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The Effect of Periosteal Resection on Tibial Growth Velocity Measured by Microtransducer Technology in Lambs

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Abstract

Background—Disruption of the periosteum, whether traumatic or elective, has long been known to accelerate growth in the developing skeleton. However, the extent, timing, and mechanism of the resultant increase in growth velocity (if any) remain undefined. The primary research questions were: Does periosteal resection result in a change (increase) in growth velocity of a long bone at the growth plate? When after the resection does the effect start and for how long? Finally, which of several cellular mechanisms is most likely responsible for the change in growth velocity.

Methods—Five lambs underwent proximal tibial growth plate periosteal resection with subsequent measurement of growth velocity by implantable microtransducers or fluorochrome labeling. This former technique provided real-time growth velocity data with a resolution of about 10µm (width of a proliferative zone chondrocyte). These measurements were accurate at up to four weeks postoperative, as verified by fluorochrome labeling, and radiographic measurement. Two lambs were continued on the study for an additional three weeks. Histomorphometric and stereological assessment of chondrocytic kinetic parameters was performed on control and experimental tibiae following euthanasia.

Results—Periosteal resection increased growth velocity in every lamb, at every time point, and in a consistent and sustained manner. Histomorphometric correlation to this phenomenon indicated that the cellular basis of this acceleration was most likely the result of hypertrophic chondrocyte axial elongation, rather than changes in chondrocyte proliferation, magnitude of hypertrophic chondrocytic swelling, or increased matrix production.

Conclusions—Periosteal resection creates immediate and sustained acceleration of growth resulting from axial elongation of the hypertrophic chondrocyte. While the increase in growth velocity was consistent, the absolute magnitude of the acceleration suggests that periosteal resection be considered as an adjunct to other primary procedures. Periosteal resection may serve as a useful clinical adjunct to provide a modest growth stimulus in cases of hemihypertrophy or angular limb deformity, or to counteract the growth inhibition seen when performing distraction osteogenesis.

Keywords

growth plate; physis; growth; growth velocities; hypertrophic chondrocyte

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INTRODUCTION

Longitudinal growth of the developing skeleton is an intricate process originating at the epiphyseal plate and regulated by a complex interplay of local, systemic, and mechanical factors. The periosteum is one factor thought to exert a vital influence upon physal growth. A relationship between the physis and the periosteum has long been postulated and was described first by Ollier in 1867, when he attributed posttraumatic skeletal overgrowth to disruption of the periosteum¹. Various investigators have tried to apply this hypothesis, thus employing periosteal resection in both veterinary and human medicine to treat angular limb deformities and limb length discrepancy^{2, 3, 4, 5, 6, 7}.

When faced with length discrepancy or angular deformity; clinicians can retard the entire physis (to equalize to a short contralateral limb) or portions of a growth plate (in order to correct angular deformity in the ipsilateral limb). It seems counter-intuitive to surgically alter the normal leg in order to accommodate deformity or length discrepancy. However, methods to increase growth in the affected limb are not reliable and are uncommonly performed. Although periosteal resection is a known mechanism to increase growth; results are not predictable; it is not known if increased growth occurs instantaneously and how long it is maintained. Furthermore, uncertainty remains about the underlying mechanism and pattern of altered growth that follows periosteal disruption.

The purpose of our study, using microtransducer-telemetry technology, was to measure changes in tibial growth velocity following periosteal resection in an adolescent lamb model. In addition to measuring changes in the temporal pattern of growth velocities, we also were interested in measuring chondrocytic kinetic parameters that correlate with observed changes in growth. We were interested if an observed change in growth velocity was the result of changes in the kinetics of chondrocyte proliferation, hypertrophic chondrocyte swelling, cell shape changes, or some combination of these.

MATERIALS AND METHODS

Five mixed-breed lambs were used in this study. Lambs were acquired as 7–8 week old weanlings, were housed in a large pen in which free-ambulation was encouraged, and experienced standard husbandry. All studies were approved by the Institutional Animal Care and Use Committee.

Lambs as Animal Models

In comparison with other animal models (rabbits, dogs, pigs, rats, mice), adolescent lambs have relatively slow growth velocities and are chronologically older at skeletal maturation reaching adult height at about 42 weeks of age^{8, 9, 10}. Using approximate age/body weight scaling, our lambs' age correspond to children about 6 – 7 years of age. At 10 weeks of age these lambs weigh on average 26 kg, while 6 year-old boys on average weigh about 20 kg.^{11, 12} The proximal tibial growth plate is elongating at about 200µm/day, a modest growth velocity in comparison with rabbits (500µm/day) or rats (400µm/day). Lambs cycle through, on average, 31 recumbency *versus* standing intervals per day with the average standing/ambulation interval of 16.9 minutes and an average recumbency interval of 29.8 minutes. When converted to total hours per day, lambs stand for about 8.6 hours/day and are recumbent for 15.4 hours/day¹³. In a recent study of hundreds of 8 year-old children, these children averaged 5.4 hours/day in standing/ambulation and 19.6 hours/day in recumbency^{14, 15}.

Periosteal Resection and Microtransducer Placement

At 10–12 weeks of age, we performed a circumferential resection of periosteum on one (randomly selected) tibia. The periosteal strip was 10 mm wide and 1 cm below the proximal tibial growth plate. The opposite tibia received a sham operation in which the same wound was produced and the periosteum was exposed without resection.

In order to measure immediate real time effect of this periosteal resection of growth velocities, we implanted microtransducers across both the experimental and control proximal tibial growth plates of three lambs (Figure 1). The remaining two lambs underwent the same experimental protocol but did not have microtransducers placed in order to optimize fixation methods for subsequent histomorphometric and stereological analysis. Importantly, a previous study demonstrated that implantation of this microtransducer has no significant effect on the normal growth velocities or normal chondrocytic kinetic parameters¹³. In brief, this microtransducer (energized by an implantable battery with growth velocity data sent by telemetry) is a linear displacement transducer with a resolution of about 5 to 10 μ m (about the height of one proliferative zone chondrocyte). Beginning 48 hours after surgery, we compare proximal tibial growth plate growth velocity from the limb with periosteal resection with the control limb. We are able to make this comparison essentially continuously (every 167 seconds), hour by hour, day by day, and week by week over an extended interval of the lambs' growth period.

Fluorochrome Labeling to Measure Growth Velocity

At the time of injection the alizarine fluorochrome label is permanently incorporated and fixed in a narrow band at the mineralizing front at the chondro-osseous junction. With time and continued new endochondral ossification, the chondro-osseous junction separates from the fluorochrome label by a *distance* equal to the *growth velocity* divided by time interval. Twenty-four hours after the alizarine label, the oxytetracycline label is given, and 24 hours later the animal was euthanized. The distance between the leading edge of the alizarine and OTC labels is the length of growth between the times of the two different labels. Distance between the leading edge of the OTC label and the chondro-osseous junction is the length of growth between the time of the OTC label and euthanasia. Measurements were made parallel with the alignment of the columns of growth plate chondrocytes. Time intervals must be a multiple of 24 hours to cancel out any circadian effect. Since in this study the time in both cases was 24 hours, the two measures of growth velocities are redundant. Other metaphyseal fluorescence is autofluorescence. In this lamb, growth velocity was estimated at 245 μ m/24 hours $\pm$ 23.

Lambs were euthanized four weeks (three of the lambs) to seven weeks (two of the lambs) after periosteal resection (the design of the microtransducer is such that after 4 weeks they will no longer be able to transmit growth velocity data). Three days prior to euthanasia and one day prior to euthanasia all five lambs received fluorochrome labels, alizarin complexone (10 mg/kg iv) and oxytetracycline (OTC 1mg/kg iv); respectively. Although fluorochrome labeling procedures allows us to determine growth velocity on the last days of the study, it is a verification of the measures obtained via microtransducers. As further verification of the microtransducer measures of growth velocities, at necropsy the total displacement between the screws over the course of the study was verified by direct measurement and by measuring the distances on anteroposterior radiographs.

Kinetics of Growth Plate Chondrocytes

Assessing which chondrocytic kinetic parameters are primarily responsible for alterations in growth velocity; we used a number of stereological measures of growth plate chondrocytes from both the resected and control limbs of the two lambs who did not have microtransducer

placement. Details of these stereological parameters and procedures have been reported previously^{13, 16, 17, 18}. In brief, growth plate cartilage was trimmed into 1×1×3mm blocks which included some epiphyseal and some metaphyseal bone. Cartilage was fixed in the presence of 0.7% ruthenium hexamine trichloride and embedded in plastic. 1.5µm thick sections were cut, mounted, stained with methylene blue/ azure II/ basic fuchsin on plastic embedded sections^{19, 20, 21}.

We assumed that alteration in rate of growth is achieved by changes in one (or more) of the following:

- volume of hypertrophic chondrocytes ($V_{(C) \text{ TERMINAL HYPERTROPHIC CHONDROCYTES}}$)
- modulation of the shape of hypertrophic chondrocytes, long axis / short axis, (eccentricity index)
- density (number per mm³) of chondrocytes ($N_{V \text{ HYPERTROPHIC ZONE}}$), which indirectly measures matrix volume/production
- number of chondrocytes produced or turned over per day ($N_{\text{TURNED OVER}}$)

In this study growth plates are normalized as an idealized unit cylinder with diameter = 1mm (diameter is arbitrary) located in the central axis of a bone. With this normalization procedure we maximize the study of axial elongation and we minimize the confounding effect of lateral growth (*Wilsman et al 1996; Wilsman et al. 2007*).

The mean volume of hypertrophic chondrocytes was determined using the point sampled, cubed, mean linear intercept method^{22, 23}, applying eight different angles of intercept. The eccentricity index of hypertrophic chondrocytes, was estimated by the mean intercept length parallel to the axis of growth (applied intercept angle of 0°) divided by the mean intercept length perpendicular to the axis of growth (applied intercept angle of 90°).

Density hypertrophic zone chondrocytes was calculated by first measuring the volume fraction of hypertrophic zone chondrocytes ($V_{V \text{ TERMINAL HYPERTROPHIC ZONE}}$). Then :

$$N_{V \text{ HYPERTROPHIC ZONE}} = V_{V \text{ TERMINAL HYPERTROPHIC ZONE}} / \overline{V_{(C) \text{ TERMINAL HYPERTROPHIC CHONDROCYTES}}}$$

This indirectly measures matrix production, as volume fraction decreases in the event of an increase in matrix volume.

Number of chondrocytes produced or turned over per day ($N_{\text{TURNED OVER}}$) is estimated by:

$$N_{\text{TURNED OVER}} = N_{V \text{ HYPERTROPHIC ZONE}} * [\pi * 0.5^2] * [\text{elongation velocity} / 24_{\text{HOURS}}]$$

Statistical analysis

From each lamb (three lambs were used for this stereological analysis) from each limb (resected or control) 4 blocks were selected randomly. From each block 2 fields from each of 2 independent sections were measured. The mean (or median) of 16 measures was our estimate for a limb. In this experimental design each lamb is its own control and thus the paired t-test is an appropriate statistical analysis of all data.

RESULTS

In every lamb at every time point, by all measuring methods, the effect of periosteal resection was to increase growth velocity ($p < 0.05$). Overall average growth velocity in all lambs, (Table 1) as measured by microtransducers, was $273\mu\text{m/day}$ *versus* $201\mu\text{m/day}$ in the control limbs of the same animal. These measurements of the positive effect on growth velocity by periosteal resection were confirmed by fluorochrome labeling and at necropsy by measuring directly the inter-screw distance, we estimated overall velocity in the periosteal resected limb at $263\mu\text{m/day}$ *versus* $210\mu\text{m/day}$ in the control limb. (Figure 1 and Table 1).

The increase in growth velocity occurred immediately, and over the course of the first week the average periosteal resected limb's growth velocity was $40\mu\text{m/day}$ faster. This accelerated growth velocity was consistent during the remaining 3 weeks of the study and averaged between $50\text{--}70\mu\text{m/day}$. (Figure 2).

Although not a part of the current investigation, this study is consistent with previous data 13, again demonstrating that elongation of the tibia (both experimental and control) takes place primarily when the lambs are in recumbency. The signal during standing represents growth plate compression and recovery from compression but there is essentially no elongation. The data point that ends of one recumbent period is statistically at the same level as the first data point of the subsequent recumbent period 13.

How long does the resection have an effect? The microtransducer data indicates that 4 weeks after resection growth velocity still was $70\mu\text{m/da}$ faster. In addition, while the microtransducer data is limited (by the design of the microtransducer) to 4 weeks, we continued to follow two lambs for an addition 3 weeks. In these two lambs, 7 weeks after the periosteal resection, the growth velocity by fluorochrome labeling remained $30\mu\text{m}$ faster in the resected limb ($230\mu\text{m/da}$. *versus* $200\mu\text{m/da}$.).

The effect of periosteal resection appeared limited to the proximal growth plate as growth velocities measured by fluorochrome labeling of the distal tibial growth plates were not significantly different between resected and control for all 5 lambs.

Chondrocytic Histomorphometry and Stereology

Of the four potential mechanisms (chondrocytic enlargement, chondrocytic proliferation, matrix production, or eccentricity index) that could explain the increase in growth rates, only the eccentricity index approached statistical significance ($p = 0.08$). The eccentricity index in the resected limb (1.6) was 14% higher than the eccentricity index in the control growth plate; in other words the hypertrophic cells were much taller [rectangular shaped] in the resected limb as opposed to the control limb where the cells were wider but not as tall [cuboidal shaped] (Figure 3).

Parameters associated with hypertrophy, the chief engine of elongation 24, 25, 26, were similar in both experimental and control growth plates. Volume fractions (an indirect measure of matrix production) were essentially the same (0.45 *versus* 0.43) as were hypertrophic chondrocyte volumes ($8,607\text{mm}^3$ *versus* $8,499\text{mm}^3$). In addition parameters associated with numbers of chondrocytes turned over as an index of chondrocytic proliferation also were similar in the experimental and control growth plates. Chondrocytic densities were similar ($53,200\text{cells/mm}^3$ *versus* $51,600\text{cells/mm}^3$) as were the number of chondrocytes turned over/produced per 24 hours ($10,800\text{cells / day}$ *versus* $9,800\text{ cells per day}$) This data is summarized in Table 2.

DISCUSSION

The primary goal of our study was to measure the extent and timing of changes in growth velocity following periosteal resection. Although generally thought to increase growth velocity; in 1991, Garces, et al. 27 wrote, “A review of previous papers shows no agreement concerning the onset and duration of the stimulus to overgrowth following periosteal stripping, nor the extent expected”

What makes our study unique is that, in comparison with previous studies that measured bone lengths, or at best fluorochrome labeling 2, 4, 27, 28, 29, 30, 31, 32 ; we are able to measure this effect with near real-time resolution with high precision over a substantial period of time. This technology (because of the physical dimensions/displacement of the microtransducer) allows 4 weeks of measurements, and fluorochrome labeling at 7 weeks confirm a continued effect of increased growth. Although this study can not document how long, past 7 weeks, and to what degree the effect of increased growth continues (and at which point the growth velocity normalizes) 7 weeks is a substantial fraction of a lamb's typical 42 week growth period.

Though many authors debate the mechanisms of post-periosteal resection overgrowth at the physal level, it is generally accepted that the periosteum acts as a mechanical restraint, providing axial compression on the growth plate 3, 29, 30, 31, 33, 34, 35, 36, 37, 38, 39, 40. By circumferential division of the periosteal tissue, then, this restraint is released, eventuating in increased longitudinal growth 30, 31, 37, 38, 39, 40. Although a number of investigators have examined the organ level mechanics of the observed overgrowth, fewer have attempted to do so at the cellular level 41, 42, 43. Both Hernandez, et al. and Houghton, et al. found no significant differences in histomorphometric parameters between stripped and control growth plates 41. Taylor, Warrell, and Evans reported an increase in the number of proliferative cells per column and concluded that the observed increase in growth following circumferential periosteal resection in rats was due to a relative increase in the rate of cellular proliferation 42. We did not find changes in proliferative zone chondrocytes in our study in lambs.

This study, using optimal (RHT) chemical fixation and real time kinetic parameters provides insight into the means by which resection of the periosteum is translated to increased elongation velocity at the level of the hypertrophic chondrocyte. Resection of the periosteum appears to release axial compression, and allow cellular elongation of the hypertrophic chondrocytes in an longitudinal direction, thereby increasing growth velocity. This is reflected in our histomorphometric data in which the eccentricity index is the primary difference between experimental and control physes. Periosteal resection appears to have no effect on chondrocyte or matrix production, and it also does not appear to affect the regulation of cellular swelling by hypertrophic chondrocytes as the mean cellular volumes in the resected tibia and the control tibia were almost identical— the only difference is the alteration of hypertrophic chondrocyte shape change. This mechanism for modulating growth rate has been reported before 10.

While the effect of periosteal resection measured in this study clearly is to increase growth velocity, the question remains if periosteal resection, alone, is sufficient to effectively accelerate growth in a clinically relevant manner. In this study, we are concerned that the measured increase in growth velocity of 10% to 20% may be misleading when translated to the clinical patient. In our study after 7 weeks, the overall length of the tibia with the resection was about 2 mm longer than the control. Since the overall length of the lambs' tibia at this age is 197mm, the effect of periosteal resection, alone, is to increase length by 1–2% over a 4 to 7 week time interval. If the same growth increase could be maintained for

several years then this procedure might have clinical utility for smaller discrepancy. While our study provides a longer time window than previous studies, our study can not confirm how long such an effect would exist. We envision that as the growth plate continues to grow the location of periosteal resection migrates away from the growth plate. This could lead to either a re-tethering of the periosteum or a diminished effect as the distance between the previous resection and the physis, with time, continues to increase. Therefore, if sustained growth is needed clinically, release of the periosteum as close to the physis as possible or repeat release of periosteum may be needed, and this may be hard to justify in a patient setting.

If such a growth stimulus is too diminutive to be useful as a primary treatment modality; it may prove useful if employed as an adjunctive procedure. For instance, in the clinical case of hemihypertrophy [with predicted discrepancy from 2 to 5 centimeters]; the standard method of treatment is to perform epiphysiodesis of the long leg. Perhaps a single stage operation where combination of periosteum release on the short leg and growth arrest of the long leg would be beneficial when insufficient growth remains to completely equalize limb length. Similarly, periosteal resection may have some utility in cases of angular deformity where the treatment plan includes guided growth. The ability to correct deformity with a staple or other modular implant implanted on the convexity may be accentuated when a periosteal resection is performed around the circumference of the metaphysis or perhaps when a hemi-resection of the periosteum is concurrently performed on the concavity. This later methodology is not proven as of yet but is the focus of future studies.

Distraction osteogenesis is an effective means to lengthen a limb but concerns exist regarding growth inhibition in the now lengthened limb after the process is over 44· 45· 46· 47. Distraction osteogenesis exposes the growth plate to high axial compression forces, owing primarily to increased tension in the surrounding soft tissues and periosteum 48· 49· 50· 51. Such forces alter mechanical loading of the epiphyseal cartilage, and may result in compressive inhibition of longitudinal growth, as described by the Hueter-Volkman principle 52· 53· 54· 55· 56· 57· 58. Some have proposed release of tightened soft tissues in order to decrease compressive forces on the growth and some research has been performed to test this hypothesis 46· 59· 60. In addition to soft tissue release, periosteal resection also may work to decrease axial loading on the physis, thereby counteracting the effects of compression 40· 61. Our data suggest that periosteal release confers a physeal growth stimulus by way of reducing axial compression, and as such, may be suitable to counteract the compressive forces that sabotage the clinical goals during distraction osteogenesis.

In summary, this study utilizes novel methods to assess growth velocity in real time and high precision. We demonstrate that increased growth from periosteal stripping can be expected immediately and will continue for at least 7 weeks of the 42 week growth period in the lamb model with about a 1% gain in limb length. We further propose that release of the periosteum allows the hypertrophic chondrocytes to “spring” into a more longitudinal configuration with the sum effect of accelerating growth. We can not confirm how long the total effect remains and therefore use of periosteal resection as a primary means to correct large limb length discrepancy remains questionable. This concept of increased growth from resection may however have clinical utility in other conditions.

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Fig. 1.

Illustration of the location of the strip of resected periosteum and the implantation of the microtransducer. A circumferential strip of periosteum 10 mm wide, was resected 1 cm distal to the proximal tibial growth plate. In the illustration this is procedure is about 1/5th complete. Subsequently, a microtransducer (*Microstrain, Burlington VT*) is implanted. The leads to and from the transducer are illustrated. The transducer is energized by an implanted battery and the signals of the current position of the transducer are sent by telemetry. Briefly, with bone elongation at the level of the growth plate, the epiphyseal screw/clamp and the metaphyseal screw/clamp move further apart. The microtransducer is likened to a tiny syringe where the barrel of the syringe (in realty a cylinder of electrified wires) is fixed to the metaphysis with the metaphyseal clamp/screw. The thin barrel of the syringe (in realty a solid metal core) is fixed to the epiphysis with the epiphyseal clamp/screw. With growth the barrel is pulled out of cylinder (designed to be "frictionless"). A silconized sleeve (omitted from the drawing) surrounds the transducer to guard against connective tissue infiltration.

Fig. 2.

Telemetry signals from microtransducers spanning the proximal tibial growth plate from the periosteal resected limb (above) and the control limb (below). In this Figure we illustrate the signals over a 24 hour period (from 120 to 144 hours after periosteal resection and implantation of microtransducers). Each dot is the distance between the epiphyseal screw and the metaphyseal screw (see 100 μ m scale bar). This distance has a resolution of 5 to 10 μ m about the diameter of one proliferative zone chondrocyte. The long dotted arrows are a regression of all 517 the data points collected over the 24 hours. The regression line for periosteal resected limb has a greater slope (higher growth velocity) than the control. The detailed nature of signals from both limbs depends on if the lamb is recumbent (red) or is standing/ambulation (black). In a previous study¹³ it was demonstrated that most bone elongation occurs when a lamb is recumbent. The apparent noisy nature of the signal during standing/ambulation is hypothesized to be caused by compression and rapid recovery from compression as the lamb stands/walks/trots.

Fig. 3. Micrographs of the hypertrophic zone chondrocytes from the proximal tibial growth plate of the control and the periosteal resected growth plates. The hypertrophic chondrocytes from the periosteal resected tibia are “taller,” that is the eccentricity index for these hypertrophic chondrocytes is greater ($p = 0.08$).

Table 1

Comparison of Growth Velocities (in $\mu\text{m}/\text{day}$) in Periosteal Resected Limbs *versus* Control Limbs — Proximal Tibial Growth Plate

	limb		p-value
	periosteal resected $\pm\text{sd}$	control $\pm\text{sd}$	
measured by microtransducers			
week 1	206 $\pm$ 20	176 $\pm$ 15	0.03
week 2	255 $\pm$ 65	186 $\pm$ 60	0.05
week 3	312 $\pm$ 83	246 $\pm$ 117	0.02
week 4	315 $\pm$ 82	266 $\pm$ 78	0.01
overall measured by microtransducers (n=3)	273 $\pm$ 72	201 $\pm$ 68	0.03
measured by fluorochrome labeling (n=5)	236 $\pm$ 46	211 $\pm$ 32	0.01

Table 2

Comparison of Morphometrical/Stereological Parameters in Periosteal Resected Limbs *versus* Control Limbs
— Proximal Tibial Growth Plate

	limb		p-value
	periosteal resected $\pm$ sd	control $\pm$ sd	
growth velocity (μ m/day) measured by microtransducers	273 $\pm$ 8.5	201 $\pm$ 8.3	0.03
eccentricity or axial ratio (width/height)	1.6 $\pm$ 0.65	1.3 $\pm$ 0.85	0.08
volume fraction	0.45 $\pm$ 0.03	0.43 $\pm$ 0.03	0.14
hypertrophic chondrocyte volume (μ m ³)	8,607 $\pm$ 402	8,499 $\pm$ 80.3	0.33
chondrocytic density (cells/mm ³)	53,216 $\pm$ 6,200	51,618 $\pm$ 2,450	0.28
chondrocytes turned over/24 hrs	10,800 $\pm$ 758	9,790 $\pm$ 552	0.12