Published in final edited form as:

Bone. 2007 February ; 40(2): 419–424.

The effect of a short-term delay of puberty on trabecular bone mass and structure in female rats: A texture-based and histomorphometric analysis.

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Abstract

Accrual of bone mass and strength during development is imperative in order to reduce the risk of fracture later in life. Although delayed pubertal onset is associated with an increased incidence of stress fracture, evidence supports the concept of “catch up” growth. It remains unclear if deficits in bone mass associated with delayed puberty have long term effects on trabecular bone structure and strength. The purpose of this study was to use texture-based analysis and histomorphometry to investigate the effect of a delay in puberty on trabecular bone mass and structure immediately post-puberty and at maturity in female rats. Forty-eight female Sprague Dawley rats (25 days) were randomly assigned to one of four groups; 1) short-term control (C-ST), 2) long-term control (C-LT), 3) short-term GnRH antagonist (G-ST) and 4) long-term GnRH antagonist (G-LT). Injections of either saline or gonadotropin-releasing hormone antagonist (GnRH-a) (100 µg/day) (Cetrotide™, Serono, Inc) were given intraperitoneally for 18 days (day 35–42) to both ST and LT. The ST groups were sacrificed after the last injection (day 43) and the LT groups at 6 months of age. Pubertal and gonadal development was retarded by the GnRH antagonist injections as indicated by a delay in vaginal opening, lower ovarian and uterine weights and suppressed estradiol levels in the short-term

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experimental animals (G-ST). Delayed puberty caused a transient reduction in trabecular bone area as assessed by histomorphometry. Specifically, the significant deficit in bone area resulted from a decreased number of trabecula and an increase in trabecular separation. Texture analysis, a new method to assess bone density and structural anisotropy, correlated well with the standard histomorphometry and measured significant deficits in the density measure (M_{Density}) in the G-ST group that remained at maturity (6 months). The texture energy deficit in the G-ST group was primarily in the 0° orientation (-13.2%), which measures the longitudinal trabeculae in the proximal tibia. However, the deficit in the G-LT group was in the 45° and 135° orientations. These results suggest that any “catch-up” growth following the cessation of the GnRH-antagonist injection protocol may be directed in trabeculae oriented perpendicular to 0° at the expense of trabeculae in other orientations.

Keywords

Delayed puberty; Textural Analysis; Bone adaptation; Trabecular bone structure; Rat model

Introduction

Suboptimal bone strength in individuals who do not reach peak bone mass during childhood or adolescence may affect the development of fractures later in life [1]. Multiple factors have a crucial effect on the accrual of bone mass and strength during the developmental years including heritable factors, physical activity, nutritional factors and hormonal status [1]. In particular estrogen levels during growth are an important factor in the pathogenesis of bone fragility and ultimately fracture [2]. Decreased estrogen levels during adolescence and decreased peak bone mass are associated with a delay of menarche and infrequent menstrual cycles [3,4,5]. Warren et al. [6] found the age of menarche to be more correlated to stress fracture occurrence than bone mineral density (BMD). While increased incidence of stress fractures may increase the risk of osteoporotic fractures later in life, evidence exists for “catch-up” growth [7,8]. That is, bone inhibiting and bone enhancing strategies early in life may not have long-term effects on bone strength due to the continued growth occurring in juvenile bone. Therefore it is unclear if a decrease in bone mass associated with delayed puberty (menarche) affects the strength of trabecular bone at maturity.

Bone strength has been shown to depend on both its density and structure [9], which may or may not be interdependent. Density measures alone, although widely used clinically, cannot identify osteoporotic subjects who will sustain fractures, due to the large overlap in bone mass measures in individuals with fractures and those without fractures [10]. Other factors including bone size, architecture and material properties must be considered [9]. Multiple approaches such as Mean Intercept Lengths (MIL) [10] and the Fourier power spectrum [11,12,13,14] have been used to characterize the structure of trabecular bone, particularly in describing orientation disparity or anisotropy. We have recently developed a texture analysis approach using Gabor filters, which is capable of providing insight into bone structure from localized texture information on a pixel level [15]. Specifically, texture analysis can analyze both the global image at a low resolution and a detailed view at high resolutions. In summary, texture analysis yields information regarding anisotropy, allows for the analysis of irregularly shaped regions and simplifies series-type analysis [16]. The wavelet approach is therefore a potentially powerful tool in analyzing trabecular bone texture where orientation, shape and architecture as well as density are the fundamental components.

The purpose of this study was to use texture-based analysis and histomorphometry to investigate the effect of a delay in puberty on trabecular bone mass and structure immediately post-puberty and at maturity in female rats. We investigated the hypothesis that administration

of a GnRH-antagonist prior to the onset of the first estrus cycle would suppress the increase in estrogen levels associated with the onset of puberty and impede the development of trabecular bone mass and trabecular orientation in the proximal tibia at 6 weeks and at maturity (6 months).

Materials and methods

Forty-eight female Sprague-Dawley rats (23 days-of-age) (Charles Rivers Laboratories, Wilmington, MA, USA) were housed 3 per cage and provided standard rat chow and water ad libitum. The animals were housed in a 12 hour light-dark cycle. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Brooklyn College (City University of New York).

The animals were randomly assigned to one of four groups; 1) short-term control (C-ST) (n=12), 2) long-term control (C-LT) (n=12), 3) short-term GnRH antagonist (G-ST) (n=12) and 4) long-term GnRH antagonist (G-LT) (n=12). At 25 days-of-age, daily injections of a gonadotropin-releasing hormone antagonist (GnRH-a) (Cetrotide™, Serono, Inc.) were used to delay the onset of puberty. Gonadotropin-releasing hormone antagonists (GnRH-a) have successfully delayed the onset of puberty in female rats and have the advantage that normal hypothalamic-pituitary function is restored after cessation of injections [17]. Injections (0.2 ml) of either saline or the GnRH-a (100 µg/day) (Cetrotide™, Serono, Inc.) were given intraperitoneally. Both short-term and long-term groups received the GnRH-a for a duration of 18 days (day 25–42). However, the short-term groups were sacrificed after the last injection (day 43) and the long-term groups at 6 months of age. All animals were sacrificed during the proestrus phase of their cycle as determined by cytology of vaginal smears. The proestrus phase is predominated by cells with a very high nuclear to cytoplasm ratio.

All animals were monitored daily for vaginal opening, an indicator of pubertal onset. Body weights were measured every 5 days during the 18 day injection period and weekly thereafter. On the day of sacrifice, animals were anesthetized by intraperitoneal injection of ketamine (80 mg/kg) and xylazine (16 mg/kg). Blood was taken through cardiac puncture, after which the animals were killed by overdose of pentobarbital. Serum estradiol was measured using a radioimmunoassay (3rd Generation Estradiol RIA, DSL-39100, Diagnostic Systems Laboratories, Inc. Webster, TX, USA). Inter-assay coefficient of variation was less than 6% and sensitivity was 0.6 pg/ml. After sacrifice, uterine and ovarian tissues were harvested and weighed. The left tibia were removed and cleaned of soft-tissue and were processed for histomorphometric and textural analysis.

Histomorphometry

Left tibia were immersion-fixed in 10% neutral buffered formalin for 48 hours, dehydrated with ethylene glycol monoethyl ether (Fisher, Fair Lawn, NJ), cleared in methyl salicylate (J.T. Baker, Phillipsburg, NJ) and embedded using methyl methacrylate with 15% dibutyl phthalate (Fisher Scientific) [18]. Undecalcified thin (5 µm) frontal sections of the proximal tibia were cut using a Polycut microtome (Leica, Nusseloch, Germany). After deplasticizing, sections were stained with von Kossa for analysis of bone mass and architecture [18]. Trabecular bone mass and structure were assessed using bright field microscopy (objective magnification 10x) and standard histomorphometric methods. Measurements were made on the proximal tibial metaphysis, 1–4 mm distal to the growth plate-metaphyseal junction, using the OsteoMeasure system (Osteometrics, Atlanta, GA, USA). The structural (static) properties measured included bone area (B.Ar; mm), bone perimeter (B.Pm; mm), total tissue area (T.Ar; mm). These indices were used to calculate the following parameters based on the plate model; percent trabecular bone (Tb.Ar/T.Ar; %), trabecular number (Tb.N; /mm), trabecular width (Tb.Wi; µm) and

trabecular separation (Tb.Sp; μm) [19,20]. These parameters were used to assess bone mass and architecture.

Textural Analysis

Proximal tibia slices were scanned under 2x objective magnification and saved as BMP files. The images were assembled (Panavue Image Assembler, Greensburg, PA, USA) and the gray scales were converted to black and white via a uniformly chosen threshold (Adobe Photoshop v6.0). The cortical regions were erased to avoid the potential artifacts in texture energy which could be induced due to the sharp transition of black and white along the cortical region of the bone slices (Figure 1). The original image was convoluted with a bank of Gabor filters to produce a series of output images containing Gabor coefficients $g_{mn}(x, y)$ where m ranges over the sequence of filter center frequencies (scales) ($1 \sim M$) and n ranges over the number of orientations ($1 \sim N$). The higher scales correspond to a non-detailed global view of the image whereas a lower scale yields detailed information in the image. For this analysis the number of scales was 3 ($M=3$) and the total number of orientations was 4 ($N=4$). The four orientations included 0° , which was perpendicular to the longitudinal trabeculae of the proximal tibia, 45° , 90° and 135° (Figure 1). Three properties of the bone images were defined that could be directly related to the texture features derived from the Gabor wavelets [15]:

1. Density measure (M_{Density}): This measure described the amount of bone in the image relative to the total area being analyzed. This measure is similar to the histomorphometric measure, trabecular bone area (Tb.Ar/T.Ar, %).
2. Textural energy measure at each orientation 0° , 45° , 90° , 135° (Texture Energy $0^\circ-135^\circ$): This measure described the amount of bone in the image at 4 orientations (Figure 1). The density measure (M_{Density}) is the average of these four texture energy values.
3. Anisotropy measure ($M_{\text{anisotropy}}$): This measure is the difference between the texture energy values of the maximum and minimum orientations, normalized by the averaged texture energy which is M_{density} .

Data Analysis

A Student's t-test assessed differences between the control and experimental groups at a significance level of $p < 0.05$ (Sigma Stat 3.0, SPSS Chicago, IL, U.S.A.) in both the short-term and long-term age groups. If the normality or equal variance assumptions were violated, Mann-Whitney Rank Sum Test assessed group differences (trabecular separation, trabecular width, M_{Density} (LT), texture energy $_{90^\circ}$, texture energy $_{0^\circ}$). Pearson Product Moment Correlations compared relationships between M_{Density} and trabecular bone area (Tb.Ar / T.Ar; %) and between texture energy $_{0^\circ}$ and trabecular separation (Tb.Sp; μm) parameters. Prior to statistical evaluations all outcome measures were plotted versus body weight and the slope was used to normalize the data using a linear regression-based correction [21]. The correction decreased the variability in the data. Results are presented as mean (SD) values.

Results

There were no differences in body weights between the groups at sacrifice in both the short-term and at maturity (Table 1). However, the 18-day GnRH-a injection protocol resulted in a significant delay in the timing of vaginal opening. Pubertal onset was delayed an average of 8 days (2 estrus cycles) in the short-term GnRH-a (G-ST) group and 10 days (2.5 estrus cycles) in the long-term (G-LT) group (Table 1). (Five animals in the G-ST group never had a vaginal opening therefore 42 days was the date of vaginal opening for statistical analysis. A comparison of histomorphometric variables (bone area and trabecular number) did not reveal any

differences in bone phenotype between the animals not reaching puberty by day 42 compared to those who reached puberty prior to day 42 but still received GnRH-a injections.) The delay in pubertal development was also confirmed by decreased uterine weights (−74 %) and ovarian weights (−77 %) in the G-ST group (Table 1). The uterine and ovarian weights were normal in the G-LT group (Table 1). Estrogen levels were 27 % lower after the GnRH-a injection protocol in the G-ST group ($p = 0.044$) but estradiol levels recovered at 6 months of age in the long-term groups (Table 1).

The delay in pubertal timing significantly affected trabecular bone structure in the G-ST group. Trabecular bone area (Tb.Ar / T.Ar; %) was significantly less (−16.5 %) compared to the C-ST group ($p < 0.05$) (Table 2). The lower bone area was due to a 20.7 % larger trabecular separation (Tb.Sp; μm) ($p = 0.068$) and a significantly lower (−12.5 %) number of trabeculae (Tb.N; /mm) in the G-ST group (Table 2). Bone perimeter (B.Pm; mm) was also significantly lower in the G-ST groups, however, no difference was found in trabecular width (Tb.Wi; μm) between the short-term groups (Table 2). The structural (static) measurements were not significantly different between the C-LT and G-LT groups at 6 months of age. Trends toward lower trabecular bone area (−17.9 %) ($p = 0.115$) and larger trabecular separation (+34.5 %) ($p = 0.166$) were found in the G-LT group compared to the C-LT group (Table 2). Trabecular separation values decreased with aging in both groups, control (−18.1 %) and GnRH-antagonist (−8.7 %), however the G-LT group only had a decrease in trabecular separation to the level of the C-ST group (Table 2).

The density measure, M_{Density} , from the texture analysis was highly correlated to trabecular bone area ($R^2 = 0.854$, $P < 0.05$) (Table 3). The texture energy for the 0° orientation was negatively correlated with the histomorphometric variable, trabecular separation (Tb.Sp; μm) ($R^2 = -0.862$, $P < 0.05$) (Table 3). As a result the outcome measures of the texture analysis yielded similar results to the histomorphometric outcome measures. A lower density measure, M_{Density} in the G-ST group compared to the C-ST group ($p = 0.095$) resulted from the 18 day GnRh antagonist injection period (Table 4). Specifically, the texture energy in the 0° orientation (Texture Energy $_{0^\circ}$), which is perpendicular to the longitudinal orientation of the distal tibia, was 13.2 % lower in the G-ST group. At maturity, a lower M_{Density} (−9.7 %) remained in the GnRH-a group compared to control, however the source of the deficit was changed. In the short-term the texture energy (M_{Density}) was significantly lower in the 0° orientation but in the long-term, the M_{Density} deficit was due to the significantly lower texture energies in the 45° and 135° orientations ($p < 0.05$) (Table 4).

The texture energy in all orientations and in both control and experimental groups increased between day 42 and 6 months of age. In the control group there was less of an increase in the 0° orientation compared to the other orientations which resulted in a decrease in the $M_{\text{Anisotropy}}$ measure between day 42 and 6 months of age. The proximal tibia architecture became more isotropic with age (Table 4). The group difference in the texture energy at 0° narrowed from 13.2 % in the short-term to 7.9 % in the long-term. However, the texture energy difference at all other orientations (45° , 90° , 135°) increased between C-LT and G-LT. For example, in the 45° orientation there was a 7.5% group difference in the short-term and 13.4% in the long-term. These results suggest an altered growth pattern in the GnRH-a group due to the delayed onset of puberty, specifically an increase in mass of the longitudinally oriented trabeculae and less of an increase in trabeculae in other orientations (Figure 2).

Discussion

An 18 day GnRH antagonist injection protocol that delayed puberty caused a transient reduction in trabecular bone area and structural variables and may affect bone orientation in the long-term. Specifically, the significant deficit in trabecular bone area resulted from a

decreased number of trabecula and an increase in trabecular separation. The texture analysis correlated well with the standard histomorphometry variables and a significant deficit in the density measure (M_{Density}) in the G-ST group remained at maturity (6 months). The texture energy in the 0° orientation was significantly less (-13.2%) in the G-ST group and remained lower at maturity (-7.9%) compared to the C-LT group but did not reach statistical significance ($p=0.106$). However, the texture energies in the 45° and 135° orientations were significantly less in the G-LT group. These results suggest that any “catch-up” growth from day 42 to 6 months, following the cessation of the GnRH-antagonist injection protocol, may be directed in trabeculae oriented to best resist loading (0°) at the expense of trabeculae in the other orientations (45° and 135°).

GnRH antagonist injections at a dosage of ($100\text{ }\mu\text{g/day}$) retard pubertal and gonadal development as indicated by the delay in vaginal opening, lower ovarian and uterine weights and lower estradiol levels in the experimental animals in the current and in previous studies [17]. However, the GnRH antagonist injections did not completely suppress the onset of puberty in all animals. Fifty-eight percent of the GnRH-a animals had vaginal openings by day 42 while receiving a dosage of $100\text{ }\mu\text{g/day}$ of the GnRH antagonist (CetrotideTM), similar to the findings of Roth et al. [17] that report 41% of the GnRH-a animals reached puberty by 37 days of age. However a comparison of histomorphometric variables (bone area and trabecular number) did not reveal any differences in bone phenotype between the animals not reaching puberty by day 42 compared to those who reached puberty prior to day 42 but still received GnRH-a injections.

The 18-day GnRH-antagonist injection protocol resulted in a similar pattern of trabecular bone loss as ovariectomy in rats. The 16.5% decrease in trabecular bone mass in the short-term experimental group (G-ST) was due to a lower trabecular number and an increased trabecular separation as opposed to a thinning of trabeculae. Ovariectomy results in decreased trabecular number [22,23,24,25] and an increase in trabecular separation [26,27] in both young and mature animals. At maturity (6 months) no statistical difference in the number of trabeculae in the long-term groups was found suggesting a recovery of the trabecular structure after the cessation of the GnRH-a injections. However, there was a trend towards a difference in the trabecular separation ($+34.5\%$) ($p=0.166$) and trabecular bone area (-17.9%) ($p=0.115$) between the C-LT and G-LT groups. An 18.1% decrease in trabecular separation was found in the control group from day 42 to 6 months however, the GnRH-a group had only an 8.7% decrease in trabecular separation. The G-LT group had similar trabecular separation values as the C-ST group suggesting that “catch-up” growth may not completely reverse the structural changes following 18 days of GnRH-antagonist injections.

To our knowledge only one other study investigated the effect of delayed puberty by GnRH antagonist injections on bone properties in rats. Rankover et al. [28] reported a suppression of estradiol after 4 weeks of GnRH-antagonist injections at a dosage of $125\text{ }\mu\text{g/day}$. Femoral BMD and trabecular density were lower after the 4-week injection period in the experimental group compared to the control group. Fourteen weeks after the end of the injection period, femoral BMD and trabecular density were still lower in the experimental animals compared to control. These results and the current study suggest long-term effects from a delay in pubertal onset. However, “catch-up” growth has been reported in other animal models of low bone mass during growth [8,7]. Gafni et al. [8] report a complete recovery in BMD, bone volume and trabecular number in rabbits after a 5-week protocol of dexamethasone injections (a protocol resulting in a significant bone mass reduction). Yet the protocol, not intended to delay puberty, was initiated at 5-weeks of age during the pre-pubertal (childhood) stage of rabbit development and was ended close to the onset of puberty. In humans, compensation for a delay in pubertal growth by a late acceleration of linear growth has been found in elite female athletes [29], however other studies involving athletes suggest bone mass cannot be regained after delayed puberty

and amenorrhea during young adulthood [6,30]. Specifically, amenorrheic dancers receiving hormone replacement for 2 years had no increase in BMD compared to the placebo or control groups. Therefore, “catch-up” growth may depend on the age of onset and severity of the condition affecting normal growth [7].

The density measure, M_{Density} , from the texture analysis correlated well with trabecular bone area measurements ($R^2 = 0.854$, $p < 0.05$). M_{Density} is a composite measurement averaged over the different orientations representing bone area. However, the texture energy for each orientation (0° , 45° , 90° , 135°) can yield information regarding trabecular bone orientation and anisotropy. The texture energy at 0° orientation correlated well with the trabecular separation variable ($R^2 = -0.862$, $p < 0.05$). An increase in trabecular separation was equivalent to a decrease in the texture energy in the 0° orientation. Histomorphometric parameters do not yield direct information regarding trabecular structure specifically the shape and orientation of the trabecular elements [16]. The texture analysis not only detected deficits in the amount of bone but the orientations in which the deficit occurred. The region of bone loss in trabecular bone affects both the mechanical properties as well as the ability to recover bone strength. Random loss of trabecula seems to be more detrimental to the ultimate strength of bone compared to a thinning of trabecula [31,32]. The number of trabecula aligned in the loading direction is important in the maintenance of the mechanical properties of trabecular bone [33]. In the current study, “catchup” growth seemed to be directed in the 0° orientation which is more able to resist the loading placed on the proximal tibia. The bone mass deficit in the short-term group resulted from a significant decrease (-13.2%) in the texture energy in 0° orientation, the longitudinally oriented trabeculae of the tibial metaphysis. Non-significant decreases in texture energy ranging from 3.7% to 7.5% were measured at the other orientations as well as a decrease in anisotropy of the proximal tibia. However, at maturity (6 months of age) there was a significant deficit in M_{Density} in the G-LT group yet the deficits occurred in orientations other than 0° . Texture energies at 45° and 135° were significantly lower, 13.4% and 9.6% respectively, than the C-LT group (Figure 2) and the anisotropy remained lower. The data reflect a more anisotropic structure during puberty in control animals however animals subjected to a delayed puberty have a more isotropic structure. The recovery of mechanical strength from bone loss following OVX seems to be predicated on the duration and extent of bone loss however the loss or thinning of longitudinally oriented trabeculae has more affect on bone mechanics than the loss of horizontally oriented trabeculae [34]. Therefore the loss of bone in the 0° orientation from a delay in pubertal timing may have a significant effect on peak bone strength; more investigations are needed into the timing and duration of pubertal delay on peak trabecular bone strength.

In summary, a recovery of trabecular bone mass following a delay in puberty using GnRH-antagonist injections may not indicate a recovery of mechanical strength. There is currently a large overlap in bone mass measures in individuals with fractures and those without fractures [35]. While traditional histomorphometry can yield indirect measures of trabecular width, separation and number, textural analysis offers a method to assess bone mass in different orientations. The delay in puberty by GnRH antagonist injections resulted in transient deficits in bone mass and structure but the histomorphometry variables did not reveal significant results at maturity. The textural analysis did suggest lingering bone deficits at orientations which may have an effect on bone strength.

Acknowledgements

This study was funded by the National Institutes of Health (R15 AG19654-01A1) (VY) and The City University of New York PSC-CUNY Research Award Program (64293-00 33) (VY), NIH grant AR41210 and the National Space Biomedical Research Institute through National Aeronautics and Space Administration Grant NCC 9-58 (MS).

The authors would like to thank Damien Laudier (The Leni and Peter W. May Department of Orthopaedics, Mount Sinai School of Medicine) for his histological expertise.

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Figure 1.

Illustration of the four orientations, 0°, 45°, 90°, 135° that were used in the Gabor filter wavelet analysis. The 0° orientation, which was perpendicular to the longitudinal trabecula and the 90° orientation analyzed the horizontal trabecula. The 45° and 135° orientations assessed trabecula mid-way between the dominant trabecula orientations.

Figure 2.
Texture energy at the four orientations, 0° , 45° , 90° , 135° , for the four groups, C-ST, G-ST, C-LT, G-LT.

Table 1

Summary of group differences in uterine and ovarian weights, day of vaginal opening (VO), Estradiol levels and body weights at sacrifice in both short-term and long-term groups. Mean (SD).

Parameters	Groups			
	C-ST	G-ST	C-LT	G-LT
Uterine Weight (g)	.608 ± .105	.156 ± .059 [*]	.676 ± .090	.654 ± .082
Ovary Weight (g)	.235 ± .227	.053 ± .030 [*]	.165 ± .027	.167 ± .053
VO (day)	31.1 ± 1.2	38 ± 4.2 ^{‡§}	31.6 ± 0.8	41.1 ± 8.6 [‡]
Estradiol pg/ml	34.48 ± 11.19	25.28 ± 13.83 [‡]	48.84 ± 34.72	43.22 ± 14.43
Body Weight at Sacrifice (g)	183.8 ± 16.3	195.1 ± 17.0	337.6 ± 27.9	366.2 ± 57.7

^{*} p<0.001 versus C-ST,

[‡] p<0.05 versus C-ST,

[‡] p<0.05 versus C-LT,

[§] Five animals did not have a VO prior to sacrifice and 42 days was used for statistical comparisons.

Table 2

Static histomorphometry measures at the tibial metaphysis for the short-term and long-term groups. Mean (SD).

Parameters	Groups			
	C-ST	G-ST	C-LT	G-LT
B.Ar/T.Ar (%)	22.18 ± 4.1	18.51 ± 4.71 [*]	36.19 ± 7.75	29.70 ± 10.27 [§]
Tb.Sp (μm)	167.7 ± 32.4	202.4 ± 61.2 ⁺	137.4 ± 33.7 [*]	184.8 ± 87.9 [†]
Tb.N (mm ⁻¹)	4.8 ± 0.6	4.2 ± 0.7 [*]	4.8 ± 0.8	4.4 ± 1.2
Tb.Wi (μm)	46.2 ± 5.4	43.2 ± 6.4	72.3 ± .8137	69.5 ± 12.3
B.Pm (mm)	99.15 ± 14.23	84.51 ± 16.21 [*]	87.23 ± 26.97	89.49 ± 31.07

^{*} p<0.05 versus C-ST,

⁺ p=0.068 versus C-ST

[§] p=0.115 versus C-LT,

[†] p=0.166 versus C-LT

Table 3

Correlation analyses between histomorphometry measurements and texture analysis measurements.

Parameter 1	Parameter 2	R ²	Significance
Bone Area (B.Ar / T.Ar, %)	M _{Density}	0.854	P < 0.001
Trabecular Separation (Tb. Sp, μm)	Textural Energy 0°	−0.862	P < 0.001

Table 4

Textural analysis outcome measures at the tibial metaphysis for the short-term (ST) and long-term (LT) groups. Mean (SD).

Parameters	Groups			
	C-ST	G-ST	C-LT	G-LT
M _{Density}	5.95 ± 0.44	5.44 ± 0.95 [‡]	7.35 ± 0.39	6.64 ± 0.98 [‡]
Texture Energy _{0°}	9.35 ± 1.0	8.12 ± 1.71 [*]	10.12 ± 0.77	9.32 ± 1.44 [§]
Texture Energy _{45°}	5.35 ± 0.51	4.95 ± 0.97	7.09 ± 0.54	6.14 ± 1.07 [‡]
Texture Energy _{90°}	3.83 ± 0.36	3.69 ± 0.66	5.41 ± 0.29	4.97 ± 0.90
Texture Energy _{135°}	5.28 ± 0.59	5.00 ± 0.76	6.76 ± 0.47	6.11 ± 0.81 [‡]
M _{Anisotropy}	0.92 ± 0.15	0.80 ± 0.17	0.64 ± 0.08 [*]	0.66 ± 0.14

^{*} p<0.05 versus C-ST,

[‡] p=0.095 versus C-ST,

[‡] p<0.05 versus C-LT,

[§] p=0.106 versus C-LT