Nutritional Catch-Up Growth

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Abstract

Malnutrition, marked by variant nutrient deficiencies, is considered a leading cause of stunted growth worldwide. In developing countries, malnutrition is caused mainly by food shortage and infectious diseases. Malnutrition may also be found in the developed world, where it is due mostly to prematurity, chronic diseases, and anorexia nervosa. In most cases, when food consumption is corrected, spontaneous catch-up (CU) growth occurs. However, CU growth is not always complete, leading to growth deficits. Therefore, it is important to understand the mechanisms that govern this process. Using a rat model of food restriction followed by refeeding, we established a nutrition-induced CU growth model. Levels of leptin and insulin-like growth factor-1 were found to significantly decrease when food was restricted and to increase already 1 day after refeeding. Gene expression analysis of the growth plate revealed that food restriction specifically affects transcription factors such as the hypoxia inducible factor-1 and its downstream targets on the one hand, and global gene expression, indicating epigenetic regulation, on the other. Food restriction also reduced the level of several microRNAs, including the chondrocyte-specific miR-140, which led to an increase in its target, SIRT1, a class III histone deacetylase. These findings may explain the global changes in gene expression observed under nutritional manipulation. We suggest that multiple levels of regulation, including transcription factors, epigenetic mechanisms, and microRNAs respond to nutritional cues and offer a possible explanation for some of the effects of food restriction on epiphyseal growth plate growth. The means whereby these components sense changes in nutritional status are still unknown. Deciphering the role of epigenetic regulation in growth may pave the way for the development of new treatments for children with growth disorders.

Linear growth is the product of an elaborate cascade of events that takes place in the cartilaginous growth center of the long bones, termed the epiphyseal growth plate (EGP). It is controlled by complex interactions among hormones, local growth factors, and components of the extracellular matrix. The growth of the human skeleton requires an adequate supply of many different nutritional factors. Some, such as proteins, lipids, and carbohydrates, form the 'building materials'; others play regulatory roles. However, the exact signaling system for growth or attenuation of growth is still unclear.
On the cellular level, linear growth involves the sequential replacement of chondrocytes of the EGP by osteoblasts in a process called endochondral ossification. It begins with the proliferation of early chondrocytes, followed by their alignment in columns, and, finally, their maturation into hypertrophic chondrocytes. The hypertrophic cells cease dividing, increase in volume by 5- to 10-fold, and secrete extracellular matrix components such as collagen type X, as well as matrix vesicles. Thereafter, the chondrocytes undergo programmed cell death, with calcification of the extracellular matrix, enabling invasion of blood vessels and osteoblasts. The cartilage scaffold is thus replaced by bone tissue.

Malnutrition, marked by variant nutrient deficiencies, is considered a leading cause of low weight and short stature. In developing countries, an average of 33% of all children younger than 5 years have malnutrition-induced linear growth retardation or stunting caused by food shortage and infectious diseases. Malnutrition may also occur in developed countries, where it is attributable mostly to prematurity, chronic diseases, and anorexia nervosa. In most cases of growth inhibition due to malnutrition, when food consumption is corrected, spontaneous catch-up (CU) growth occurs. CU growth is defined as ‘height velocity above the normal statistical limits for age and/or maturity during a defined period of time following a transient period of growth inhibition’ [1]. However, CU growth is not always complete, leading to growth deficits. Therefore, it is important to understand its underlying mechanisms.

CU growth is probably an endogenic process, involving reduced senescence in the growth-arrested EGP [2]. To study the factors controlling nutrition-induced CU growth, we established an experimental model in which young rodents were subjected to 40% food restriction for 10 days, followed by removal of the restriction [3]. During the food-restriction phase, the animals gained 1.2 g/day, whereas a control group of animals fed ad libitum gained 6.5 g/day. When normal food consumption was reinstituted, the study group showed an immediate increase in weight, with the largest rise on the first day (10–15 g). Tibial EGP height showed a significant reduction compared to controls during food restriction (404 vs. 729 μm, p < 0.01) and a significant increase from the food-restricted condition after one day of refeeding (to 472 μm, p < 0.05). However, a significant increase in bone length was observed only after 7 days [3].

Systemic Factors

**Growth Hormone and Insulin-Like Growth Factor-1**

During childhood, linear growth is predominantly regulated by growth hormone (GH). GH affects growth both directly, by binding to its receptors in the EGP, and indirectly, via insulin-like growth factor (IGF)-1. IGF-1 serves both as the main mediator of GH action and as a GH-independent growth factor, especially in utero [4]. It also stimulates cell proliferation and differentiation and protects cells from apoptosis.
GH and IGF-1 concentrations are responsive to changes in the nutritional status [5]. Fasting induces a GH-resistant state; whereas it increases serum GH levels in humans, it reduces serum GH levels in mice and rats; nevertheless, in all animals examined, IGF-1 levels were reduced. Our experimental studies in mice revealed a dramatic reduction in the level of GH receptor (GHR) in the EGP during food restriction [6]. In addition, the level of IGF-1 decreased during food restriction and, increased already after one day of refeeding [7, 8]. We speculate that these concomitant responses are part of an adaptive mechanism by the body to shunt calories away from nonessential processes, including growth, during periods of malnutrition.

Leptin
Leptin is a hormone predominantly produced by adipocytes [9]. It was originally described as a circulating hormone involved in feeding behavior and energy homeostasis. More recent studies showed that it also has numerous peripheral effects [3, 10–12]. Leptin directly stimulates GH secretion [13] and significantly improves the structural properties and elongation rate of bones [10] by directly stimulating EGP cartilage proliferation and differentiation [11, 14, 15]. In our experimental studies, treatment of food-restricted rodents with leptin increased longitudinal growth and EGP height [11]. Leptin level is significantly reduced by food restriction [6, 7] and immediately increases with food replenishment [6]. The immediate increase may reflect a new food-restriction-induced set-up of the metabolic system and may also be associated with adult-onset metabolic disorders.

Local Molecular Mechanisms
Several factors that regulate the translation of energy within cells may be important for linear growth. These include transcription factors such as hypoxia-inducible factor (HIF)-1, epigenetic mechanisms, microRNAs (miRNAs) [8], and enzymes such as mammalian target of rapamycin (mTOR) [16].

Hypoxia-Inducible Factor-1
To study the effect of food restriction on gene expression, analyses were performed on a diversity of animals and tissues. Although no common gene affected by food restriction was identified across the different species, several shared factors were found, including energy metabolism and cell growth, regulation of transcription, and stress and immune functions [17]. To the best of our knowledge, our group has conducted the only analysis so far of genes in the EGP itself. We showed that food restriction induced dramatic changes in the expression of several genes and transcription factors, among them, HIF-1α, a key subunit of HIF [3]. HIF-1α is responsible for the adaptation of chondrocytes to the low-oxygen pressure of the avascular and relatively
hypoxic tissue in which they are located. Its significance to chondrocyte survival, and its involvement in chondrocyte proliferation, differentiation and growth arrest, is well recognized [18]. HIF-1α was also found to be a major factor in anaerobic glycolysis, which supplies most of the energy requirements of the chondrocytes, as well as extracellular matrix production which is essential for proper growth and development [19]. The activity of HIF-1α affects the expression of many genes that code proteins involved in angiogenesis, cell metabolism, proliferation, motility, adhesion, and survival [3]. In rats, refeeding and CU growth were associated with a reversal of the decrease in HIF-1α and its gene targets [3].

**Epigenetic Regulation**

Epigenetics is defined as changes in gene function caused by mechanisms other than changes in the genomic DNA sequence. These may include chromatin structure remodeling together with chemical modifications of DNA and associated proteins, such as histones, mediated by histone acetyltransferases, histone deacetylases (HDACs) and other enzymes. In general, histone acetylation leads to transcriptional activation whereas removal of acetylation by HDACs is associated with transcriptional repression.

Our studies have shown that food restriction induces a global shut-down in gene expression [3], suggesting epigenetic regulation. The involvement of HDAC was suggested by the significant increase in the protein level of SIRT-1 [8] with food restriction and its immediate reduction upon refeeding. SIRT-1 is a member of the sirtuin family of class III HDACs which utilize nicotinamide adenine dinucleotide (NAD+) to deacetylate a number of histone and non-histone substrates. The founding member of this family, silent information regulator 2 (SIR-2), promotes longevity in yeast by repressing gene expression and stabilizing chromatin. SIRT-1 was found to regulate the proliferation, senescence, and apoptosis of cells via the regulation of several transcription factors that govern metabolism and endocrine signaling [20]. It is widely implicated in the response to calorie restriction in numerous tissues, in a tissue-specific manner [21–23].

**MicroRNAs**

MicroRNAs (miRNAs) are non-protein-coding RNAs, measuring approximately 19–23 nucleotides in length, which negatively regulate the expression of a large portion of the protein-encoding and non-protein-encoding genes at the transcriptional or post-transcriptional level. Mature miRNAs are derived from two major processing events driven by sequential cleavages by the RNAse-III enzymes, Drosha and Dicer, and subsequently incorporated into the RNA-induced silencing complex (RISC) that guides it to its target. Each miRNA can regulate one to several mRNA transcripts, and conversely, a single mRNA may be regulated by one to several miRNA sequences [24, 25]. In mammals, miRNAs regulate several systems; their dysregulation is associated with such diseases as cancer and diabetes [26].
The central role of miRNAs in mammalian development was first reported by Bernstein et al. [27] who found that development was arrested in E7.5 mice devoid of Dicer. In addition, mice lacking Dicer in their cartilage had many skeletal defects [28]. Dicer has been found to significantly affect limb size and morphogenesis, and its absence leads to a delay in the expression of limb-patterning genes [29]. In our study, a high-throughput miRNA microarray technology was used to identify the battery of miRNAs expressed in the mature EGP and to test their response to nutritional cues [8]. We also found a direct link between miR-140, a chondrocyte-specific miRNA [30, 31], and SIRT-1 in the EGP. Calorie restriction reduced the level of miR-140 and relieved the inhibition on SIRT-1 translation [8], leading to an increase in the SIRT-1 protein level. These observations expanded the earlier findings of a SIRT-1 increase during long-term calorie restriction [21–23] to the context of linear growth. Interestingly, SIRT-1 was recently found to deacetylate HIF-1α and reduce its activity [32].

**Mammalian Target of Rapamycin**

mTOR is an evolutionarily conserved serine/threonine protein kinase which serves as a key regulator of cell metabolism. It is inactivated when nutrient levels are inadequate. Inhibition of mTOR leads to the inhibition of protein synthesis, arrest of cell growth, inhibition of HIF-1α and glycolysis, and degradation of autophagic protein [33]. Accordingly, we propose the following paradigm: Calorie restriction reduces miR-140 levels by a still-unknown mechanism (involving mTOR?), relieving the translational inhibition of SIRT-1. This leads to an increase in SIRT-1 levels and activity and, in turn, to deacetylation and reduced activity of HIF-1α and its downstream targets [8]. Our paradigm suggests a cross-talk between nutrition, miR-140, SIRT-1, and HIF-1α. It would be interesting to study the role of mTOR in this context (fig. 1).

**Conclusion**

Malnutrition is known to impair linear growth, but the specific processes that regulate the effect of nutrition on growth remain controversial. Several possible mediators are beginning to emerge. We suggest that multiple levels of regulation including transcription factors, epigenetic mechanisms and miRNAs respond to nutritional cues. These findings may offer an explanation for some of the effects of food restriction on EGP growth. The mechanisms by which SIRT-1 and miRNAs sense and respond to the change in nutritional status are still not known. Deciphering the role of epigenetic regulation in growth may open a new era of research and pave the way for the development of new treatments for children with growth disorders.
Fig. 1. Calorie restriction effect on the EGP: a suggested model. Calorie restriction reduces the level of miRNA-140-3p by an as yet unknown mechanism. This reduction relieves the inhibition on SIRT1 translation, leading to an increase in SIRT1 protein levels and a possible concomitant increase in deacetylation of HIF-1α. The reduction in its level and activity leads to a reduction in glycolysis, chondrogenesis and proliferation. Deacetylation of additional proteins such as histones and transcription factors may contribute to the EGP growth attenuation. Reprinted from Pando et al. [8], with permission from Elsevier.

References


