Linear growth is a hallmark of childhood; in most cases it proceeds without interference until final height is achieved after puberty. However, numerous genetic and environmental factors may interfere with the normal process. One of the most common environmental factors that may affect linear growth is malnutrition.

Due to the significant influence of nutrition on childhood’s growth, the identification of good nutritional biomarkers for growth outcomes will potentially help the pediatrician to improve diagnosis and treatment of nutritional growth attenuation. This paper will discuss the following questions: What is a good nutritional biomarker for growth outcomes? Which nutritional biomarkers are already known? What additional biomarkers does the endocrinologist need?

What Is a Good Nutritional Biomarker for Growth Outcomes?

Generally, the clinical definition of a biomarker is a biological characteristic that can be measured objectively, and that serves as an indicator of normal biological or pathogenic process, or response to therapeutic interventions [1]. Biomarkers should
assess reserves, body pool size and tissue amounts of the nutrient, should have standardized measuring methodologies, should have evidence-based cutoffs to distinguish between ‘normal’ status and varying degrees of deficiency or excess, and should reflect a biologically relevant response to an intervention, thus it should be both sensitive and specific. In addition, biomarkers should be practical for sample collection and storage [1].

Specifically, nutritional biomarker for linear growth should answer the following questions:
1. What is the nutritional status of the child?
2. Is the short stature secondary to nutritional problem?
3. What nutritional components are deficient?
4. Does the nutritional intervention ‘work’?

**Which Nutritional Biomarkers for Growth Outcomes Are Already Known?**

*Biomarkers that Measure Physical Characteristics*

Several anthropometric measurements are being used as biomarkers for childhood nutrition in epidemiological studies and in the clinic.

The most extensively used nutritional biomarker is weight gain. It has long been acknowledged that adequate weight gain is necessary for the progression of normal linear growth whereas poor weight gain is associated with delayed linear growth and, if severe, with ultimate stunting of adult height [2]. The beneficial effect of weight gain in improving growth rate has been well documented in studies of children suffering from various disorders [3–5].

Other anthropometric biomarkers include different length measurements, such as trunk length, leg length and relative lower leg length. Relative leg length (leg length as a proportion of stature) is increasingly used as a biomarker of childhood nutrition in epidemiological studies [6]. The theoretical basis for its use was provided >50 years ago by Leitch [7] who used animal models and the cephalocaudal gradient in mammalian growth to argue that ‘children continuously underfed would grow into underdeveloped adults with normal or nearly normal size head, retarded trunk and relatively short legs’. Others have suggested that relative lower leg length may be even more sensitive as a biomarker of childhood nutrition than leg length [8, 9]. However, despite its obvious importance as a tool for epidemiological research and evaluation of nutrition policy, evidence that the cephalocaudal gradient in human growth is modifiable by nutritional influences in childhood remains circumstantial or weak [6].

Another potential anthropometric biomarker is the adipose tissue, as in both appropriately grown for gestational age (AGA) and growth-restricted (GR) infants, adiposity (measured by MRI) was associated with accelerated linear growth [10].
**Biomarkers that Measure Biochemical Agents**

**Biomarkers for Micronutrients Status**

Classical nutrient deficiencies may lead to stunting (energy, protein, vitamin A, iron, zinc), rickets (vitamin D), and other bone abnormalities (copper, zinc, vitamin C) [9]. Most studies of single micronutrients in the field of nutrition and growth focus on zinc, iron and vitamin A. In observational studies, mostly on malnourished children in developing countries, positive correlations were found between the status of these micronutrients and growth. However, the results of randomized control trials, especially with regards to vitamin A and iron, were controversial [11]. To date, zinc is the only single micronutrient with conclusive evidence linking its intake to growth [11, 12]. However, its value as a single biomarker is very limited [1] because its level is influenced by numerous confounding factors and it is limited for detection of mild depletion only. The combination of several micronutrients biomarkers will potentially have a better predictive value for growth outcomes; however, the best combination will have to be further established.

**Leptin**

Leptin, a hormone predominantly produced by adipocytes, was originally described as a circulating hormone involved in feeding behavior and energy homeostasis. Later, it was found that leptin has numerous peripheral effects including bone growth and remodeling.

A direct link between leptin and linear growth was suggested by findings that leptin administration to the leptin-deficient Ob/Ob mice corrected their metabolic abnormalities and also led a significant increase in femoral length [13]. Leptin was also found to directly stimulate GH secretion [14]; lower levels of GH were found in both leptin-deficient Ob/Ob mice and humans with a mutation of the leptin receptor [15, 16].

Leptin was shown to exert a stimulatory effect on growth-plate cartilage proliferation and differentiation (for review, see [17]).

In children, the involvement of leptin in growth was supported by a series of studies suggesting that periods of fast growth such as fetal life [18] and adolescence [19] require a certain level of leptin.

The results of several observational studies suggest that leptin might serve as a nutritional biomarker for linear growth. For example, stunted 1-year-old South African children had lower leptin levels as compared to normal height children [20]. In another study, differences in leptin values were correlated with the nutritional status in neonates: At birth, circulating leptin concentrations were higher in AGA than in small for gestational age (SGA) babies, similar at the 1st and the 6th months of age, but then again increased in SGA from 6 months to 1 year during their catch-up growth [21].
IGF-1 and IGFBPs

IGF-1 serves as both the main mediator of GH action and as a GH-independent growth factor. Many studies have demonstrated that GH and IGF-1 concentrations are responsive to changes in nutritional status [22].

IGF-binding proteins (IGFBP) regulate the bioavailability of IGF-1. IGFBP-1 levels rise and fall in response to hepatic portal blood insulin, forming a link between dietary ingestion, glucose metabolism and the IGF axis. IGFBP-3 is the principal regulator of circulating IGF-1, binding approximately 90% of the IGF-1 present in serum [20]. Even though primarily dependent on GH, serum concentrations of IGF-1 and IGFBP-3 are also under long-term regulation by nutritional status [20, 23]. The IGF system is extremely sensitive to metabolic alterations in dietary protein levels and energy from carbohydrates, [20].

Early weight gain and subsequent linear growth were associated with early increment in IGF-1 and IGFBP3 in infants with malnutrition [24]. Similarly, nutritional recovery had a significant effect on IGF-1 and IGFBP-3. In malnourished children <48 months of age [25]. In children with congenital heart disease postoperatively over a period of a year, the parallel increase of weight gain and IGF-1, IGFBP-3 levels were the best evidence that these parameters are good nutritional indicators [26]. In addition, Mamabolo et al. [20] found higher IGFBP-1 levels in stunted South African children at 1 year as compared to normal-height children, and IGFBP-1 correlated negatively with length, body weight and weight gain at 1 year. Several interventional studies suggest associations between nutritional components that may improve linear growth and changes in serum IGF-1 and IGFBP-3. In one such study in prepubertal boys, milk consumption, and specifically casein, increased serum IGF-1 levels [27]. In another study, serum IGF-1 and IGFBP-3 levels were decreased in children with zinc deficiency, and were increased after zinc supplementation [28].

Markers of Bone and Collagen Formation

Procollagen type I C-terminal propeptide (PICP) and type III N-terminal propeptide (P3NP) are cleaved and released into the circulation during the final stages of collagen biosynthesis. Type III collagen is widely distributed in most soft tissues but not in bone; circulating P3NP levels therefore reflect soft tissue collagen synthesis. Type I collagen is the predominant form in bone: circulating PICP levels therefore largely reflect bone formation. Bone alkaline phosphatase is a specific marker of differentiated osteoblasts and responds more slowly to altered clinical states than does PICP which is produced by proliferating osteoblasts [24]. Several observational studies indicate that those markers of bone and collagen formation may be useful nutritional biomarkers for growth outcomes. One example is the observation of Doherty et al. [24] that in 6- to 36-month-old severely malnourished children early weight gain and subsequent linear growth were associated with early increment in bone alkaline phosphatase, PICP and P3NP. Similarly, changes in weight had a strong positive correlation with PICP in low birth weight infants, over the 6 weeks of life [29].
Other bone related biomarkers that were evaluated were osteocalcin (OC), the collagen-related peptides (deoxypyridinoline (DPD), pyridinoline (PYD)) and non-collagenous proteins [30]. However, all were found to have poor diagnostic value. The changes observed were often modest, with a low correlation to the growth state of the child.

**What Additional Biomarkers Do the Endocrinologists Need?**
Most of the biomarkers available to us today are not sensitive to the immediate changes. Hence, there is an urgent need for the establishment of additional, more sensitive surrogate biomarkers, which will reflect immediate changes in both the metabolic and growth status of a child.

Understanding the molecular mechanisms mediating the link between nutrition and growth may discover novel biomarkers.

A rat model of food restriction induced growth attenuation, followed by nutritional induced catch up growth was therefore established. In rats, 40% food restriction for 10 days induced dramatic changes in the expression of numerous genes within the growth plate. Several transcription factors were affected [31] as well as micro RNAs and proteins involved in epigenetic regulation [32]. Interestingly, one of the transcription factors was found to be HIF1α, a key subunit of HIF, which serves as a master transcription factor regulating the expression of several genes that code for proteins involved in proliferation, metabolism, angiogenesis, motility, adhesion, and survival [31].

Significant changes in several microRNAs (miRNAs) were also found. These are small non-protein-coding RNAs, measuring approximately 19–23 nucleotides in length that negatively regulate the expression of a large portion of the protein-encoding and non-protein-encoding genes at the post-transcriptional level.

A link between nutrition, miRNA and growth is supported by several recent observations: (1) Several miRNAs were shown to be associated with glucose-induced insulin secretion [33] and metabolic control. (2) It was recently shown that the chondrocyte-specific miR-140 is reduced in the EGP of food-restricted rats. Furthermore, a direct link between miRNA-140 and SIRT1 was established, showing that while food restriction leads to reduced level of miR-140, it concomitantly relieves the inhibition on SIRT1 synthesis [32]. SIRT1, of the class III histone deacetylases (HDAC), is a highly conserved enzyme that utilizes nicotinamide adenine dinucleotide (NAD+) to deacetylate a number of histone and nonhistone substrates shown to be implicated in the response to calorie restriction (CR) [34]. Studies found that SIRT1 was induced in vitro by nutrient deprivation and in vivo after long-term CR. SIRT1 may regulate cell proliferation, senescence and apoptosis by regulating several transcription factors that govern metabolism and endocrine signaling.

Understanding the mechanism linking nutrition and growth will hopefully provide novel sensitive and specific new molecular biomarkers.
Conclusions

Currently, there is a quite long list of potential nutritional biomarkers for growth outcomes. However, no single biomarker meets the pediatric endocrinologist’s needs of good nutritional biomarkers for growth outcomes.

Pediatric endocrinologists might have to compromise on a combination of markers, which will provide the most complete picture during the diagnosis, and enable the best short-term prediction for the treatment outcome. The present challenge is to establish the optimal combination which will also be practical for clinical use.

References


