 months was better in the cereal group. The design was surprising given that higher indices of Fe status from the meat was a secondary hypothesis. However, the extent of stunting worsened from a length-for-age Z score of  $-1.4$  at baseline to  $-1.9$  at 18m with no difference between the two groups. In fact, only maternal height and education emerged as (positive) influences on length growth and the authors commented that this may be a surrogate for better socio-economic status and favourable practices such as hygiene and medical care<sup>(162)</sup>. Importantly, handwashing and use of boiled water for food preparation were emphasised in the trial.

The majority of ASF interventions have involved milk, starting with studies in the 1920–1930s in the UK and India and in the 1950s in USA<sup>(212)</sup>. Whilst all the studies leave much to be desired in terms of their design and analysis, they resulted in the identification of milk as an important determinant of height growth in children, and they were instrumental in the subsequent legislation in the UK in 1934, 1940 and in 1945 when free school milk was introduced<sup>(213)</sup>. Since these early studies the influence of cows' milk intake on height growth post-weaning has been repeatedly confirmed in a large variety of cross-sectional, observational and intervention studies in healthy children in the UK, Denmark, USA and Japan<sup>(214–219)</sup> and in stunted children in developing societies.

In healthy Danish preschool children, all with protein intakes well above current protein requirements, with  $>60\%$  from ASF, height was positively associated with intakes of total animal protein and milk, but not with intakes of vegetable protein or meat<sup>(175)</sup>. In New Zealand, long-term avoidance of cows' milk is associated with small stature in pre-pubertal school children which was argued to reflect the simple chronic avoidance of milk, rather than health problems associated with milk allergy or intolerance<sup>(220)</sup>. In the USA, analysis of National Health and Nutrition Examination Survey (NHANES) 1999–2002 indicated that adult height was positively associated with milk consumption at the ages of 5–12 years and 13–17 years, after controlling for sex, education and ethnicity<sup>(219)</sup>. However, given the complexity of height growth regulation, it would be very surprising if milk intake explained all the variation and Wiley<sup>(219)</sup> reports on five intervention studies in the UK,

Switzerland, New Zealand and the USA which report no significant effect on height in mainly adolescent girls. Nevertheless, it is undeniable that milk, which has the function of supporting rapid postnatal growth, can also influence growth throughout childhood and is a determinant of adult height.

At the outset of these studies milk was viewed simply as a nutrient-rich food providing for growth in stature, with elaborations of this view into a 'Milk hypothesis' predicting that a greater consumption of milk during infancy and childhood will result in greater stature in adult life<sup>(218)</sup>. It is the case that milk consumption and lactose digestion after weaning are exclusively human mammalian traits made possible by lactase persistence (LP), the continued production of the enzyme in the post-weaning period into adulthood. LP post-weaning is not the ancestral condition in humans but is made possible by a relatively recent SNP of the *LPH* (lactase-phlorizin hydrolase) gene<sup>(221)</sup>. Whilst the emergence of LP was advantageous amongst early pastoralists with a constant source of fresh milk, consumption in industrialised urban societies was very limited until the pasteurisation of the milk supply to cities in the late 19th and early 20th centuries and the widespread use of refrigeration. This means that the marked increase in milk consumption by urban populations in northern Europe and North America is a 20th-century phenomenon.

It has certainly become clear in recent years that because the specific biological function of milk is to enable rapid post-natal growth, it differs in many ways from all other human foods in having growth-promoting properties not found in meat or other ASF, although these properties are by no means understood. Also the widely assumed beneficial role of milk in the human diet after weaning is being questioned<sup>(214)</sup>. Melnik *et al.*<sup>(222)</sup> identifies the presence of microRNA in milk exosomes which could possibly act as anabolic signals for the stimulation of cellular growth and proliferation. Also, milk has uniquely high levels of amino acids known to be involved in anabolic signaling, especially tryptophan with concentrations in milk proteins uniquely high, 40% higher than egg and double that of meat. Tryptophan is said to activate the GH-IGF-1-mTORC1 pathway either directly or through gastrointestinal glucose-dependent insulinotropic peptide production. The caseins and lactoglobulins contain high concentrations of leucine, although this is not unique to milk, with higher levels in maize and sorghum. As indicated above, leucine is an upstream activator of the mTORC1 signalling pathway, important for cell replication and growth<sup>(77)</sup>. These features may explain why Hoppe *et al.*<sup>(175)</sup> showed, in a cross-sectional study of Danish preschool children, that serum IGF-1 levels and overall height were significantly related to milk but not meat intake. They also showed in an intervention study with a high protein supplement of either milk or beef in 8-year-olds that milk, and not meat, increased concentrations of serum IGF-1 and the serum IGF-1:serum IGFBP-3 ratio significantly<sup>(223)</sup> and that fasting insulin levels and consequently insulin resistance (calculated with the homeostasis model assessment) were higher in the milk- but not meat-supplemented children<sup>(224)</sup>. This combination of increased growth and insulin resistance raises the question of whether these effects of milk in these otherwise healthy children are of benefit or not<sup>(214)</sup>. The benefit of tallness is said to include a decreased risk of some diseases (cardiovascular and respiratory



diseases) but an increased risk of colorectal, postmenopausal breast and ovarian cancers, and possibly pancreatic, prostate and premenopausal breast cancers<sup>(3)</sup>. Furthermore, it has been known for some time that societies with high rates of milk consumption and with tall adults generally exhibit poor bone health in old age with higher fracture rates compared with less developed countries with much less milk consumption<sup>(225)</sup>. Why this should occur is not known but the possibility that the more rapid growth of childhood and earlier puberty results in a bone architecture which becomes more fragile in old age deserves investigation.

As for children in developing countries, data on preschool children from seven countries in Central and South America indicate that milk intake was significantly associated with higher HA Z score in all countries, whereas meat and egg/fish/poultry intakes were only associated with height in one of the countries<sup>(226)</sup>. A comparison of the heights and weights of children of the Ugandan Karamoja, a cattle-herding, milk-consuming people, with those of the Buganda, farmers growing plantain, sweet potatoes and cassava, showed that the Karamoja children exhibited normal height growth but low weight for height, whilst the Bugandan children were stunted but of normal weight for height<sup>(227)</sup>.

Malcolm's dietary interventions of children of the Bundi people of the highlands of Papua New Guinea with skimmed milk increased their height<sup>(163–165)</sup>. The growth rate of the Bundi children, with their diet of mainly sweet potato and taro providing 3% dietary protein energy, was slower than that of any other reported population in the world<sup>(165)</sup>. At 7 years they were 9.2 and 5.7 cm shorter than Guatemalan boys and girls studied in the INCAP study<sup>(228)</sup>. His 3- to 8-month interventions in preadolescents involved three studies comparing their usual diet with supplemental skimmed milk powder or additional energy as margarine or additional food<sup>(164)</sup>. In all three studies some linear growth was observed with the usual diet which was slightly increased by more of the usual diet. However, the growth rate in length was doubled by the skimmed milk in all three experiments. Although the design of these studies leaves much to be desired compared with best current practice they remain unique in terms of the detailed description of the community in which they were carried out<sup>(165)</sup>. Nevertheless, the outcome of these studies is consistent with current knowledge of the role of milk in linear growth regulation. As with all such interventions with milk, although these studies are reported as protein supplementations, they provide increases in minerals, phosphate and other key nutrients. For example, from the published composition of skimmed milk powder, extra Zn in Malcolm's studies would range from 1.2 to 3 mg/d, not insignificant accounts given that most Zn supplementation studies involve 5 mg/d. Extra iodine would range from 40 to 100 µg/d. The WHO recommends a daily intake of iodine of 120 µg for school children<sup>(229)</sup> so that if these children were iodine deficient, the milk intervention may have met their iodine requirements. Malcolm makes no mention of iodine deficiency in his monograph on the Bundi people<sup>(165)</sup>. However, the Highlands of New Guinea were known to be an area of iodine deficiency from the 1960s and were where a classic iodised oil supplementation study occurred<sup>(230)</sup>, aimed to prevent endemic cretinism. This means that the growth

response observed by Malcolm could have reflected an improvement of their protein, Zn and iodine status in addition to any of the other milk effects described above.

### Infection and poor linear growth in children

The combined and interactive effects of infections, environmental factors and malnutrition as possible determinants of stunting in children have long been thought to be of great importance but were first articulated in a systematic way by Scrimshaw *et al.*<sup>(15)</sup>. Both serious acute infections, particularly those that involve the gastrointestinal tract, and chronic infections impair linear growth<sup>(88,231)</sup>. Symptomatic infection is common during the first years of life in low-income countries and repeated episodes of diarrhoea or parasitic infection are associated with increased risk of stunting<sup>(206,232)</sup>. As a result of many intervention trials and observational studies, the idea that infectious diseases are the likely causes of a large share of stunting, together with poor diet, is now the mainstream public health view<sup>(185)</sup>. The stunting syndrome as illustrated in Fig. 1 identifies interactions between malnutrition and infection throughout the maternal, infant and child life cycle inhibiting growth<sup>(10)</sup>. These interactions are mutually reinforcing through infection exacerbating any malnutrition, because of appetite suppression and reduced food intake, and any malabsorption reducing nutrient intake, while malnutrition reduces immune defence systems, thereby worsening the adverse influence of infections.

The relationship between appetite and inflammation in terms of CRP, TNF- $\alpha$  and IL-6 has been clearly identified in adult dialysis patients<sup>(233)</sup> and in cancer patients<sup>(234)</sup>, although such relationships would be difficult to detect in stunted children with low-grade inflammation. However, it is very clear that infection and associated inflammation have direct inhibitory influences on anabolic processes throughout the organism, including the growth plate, which are additional to growth inhibition through malnutrition. Some of the evidence in children is discussed above (see the Inflammation and endochondral ossification section above). HIV-infected children exhibit impaired growth, and reduced height velocity is more strongly associated with disease progression than reduced weight velocity<sup>(235)</sup>. While reduced appetite and food intake account for much of the failure of weight growth in such children<sup>(236)</sup>, with weight improved by supplemental enteral and tube feedings<sup>(237)</sup> or treatment with megestrol, a progestational agent used as an appetite stimulant<sup>(238)</sup>, linear growth remains depressed in each case.

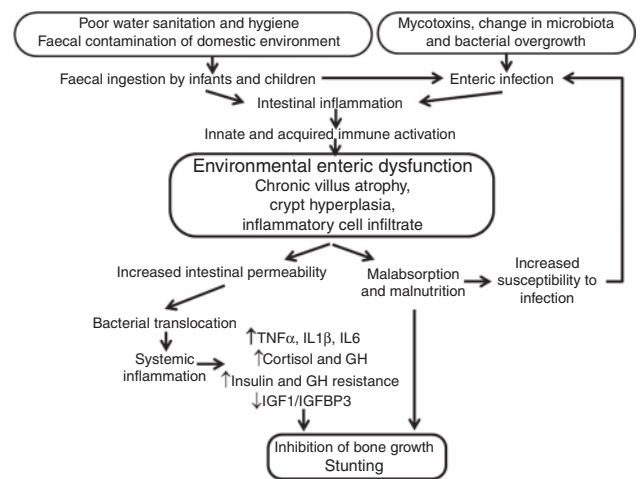
Endogenous low-grade inflammation associated with increased adiposity may also contribute to stunting in older children and adolescents. Serum TNF- $\alpha$ , IL-6 and CRP concentrations were positively associated with measurements of overweight in children in Europe<sup>(239)</sup> and among South-African township adolescents, of whom 18% were stunted, and, of these, more had a body fat percentage above the normal cut-off points than non-stunted adolescents<sup>(240)</sup>. Of the stunted girls, 50% had excess body fat, serum TNF- $\alpha$  was higher than in non-stunted girls and cluster analysis showed serum IL-6, waist:hip ratio and TNF- $\alpha$  clustered together. While in this cross-sectional study the association between low-grade inflammation and stunting does not

indicate causality, it may indicate an inability to catch up from stunting in earlier childhood. In 6- to 24-month-old Brazilian infants, higher levels of high-sensitivity CRP were associated with higher levels of GH and lower levels of IGF-1 and IGFBP-3 (GH resistance), with latter changes associated with shorter stature<sup>(95)</sup>. Low-grade inflammation in the first year of life and perturbation of the GH-IGF axis are associated with stunting in an 18-month longitudinal study of apparently healthy Zimbabwean infants<sup>(94)</sup>. The stunting at 18 months reflected both impaired intra-uterine growth, implicating poor maternal health and low *in utero* IGF-1 levels, and impaired postnatal growth associated with chronic inflammation, starting very early in infancy, supporting the concepts shown in Fig. 1.

### Environmental enteric dysfunction

In the context of the stunting syndrome, infection includes specific diagnosed infections such as parasitic infections, especially malaria and intestinal helminths, and diarrhoea, particularly in conditions of poor sanitation and hygiene. One study suggested that 25% of stunting was attributed to five or more episodes of diarrhoea<sup>(241)</sup>. However, while all agree that diarrhoea is implicated as a cause of poor growth, there has been a debate about its relative importance<sup>(242,243)</sup>. An analysis of seven longitudinal cohort studies conducted in four low-income countries indicated that the average child's diarrhoea burden between birth and 2 years only accounted for a clinically modest reduction in height growth<sup>(244)</sup>. In Gambian infants in which growth faltering in terms of weight and height growth was reported to become apparent in the 2nd year of life, although diarrhoea did occur, its frequency did not explain the growth faltering<sup>(245)</sup>. Direct measurements of an enteropathy associated with increased intestinal permeability, as indicated by the handling of oral lactulose and mannitol, were a much better predictor of impaired weight and height gain. Increased gut permeability implies a failure of normal gut barrier function, which normally prevents translocation of pathogenic organisms and endotoxins, and which can, in extreme cases, be a route to the development of sepsis in patients in intensive care. Subsequent studies in Gambian infants indicated endotoxin translocation in terms of increased plasma concentrations of total IgG and IgG-endotoxin-core antibody (EndoCAB) and these responses were correlated with increased intestinal permeability. In fact, intestinal permeability, plasma IgG concentration and EndoCAB together accounted for 56% of the growth inhibition<sup>(246)</sup>. Mucosal biopsies of these children indicated chronic T cell-mediated enteropathy with crypt hyperplasia, villous stunting and high numbers of intra-epithelial lymphocytes in all Gambian children, regardless of nutritional status<sup>(247)</sup>. However, with worsening nutrition, mucosal cytokine production became biased toward a pro-inflammatory response, i.e. a dominance of interferon- $\gamma$  over TGF- $\beta$  expression and with increased TNF- $\alpha$ -producing cells in the mucosa. These authors concluded that in these Gambian children translocation of immunogenic luminal macromolecules across a compromised gut mucosa had resulted in stimulation of systemic immune/inflammatory processes and subsequent growth impairment. On the basis of this and other evidence,

Prendergast & Humphrey<sup>(10)</sup> argued that subclinical infection with enteric pathogens is common, even in the absence of diarrhoea<sup>(248)</sup>. Humphrey<sup>(249)</sup> identified this subclinical infection due to enteric pathogens as tropical enteropathy, characterised by villous atrophy, crypt hyperplasia, increased permeability, inflammatory cell infiltrate, and modest malabsorption caused by faecal bacteria ingested in large quantities by young children living in conditions of poor sanitation and hygiene. She suggested it to be the primary causal pathway from poor sanitation and hygiene to undernutrition (and by implication stunting), rather than diarrhoea. Subsequently, Korpe & Petri<sup>(250)</sup> reviewed the distinction between this environmental enteropathy and other non-infectious tropical malabsorption syndromes such as tropical sprue, coeliac sprue and Crohn's disease, and Keusch *et al.*<sup>(251)</sup> introduced the term environmental enteric dysfunction. Its relevance to child health has been recently reviewed<sup>(252)</sup>. Importantly, in the context of the present review, Prendergast & Humphrey<sup>(10)</sup> wrote: 'We therefore view stunting as an inflammatory disease arising, in part, from primary gut pathology. Since gut damage also occurs with recurrent (especially persistent) diarrhoea, severe acute malnutrition, HIV infection and micronutrient deficiencies, there are multiple overlapping causes of enteropathy in settings of poverty which may exacerbate the growth failure arising from EED (environmental enteric dysfunction)'. The EED pathway between the poor environment and stunting is illustrated in Fig. 3<sup>(249-252)</sup>. One potential link between WASH and the development of the enteropathy is a detrimental change in the intestinal microbiota<sup>(252)</sup>, with evidence from longitudinal studies of twin cohorts of stunted children from Malawi



**Fig. 3.** Stunting as a predominantly inflammatory disease resulting from poor hygiene and environmental enteric dysfunction. The pathway between an unsanitary environment, dietary mycotoxins and other potential pathogens, an abnormal intestinal microbiota and stunting includes intestinal inflammation and pathological changes to the GI mucosa. This results in a failure of barrier function allowing translocation of pathogens and endotoxins resulting in a systemic inflammatory response which inhibits bone growth. In addition, nutrient malabsorption occurs exacerbating any dietary insufficiency, worsening malnutrition and increasing susceptibility to infection through its adverse effect on the immune system. This also contributes to the bone growth inhibition. GH, growth hormone; IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein. Adapted from Humphrey<sup>(249)</sup>, Korpe & Petri<sup>(250)</sup>, Keusch *et al.*<sup>(251)</sup> and Crane *et al.*<sup>(252)</sup>.

and Bangladesh<sup>(253)</sup> in whom the relative abundance of *Acidaminococcus* sp. was shown to be associated with future linear growth deficits. Because *Acidaminococcus* sp. can utilise glutamate as their sole source of carbon and energy, with glutamate usually a key source of fuel for the healthy enterocyte, the authors speculate that overgrowth of bacteria that can ferment glutamate may impair barrier function with the consequent deleterious effect on linear child growth. Other recent work points to a specific deleterious role of frequent exposure to mycotoxins<sup>(254)</sup>. These contaminate a wide range of staple foods, including maize and groundnuts, especially aflatoxin, fumonisin and deoxynivalenol. The authors argue that several billion individuals in predominantly developing countries may be at risk of exposure, with epidemiological evidence from multiple countries suggesting that exposure in pregnant women, infant cord blood, and young children is widespread during early life and associated with impaired infant growth velocity. Although aflatoxins have been most widely studied in relation to liver cancer<sup>(255)</sup>, all three mycotoxins can mediate intestinal damage through distinct pathways resulting in increased local and systemic pro-inflammatory cytokines with immunomodulation toward an inflammatory state, potentially interfering with the IGF-1 axis. Some years ago Hendrickse *et al.*<sup>(256)</sup> identified a link between aflatoxicosis and kwashiorkor in Sudanese children. Although there was little evidence of aflatoxicosis in malnourished Jamaican children<sup>(257)</sup>, where aflatoxicosis occurs, a link between the induced inflammation and kwashiorkor is consistent with the concept that kwashiorkor involves the catastrophic impact of an external noxious insult on children with inadequate micronutrient protection unable to counter the consequent oxidative stress<sup>(257)</sup>.

Recent direct evidence for the EED concept comes from studies in pre-school rural Bangladeshi children<sup>(258)</sup>. Objective measures of lower water quality, poor sanitary/handwashing infrastructure, lower gut permeability and lower IgG EndoCAB titres were related to lower HA Z score.

One important consequence of a widespread occurrence of EED is that it would explain the largely disappointing nature of nutritional intervention trials: i.e. the fact that only in a very few studies has the provision of supplements of either specific nutrients, multi-nutrient mixes or foods been shown to restore growth to normal or to the increased rates which might be expected if catch-up were occurring. Whilst a review by Dewey & Adu-Afarwuah<sup>(259)</sup> of thirty-eight reports of such supplementation trials indicated both weight and height gain, the latter in the range of an increase in 0.0–0.64 length-for-age Z score by 12 to 24 months, Humphrey<sup>(249)</sup> commented that none of children studied in these various interventions achieved normal growth. In fact, the height growth achieved in the most successful of these studies was equivalent to only about a third of the average deficit of Asian and African children. Dewey & Adu-Afarwuah<sup>(259)</sup> did argue that although changes in mean HA Z scores were small, nevertheless the impact on the lower tail of the distribution – that is, on stunting rates *per se* – could be considerably larger (for example, a fall from 15.8 to 4.7% in the intervention group). However, they concluded that complementary feeding interventions, by themselves, cannot

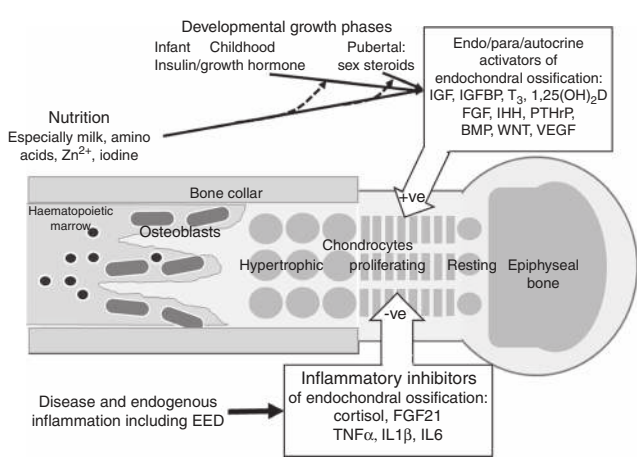
change the underlying conditions of poverty and poor sanitation that contribute to poor child growth. A typical recent example of the failure of a nutritional intervention is the randomised controlled trial of a meat supplement undertaken by Krebs *et al.*<sup>(162)</sup> discussed above. The rural, semi-rural and urban communities involved in that study were all likely to be exposed to poor hygiene and may well have suffered from EED. Also as discussed above, the disappointing outcomes of many of the LNS within the International Lipid-Based Nutrient Supplements programme has also been attributed to asymptomatic infections, EED and/or an unfavourable composition of intestinal bacterial microbiota<sup>(192)</sup>.

On the basis of the existing data, two major randomised studies are now investigating the concepts shown in Fig. 3 in terms of the potential role of EED in childhood stunting. WASH Benefits<sup>(206)</sup> is testing, in cluster-randomised trials, improvements in water quality, sanitation, handwashing and child nutrition, alone and in combination, on length growth over the first 12 and 24 months at multiple sites in Bangladesh and Kenya. The Sanitation, Hygiene, Infant Nutrition Efficacy (SHINE) Project<sup>(207,260,261)</sup> is a proof-of-concept cluster-randomised trial underway in Zimbabwe testing the protecting of babies from faecal ingestion with a WASH intervention and optimising nutritional adequacy of the infant diet with an infant and young child feeding intervention on length growth and anaemia. In each trial LNS are used, described as a next-generation version of Nutributter, which are identical except that the SHINE trial includes less Fe (6 mg compared with 9 mg) than in WASH Benefits, to mitigate concerns about the potential effect of supplemental Fe on infections<sup>(261)</sup>.

The role of nutrition and inflammation on the regulation of endochondrial ossification incorporating what has been discussed in this review is illustrated in Fig. 4<sup>(20)</sup>.

## Conclusions

It has been commented<sup>(8)</sup> that even though more than a quarter of the world's children are stunted, stunting often goes unrecognised in children who live in communities where short stature is so common that it seems normal. Nevertheless, for these communities because of the cycle of adverse consequences identified in the stunting syndrome (Fig. 1), few will be able to experience the same sense of wellbeing experienced by many in developed societies. While appropriate activation of growth-plate endochondrial ossification and consequent linear growth require adequate nutrition, of which the influences of dietary energy, iodine, amino acids and Zn and evidence for their deficiencies in the aetiology of stunting have been discussed here, it remains to be discovered what the minimum nutritional requirements are for acceptable linear growth and especially whether the plant-based diets of the most deprived communities can be sufficiently improved to meet such requirements. Assuming that the latest generation of nutritionally complete supplements prove to be effective, their use will not answer this question. As for the nutritional interventions which have improved linear growth, milk stands out in its growth-promoting actions. The evidence of more rapid linear growth and earlier puberty with high milk intakes during childhood suggested by the studies on Scandinavian children



**Fig. 4.** Role of nutrition and inflammation in the regulation of endochondral ossification. During the three developmental growth phases of the infancy, childhood and puberty (ICP) model of Karlberg<sup>(20)</sup>, the primary endocrine activators of growth are insulin (infancy), growth hormone (childhood) and the sex steroids (puberty) (although current evidence suggests that growth hormone may also be involved in the infancy stage). These act on the paracrine/autocrine system within the growth plate which mediate endochondral ossification. However, a suitable nutritional anabolic drive is necessary for this process to occur involving both type I nutrients such as iodine (to enable adequate thyroid hormone production) and type II nutrients of which amino acids and zinc are particularly important in stimulating the endocrine/paracrine system and in having direct regulatory influences, although this is poorly understood at the molecular level. Milk also appears to have a specific influence not observed with other animal-source foods. Elevated levels of glucocorticoids, inhibitory fibroblast growth factors (for example, FGF21), pro-inflammatory cytokines, especially TNF $\alpha$ , IL-1 $\beta$ , IL-6 and other inflammatory mediators associated with infection and inflammation and environmental enteric dysfunction as in Fig. 3, block the nutritional anabolic drive and inhibit endochondral ossification. How these interactions between nutrition and infection occur at the cellular and molecular level is poorly understood. IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; T<sub>3</sub>, triiodothyronine; 1,25(OH)<sub>2</sub>D, 1,25-dihydroxyvitamin D; IHH, Indian hedgehog; PTHrP, parathyroid-hormone-related protein; BMP, bone morphogenetic protein; WNT, wingless/integrated protein; VEGF, vascular endothelial growth factor; EED, environmental enteric dysfunction.

raises important but as yet unanswerable questions about the long-term consequences for health within milk-drinking developed communities.

However, it is clear that the dietary anabolic drive on linear growth is seriously compromised in the presence of inflammation, which appears to be widespread. It is encouraging and informative that there are success stories. Thus de Onis *et al.*<sup>(8)</sup> point out the dramatic improvements in Brazil's Northeast where stunting decreased from 34% in 1986 to 6% in 2006 as a result of rising incomes and increased access to schools, clean water, sanitation and basic health care. This would reinforce the view of stunting as an inflammatory disease<sup>(10)</sup>, in which case public health programmes focusing on WASH in order to minimise environmental enteric dysfunction are urgently required. It is to be hoped that lessons learned from the two WASH and nutrition trials indicated above<sup>(206,207)</sup>, which include both education about and physical improvements in WASH, will enable cost-effective strategies to be designed.

**Acknowledgements**

No financial support was received for the preparation of this paper. There are no conflicts of interest.

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