The femoral distal epiphysis of ovariectomized rats as a site for studies on osteoporosis:
Structural and mechanical evaluations

G. Giavaresi[1], M. Fini[1], S. Gnudi[2], F. De Terlizzi[3], A. Carpi[4], R. Giardino[1,5]

[1]Department of Experimental Surgery and [2]Department of Internal Medicine, Istituti Ortopedici Rizzoli, Bologna; [3]IGEA SRL, Carpi – Modena; [4]Department of Ageing and Reproduction, University of Pisa, Pisa; [5]Department of Surgical Pathophysiology, University of Bologna, Bologna, Italy.

Abstract

Objective

To investigate in detail the mechanical and structural characteristics of cancellous bone from the femoral distal epiphysis of normal and ovariectomized rats, and to provide reference values in order to improve experimental research on osteoporosis by characterising an alternative and complementary anatomic site.

Methods

40 female Sprague-Dawley rats (10 months old) were randomly divided into 4 groups of 10 each: baseline, ovariectomized (Ovx), sham-operated (Sham-Ovx) and sham-aged (Sham-Aged). Baseline animals were sacrificed at the beginning of the study. Ovx and Sham-Ovx animals were sacrificed 16 weeks after surgery, whereas Sham-aged rats were killed when aged 14 months. Femurs were excised and densitometric, ultrasonographic, mechanical and histomorphometric analyses were performed.

Results

When comparing the Ovx group with the others, ultrasonographic and densitometric measurements showed significant decreases ($p < 0.0005$) amounting to 3-5% in the amplitude dependent speed of sound (AD-SOS) and 13-20% in the BMD, respectively. Significant decreases were also seen in the femoral condyle Max. Load (28-31%; $p < 0.0005$) and Elastic Modulus (19-25%; $p < 0.005$) in the Ovx group in comparison with the Sham-Ovx and Sham-Aged groups. Histomorphometric analysis showed a significant cancellous bone loss ($p < 0.0005$). Densitometric ($p < 0.01$), histomorphometric ($p < 0.01$) and mechanical ($p < 0.05$) parameters were correlated with AD-SOS. Among the histomorphometric parameters, stepwise regression analysis showed that the trabecular bone volume (BV/TV) and Max.Load correctly predicted AD-SOS ($p < 0.0005$) and BMD ($p < 0.0005$).

Conclusion

The data from this study characterize osteopenia occurring in the rat distal femur 16 weeks after ovariectomy and provide methodology and reference values for further investigations on osteoporosis and bone-implant osteointegration in osteopenic bone.

Key words

Osteoporosis, ovariectomized rat, femur, dual X-ray absorptiometry, quantitative ultrasound.

Gianluca Giavaresi, MD; Milena Fini, MD; Saverio Gnudi, MD; Francesca De Terlizzi, BPhys; Angelo Carpi, MD; Roberto Giardino, MD.

Please address correspondence and reprint requests to: Gianluca Giavaresi, MD, Servizio di Chirurgia Sperimentale, Istituto di Ricerca Codivilla-Putti, IOR, Via di Barbiano 1/10, 40136 Bologna, Italy. E-mail: gianluca.giavaresi@ior.it

Introduction

Osteoporosis and related fracture risk represent a public health and socio-economic problem due to the associated morbidity, disability and decreased quality of life in an ageing postmenopausal female population. In addition, during inflammatory arthritis local factors are responsible for the development of a generalized bone loss of the axial and appendicular skeleton together with juxta-articular osteopenia and focal erosion of marginal and subchondral bone (1, 2). The presence of bone rarefaction is widely accepted as one of the most predictive negative factors for biomaterial osseointegration, as it is associated with implant failure (3,4). The synthesis instrumentation and prosthetic devices used to treat most of the osteoporotic fractures and altered joints are usually made of biomaterials whose biocompatibility and osseointegration were evaluated on healthy animals following standardised procedures (ISO 10993-6), without consideration of the quality and quantity of the host bone. For these reasons, investigations on implant osseointegration in osteopenic bone using ovariectomized animal models have been recognized to be mandatory (5-11).

The ovariectomized rat model is widely used to study the development of osteopenia, even though some differences are recognized between the rat and human skeleton (12-17). Extensive literature is available on the rat femoral proximal epiphyses, which share many histo-anatomic similarities with humans, and on the effect of ovariectomy on rat long bones, providing detailed descriptions of the changes occurring both in the trabecular bone mass and in the cortical bone dimensions and remodelling of the diaphysis (12, 14, 16). As far as we know, and with respect to the trabecular component of the femoral bone, few studies are available on the distal femur (6,9,16,18-20) and, moreover, a complete study by means of densitometric, ultrasonographic, mechanical and histomorphometric evaluations and relative correlation has never been performed. The association of dual x-ray absorptiometry (DXA) (21, 25, 28) and quantitative ultrasound (QUS) (22-24, 26, 29) with destructive methods such as biomechanical tests (15, 20) and histomorphometric analyses (12, 19, 27) has markedly improved the accuracy of experimental research on osteoporosis and may allow the adequate assessment of bone modifications.

The characterization of the effect of ovariectomy on the distal femur could be extremely useful not only for studies on the pathophysiology and treatment of osteoporosis, but also for surgical studies on biomaterial osseointegration in osteopenic bone. Results from past studies which demonstrate that ovariectomy causes an imbalance in bone remodelling and structural characteristics as it occurs in other sites (i.e., the proximal femur or vertebrae) may provide researchers with an additional site of investigation. Consequently, more information on bone remodelling may be obtained from the same animal model. Regarding the latter studies, the distal femur may make it possible to investigate bone-implant osseointegration in osteopenic bone (5, 6, 9).

The aim of this study was to investigate the mechanical and structural characteristics of cancellous bone from the femoral distal epiphysis in normal and ovariectomized rats, and to provide reference values in order to improve experimental research on osteoporosis by characterising an alternative and complementary anatomic site. The advantage of using the femoral distal epiphysis is the opportunity to measure cortical and cancellous bone parameters, as well as mechanical properties and to have an easy surgical pathway for the evaluation of miniaturized prosthetic device osseointegration.

Materials and methods

The study was performed in compliance with the European and Italian Laws on animal experimentation and with the Animal Welfare Assurance regulation no. #A5424-01 of the National Institute of Health (NIH, Rockville, Maryland USA). The animal research protocol was approved by the responsible public authorities as requested by the Italian Law according to the EC rules (Law by Decree, 27 Janu-

ary 1992, No. 116).

Forty retired breeder female Sprague-Dawley rats, 10 months old and 450 ± 50 g, were housed under controlled conditions (temperature $20° \pm 0.5°C$; relative humidity $55 \pm 5\%$; 12 hours light and 12 hours darkness) and were supplied with food 250 g/rat/wk (4RF18, Mucedola SRL, Settimo Milanese MI, Italy) and water *ad libitum*. At the beginning of the study, a group of 10 rats were anesthetized, then sacrificed by an i.v. injection of 1 ml Tanax (Hoechst AG, Frankfurt-am-Main,Germany); they served as the baseline control group (Baseline). Under general anaesthesia, 10 rats underwent bilateral ovariectomy by a dorsal approach (Ovx), while the same operation was simulated in another 10 rats (Sham-Ovx). The remaining rats were used as a sham-aged group (Sham-Aged) and killed at the end of the study.

Sixteen weeks after surgery the animals were euthanized and the femurs of each were excised, cleaned of soft tissue and used for densitometric and ultrasonographic measurements. The right femurs were then used for mechanical analyses, and the left ones for histomorphometric studies. Blinded operators carried out all of the measurements for all of the techniques.

Dual X-ray absorptiometry (DXA)
The bone mineral content (BMC) and density (BMD) of the femurs were measured using dual X-ray absorptiometry (Norland XR 26 Mark II densitometer) with a scansion speed of 1 mm/s and a resolution of 0.5 x 0.5 mm. BMC (mg) and BMD (mg/cm^2) values were obtained on the distal epiphysis below the supracondylar line and were calculated as the average of three scans for each specimen, in order to reduce the possibility of positioning errors. Before taking the measurements, the instrument was calibrated by means of a Norland phantom.

Quantitative ultrasound
The ultrasound (US) measurements were performed by means of a DBM Sonic 1200 (IGEA SRL, Carpi, Italy). The device was equipped with an electronic high-precision caliper (± 0.02 mm) on which two ultrasound probes ($\varnothing$ 12 mm) were mounted:one probe generated the ultrasound (1.25 MHz) and the other received the ultrasound beam after it had crossed the bone specimen. All osteosonogrammetry data were stored in a personal computer connected to the device. A plexiglass phantom (reference value: 2760 m/s) was used to calibrate the DBM Sonic weekly.

US measurements were assessed in the medio-lateral direction at the level of the femoral distal epiphysis. The caliper of the device was closed perpendicularly to the measurement point and rotated until scattering did not influence the number and amplitude of the peaks recorded on the screen. Coupling was achieved with a standard US gel. The amplitude-dependent speed of sound (AD-SOS m/s) through the bone was obtained for each femur by calculating the mean of 4 measurements, in order to reduce repositioning errors. The value of each AD-SOS measurement was calculated on the earliest part of the curve (first electrical maximum of the signal) obtained above the background noise (trigger point). Finally, a quantitative analysis of the pattern of the transmitted US signal was performed and the following parameters were calculated to associate each aspect of this trace with the structural and mechanical properties of the bony tissue (23, 30):

- FWA (fast wave amplitude) in mV: the amplitude of the first peak in the first part of the received ultrasound signal, *i.e.* the fastest and most impulsive peak transmitted through the mineralized bone segments which have a higher transmission speed;
- TF (time frame) in μs: the time interval between the moment when the first peak reaches its maximum amplitude and the instant when the signal reaches a reference time cut-off level corresponding to a velocity of 1700 m/s. This is related to the bone moment of inertia and resistance against load, and it turned out to be influenced by the distribution of the bone mineralized matrix;
- EN (signal energy normalized) in mV^2 x μs: the area under the signal curve representing the mechanical

energy transferred beyond the bone crossed by the signal, and depending on the type and structure of the material (the more continuous and thicker the pathways, the more energy is transferred);
- UPA (ultrasound peak amplitude) in mV: the maximum signal amplitude in the time frame;
- Slope (growing trend of peaks) in rad: the slope of the straight line joining the maximum signal peaks and indicating the signal dynamics. This index identifies quality and quantity of the ultrasound signal which crosses the bone: the greater the slope, the more compact the bony structure.

Mechanical testing
After QUS and DXA measurements were performed, the right femurs were wrapped in normal saline moistened gauze, placed in plastic bags and then stored at -20°C for a fortnight before testing. The femurs were then removed from the freezer and maintained wet in normal saline at room temperature during the subsequent stages of the mechanical testing, which were performed within 4 hours (31). The distal epiphyses were removed and processed using a cutting-grinding system (model 300 CL, Exact GmbH, Norderstedt, Germany), in order to obtain a cube of about 5 mm per side. The bone specimens were loaded to failure (Sintech-1/M, MTS Adamel Lhomargy, Ivry sur Seine, France) by a compression test along the medial to the lateral axis (20, 31). Tests were performed with a load cell of 0.5 kN at a constant speed of 1 mm/min until failure: the Maximal Load and Elastic Modulus (i.e., the slope of the stress-strain curve in the elastic region; the linear part of the curve constrained between 30% and 60% of the maximum stress value) were recorded. To stabilize the specimen, a small pre-load (5% of the average maximal load) was applied prior to actual testing.

Histomorphometric analysis
The left femurs were fixed in 4% buffered paraformaldehyde and then processed for methacrylate embedding. The blocks were cut longitudinally

with a Leica 1600 microtome (Ernst Leitz, Wetzlar, Germany) and cross sections (20 m thick) of the femoral distal epiphysis were obtained. Three serial sections, spaced 200 m apart, were polished (Struers Dap-7, Struers Tech A/S, Rodovre/Copenaghen, Denmark) and stained with Goldner's trichrome and Toluidine Blue. Then they were morphometrically analyzed using an image analyzer system (Kontron S300 v.2, Kontron Elektronik, München, Germany), and the measurements were taken semi-automatically from four equidistant sites in each section, and on three sections. The nomenclature approved by the American Society of Bone and Mineral Research (ASBMR) was used to measure and calculate the following cancellous bone parameters (32):

- trabecular bone volume (BV/TV, %): the whole spongy bone present in sampling sites, expressed as a percentage of the total tissue area converted to a volume by multiplication by the unit thickness;
- trabecular thickness (Tb.Th, m), trabecular separation (Tb.Sp, m) and trabecular number (Tb.N, /mm): indices of the width, the distance between, and the density of the trabeculae, respectively;
- index of spatial connectivity (N.Nd/ N.Tm): the ratio between the number of nodes (trabecular branch points, N.Nd) and the number of termini (trabecular end-points, N.Tm) ;
- cortical thickness (Ct.Wi, m): the thickness of the cortices between the periosteal and endosteal surfaces.

Statistical analysis
Statistical analysis was performed using SPSS v. 7.5 software (SPSS/PC Inc., Chicago, Illinois). Data are reported as the means ± SD at a significance level of $p < 0.05$. After verifying the normal distribution and homogeneity of the variances, analysis of variance (ANOVA) and Scheffé's post hoc multiple comparison tests were carried out. Linear regression analysis was used to detect the associations between those variables yielding correlation coefficients. Finally, stepwise linear regression analysis was performed to determine the variable that best predicted QUS and DXA (variables selected to enter the model were significant with at least $p < 0.001$).

Results
No significant differences between the groups for final body (Baseline: 438 ± 33 g; Ovx: 452 ± 45 g; Sham-Ovx: 436 ± 48 g; Sham-Aged: 444 ± 31g) and femoral (Baseline: 1.33 ± 0.19 g; Ovx:

1.18 ± 0.06 g; Sham-Ovx: 1.24 ± 0.11 g; Sham-Aged: 1.20 ± 0.08 g) weights were found.

Table I shows densitometric and ultrasonographic parameters of Baseline, Ovx, Sham-Ovx, and Sham-Aged rats. Multiple comparison tests showed that femoral densitometric data registered significant decreases in BMD of the Ovx group by 20%, 13% and 15% when compared to the Baseline ($p < 0.0005$), Sham-Ovx ($p < 0.01$) and Sham-Aged ($p < 0.005$) groups, respectively. The AD-SOS of the Ovx group decreased significantly ($p < 0.0005$) by 5%, 3% and 5% compared to the Baseline, Sham-Ovx and Sham-Aged groups, respectively. Of the calculated parameters of the US signals, TF, EN and Slope showed significant differences in the Ovx (TF = -0.17 s; EN = -14.2 mV^2 x s; Slope = -1.23 rad) and Sham-Ovx (EN = -10.8 mV^2 x s; Slope = -0.84 rad) groups in particular, when compared to the Baseline Group. In addition, the TF and Slope data in the Ovx group decreased by 28% ($p < 0.05$) and 84% ($p < 0.001$) when compared to the Sham-Ovx and Sham-Aged groups, respectively.

The intraoperator precision of the DXA and QUS techniques was tested. The intraoperator figures for AD-SOS and DXA were 0.7% and 1.2%, respective-

Table I. Densitometric and ultrasonographic parameters of normal and ovariectomized rats (Mean ± SD, n=10 replicates for each group).

Parameter	Unit	Baseline	Ovx	Sham-Ovx	Sham-Aged	ANOVA	
						F	p
Densitometric							
BMD	mg/cm^2	199 ± 21	158 ± 8 [a,b,c]	181 ± 15	185 ± 15	12.28	<0.0005
BMC	mg	138 ± 19	126 ± 9	139 ± 17	140 ± 9	2.02	ns
Ultrasonographic							
AD-SOS	m/s	2070 ± 35	1960 ± 34 [d]	2027 ± 27	2056 ± 25	24.59	<0.0005
FWA	mV	74.5 ± 19.4	57.3 ± 8.9	60.4 ± 20.2	65.04 ± 20.61	1.03	ns
TF	μs	0.59 ± 0.08	0.43 ± 0.08 [e]	0.59 ± 0.10	0.57 ± 0.11	4.30	<0.05
EN	mV^2 x μs	17.1 ± 9.5	4.5 ± 2.4 [f,g]	9.6 ± 3.7	11.16 ± 6.79	5.63	<0.005
UPA	mV	124.6 ± 55.2	73.5 ± 17.4	98.1 ± 39.9	96.00 ± 42.56	1.54	ns
Slope	rad	1.39 ± 0.28	0.17 ± 0.30 [h,i]	0.56 ± 0.40 [l]	1.09 ± 0.29	17.56	<0.0005

BMD: bone mineral density; BMC: bone mineral content; AD-SOS: amplitude dependent speed of sound; FWA: fast wave amplitude; TF: time frame; EN: signal energy normalized; UPA: ultrasound peak amplitude; Slope: growing trend of peaks.
Scheffé's post hoc multiple comparison test:
BMD: [a]Group Ovx versus group Baseline (p<0.0005); [b]Group Ovx versus and Sham-Ovx (p<0.01); [c]Group Ovx versus Sham-Aged (p<0.005).
AD-SOS: [d]Group Ovx versus groups Baseline, Sham-Ovx and Sham-Aged (p<0.0005);
TF: [e]Group Ovx versus groups Baseline and Sham-Ovx (p<0.05).
EN: [f]Group Ovx versus group Baseline (p<0.01); [g]Group Sham-Ovx versus group Baseline (p<0.05).
Slope: [h]Group Ovx versus group Baseline (p<0.0005); [i]Group Ovx versus groupSham-Aged (p<0.001); [l]Group Sham-Ovx versus group Baseline (p<0.005).

Table II. Mechanical parameters in normal and ovariectomized rats (Mean ± SD, n=10 for each group).

Parameter	Unit	Baseline	Ovx	Sham-Ovx	Sham-Aged	ANOVA F	p
Max.Load	N	133 ± 13	98 ± 16[a,b]	142 ± 24	137 ± 11	13.82	< 0.0005
Elastic Modulus	N/mm^2	292 ± 31	254 ± 40[c]	341 ± 61	315 ± 59	4.39	< 0.05

Scheffé's post hoc multiple comparison test:
Max.Load: [a]Group Ovx versus group Baseline (p<0.001); [b]Group Ovx versus groups Sham-Ovx and Sham-Aged (p<0.0005).
Elastic Modulus: [c]Group Ovx versus group Sham-Ovx (p<0.05).

ly. When a dynamic range was used for AD-SOS [dynamic range = maximum AD-SOS value measured in the rat population - minimum AD-SOS value measurable (1570 m/s)], the intraoperator precision results for AD-SOS increased up to 1.2%.

The femoral condyle Max.Load of the Ovx group was significantly reduced when compared to the Baseline (p < 0.001), Sham-Ovx (p < 0.0005) and Sham-Aged (p < 0.0005) groups, while the Elastic Modulus of the Ovx group presented a significant (p < 0.005) decrease in the femoral condyle compared to the Sham-Ovx group (Table II).

Table III reports the static histomorphometric parameters in normal and ovariectomized rats. BV/TV and Tb.Sp of the Ovx group revealed significant (p < 0.0005) changes in comparison with the Baseline (BV/TV: -33%; Tb.Sp: 61%), Sham-Ovx (BV/TV: -31%; Tb.Sp: 49%) and Sham-Aged (BV/TV: -28%; Tb.Sp: 56%) groups. Significant decreases in Tb.Th were found in the Ovx group compared to the Sham-Ovx and Sham-Aged groups (about 16%). Tb.N in the Ovx group decreased by 21%, 17% and 20% compared to the Baseline (p < 0.005), Sham-Ovx (p < 0.05) and Sham-Aged (p < 0.005) groups, respectively. The N.Nd/N.Tm of the Ovx group showed significant decreases in comparison

Table III. Static histomorphometric parameters in normal and ovariectomized rats; sections observed at a magnification of 12.5x (Mean ± SD, n=10 for each group).

Parameter	Baseline	Ovx	Sham-Ovx	Sham-Aged	ANOVA F	p
BV/TV (%)	43.30 ± 1.99	29.07 ± 3.44 [a]	42.07 ± 2.67	40.49 ± 8.91	16.73	< 0.0005
Tb.Th (μm)	108.39 ± 8.15	92.19 ± 5.28 [b]	110.64 ± 11.56	110.03 ± 25.10	4.09	< 0.05
Tb.N (# /mm)	4.02 ± 0.39	3.15 ± 0.40 [c,d]	3.81 ± 0.38	3.95 ± 0.51	8.60	< 0.0005
Tb.Sp (μm)	142.53 ± 16.95	229.19 ± 42.52 [a]	153.79 ± 22.47	146.61 ± 31.46	18.76	< 0.0005
N.Nd / N.Tm	4.20 ± 1.08	2.58 ± 0.52 [e]	3.75 ± 0.92	3.64 ± 1.00	5.70	< 0.005
Ct.Wi (μm)	144.6 ± 30.6	160.7 ± 47.5	147.7 ± 39.5	156.89 ± 32.18	0.39	ns

BV/TV: trabecular bone volume; Tb.Th: trabecular thickness; Tb.N: trabecular number (number of *trabeculae* /mm); Tb.Sp: trabecular separation; N.Nd/N.Tm: index of spatial connectivity; Ct.Wi, width of cortical bone.
Scheffé's post hoc multiple comparison test:
BV/TV and Tb.Sp: [a]Group Ovx versus groups Baseline, Sham-Ovx and Sham-Aged (p<0.0005).
Tb.Th: [b]Group Ovx versus group Sham-Ovx (p<0.05).
Tb.N: [c]Group Ovx versus groups Baseline and Sham-Aged (p<0.005); [d]Group Ovx versus group Sham-Ovx (p<0.05).
N.Nd/N.Tm: [e]Group Ovx versus groups Baseline and Sham-Ovx (p<0.005).

Table IV. Correlation between densitometric[*], ultrasonographic[°], mechanical[#] and histomorphometric[§] parameters (n=40).

Parameter	BMD[*]	BMC[*]	AD-SOS[°]	EN[°]	TF[°]	Slope[°]	Max.Load[#]	Elastic Mod. [#]	BV/TV[§]	Tb.Th[§]	Tb.N[§]	Tb.Sp[§]
BMC[*]	0.632[a]											
AD-SOS[°]	0.658[a]	0.396										
EN[°]	0.380	0.040	0.667[a]									
TF[°]	0.396	0.074	0.485[b]	0.257								
Slope[°]	0.537[b]	0.272	0.679[a]	0.714[a]	0.358							
Max.Load[#]	0.550[b]	0.371	0.595[a]	0.313	0.420[b]	0.342						
Elastic Mod.[#]	0.189	0.023	0.459[b]	0.212	0.452[b]	0.103	0.597[a]					
BV/TV[§]	0.507[b]	0.314	0.687[a]	0.403[b]	0.639[a]	0.462[b]	0.543[b]	0.398				
Tb.Th[§]	0.181	0.070	0.410[b]	0.489[b]	0.327	0.443[b]	0.318	0.280	0.679[a]			
Tb.N[§]	0.595[a]	0.305	0.513[b]	0.153	0.718[a]	0.307	0.536[a]	0.299	0.548[b]	0.026		
Tb.Sp[§]	-0.585[a]	-0.317	-0.643[a]	-0.389	-0.721[a]	-0.518[b]	-0.613[a]	-0.398	-0.784[a]	-0.397	-0.903[a]	
N.Nd/N.Tm[§]	0.314	0.092	0.446[b]	0.460[b]	0.250	0.266	0.356	0.192	0.444[b]	0.258	0.297	-0.378[a]

Number are the correlation coefficients (Pearson's correlation test). Statistical significance: [a]p < 0.01; [b]p < 0.05.

with the Baseline (-39%) and Sham-Ovx groups (-31%).

Significant correlations between the densitometric, ultrasonographic, mechanical and histomorphometric data are reported in Table IV. A correlation was found between AD-SOS and BMD (p < 0.0005), as well as between AD-SOS and almost all of the histomorphometric and mechanical parameters of the trabecular bone. In addition, a significant correlation was observed between BMD and some of the histomorphometric parameters and Max.Load. A significant correlation between histomorphometric and mechanical parameters was registered, as well. Regarding stepwise regression, BV/TV and Max. Load were the parameters that predicted AD-SOS correctly (adjusted r² = 0.517; F= 21.89, p < 0.0005) and BMD (adjusted r² = 0.388; F = 13.35, p < 0.0005).

Discussion

The aim of this study was to quantify structural and strength changes in the femoral distal epiphysis of ovariectomized rats, in order to evaluate whether this area may be a suitable site to conduct experimental studies on human osteoporosis and biomaterial osseointegration in osteoporotic bone by means of densitometric, ultrasonographic, mechanical and histomorphometric analyses. The estrogen-deficient osteopenia induced in the trabecular bone of the femoral distal epiphysis, as well as the correlation between bone mass, structure and strength changes at 16 weeks after ovariectomy were investigated. To the present authors' knowledge there are no previous similar studies available in the literature.

Both the densitometric and ultrasonographic data demonstrated a significant decrease in bone density at the level of the distal epiphysis, thus confirming the occurrence of structural changes and the usefulness and accuracy of both QUS and DXA, as already observed by other authors in animal studies on other sites (21, 22). In the present study, the QUS results were also confirmed by the analysis of the osteosonographic curves, whose parameters revealed the same trend as the mechanical and histo-morphometric findings. In particular, the TF decreased by approximately 27%,the EN (affected by structural and compositional factors) decreased by 66% on average, and the Slope parameter (which provides a quality and quantity index of the signal crossing the bone) decreased by 82% (33).

Mechanical and histomorphometric findings showed significant strength reduction and cancellous bone loss in the femoral distal epiphysis of the Ovx Group, when compared to the Baseline and Sham Groups. The current strength reduction in the cancellous bone was mainly due to an approximate 23% decrease in Max.Load and to a 19% decrease in the Elastic Modulus. These findings were not compared to those of other authors because of the lack of similar data on the distal femur in the literature. However, the mechanical results obtained for the distal femur here are similar to those achieved in a previous study in which the proximal femurs of normal and ovariectomized rats were subjected to a compression test (34). Although there are some obvious differences in mechanical characteristics, it may be stated that there are no differences between the two anatomical sites in terms of CV (~14%) and the percentage decrease observed in the ovariectomized versus sham-ovariectomized groups (30-36% for Max.Load and 22-25% for Elastic Modulus). Peng et al. demonstrated that the bone strength of the femoral neck of sham-ovariectomized rats was positively correlated with the percentage of BV/TV in the distal femur. They explained the lack of correlation observed for the ovariectomized rats with the geometrical changes occurring in cortical bone by suggesting that ovx-induced trabecular bone loss also occurs in the femoral neck. However, these hypotheses were not confirmed by a histomorphometric analysis (16).

The cancellous bone loss in this study was mainly due to a 32% decrease in BV/TV, a 19% decrease in Tb.Th and Tb.N, a 35% decrease in N.Nd/N.Tm, and a 55% increase in Tb.Sp, thus suggesting that both mechanisms (trabecular rarefaction and trabecular perforation) should be considered in the expla-nation of bone loss in Ovx rats, as observed by Lauritzen et al. (20). In fact, the reduction in the histomorphometric variables related to the trabecular volume and trabecular connectivity were both significantly changed in the Ovx group. In female rats ovariectomized at the age of 3 months, Peng et al. observed a significant decrease in the distal femur BV/TV, when they compared ovariectomized and sham-ovariectomized groups 10 to 20 weeks after surgery (16). In a rat model ovariectomized at 6 months, Durbridge et al. also found a significant decrease in the distal femur BV/TV, when ovariectomized and sham-ovariectomized groups were compared 9 weeks after surgery (18). A percentage trend similar to the current results for Tb.Th (decrease in Tb.N and increase in Tb.Sp) was observed in the femoral neck of ovariectomized versus sham-ovariectomized and baseline rats by Bagi et al., even if the younger age of the rats used (about 3 months) may be considered to be responsible for the greater bone rarefaction observed (12).

Our correlation study showed that the histomorphometric parameters correlated significantly only to Max.Load. To our knowledge, no other authors have correlated these structural and mechanical parameters recorded on the femoral distal epiphysis. AD-SOS and BMD were highly correlated (r= 0.658, p < 0.01), almost in the same range of r-values according to various studies (26, 29), and their correlation with mechanical and histomorphometric parameters almost reached the same significance levels (Table III). In particular, only AD-SOS correlated significantly with the Elastic Modulus (p < 0.05), while they were both correlated to Max.Load, BV/TV, Tb.N and Tb.Sp, with the same p-level. The correlation of TF, EN and Slope with microarchitectural parameters confirmed that AD-SOS derived parameters were mainly affected by the amount and structural properties of the bone, as observed by Barkman et al. (35). In addition, stepwise multiple regression suggested and confirmed that the structural (BV/TV) and mechanical (Max.Load) aspects had some influence on AD-SOS and

BMD: 52% and 39%, respectively. Therefore, both of the methodologies investigated focused more on cancellous bone microarchitectural changes and less on mechanical properties, thus showing their limited capability to detect bone stiffness and strength modifications. In our opinion, this result could depend on the choice to perform destructive mechanical tests only in one direction, rather than non-destructive tests in axial, saggital and coronal directions (36, 37).

The current authors had previously focused their attention on the capability of QUS and DXA to determine bone loss and predict the mechanical and microarchitectural properties of vertebral cancellous bone in ovariectomized rats, and they demonstrated that QUS seemed to depend more on cancellous bone microarchitectural changes than on bone strength modifications (38). However, various authors have failed to demonstrate any significant correlation between ultrasonographic and densitometric parameters and microarchitecture (25, 36, 40, 41), and the present findings are partially in accordance with some *in vitro* studies reporting a significant correlation between QUS and various mechanical parameters (29, 37).On the other hand, Hans *et al.* observed that the variability of the speed of sound was mainly due to density (88-93%) and, to a small extent, to elasticity or anisotropy (36); they stated that a possible explanation for their data could be the ultrasound frequency of 1.25 MHz (the same frequency used for this study), which could yield limited information about human trabecular bone microarchitecture (29, 41).

In conclusion, the present data demonstrate the development of quantifiable osteopenia in the rat distal femur 16 weeks after ovariectomy. The measurement of the speed of sound through the bone with QUS has proven to be reliable and well correlated with the absorptiometric assessment of BMD. Both the fact that QUS and DXA measures were taken *in vitro* and the difficulty of correlating the present findings with those obtained *in vivo* should be taken into consideration. However, *in vivo* studies offer the possibility to per-

form destructive tests such as histomorphometry and biomechanics. Consequently, researchers may try to collect more information on ovariectomy-induced modifications by means of post-mortem ultrasonographic and densitometric assessments. So far the only sites which have proved reliable for QUS measurements in the rat model has been the first caudal vertebrae (38, 39). However, this *in vivo* study shows that QUS measures can be used to obtain more complete information on rat femoral condyles. The distal femur could make it possible to easily perform both structural and mechanical investigations on bone thanks to its geometry and the greater amount of tissue available for sampling. Finally, implantation in osteopenic bone may be easily carried out to investigate and develop new biomaterials for the reduction of the aseptic failure, which occurs more frequently in patients with poor bone stock secondary to various metabolic or inflammatory diseases.

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