

Effect of Short-Term Fasting on Bone Elongation Rates: An Analysis of Catch-up Growth in Young Male Rats

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ABSTRACT

Bone elongation in the postnatal animal is a result of cellular activity during endochondral ossification. Growth plate chondrocytes undergo a differentiation cascade involving stem cell clonal expansion and cellular enlargement during hypertrophy. Nutritional status has a significant effect on rates of bone growth, and a period of accelerated growth will occur if nutritional stunting of growth in early childhood can be corrected. This study focuses on changes in rates of increase in bone length in a model of catch-up growth in 4-wk-old male rats. Animals fasted for 3 d reached a weight ~60% of the control littermates. By 28 d postfasting, fasted animals had regained weight to 95% of control levels. A 3-d fast caused an immediate and profound decrease in rate of growth in the proximal tibial growth plate to only 30% of that of control animals, while *stopping* growth in the distal tibial growth plate. During the rapid initial rate acceleration of bone

elongation, growth rate in both growth plates reached that of the control littermates by 7 d postfasting. The proximal tibial growth plate then maintained rates that were 10–15% higher than control over the rest of the experimental period. By 10 d postfasting, the previously fasted animals were on the same weight/rate trajectory as the control littermates. Changes in elongation rates were reflected by dramatic changes in growth plate morphology in all cellular zones. This is the first study to directly correlate weight recovery during catch-up with growth rate responses at the level of the growth plate. (*Pediatr Res* 53: 33–41, 2003)

Abbreviations

OTC, oxytetracycline
PTU, propylthiouracil
RHT, ruthenium hexamine trichloride

In both the prenatal and the postnatal animal, there is a complex interplay between overall nutritional status and linear bone growth. There is experimental evidence that GH/IGF-1 concentration is responsive to changing nutritional status (1–5), and growth responses to food deprivation also have been shown to involve the hormone leptin, which is secreted by adipocytes (6–8). However, how undernutrition is translated into changing cellular activity during bone elongation has not been studied. This is significant for understanding cellular mechanisms behind the complex phenomenon of catch-up growth, which can occur to different extents, and at different rates, depending upon whether nutritional deprivation occurs *in utero*, during the early neonatal period of nursing, or later in postnatal growth (4–6, 9–12). Catch-up growth, as first described by Prader in 1963 (13), is characterized by a period of

growth accelerated above normal during recovery from a period of previous deprivation, which allows the child to accelerate toward, and even resume, his/her preillness growth curve (14). A recent definition is “height velocity above the statistical limits of normality for age and/or maturity during a defined period of time, following a transient period of growth inhibition (9).”

Multiple rodent models of catch-up growth have been investigated, differing in the timing of, and the nature of, the stimulus for growth retardation. The most common pharmacologically induced models to rapidly slow growth use either the anti-thyroid agent PTU (15–17) or dexamethasone (5, 14, 17–19). Models in which manipulation of nutrition is the primary variable include 1) food restriction to the mother during gestation and/or lactation (20, 21); 2) bilateral uterine artery ligation during late gestation (22, 23); 3) manipulation of litter size during weaning, contrasting litters of four pups to litters of 16 or more pups (11, 18, 24); and 4) short periods of fasting to the postweaning animal (3, 5, 17, 25–29). These experiments have demonstrated that the timing and the duration of the stimulus are critical, with differing outcomes in

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prenatal/postnatal and preweaning/postweaning experiments (27).

The purpose of this study was to analyze changes in long bone growth rates at the level of the growth plate in short-term-fasted rats post refeeding over a 28-d interval. The model was 4-wk-old male rats in pairs with one having a 3-d fast and the littermate used as a control. This is a model intended to understand growth plate cellular response to, and catch-up of bone elongation after, a period of short-term malnutrition and/or total food deprivation during the rapid growth phase of childhood/adolescence. Growth rates in the proximal and distal tibial growth plates were measured using the fluorescent calcium chelator, oxytetracycline, which allows an estimate of the rate of growth in the 24-h period before euthanasia (30). Body weights and food consumption in the catch-up period also were monitored. The hypothesis was that 24-h rates of tibial growth initially would be significantly depressed in the fasted animals compared with control littermates, but that rates of growth would increase until they exceeded that of control littermates during the time course of catch-up growth. It also was hypothesized that the timing of growth plate closure would not be affected in this model. The data also allow a comparison of the trajectory of catch-up in weight to the trajectory of catch-up in rates of bone elongation, and how both of these are sustained through time.

METHODS

Sprague-Dawley rats (CD-1 IGS) were purchased from Charles River Laboratories and a small breeding colony was established at our facility for the rats used in this study. To ensure that intrauterine and preweaning litter size were not variables, only rats born into litters sizes of 10–14 animals were used. The rats were weaned at 3 wk and fed *ad lib* until 4 wk of age (peripubertal). Mothers were fed a pregnancy chow [Global 22/5 Rodent Diet (W) 8640, Harlon Teklad, Madison, WI, U.S.A.] during both pregnancy and nursing. All animals were kept under circadian lighting conditions, with 12 h light/12 h dark. Male littermates were assigned to groups as pairs, one to continue to be fed a commercial rat diet (Teklad Global 16% Protein Rodent Diet, 2016) *ad libitum* (control), and the other to be fasted for 3 d with water provided (fasted). All animals had access to a large tube for shelter in each individual cage. During the fasting period, the animals were observed daily both by ourselves and by a staff veterinarian; the fasted rats remained bright, alert, and active. There was no evidence of spontaneous disorders in any animal throughout the study. Male rats were used because it is well documented that there is a sexual dimorphism in the response to GH, male rats gain weight faster than female rats in the postweaning period, and male rats reach a final large body size (26, 30–32).

Time zero was defined as the end of the fast, and collections of proximal and distal tibial growth plates were made at 0, 1, 2, 3, 7, 14, 21, and 28 d. Each time group consisted of six animals from different litters. This experimental design involved 96 animals total (48 controls and 48 fasted) representing 22 litters. Animals were injected with the calcium chelator OTC (5 mg/kg) 24 h before euthanasia. OTC is laid down

initially in areas of high calcium turnover, which includes the calcified septa of the growth plate in the distal hypertrophic cell zone and the newly formed metaphyseal bone (33). Incorporated OTC after 24 h can be viewed as a fluorescent band in the metaphyseal bone. All procedures were approved by the Institutional Animal Care Committee.

The two growth plates of the tibia were chosen for analysis. In the 4-wk-old rat, the proximal tibial growth plate and is one of the fastest growing in the animal, and it is growing at approximately three times the rate of the distal tibial growth plate (34). In addition, the proximal growth plate will continue in active growth for several weeks longer than the distal growth plate (35). Therefore, these two growth plates are representative of differential growth rates occurring in the animal both at one point in time and through time.

Growth plate collections were made 1 h after lights on. After euthanasia with a lethal dose of pentobarbital (300 mg/kg, i.p), the two growth plates were collected, rapidly trimmed, and fixed for 3 h in 2% paraformaldehyde/2%glutaraldehyde in 0.1M cacodylate buffer, pH 7.3, with 0.7% RHT, and embedded in Epon-Araldite using standard techniques (34). RHT is a small molecular weight cationic dye that diffuses rapidly into the cartilaginous matrix, stabilizing both the negatively charged proteoglycans of the matrix as well as the interface between the chondrocytic plasma membrane and the pericellular matrix. RHT has been demonstrated to be required for satisfactory preservation of growth plate chondrocytes similar to their *in vivo* morphology, and therefore RHT-fixed growth plates are considered to give optimal fixation (36).

OTC fluorescence was visualized under epifluorescent microscopy on 1-micron-thick sections with an excitation filter of 365–380 nm and a barrier filter of 530–540 nm. To estimate the elongation over 24 h, the distance between the leading edges of the fluorescent band in the metaphyseal bone and the chondro-osseous junction was measured on video-captured images (3CCD color Sony Progressive video camera, Sony, Teaneck, NJ, U.S.A.). The chondro-osseous junction was identified and a line was drawn traversing it. Next, the leading edge of the fluorescent OTC band was identified, and a second line drawn that represented its location in the metaphyseal bone. Both of these lines were drawn with a relatively thick drawing tool such that each line could represent the general form of the transition it was identifying, minimizing minor irregularities. Elongation was determined by averaging 10 measurements equally divided from sections from two different blocks from the same animal. Measurements were made by individuals blinded as to the specific identity of the slide they were measuring, to eliminate the potential for systematic bias. We have found this method of measurement to give consistent results among different individuals, and we as well as others have used it in previous experimental situations (34, 35, 37–39). Color staining of one-micron-thick sections was with basic fuchsin/methylene blue/azure 2 (40).

Animals were weighed five to seven times a week from d 0 throughout the experimental time period. Food consumption was monitored at similar time intervals. For all subsequent calculations, differences between groups were analyzed by ANOVA, at $p < 0.05$, with *a posteriori* pair-wise comparisons.

RESULTS

A 3-d fast led to a dramatic weight difference between the control and fasted group, as seen in Figures 1 and 2. Starting from initial weights that were essentially identical, control rats increased in weight by 16.7% (102.4 g at d -3 versus 119.5 g at d 0), whereas fasted rats lost weight by 29.8% during the fasting period (104.0 g at d -3 versus 73.0 g at d 0). Thus, by the end of the 3-d fasting period, weights of the fasted animals averaged only 60.0% of their matched littermates ($p < 0.01$). Weight gain as a function of time postfasting is shown in Figure 3A. Fasted animals weighed less than control animals throughout the experimental period, but the magnitude of the difference continued to lessen over time. Starting at weights 60% of their control littermates immediately postfast, the fasted animals reached 95% of the control weight after 28 d. The differences in weight were statistically significant at $p \leq 0.02$ for each of the time periods, except at 28 d ($p = 0.06$). In Figure 3B, cumulative percent weight gain is plotted against

days from the end of the fast. Over the 4-wk experimental period, control animals increased in weight $\sim 275\%$; in the same period of time, fasted animals increased in weight by $\sim 450\%$. For the first 3 d of the experimental period, daily weight gain for the fasted animals averaged 12.4 ± 1.3 g compared with 8.6 ± 0.8 for the controls ($p < 0.01$). By comparison, for the last 3 d of the experimental period, the weight gain for the fasted animals (6.3 ± 1.6 g) was not statistically different from the controls (5.2 ± 2.4 g) ($p = 0.30$).

Figure 4 plots food consumed per day in relationship to body weight for the two groups throughout the experimental period. For the first 2 wk, the fasted animals consumed more food in relationship to body weight than did the controls; at the time points of 3 and 4 wk, the rates for the two groups were identical. Therefore, during the period in which the most rapid changes were occurring, the previously fasted animals were consuming significantly more food for their body size than were controls.

Figure 5 demonstrates the method by which growth rates were calculated from histologic sections showing OTC fluorescence. On each section, the chondro-osseous junction of the growth plate was identified (proximal arrowhead in the left margin of each micrograph). The distance from the chondro-osseous junction to the leading edge of the fluorescent band (distal arrowhead) represented growth over the last 24 h. It is readily seen in Figure 5, comparing the proximal tibial growth plate from a control animal at 0 time to the proximal tibial growth plate from a fasted animal at 0 time, that there was a significant retardation of growth rates as a result of fasting, a reduction to a rate that was $\sim 30\%$ of control immediately after the fasting period.

Actual growth rates for both the proximal and the distal tibial growth plates at all time periods are plotted in Figure 6. In the control animals starting at d 3, there is a clear constant slowing of growth rate in the proximal growth plate over the experimental time period, from $\sim 350 \mu\text{m/d}$ and declining to $\sim 180 \mu\text{m/d}$. This represents a decline of approximately 50% over the 4-wk period. These rates agree well with previously recorded rates for this growth plate and demonstrate the high but steadily declining rate of growth that is characteristic of rodent adolescence (30, 34, 35). The distal tibial growth rate mirrors this picture, albeit beginning at a rate less than one third of the proximal growth plate and effectively ending statistically significant growth 21 d into the experimental period (7 wk of age), with rates that are just at the limit of reliable measurement ($< 15 \mu\text{m/d}$).

Rates of growth in the proximal tibial growth plate of the fasted animals began at approximately one third of that of controls immediately postfasting, and actually fell by the first postfasting (difference control versus fasted $p < 0.01$ for all four time points) (Fig. 6). Then, however, there was a very steep rise to the growth rate curve so that by d 7 the rate was identical to that of the control animals ($p = 0.43$). For the remainder of the experimental period, the growth rate of the proximal tibia remained above that of the control, sometimes by as much as 15% ($p < 0.03$ at 21 d). Even after 28 d, the proximal tibial growth plate of the fasted animals was still

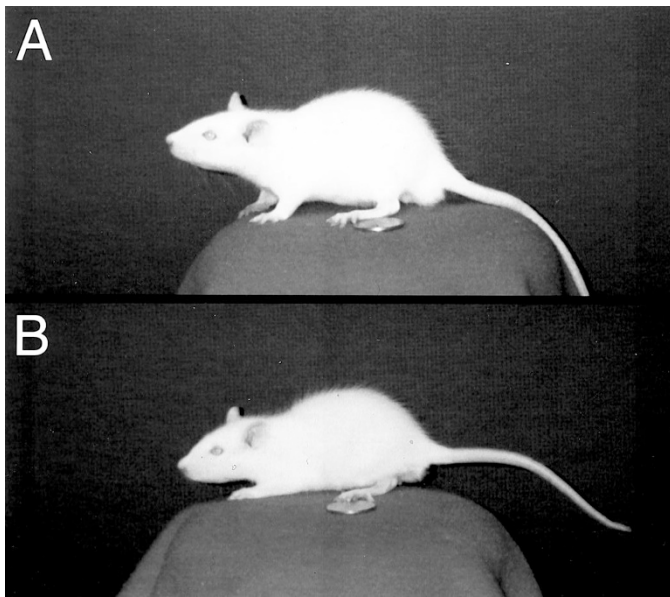


Figure 1. Size effects of a 3-d fast. The rats in *A* and *B* are male littermates. *A* shows a control animal and *B* is his littermate, who was fasted for 3 d at 4 wk of age. Photos were taken immediately after the fasting period.

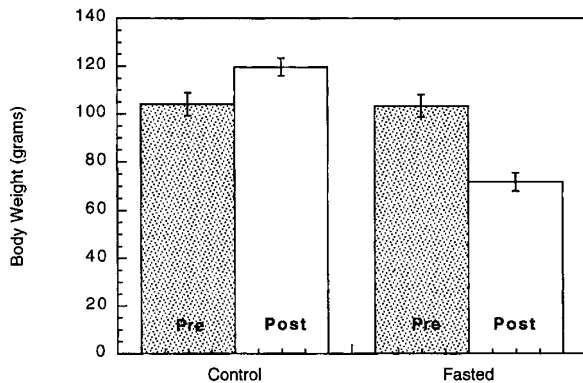


Figure 2. Weight effects of a 3-d fast. At 4 wk of age, all rats weighed slightly over 100 g. After a 3-d fast, the fasted animals averaged only $\sim 60\%$ of the weight of the control animals.

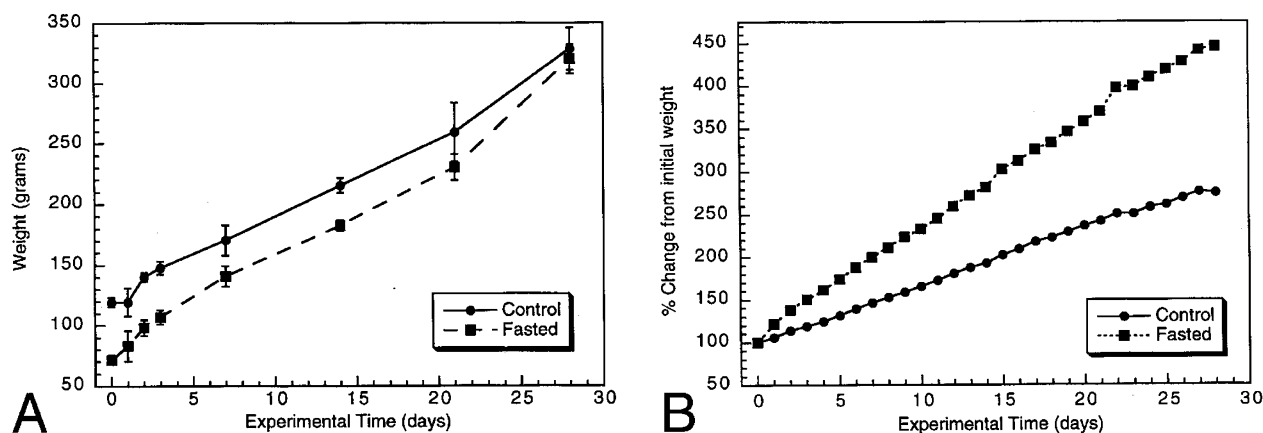


Figure 3. Weight gain of fasted and control rats. *A* plots the weight gain in both control and fasted animals over a 4-wk period postfasting. Immediately after the fast, fasted animals weighed approximately 60% as much as their control littermates compared with 95% as much at the end of the 4-wk experimental period. *B* demonstrates that control animals increased in weight by ~250% over the 4 wk, whereas fasted animals increased their weight by over 425% in the same time period.

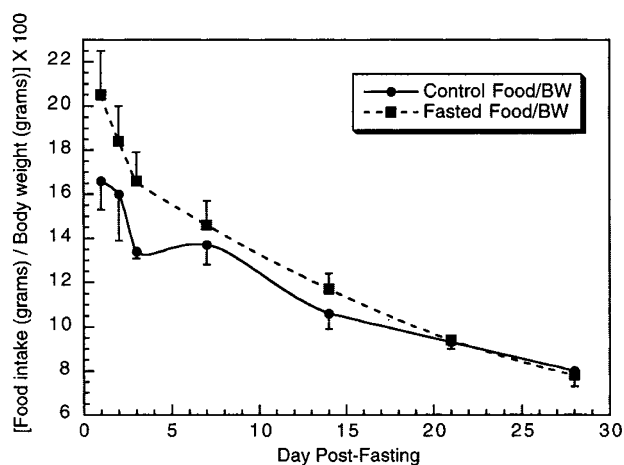


Figure 4. Weight gained to food consumed: comparisons of fasted and control animals. For the first 2 wk, fasted animals consumed more food per day in relationship to their body weight than their control littermates. However, by wk 3, food consumption in relationship to body weight of the fasted animals was identical to that of control animals.

growing >10% over that of the controls, which is as much as 25 μm of additional daily growth ($p < 0.06$). This is shown on the micrographs of Figure 7*A*, which demonstrate typical OTC data for the proximal tibial growth plate of control and fasted animals at 28 d postfasting. However, it should be noted that never during the catch-up period did rates in the proximal tibia of fasted animals reach the level of controls at their fastest.

Data for the distal growth plates as shown in Figure 6 mirror the findings for the proximal growth plate with two important exceptions. First, at d 0, essentially all growth had stopped in the distal tibial growth plate of the fasted animals (difference fasted from control, $p < 0.002$). Secondly, although growth rapidly resumed and actually was above control by d 7 ($p = 0.10$), growth in this growth plate approximated control values by d 21 and, like that in the control animal, essentially was 0 by the end of the 28-d experimental period (difference fasted from control, $p = 0.22$). Although it might be premature to generalize based on the data from this one growth plate, these data would suggest that catch-up occurred because of higher

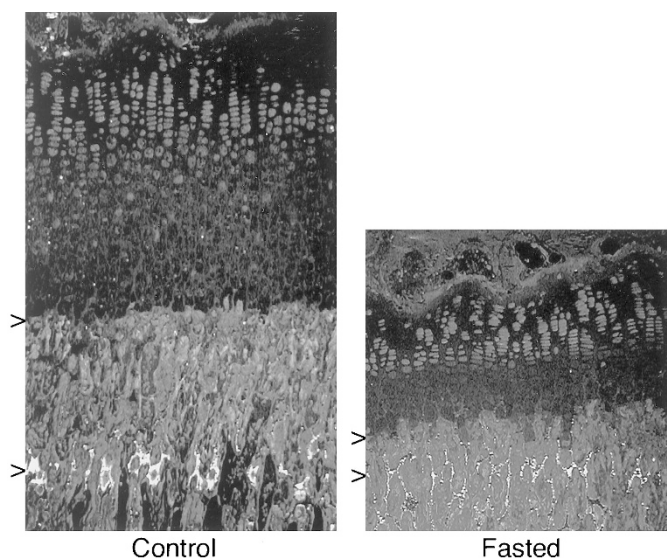


Figure 5. Growth rates in the proximal tibia measured by OTC fluorescence. The method for measuring growth rate by OTC fluorescence by identifying the distance between the chondro-osseous junction of the growth plate (proximal arrow in each micrograph) and the leading edge of the fluorescent band seen in the metaphyseal bone is demonstrated. This distance represents the amount of growth in 24 h. A comparison of the proximal tibial growth plate from a control animal after the 3-d fasting period with that from a fasted animal shows that fasting had reduced growth in this growth plate to approximately one third of normal. Bar = 100 μm .

rates immediately after the fasting period but without altering the timing of cessation of growth. The micrographs in Figure 7*B* show this comparison: a control distal tibial growth plate at the left in Figure 7*B* compared with the same from the fasted animal (center). The latter shows all fluorescence at the chondro-osseous junction, indicating cessation of growth. The micrograph to the right in Figure 7*B* demonstrates the resumption of growth in the distal tibial growth plate of a fasted animal by d 7, to a value actually higher than that of the control at the same time period (latter not shown).

Figure 8*A* compares the response trajectories for weight and proximal tibial growth rate in the fasted and control groups by plotting the ratio of fasted/control for these two variables as a

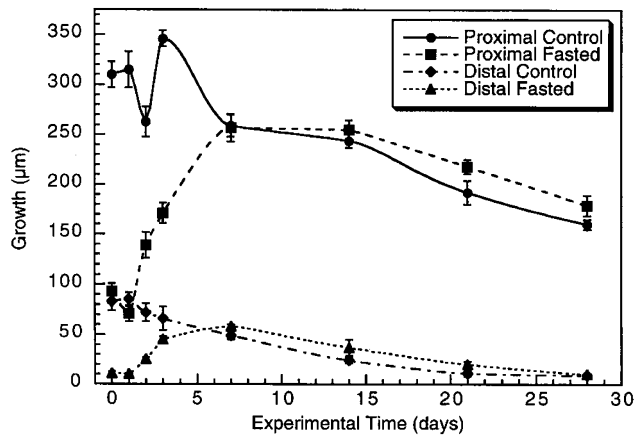


Figure 6. Comparisons of 24-h growth rates in growth plates from control and fasted animals. Growth rate of the proximal tibia in fasted animals, although beginning at a level 30% that of control animals, reached the level of controls by the end of the first week and remained 10–15% higher than control for the remainder of the experimental period. Results for the distal tibial growth plate mirrored this but were more dramatic. Fasting caused cessation of growth in the distal growth plate, growth rate caught up before d 7, and by d 21 there was no statistical difference in rate between control and fasted animals. In both groups, the distal tibial growth plate essentially had stopped growing by wk 3 of the experimental period.

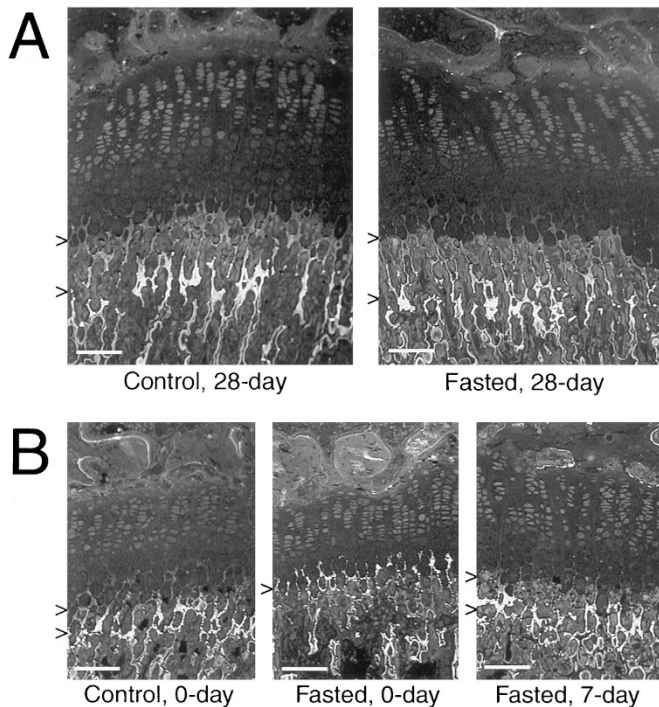


Figure 7. Growth rates in fasted and control animals over time by OTC fluorescence. *A* demonstrates that, by the end of the experimental period, growth rate had slowed dramatically in both groups, but remained slightly higher in the previously fasted animals. *B* makes important comparisons for the distal tibial growth plate. It is clear that immediately after the fast, growth in the distal tibial growth plate had ceased in the fasted animals, whereas there was significant growth in the controls. By d 7, growth had resumed in the distal tibial growth plate of fasted animals. Bar = 100 µm.

function of time. Although in the most literal sense the fasted animals never completely caught up in weight to the controls, in actuality they went from a weight that was approximately 60% of that of controls to a weight that was approximately

95% of controls. Growth rate in the proximal tibia was reduced to approximately 30% of control after 3 d of fasting, but caught up to control rates in only 7 d. It remained at a rate higher than controls for the rest of the experimental period. Figure 8*B* plots the correlation between animal weight and rate of growth in the proximal tibial growth plate. The curve for the controls demonstrates that, as expected, as animals increase in weight, the rate of bone elongation in the growth plate decreases. For the fasted animals, in contrast, during the catch-up phase as weight increases, rate of bone elongation also increases. There is then an abrupt change when the trajectory of the fasted animals joins that of the controls, as weight increases and rate of elongation decreases in the last 3 wk of the experiment. The growth trajectory for the two groups of animals is essentially identical during the last 3 wk (superimposed curves), despite their differences in weight.

The histologic appearance of growth plates from pairs of animals at different time periods are illustrated in Figure 9, with control on the left in each figure and fasted on the right. Figure 9*A* (left) demonstrates the general histologic organization of the proximal growth plate in control animals at the start of the experiment, showing the characteristic columnar arrangement of cells in a differentiation cascade of proliferation, hypertrophy, and cellular death with vascular invasion at the chondro-osseous junction. In animals fasted for 3 d (Fig. 9*A*, right) there was a very dramatic reduction in overall growth plate height, which included height changes in all cellular zones. Not only was there an apparent reduction in overall cellular number, but also in the final volume of hypertrophic chondrocytes. No disorganization of the characteristic spatial organization of growth plate chondrocytes was seen in growth plates from fasted animals. Figure 9*B* demonstrates the similar comparison for the distal tibial growth plates. Distal growth plates in the two groups do not differ as dramatically, but in the fasted animals there is still a clear reduction in overall growth plate height, with an apparent decrease in cellular number and hypertrophic cell volume. Although not an absolute correlate, the overall difference in height between the proximal and distal tibial growth plates of the control animals reflects their very different growth rates, with the proximal growth plate growing at approximately three times the rate of the distal growth plate (34, 41). By d 7, growth plates from the fasted and the control animals were growing at the same rate, and this was mirrored by their histologic appearances. The proximal growth plate of the fasted animals was essentially identical morphologically to that of the controls (Fig. 9*C*), and this was also true of the distal growth plates (Fig. 9*D*).

DISCUSSION

Whereas most studies follow catch-up growth in relationship to body weight or overall body length, many fewer studies have looked at the catch-up response specifically in relationship to the rate of linear growth of long bones of the limbs (14, 16, 42). Postnatal bone growth occurs by endochondral ossification in growth plates at the ends of long bones. Within the growth plate, the spatial organization of chondrocytes reflects a temporal progression of chondrocytic differentiation (life

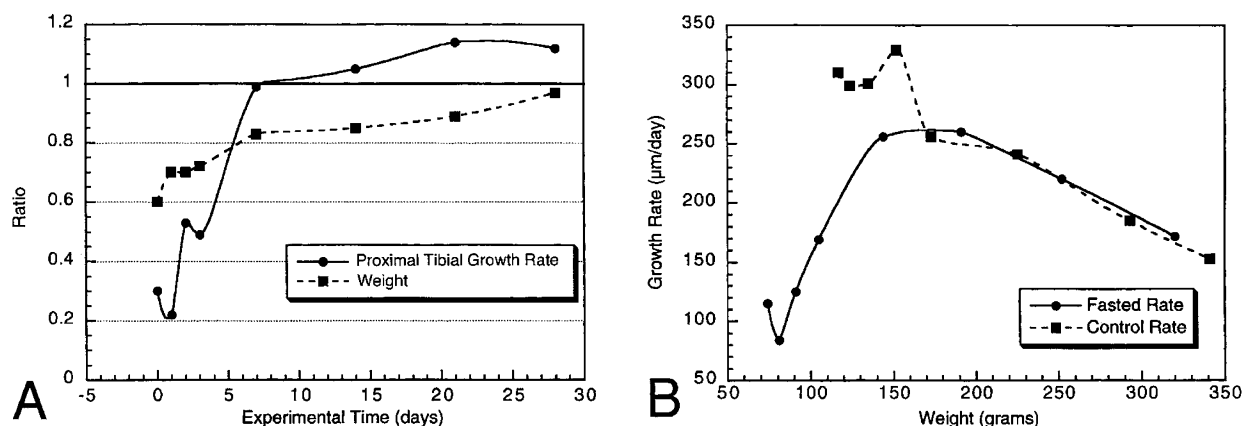


Figure 8. Growth rate in the proximal tibial growth plate and animal weight. *A* plots ratios of fasted to control animals for weight gain and gain in growth rate in the proximal tibia during catch-up growth. Maintenance of a growth rate consistently higher than controls means that tibial elongation is still continuing. *B* plots the correlation between growth rate in the proximal tibia and animal weight over the experimental period. As control animals gained weight, their rate of growth generally slowed. This was most apparent for the last 3 wk of the experiment. However, fasted animals showed two trajectories of bone elongation rate. During the catch-up period (ascending in *B*), rate of growth increased as body weight increased. For the last 3 wk of the experiment, the trajectory of the fasted animals essentially mirrored that of controls.

history). The two primary rate dependent variables that interact to determine quantitative increases in long bone growth are mitotic activity in the proliferative zone and cellular volume increase and shape change during chondrocytic hypertrophy (34, 35, 37, 43). Conceptually these can be considered to be bounded by three important gates—the transition from the germinal chondrocyte's entrance into the cell cycle to start clonal expansion in the proliferative zone; the transition between the exit from the cell cycle to the initiation of cellular volume increase in the hypertrophic zone; and the transition from the terminal chondrocyte to vascular invasion (44). At each of these transitions there are major changes in gene expression. Rates of linear bone growth can be altered both by rate changes during proliferation and hypertrophy, as well as by rate changes at any of the three transition points (44,45).

During the chondrocytic differentiation cascade that results in increased linear growth, there is a complex interplay between systemic, autocrine, and paracrine regulators of overall rate of growth. This is played out on the growth plate template that was patterned early in prenatal development and that has intrinsic capabilities and limitations, as well as a limited life span (closure occurs at the end of adolescence). Therefore, the growth plate is a particularly interesting organ to analyze in relationship to catch-up growth, not only because it is the organ that controls the rate and extent of linear growth, but also because it represents an organ where multiple levels of regulation, from that of embryological patterning to that of systemic and nutritional regulators at the level of the whole animal, are integrated.

This is the first study to measure precise growth rate responses to short-term fasting at the level of individual growth plates, rather than weight changes and/or changes in overall body or limb length. Growth rate in the proximal tibial growth plate of the fasted animals reached that of the control limb at 1 wk postfasting, and remained sustained at a higher rate of growth throughout the 4-wk experimental period. Therefore, the initiation of the catch-up response can be considered

immediate and profound. Fasting led to immediate changes in cellular activity in the growth plate, such that longitudinal growth rate was reduced to approximately one third of control level in the proximal growth plate, and was essentially brought to zero in the distal growth plate. By examining two growth plates growing at very different rates, it is possible to generalize that the response is through an immediate change in growth rate at both ends of the limb, and therefore is a response that should be considered in light of what is known about the control of differential limb growth in the postnatal animal (34, 37, 43, 45).

Previous investigators, using several different fasting-based models of catch-up growth in which weight changes have been measured as the end point, have concluded that deprivations postweaning are much less serious than those preweaning, and that there is progressively less effect as the expected rates of growth in controls decreases (27, 28). Therefore, when examining the magnitude of the response in the present study, it is important that comparisons be made with other investigations in which the age, sex, species (and even strain) of the animals, and duration of the fast, are comparable. The percent weight loss that we recorded after a 3-dfast (29.8%, starting with male rats weighing ~100 g) was comparable to that seen in several previous similarly designed studies: 25% loss, starting at weights of ~120 g (26); 42.8% loss, starting at weights of ~70 g (17); and 28% loss, starting at weights of ~125 g (16). Thus, this model is one of acute and severe nutritional deprivation, in which we have demonstrated that changes in bone growth rates are even more dramatic than changes in rates of weight gain/loss. Its significance for disease in children is to further the understanding that growth plates respond to *any* severe period of nutritional stress. Recovery of bone elongation rates to control levels is rapid; however, recovery in terms of achieving the prestress predicted total length of the bone is a prolonged process, even if the period of insult was of very short duration.

All variables capable of altering rate of growth must be considered as potential mediators of the catch-up response.

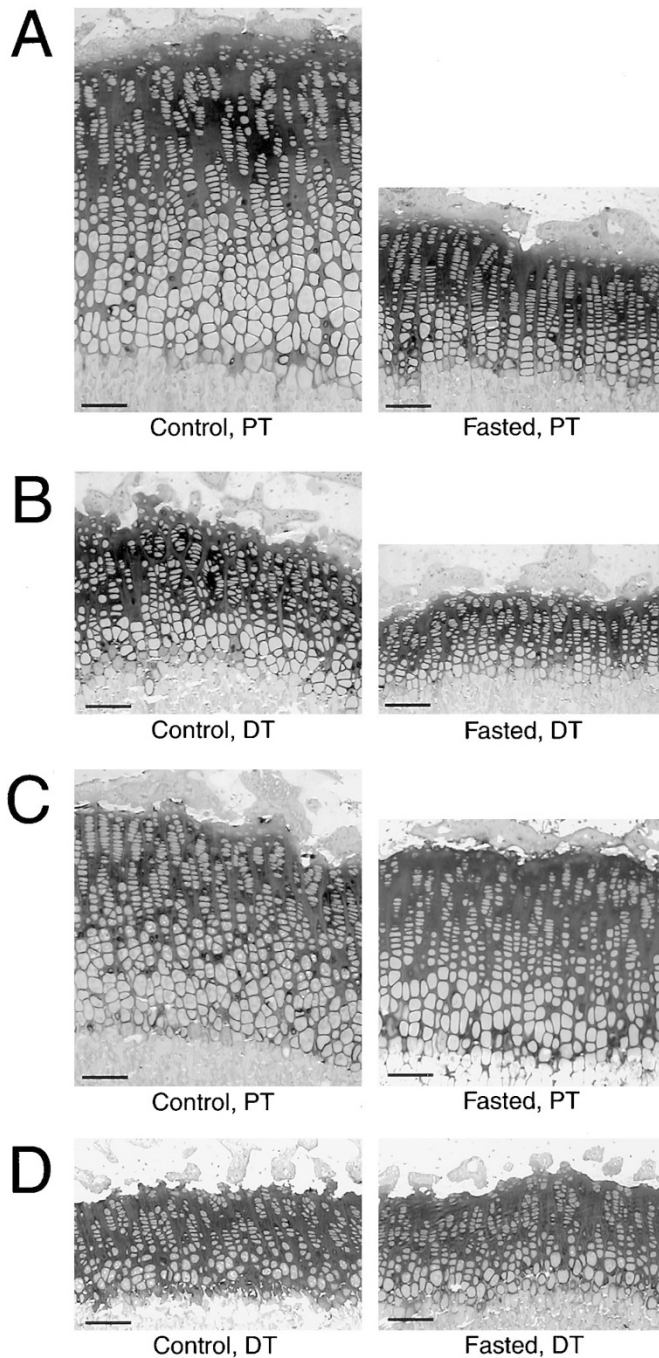


Figure 9. Histologic appearance of growth plates from control and fasted animals. *A* shows the highly organized columnar arrangement of chondrocytes of the proximal tibia of a 4-wk-old control animals. In fasted animals, columnar organization was maintained. However there was a sharp reduction in overall growth plate height, with an apparent reduction in the number of chondrocytes in all zones. There was also an apparent reduction in the final volume of hypertrophic chondrocytes. *B* makes a similar comparison for the distal tibial growth plates. *C* and *D* show the same comparisons for control and fasted littermates at d 7, when catch-up in growth rate had been achieved. Bar = 100 μ m.

These may vary depending upon the specific model. In a recent study using a dexamethasone model in rabbits, the primary alteration to the growth plate is reported to be to the proliferative cell population (14, 42). Therefore, the catch-up response in this model is consistent with hypothesizing that dexamethasone essentially causes a delay in, but not an alteration of, the

number of stem cell divisions. This has been interpreted as delaying senescence of the growth plate but not altering its total proliferative capacity, consistent with the concept of replicative senescence described in other cell types (46, 47). When dexamethasone was given to one limb rather than systemically, acceleration of growth rate during catch-up was only in the limb that received the treatment (14). This supports the hypothesis that the response in catch-up growth is “intrinsic to the growth plate,” that it involves interruption of a prepatterned program of growth, that the program can be delayed, but that ultimately an intrinsic program determines the final length of a bone, a so-called “sense of size” (48, 49). Further support for such an intrinsic program within the growth plate comes from experiments involving transplantation of growth plates. The transplanted growth plate maintains the growth rate of the donor and not of the recipient, suggesting that the transplanted growth plate carries its own program or cadence for continuing growth, despite changes in the systemic hormonal environment (50).

Models involving fasting go beyond the concept of catch-up growth as intrinsic to the growth plate and support the concept that catch-up growth involves the full panoply of variables during the differentiation cascade of the growth plate chondrocyte in the postnatal animal. Some of these are determined at the time of embryonic patterning of the bone and others are regulated by the interplay of systemic and autocrine/paracrine regulators acting throughout the period of growth. This is consistent with considering that basic mechanisms of catch-up growth are regulated through a systemic response under neuroendocrine control, with GH as a primary target since, in the presence of normal thyroid functioning, GH and IGF-1 constitute the primary hormonal axis of postnatal growth (51, 52). The degree of mismatch between the target size and the actual size is compensated for by adjusting growth rate integrated at the level of the nervous system, as originally proposed by Tanner (32, 53).

In support of this, studies have found decreases in serum GH and IGF-1 levels during fasting, and increases during the catch-up period (5, 23, 28, 54). However, a study in 5-wk-old rabbits that were fasted for 2 d demonstrated that although serum IGF-1 levels fell during the fast, serum GH levels rose. In the growth plate, the IGF-1 system was up-regulated through up-regulation of mRNA for both the GH receptor and IGF-1 (3). A study of young fasted rats indicated that, when giving additional GH in the postfasting catch-up period, tibial growth in male rats could not be enhanced above the rate involved in “normal” catch-up (26). These authors hypothesized that tibial growth in the catch-up period already was maximal for rats of that age. Although the specifics of the response still needs further investigation, there is a basic consensus that GH-mediated changes in growth rate are involved in the catch-up response to short-term fasting, regulated through a systemic response.

Leptin, the product of the *ob* gene synthesized in adipose tissue, serves as an afferent signal in a negative feedback loop regulating the size of adipose tissue mass (7, 8, 23, 55). It has been demonstrated that, although leptin given to normal adult rats does not lead to a change in spontaneous GH secretion,

leptin given to fasted rats causes a reversal of the inhibitory effect fasting causes on GH secretion (6, 56). Therefore, leptin can act as a metabolic signal connecting adipose tissue with the GH axis, and may have different roles during normal homeostasis and during fasting. When adult rats or mice were fasted for 1–3 d, there was a sharp decrease in the amount of leptin mRNA in adipose tissue (57, 58). Another study demonstrated reduction in the amplitude of leptin pulsatile secretion in rats after a 48-h fast, manifested by decreased plasma levels (59). Therefore, leptin may act as a circulating feedback signal to regulate general energy homeostasis by reflecting adipose tissue stores. Although metabolic changes mediated by leptin are not completely understood, the leptin mediated effects have always been related to weight regulation and a set-point involved with body weight (60). It is interesting to speculate that leptin, acting either directly through its own receptors on growth plate chondrocytes (61, 62), or indirectly through either GH or thyroxin (51), may be an important mediator of bone elongation during catch-up growth. This would be a significant area for future investigation.

A continuation of this study will use stereological analyses of chondrocytic performance parameters to analyze chondrocytic responses during the differentiation cascade resulting in increased rate of growth, as has been done in our previous studies using models of several different altered metabolic or biomechanical states (34, 38). It will test the hypothesis that the reduced rate of elongation after food deprivation as well as the increased rate after return to normal nutrition are regulated by altering the rate chondrocytes proceed through their differentiation cascade, amplified by volume changes of the hypertrophic chondrocyte. This would be consistent with a response mediated by changes in the GH/IGF-1 axis (39).

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