Chapter 6

Evaluation of Delayed-image Bone Scintigraphy to Assess Bone Formation after Distraction Osteogenesis in Dogs

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Abstract

The aim of our study was to quantitatively assess distraction-induced bone formation in a crural lengthening model in dogs by use of delayed-image bone scintigraphy. Twelve mature Labrador Retrievers were randomly allocated to 3 groups and a circular external skeletal fixation system was mounted on the right crus. Distal osteotomy of the tibia and fibula was performed in the first and second group dogs, followed by a lengthening procedure of 10 mm in the first group only. The third group served as sham-operated controls. Delayed-image bone scintigraphy with technetium-99m hydroxy methylene diphosphonate was performed 2, 4, and 6 weeks after surgery. Delayed image:region-of-interest, delayed image:crural, and delayed image:femoral scintigraphic activity ratios were calculated and evaluated by use of ANOVA for repeated measures. New bone formation was quantified by use of densitometric image analysis, and values for the scintigraphic ratios were compared. In the distraction and osteotomy groups, values of delayed-image:region-of-interest and crural ratios increased significantly. Although densitometric image analysis revealed increased bone formation after distraction, the region-of-interest ratios and crural ratios were similar in both groups. All dogs had increased delayed image:femoral ratios. In summary, delayed-image bone scintigraphy ratios were not effective at differentiating between the amounts of distraction-induced bone and osteotomy-induced bone. Metabolic bone activity in the adjacent femur was increased as a consequence of circular external skeletal fixator placement. Delayed-image bone scintigraphy was not adequately sensitive to quantitatively monitor bone formation, but may be useful as an early predictor of bone healing.

Introduction

Distraction osteogenesis has been used in the management of various skeletal conditions, including bone length deficits, bone deformities, bone loss after traumatic injury or radical resection, and craniofacial reconstruction.[21,33]The principle of distraction osteogenesis describes formation of new bone under conditions of controlled mechanical distraction of an osteotomy site. Distraction osteogenesis is characterized by intramembranous bone formation.[2,4] In contrast, routine healing at an osteotomy site is initiated with callus formation and proceeds via endochondral bone formation. The extent of distraction osteogenesis is determined by many factors, including the site of the osteotomy, latency period, distraction rate, soft tissue condition, local blood supply, and age of the animal. Local blood flow to the area of affected bone is closely associated with osteogenesis.[3]

Although the use of histologic, ultrastructural, and radiologic methods to characterize distraction osteogenesis has been described, those methods are either invasive or only applicable in later phases of the mineralization process.[7,27,30] Delayed-image bone scintigraphy is a non-invasive quantitative method for evaluating changes in bone metabolic activity.[12,19] In contrast to radiography, which reveals the amount of mineralization, delayed-image bone scintigraphy evaluates uptake of technetium-99m tracer by immature bone and thus precedes actual accretion of bone.[26,35] In distraction osteogenesis, delayed-image bone scintigraphy has been used successfully to predict the progression of bone formation in the early stages of the lengthening process and to assess the optimal time of bone consolidation in the later stages of bone maturation.[12,19] We hypothesized that delayed-image bone scintigraphy would be useful in the quantitative assessment of bone regeneration after a distraction osteogenesis procedure. Because distraction osteogenesis is known to increase local and regional blood flow, we also speculated that the lengthening procedure would increase the rate of bone metabolism in the adjacent long bone.[3]

Materials and Methods

Animals

Procedures were approved by the Utrecht University Ethics Committee for Animal Care and Use, and all experimentation was conducted in conformity with ethical and humane principles of research. Twelve mature Labrador Retrievers with a mean age of 19 months (range, 12 to 31 months) and mean body weight of 27 kg (range, 21 to 32 kg) were allocated to 3 groups (n = 4 each). Dogs were individually housed and fed a standard commercial dog food twice a day and had ad libitum access to water.

Surgery and distraction procedures

The lengthening procedure was performed by application of a CESF system (Imex Veterinary Inc., Longview, TX, USA). All frames were identical and consisted of 2 proximal and 2 distal full rings with a 100-mm diameter, connected by 3 treaded rods with a 1-mm pitch. Frames were assembled prior to surgery and steam sterilized. Dogs received medetomidine (Domitor, Pfizer Animal Health BV, Capelle a/d IJssel, The Netherlands) as a pre-anesthetic sedative and anesthesia was induced with propofol (Rapinovet, Schering-Plough Animal Health NV, Bruxelles, Belgium) administered IV. After intubation, inhalation anesthesia with nitrous oxide, oxygen, and isoflurane commenced. Amoxicillin with clavulanic acid (Augmentin, SmithKline Beecham Farma BV, Rijswijk, The Netherlands) was administered (20 mg/kg, IV) prior to surgery. The skin of the right hind limb was aseptically prepared in a standard fashion. A CESF was attached to the right tibia by use of two 1.5-mm diameter transosseous wires on both the proximal and distal rings and one 1.5-mm diameter transosseous wire on both central rings. An equivalent of 60 kg of tension was applied to the wires with a dynamometric wire tensioner (Hofmann SaS, Monza, Italy).

A craniomedial surgical approach to the tibia and fibula was used to facilitate circumferential elevation of the soft tissues and periosteum. In dogs in the distraction and osteotomy groups, the tibia and fibula were osteotomized in the diaphysis at the level of two-thirds of the tibial length from its proximal aspect by use of an oscillating saw. Ample volumes of fluids were used for lavage during the osteotomy procedure for thermal protection of bone and periosteum.

Dogs in the control group were sham-operated. In all dogs, the periosteum was closed with an absorbable suture material and closure of the subcutis and skin proceeded routinely. Dogs wore a protective full-leg bandage for 3 days after surgery and received buprenorphine (Temgesic, Schering-Plough, Weesp, The Netherlands) as an analgesic (10 µg/kg, SC, q 6 h) for 3 days after surgery. Full loading of the limbs was permitted immediately after surgery. After 4 days, dogs in the distraction group were subjected to lengthening via adjustment of the CESF at a rate of 0.5 mm twice daily, for 10 consecutive days. The site of bone regeneration was allowed to mature for an additional 4 weeks. Dogs were euthanatized with a barbiturate overdose 6 weeks after the initial surgery.

Delayed image bone scintigraphy

Delayed-image bone scintigraphy was performed 2, 4, and 6 weeks after surgery. Each dog received 550 MBq of ^{99m}Tc-HDP IV, 3 hours before data collection. Scintigraphic imaging was performed with a gamma camera system (Siemens Medical Systems, Den Haag, The Netherlands) equipped with a high-resolution parallel-hole collimator. The gamma camera was connected to a dedicated open workstation computer. Immediately prior to scintigraphic imaging, dogs were premedicated with medetomidine and anesthesia was induced and maintained by a continuous rate infusion of propofol delivered via an infusion pump. Dogs were positioned in dorsal recumbency with both crura placed parallel to the tabletop. The camera was positioned over both hind legs with the collimator centered over the distraction zone, osteotomy zone, or corresponding zone in the control group, perpendicular to the long axis of the right and left crura. Counts were collected during a 5-minute period by use of a 256 X 256 matrix with a pixel size of 1.68 mm. Simultaneously, 1 mL of a dilution of the injection dose (dilution, 1:1000) was counted and used as a standard. Bone metabolic activity was determined (by focusing the camera on the distraction, osteotomy, and control zones) for the region of interest, the entire crus, and the distal third of the femur. The regions of interest were selected to include the area of all mineralized callus that was radiographically visible adjacent to the osteotomy zone 6 weeks after surgery. The region of interest was centered over the osteotomy zone with an equal distribution over the proximal and distal segments of the bone. The dimensions of the region of interest were 20 X 10 pixels (5.64 cm^2). An identical region of interest was used for the opposite limb. The same regions of interest were used in the sham-operated dogs. In the distraction group, the region of interest on the right (experimental) limb was enlarged in a proximodistal direction to 20 X 16 pixels (9.03 cm^2) to include the same amount of original crural bone as was included in dogs in the osteotomy and control groups as well as the area of distraction-induced bone regeneration in the lengthening zone. The region of interest for the left limb remained identical to that used in dogs in the other groups. The precise locations of the regions of interest were chosen with reference to the radiographs of each dog so that the same anatomic area evaluated in the densitometric image analysis was accurately reflected.

Activity in the entire tibia and fibula (i.e., crural activity*)* on the right and left limbs was measured. The distal one-third of the femur *(*i.e., femoral activity*)* was delineated on the scans and activity was measured (Fig 1A and 1B). Activity in the right and left limbs was expressed as a percentage of total dose activity. The dose percentage of activity in the right limb was divided by that in the left limb to yield a ratio that expressed the difference in bone metabolic activity between the 2 limbs. Thus, the delayed image:region-of-interest ratio, delayed image:crural ratio, and delayed image:femoral ratios were determined.

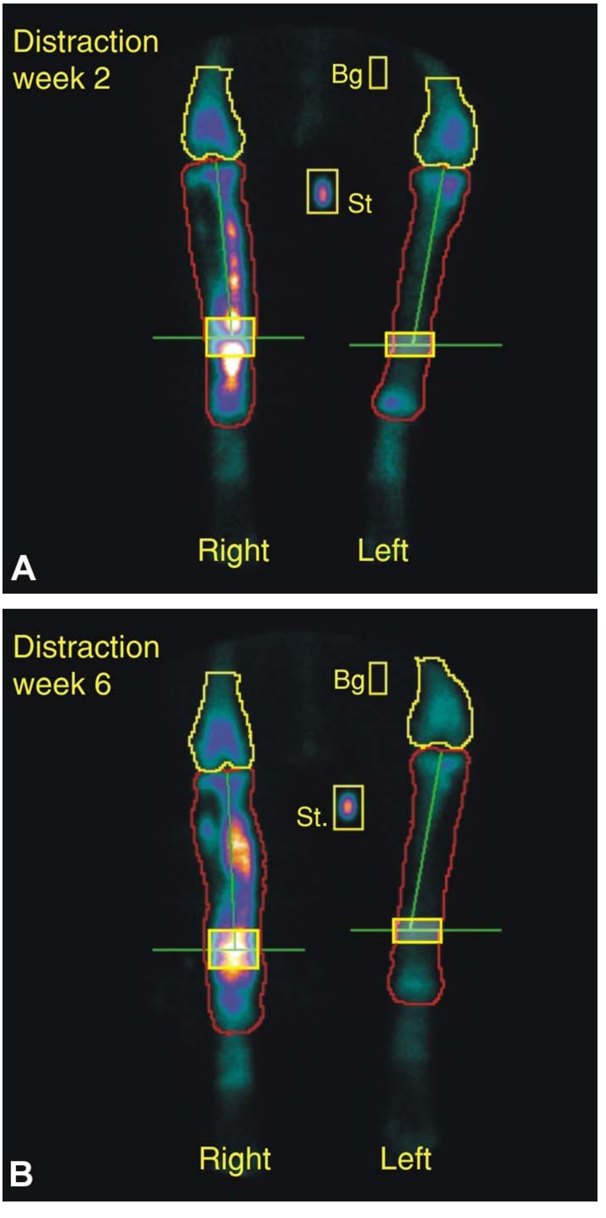

Fig 1. Delayed-image bone scintigraphic images of the right and left crura and distal portion of the femurs in a dog at the end of the active lengthening period 2 weeks postoperatively (A) and during the consolidation phase at 6 weeks after surgery (B). The region of interest (high-lighted), entire crus, and distal portion of the femur are outlined. Notice the area of high uptake of 99mTc-HDP tracer adjacent to and in the distraction zone in the distal portion of the experimental (right) tibia
St = Standard. Bk = Background.

Densitometric image analysis

Densitometric image analysis of radiographs was used to measure new bone formation.[13] Immediately after surgery and once weekly for 6 weeks thereafter, radiographs of the right crus and CESF were obtained in the caudocranial and lateromedial planes. The radiographic views included a ruler and an aluminum step-wedge consisting of ten 2-mm thick aluminum slabs mounted in an overlapping manner. Bone formation was quantified by use of a densitometric image analysis system. Radiographs were recorded with a high-resolution camera (Sony b/w CCD camera type XC-77CE, Sony Corporation, Tokyo, Japan) and digitized for image analysis (frame size, 752 X 574 pixels; 256 gray levels) with a personal computer-based system equipped with imaging software (KS400 version 3.0 software, Carl Zeiss Vision, Oberkochen, Germany). A program was developed to quantify the amount of mineralized callus observed. Each radiograph was calibrated geometrically and densitometrically by use of the image of the ruler and the aluminum step-wedge. The densitometric calibration was performed by measuring the mean optical density of a square area of 50 X 50 pixels in 6 steps of the aluminum model (i.e., 0, 2, 4, 6, 8, and 10 mm). Measurements were obtained from a median filtered image to reduce the influence of photographic grain in the film. The optical density values were a polygonal fit with the aluminum values to produce a transformation table, which enabled expression of the amount of newly formed bone in units of equivalents of cubic millimeters of aluminum. The region of interest was centered over the distraction or osteotomy zone similarly as in the delayed image bone scintigraphy procedure and included all new bone formation. The regions of interest were delineated on the digitalized caudocranial and lateromedial images and densitometric analyses (including the bone area and bone amount), were performed. For each dog, mean bone area and bone amount in the caudocranial and lateromedial images were determined and used for statistical analysis.

Statistical analysis

Delayed-image ratios were evaluated via ANOVA for repeated measures and a least significant difference post-hoc test. Densitometric data, including values for bone area and bone amount, were compared by use of a Student *t*-test. Correlations between delayed-image ratios and densitometric bone area and bone amount were examined by use of the Pearson correlation test. Power was set at 0.80 and values of $P < .05$ were considered significant. Analyses were performed by use of commercially available statistical software (SPSS 10.1 statistical package, SPSS Inc, Chicago, IL, USA).

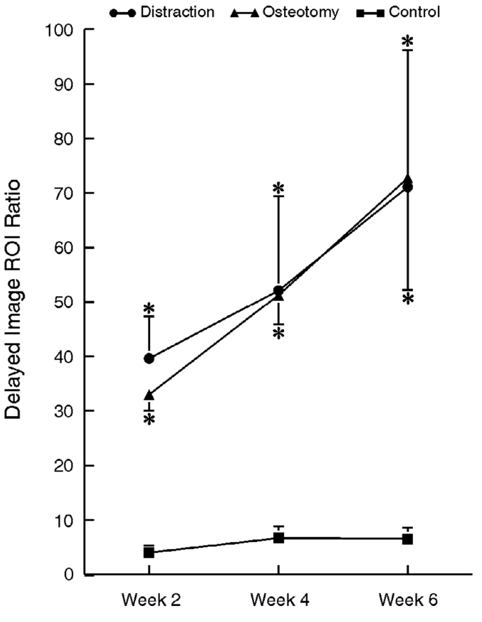

Fig 2. Changes in delayed-image: region-of-interest scintigraphic activity ratios after surgery in 12 dogs that underwent osteotomy alone (n = 4), osteotomy and distraction osteogenesis (4), or a sham procedure (4). Delayed image: region-of-interest ratios were calculated as activity in the region of interest as a percentage of total dose activity. Data are presented as mean ± SEM. *Significant increase in comparison with the controls ($P < .05$).

Results

Scintigraphy

Bone metabolic activity increased significantly on the instrumented right side in all dogs in the distraction, osteotomy, and control groups. The higher uptake of ^{99m}Tc-HDP resulted in increased delayed image:region-of-interest ratios, delayed image:crural ratios, and delayed image:femoral ratios at all time points. In the distraction group, the mean region of interest ratio initially had the largest increase, but values were not significantly different from those in the osteotomy group (Fig 2). Both the distraction and osteotomy groups had significantly higher mean delayed image:region-of-interest ratio than the controls at all time points. Although delayed image:crural ratios were highest in the distraction group, the differences between those ratios and ratios in the osteotomy group were not significantly different (Fig 3). The distraction and osteotomy groups had significantly higher delayed image:crural ratios than controls at all time points. Although delayed image:femoral ratios indicated a higher uptake of ^{99m}Tc-HDP in the femurs of instrumented limbs, compared with the contralateral limbs, no differences were observed among groups (Fig 4). Delayed-image ratios of the region of interest, crus, and femur tended to increase during the study period.

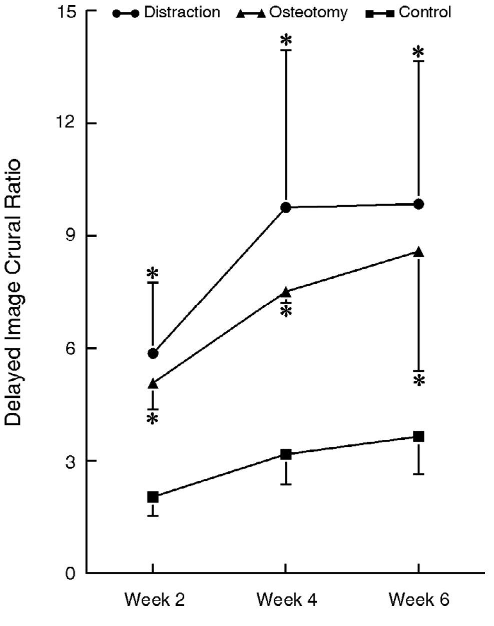

Fig 3. Changes in delayed image: crural scintigraphic activity ratios in the same dogs as in Fig 2. Data are presented as mean ± SEM. *Significant increase in comparison with controls ($P < .05$).

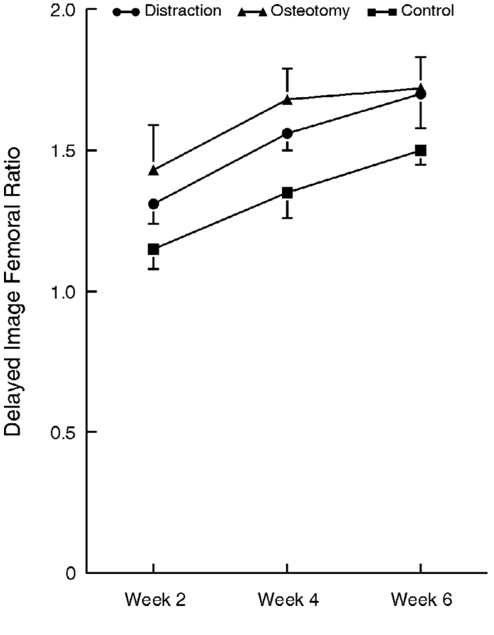

Fig 4. Changes in delayed image: femoral scintigraphic activity ratios in the same dogs as in Fig 2 and 3. Data are presented as mean ± SEM. Notice that there were no significant differences among the groups.

Densitometric image analysis

The distraction procedures were uneventful in all dogs and no complications were encountered. In dogs in both the distraction and osteotomy groups, periosteal new bone adjacent to the osteotomy site was observed radiographically as early as 1 week after surgery. The amount and density of periosteal bone increased over time. In the distraction zone, new bone formation was observed 3 weeks after surgery and periosteal bone had merged with bone in the distraction zone at 4 weeks. In the control dogs, no periosteal bone formation was detected at the site of periosteal elevation. Mineralization in the distraction and osteotomy groups had progressed to an extent that densitometric evaluation was possible beginning in week 5. In the distraction group, measurements of bone area and bone amount were significantly larger 5 and 6 weeks after surgery, compared with measurements in the osteotomy group. In dogs in the distraction group, bone area and bone amount increased during that period (Table 1).

Table 1. Summary of densitometric image data for new bone formation in dogs that underwent an osteotomy procedure alone (n = 4 dogs) or osteotomy and distraction osteogenesis (4). Measurements were taken 5 and 6 weeks after surgery.

Group	Bone area (mm^2)		Bone amount (mm^3 Al X 10^3)	
	Week 5	Week 6	Week 5	Week 6
Distraction	$277.6 \pm 39.7^{*}$	$366.6 \pm 52.8^{*,\dagger}$	$278.0 \pm 50.7^{*}$	$365.6 \pm 67.0^{*,\dagger}$
Osteotomy	120.6 ± 18.8	149.0 ± 26.7	132.9 ± 24.5	$166.5 \pm 32.7^{\dagger}$

Data are given as mean ± SEM. Values for bone area are given in units of square millimeters. Values for bone amount are given in equivalents of aluminum (Al) in mm^3 X 10^3.
* Significant differences between the 2 groups at the corresponding time ($P < .05$).
† Significant increase in time within the group ($P < .05$).

The mean delayed image:region-of-interest ratio in the distraction group was positively correlated with densitometric bone area, but not with the densitometric bone amount, at 6 weeks (R = 0.95; $P = .02$ and R = 0.84; $P = .08$, respectively). In the osteotomy group, no significant correlations were detected between the delayed image:region of interest ratio versus bone area and bone amount (R= 0.61, P = .20 and R=0.65, P = .18, respectively).

Discussion

We hypothesized that delayed-image bone scintigraphy would be useful in quantitatively assessing bone regeneration after a distraction osteogenesis procedure. We also speculated that a lengthening procedure would increase bone metabolic activity in the adjacent long bone. Delayed-image bone scintigraphy ratios were not effective in quantitatively differentiating between distraction-induced bone formation and osteotomy-induced bone formation. Metabolic bone activity in the adjacent femur was increased as a consequence of placement of the CESF device.

In our study, dogs had no prior bone disease or injury and all healed without complication, but results in a clinical setting could be different. Radiographic data revealed the lengthening procedure to be successful via analogy with previous models of distraction osteogenesis in dogs and other species.[4,14] In the present study, an oscillating saw and high-volume lavage were used during the osteotomy, a technique that has been effective in experimental and clinical settings.[7,20,22,23] Densitometric image analysis revealed a greater amount of bone formation in association with the distraction procedure, compared with bone formation after osteotomy alone.

Three-phase bone scintigraphy is a noninvasive method for semi-quantitative assessment of blood flow, blood distribution, and bone metabolic activity.[26] Angiographic imaging during 3-phase scintigraphy reflects local perfusion. Hyperemia of bone and soft tissues is revealed on the blood-pool image. Accumulation of ^{99m}Tc-HDP in the delayed image was presumed to reflect osteoblast activity and new bone formation. Many studies have revealed that blood flow increases during the processes of bone healing and distraction osteogenesis.[1,3,19,24,25,32] Although blood supply is considered to be closely related to rate of osteogenesis, blood flow, as indicated by the perfusion index, appears to be of questionable value as a predictor of new bone formation.[5,12,19] In contrast to the first 2 phases of bone scintigraphy, the value of delayed-image bone scintigraphy in predicting the outcome of bone consolidation after a distraction procedure in humans has been reported.[19] Other recent studies have revealed the role of angiogenic factors, including vascular endothelial growth factor and basic fibroblast growth factor, in distraction-induced bone regeneration.[6,9,17,28] Expression of those factors is maximal during active bone lengthening and decreased during the consolidation phase.

In the present study, we used delayed-image bone scintigraphy to determine the activity of bone metabolism and new bone formation during a lengthening procedure. Initially, the delayed image:region of interest ratio was increased most notably in dogs in the distraction group. This point in time coincided with the end of active lengthening, which is characterized by the highest expression of angiogenic and osteotropic growth factors (i.e., bone morphogenetic proteins, insulin-like growth factor-I, and transforming growth factor ß) and thus the highest rate of bone formation.[8,10,11,28,29,34] Because densitometric image analysis revealed the mean area of new bone formation in the distraction group to be more than twice the size of that in the osteotomy group 6 weeks after surgery, we expected that the delayed image:region-of-interest ratio would reveal this difference. Whether the lack of increase in the region-of-interest ratio can be attributed to a different rate of uptake of ^{99m}Tc-HDP during intramembranous bone formation is uncertain.[2,4] Another explanation is that there could have been altered distribution of tracer as a result of increased blood flow in the lengthened crus. Nevertheless, observation of increasing delayed image:region-of-interest ratios from week 2 onwards in both the distraction and osteotomy groups was consistent with the radiographic evidence of advancing bone formation.[12]

The strong correlation between the delayed image:region-of-interest ratio and densitometric bone area, but not bone amount, in the distraction group at 6 weeks was consistent with the assumption that delayed-image bone scintigraphy is not a measure of mineralization. Although delayed image:region-of-interest ratios were similar in the distraction and osteotomy groups, crural ratios tended to be higher after distraction throughout the study period. Upregulation of bone metabolism outside the lengthening zone, mediated via production of osteotropic and angiogenic growth factors during the distraction procedure, may play a role in this finding.[8,10,11,15,28,29,34] Potential significant differences between the regions of interest may have been obscured by the sample size in the present study, but differences would be less relevant, compared with results of densitometric image analysis.

In the control group, the effect of the CESF system on bone response in the right limbs suggested that the metabolic response of bone to a minimally invasive external fixator can be substantial. Whether enhanced bone metabolic activity was the result of production of angiogenic and osteotropic growth factors as a reaction to the transosseous wires is unclear. Although the local response, characterized by the delayed image:region-of-interest ratios and crural ratios, differed significantly in both the distraction and osteotomy groups, compared with controls, the distant effect on the femur was similar in magnitude in all 3 groups. In actively growing patients, the increased bone metabolism in the adjacent long bone during distraction osteogenesis or fracture healing could be responsible for the phenomenon of longitudinal bone growth stimulation.[16,18,31]

In summary, delayed-image bone scintigraphy ratios were not effective in quantitatively differentiating between the amount of distraction-induced and osteotomy-induced bone formation. Delayed image:region-of-interest and crural ratios revealed bone formation in the areas of distraction-induced activity and bone callus at the osteotomy site. In the clinical setting, delayed-image bone scintigraphy ratios may be valuable as early predictors of bone healing. Nevertheless, quantification of newly formed bone in individual patients does not appear to be feasible. Instrumentation with a CESF system appears to stimulate bone metabolism not only in the instrumented bone, but also in adjacent long bones.

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