Translational Neuroendocrinology: Control of Human Growth

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Human growth is driven by both basic cell processes as well as hormones, in particular the growth hormone (GH)–insulin-like growth factor (IGF)-1 axis. Understanding how these mechanisms are coordinated is not only critical to achieving a normal growth rate, but also to recognising potential new causes of disordered growth and how they might be treated.

We have demonstrated in healthy children that height is gained by periods of rapid growth interspersed by periods of very slow growth or even stasis. We have also shown that a lower order organism, Caenorhabditis elegans, grows in a similar manner. By contrast, secretion of GH from somatotrophs occurs on a daily basis in discrete pulses over a 24-h period. We have used the measurement of GH in urine as a surrogate marker of GH secretion to show that there are rhythms of GH output with frequencies of several days. We then assessed which attributes of these GH profiles were related to growth and found that disorderliness in the GH profile (as measured by approximate entropy) was related to better growth rate.

This feature was then tested in the dwarf rat using different GH regimens to introduce variation into the administration of daily GH injections. Better long bone growth was associated with week-to-week or even random dose variation compared to the same amount of GH delivered as a standard daily dose.

Understanding the control of growth has implications in clinical practice for modelling GH treatment regimens based on physiological principles.

Key words: growth, children, growth hormone, rhythms, therapy
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Introduction: Setting the scene – clinical drivers

The achievement of normal growth and a normal adult height requires the coordination of a number of factors: an appropriate intrauterine environment; adequate nutrition, of particular importance in the first 2 years of life; a nurturing psychosocial environment; normal amounts and patterns of GH secretion, alongside normal thyroid hormone and cortisol secretion and timely generation of sex-steroids; and the ability for peripheral tissues, including growth plates, to respond to hormone output as well as local growth factors.

All of these mechanisms must be considered when formulating a differential diagnosis in the evaluation of a short and/or a slowly growing child. It is therefore important that the underlying physiological processes that link these mechanism to a satisfactory growth rate are fully understood. This is because we need to provide an answer to the question posed by the families of a short child: can my child’s short stature be treated and how effective will that treatment be?

The process of human growth

The growth curve: infancy–childhood–puberty model

Traditional growth curves, based on cross-sectional, and, in some cases, longitudinal measurements on large numbers of children, present a smoothed growth trajectory implying that growth is achieved by the continuous accrual of height (Fig. 1). The growth curve can be divided into three distinct phases based on mathematical
Fig. 1. (A) The infancy–childhood–puberty model of growth (1); the solid line represents an average growth curve with age [with the growth velocities (GV) indicated], whereas the dashed lines represent the theoretical continuation of growth at infancy and childhood rates. Each phase of growth is determined by specific factors, defined in the boxes. (B) The average Height and Weight velocities (in cm/day and kg/day, respectively) of 43 prepubertal children measured three times each week between September and July. The nadir in height velocity in February/March coincides with a phase of increased weight velocity between January and May (4,5). These average curves are generated using ‘smoothing windows’: these were 118 days for weight and 60 days for height. This technique does lead to an impact on the average values at the beginning and end of a series, with larger variation for a longer smoothing window; hence, the low values for weight velocity in July and the high values in September. (C) Height (cm) and height velocity (cm/day) curves from one prepubertal individual measured frequently over a 300-day period. Marked fluctuations in growth rate are evident throughout the measurement period (6), including periods of stasis.

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modelling (1): the infancy phase occurs over the first 2 years and is heavily nutrition dependent. The childhood phase lasts until puberty and is dependent on GH and thyroxine. This predicts that the growth failure associated with congenital GH deficiency will start to manifest from approximately 2 years of age. Finally, the pubertal phase of growth acceleration followed by deceleration to the end of growth is driven by firstly the sex-steroid-induced amplification of GH secretion and the direct growth-enhancing effect of sex-steroids on the growth plate followed by its fusion as a result of continued exposure to sex-steroids (Fig. 1a).

Models of short-term growth

When the growth process is evaluated by frequent measurements of height and leg length in individual children, it becomes clear that growth is not a linear process (2–7); indeed, irrespective of whether growth measurements are taken at intervals of months, weeks or days, fluctuations in growth rate can be defined. First, a minor growth spurt can be seen in mid-childhood consistent with the influence of adrenal androgens secreted during adrenarche (8). Longitudinal measurements over a year can be used to define seasonal variation in growth rate with more rapid growth in spring and summer and a nadir in mid-winter (Fig. 1a) (5,9). The accrual of weight follows the opposite pattern, with most weight gained over the autumn and winter. Large but infrequent changes in weight with growth spurts of short duration were found in tall children, whereas good growth over the year was related to large but frequent gains in weight and large individual spurts in height (5). Measurements taken at frequent intervals (from weekly to daily) reveal a further layer of complexity, with irregular fluctuations in growth rate, including periods when growth is negligible: a stasis (4,5,7) (Fig. 1c). In one study of infant growth, it was proposed that these periods of growth stasis are punctuated by short-lived (lasting 1 day or less) ‘jumps’ or ‘saltations’ of a magnitude up to 2.5 cm (7). This model has not been confirmed in older pubertal children (4). One of the key issues for analysing and interpreting these data is the type of statistical modelling used because this, in itself, can impose a structure to the data. The growth spurts and stases seen in normal children were also observed in those with growth disorders such as in GH deficiency, Turner syndrome and intrauterine growth restriction (6).

We hypothesised that nonlinearity is a preserved feature of growth and that examination of this process in a simple, multicellular organism, such as the nematode roundworm Caenorhabditis elegans, would offer insights into the cellular basis of this phenomenon. Growth in C. elegans proceeds from hatching to adulthood as four defined larval stages, separated by molts, over a 3-day period (10), which makes it an ideal system in which to generate a description of growth dynamics throughout the entire growth-span of an organism. We developed a novel method for repeated, high-resolution measurements of body size in individual nematodes and measured body length of individual hermaphrodites every 15 min at 20 °C from hatching to the L4/young adult molt. The size and growth rate of worms during each larval stage and the timing of developmental cues were consistent with other reports (11), although the accuracy and frequency of the measurements were such that distinct episodes of growth were clearly evident within each larval stage (Fig. 2a,b). The growth velocity curves clearly showed nematode growth in all larval stages, to be a biphasic process, with alternating periods of rapid growth separated by periods of slower growth (Fig. 2c).
We considered two simple, potentially overlapping, hypotheses to explain the pattern of nonlinear growth in *C. elegans*. In the first, the timing of cell division is the primary determinant of the growth pattern and in the second it is periodic changes in cell size that drive the growth dynamics. Comparison of growth curves with the timing of cell division, derived from the post-embryonic cell lineage (12), indicates that spurts in nematode growth occur not only during times of mitotic activity, but also continue at a time cell division is not occurring. This is most evident in the fourth larval stage when somatic cell division ceases shortly after the molt, with the animal having attained its full complement of somatic nuclei, yet episodic growth continues throughout this larval stage. These observations suggest that changes in cell size are likely to be the main determinant of nonlinear growth, whereas cell division exerts some influence on the overall rate of growth by increasing the pool of cells available for growth.

These observations suggest that nonlinear growth dynamics may be a fundamental feature of normal organismal growth and, in some systems at least, this growth pattern may be periodic or rhythmic. In this respect, periodic oscillations in levels of total RNA, total protein and enzyme activity, as well as respiration rates, have been detected in synchronous cultures of a variety of unicellular organisms (13). We speculate that these endogenous metabolic rhythms may subserve the cyclic growth kinetics generated by individual cell division, cytoplasmic expansion and cell growth, which, combined throughout the organism, influence the kinetics of whole body growth. The precise control required for the coordination of such a process could arise from the cell itself, in an autonomous manner, or externally via an endocrine/paracrine signal. In this respect, there are a number of conserved signalling pathways in *C. elegans*, including an insulin-like signalling pathway (14), which could provide this type of control and drive nonlinear growth in the worm. It is possible that a similar model is also relevant to the growth process of higher organisms, including man.

The overall conclusion from these studies both in humans and *C. elegans* is that growth is not a linear process but one that is characterised by marked variation in short-term growth rates, including periods of growth stasis. In humans, GH is secreted from the pituitary gland on a daily basis, yet there are many days when growth is not occurring; thus, what is the exact relationship between GH and growth?

**Control of GH secretion**

GH is secreted in pulses from the somatotrophs of the anterior pituitary under the regulatory influence of bursts of hypothalamic GH-releasing hormone secretion and possibly ghrelin, in association with a brief reduction in the tonic secretory inhibitor, somatostatin (15).

**GH secretion from the pituitary**

Defining rhythms of GH excretion

Ultradian rhythms of GH secretion (frequency < 24 h) have been determined by frequent blood sampling (5–20-min intervals) over 24 h. These rhythms in GH secretion vary markedly in amplitude with a tendency for the larger pulses to be secreted overnight and, overall, approximately eight pulses are produced over 24 h (16,17). It is not possible to repeatedly make such measurements in normal children. Therefore, assays have been developed to measure the small fraction of intact GH that is excreted in urine, with this measure correlating with serum GH levels (18). Urinary GH (uGH) has been used as a surrogate ‘integrated’ marker of serum GH over the preceding hours. uGH assays have been used in diagnosis, and to determine compliance with GH treatment, although mostly to non-invasively research day-to-day GH output (19). Studies that have entailed frequent urine collections (first morning urine collected daily to three times per week) have been used to define infradian (frequency > 24 h) rhythms in GH output. Such work has demonstrated that significant rhythms can be found with periods of 3.2 and 4.7 days (19).

There is therefore both diurnal and longer-term rhythmicity in GH output, which may imply that variation in GH output has physiological significance.

**Reconciling patterns of growth and GH secretion**

We recognise that a severe deficiency in the amount of GH is associated with growth failure, as seen in classic GH deficiency. We also recognise that there is a relationship between amounts of GH secreted over 24 h and growth rate (16); however, this relationship follows an asymptotic curve, where very low amounts of GH are associated with very slow growth but, as the GH output increases, so the change in growth rate lessens. Importantly, this asymptotic relationship can also be recreated in normal children with uGH measurements (20).

This implies that, once a relatively normal amount of GH is being secreted, there must be different ‘read-outs’ in the GH output that are related to growth. Weekly growth monitoring with paired overnight urine collection revealed that the proportion of high frequency variation in uGH excretion was correlated with the amount of IGF-I excreted, which in turn was significantly reduced during periods of slow growth. This indicated that the variation in HG excretion over 2–4 weeks may be an important determinant of the human growth process (21).

**Correlating growth to GH: approximate entropy**

We have explored a number of ways that GH output and growth may be related in normal children. The coefficient of variation of the change in uGH from day-to-day is correlated with growth, such that large day-to-day changes are associated with taller stature and higher growth rates, whereas small day-to-day changes in uGH are associated with shorter stature and lower growth rates (20). This implies that information contained within the pattern of GH output relates to long-term growth performance.

This led us to examine whether the degree of randomness within the GH profile, as defined by approximate entropy (ApEn), may also relate to growth. We found that the higher the ApEn score (equating to increased disorder/randomness), the better the growth rate (Fig. 3) (22).
The effect of different growth hormone (GH) treatment regimens on (a) the body weight and (a) the femur length of GH-deficient dwarf rats. The rats were treated for 6 weeks from 6 weeks of age with a daily s.c. injection of either (1) 100 μg of recombinant bovine (r)GH (fixed dose); (2) 138 μg of rGH one week alternated with a daily dose of 62 μg of rGH the following week [square-wave dose]; (3) rGH at a dose chosen at random from the following possibilities: 5, 15, 50, 80, 130, 170 or 250 μg; these seven doses were given in a random order each week [random dose]; or (4) 0.9% saline [control]. Importantly, by the end of the experiment, the animals in groups 3 and 4 received the same total amount of GH as given by daily standard dosing; and, finally, a ‘random’ regimen in which seven daily doses were chosen at random and repeated over the 6 weeks, again to give the same total dose of GH.

Although 6 weeks of treatment with the four regimens did not result in a significant difference in the absolute weight (Fig. 4a) of the rats, GH treatment caused a significantly greater gain in weight than treatment with saline (P < 0.05) and treatment with a variable GH regimen (square and random) was more effective in stimulating weight gain than the fixed dose (P < 0.05). Most importantly, the square-wave regimen generated the longest femur length at the end of the experiment (Fig. 4b). In addition, individual organ weights were differentially affected by GH regimen: the square and random dosing having the greatest impact on muscle and kidney weight gains. This also extended to IGF-I and IGF binding protein-3 levels being highest in those receiving the square-wave dosing.

The effect of variable dose GH treatment on femur length was modest (two-fold gain compared to fixed dose). Accrual of such modest effects over the many years of treatment throughout childhood could result in a meaningful additional amount of growth with no change to the total amount (and cost) of r-hGH administered. This needs to be tested in a randomised clinical trial.

Relevance to clinical practice and drug development: long-acting GH

An increasing number of long-acting r-hGH preparations have been developed and are at various stages in the clinical trial pipeline (24–27). Most of these preparations generate high concentrations...
of GH in the blood in the first days after injection and then maintain therapeutic levels (as determined by serum IGF-I levels) for variable times thereafter. This does introduce a degree of ‘disorder’ into the treatment regimen, although whether this is occurring at the right frequency is yet to be proven. Preparations to date appear to have similar rather than superior efficacy to daily injections (26). Such drug developments do lead to the question of what is the optimal profile of GH for replacement therapy and would this differ under those conditions that are treated with r-hGH but are not associated with GH deficiency (e.g. Turner syndrome and the short child born small for gestational age)?.

Conclusions

Growth is not a linear process. In fact, it is characterised by significant fluctuations in rate from undetectable to rapid. This pattern is seen not only in humans, but also in a lower order organism such as C. elegans. As children secrete GH from the pituitary in pulses every day, there is an obvious discrepancy between this variable growth rate and the daily secretion of GH. We have shown that the pattern of GH secretion over time intervals > 24 h (infradian rhythms) carries important ‘signals’ that determine growth. When translated to the treatment of short children with r-hGH, we speculate that varying the daily GH dose may improve the growth response; we have demonstrated this is the case in a rodent model but this effect has not been investigated in human studies. Nonetheless, the variation in GH profiles associated with long-acting GH could mimic some aspects of this long-term variation, although, to date, efficacy of long-acting GH only matches (not exceeds) daily injections.

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