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Determining the biomechanical quality of normal and osteoporotic bones in rat femora through biomechanical test and finite element analysis

Normal ve osteoporotik sıçan femurunda kemiğin biyomekanik kalitesinin biyomekanik testle ve sonlu elemanlar analizi ile belirlenmesi

Ulku COMELEKOGLU,[1] Huseyin MUTLU,[2] Serap YALIN,[3] Selda BAGIS,[4] Altan YILDIZ,[5] Oya OGENLER[1]

Department of Biophysics[1] and Radiodiagnostic[5], Mersin University, Faculty of Medicine; [2]Department of Machine Engineering, Mersin University, Faculty of Engineering; [3]Department of Biochemistry, Mersin University Faculty of Pharmacy; [4]Department of Physical Medicine and Rehabilitation, Baskent University, Turkey

Amaç: Bu çalışmada, normal ve osteoporotik sıçan kortikal femurunun biyomekanik özellikleri hem deneysel biyomekanik analiz hem de sonlu elemanlar analizi (SEA) ile değerlendirildi.

Çalışma Planı: Ağırlıkları 225-250 gram arasında değişen 14 adet genç erişkin Sprague-Dawley cinsi dişi sıçan eşit sayıda iki gruba ayrıldı. Bir gruba ketamin anestezisi altında iki taraflı ovariektomi yapıldı. Ovariektomiden 14 hafta sonra, çift enerjili X ışınları absorbsiyometrisi ile tüm sıçanlarda kemik mineral yoğunluğu (KMY) ölçüldü; daha sonra yüksek dozda ketamin ile deneklerin yaşamı sonlandırılarak sağ ve sol femurları çıkarıldı. Sol femurlara biyomekanik germe testi uygulandı. Sağ femurlarda ise kortikal kemik kesiti bilgisayarlı tomografi ile görüntülendi, bilgisayara aktarılan görüntülerden kemiklerin üçboyutlu modelleri elde edildi. ANYSIS 9.0 paket programı kullanılarak SEA yapıldı. Her iki yöntemle elde edilen kemiklerin deformasyon, stres ve strain dağılımları karşılaştırıldı.

Sonuçlar: Ovariektomi grubunda KMY değerinin kontrol grubuna göre yaklaşık %29 oranında azaldığı görüldü ($p<0.05$). Ayrıca, biyomekanik testte deformasyon %39, stres %51 ve gerilme %64 düşüş gösterdi ($p<0.05$). Deneysel biyomekanik analiz ile SEA analizi sonuçları arasında anlamlı fark bulunmadı ($p>0.05$).

Çıkarımlar: Elde edilen sonuçlar ışığında, klinik uygulamada osteoporotik hastalarda SEA ile kemiğin biyomekanik kalitesinin *in vivo* olarak belirlenebileceği düşünüldü.

Anahtar sözcükler: Biyomekanik; kemik yoğunluğu; femur/anatomi ve histoloji; sonlu elemanlar analizi; osteoporoz; ovariektomi; stres, mekanik; sıçan.

Objectives: This study aimed to determine the biomechanical quality of cortical bone in normal and osteoporotic rat femora with the use of biomechanical analysis and finite element analysis.

Methods: Fourteen young adult female Sprague-Dawley rats weighing 225-250 g were randomized into two groups equal in number. One group underwent bilateral ovariectomy under ketamine anesthesia. Fourteen weeks after ovariectomy, bone mineral density was measured by dual energy X–ray absorptiometry in two groups and the rats were sacrificed under high-dose ketamine anesthesia for removal of all the femora. The right femora were subjected to biomechanical tension tests. In the left femora, cortical bone sections were visualized by computed tomography and finite element analysis was performed on computer-generated three dimensional images using the ANYSIS 9.0 software. Deformation, stress, and strain values obtained by the two analyses were compared.

Results: Compared to the control group, bone mineral density decreased by 29%, and decreases in deformation, stress, and strain values were 39%, 51%, and 64%, respectively, in the ovariectomized rats ($p<0.05$). No significant differences were found between the results of biomechanical measurements and finite element analysis ($p>0.05$).

Conclusion: Our data suggest that finite element analysis can be used *in vivo* to determine biomechanical quality of the bone in osteoporotic patients.

Key words: Biomechanics; bone density; femur/anatomy & histology; finite element analysis; osteoporosis; ovariectomy; stress, mechanical; rats.

Correspondence to: Dr. Ulku Comelekoglu. Mersin University, Faculty of Medicine Department of Biophysics, 33169 Mersin-Turkey.
Phone: +90324 - 341 28 15 / 1007 Fax: +90324 - 341 24 00 e-mail: ucomelek@yahoo.com

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Osteoporosis is a disease which is characterized by low bone mass, structurally damaged bone tissue, decreased bone strength and finally related to increased risk of bone fractures due to these weakness.[1-2]

Nowadays; osteoporotic fractures are one of the most important public health problems encountered over 30 years of age and especially older women with their morbidity, mortality, and high treatment costs. Although, measurement of bone mineral density is known to be important for the diagnosis of osteoporosis, it is reported that the determination of bone quality is more valuable than the measurement of bone mineral density.[3] The concept of bone quality includes material and geometric properties of bone. The material properties of a bone are intrinsic properties and they determine the bone strength.[4] Material properties of a bone can be determined by biomechanic tests,[5] and these tests are performed under laboratory conditions. For this purpose; tension, compress, rotation, and distortion forces are applied to bones and their strength and durability are evaluated.

Treatment of osteoporosis is quite expensive and the criterion for the evaluation of success of this treatment is bone mineral density. However, increased bone mineral density is not a sufficient criterion for the indication of decreased bone fragility. In some situations, increased mineralization may results in increased bone fragility.[4] For all of these reasons, determining the biomechanical quality of a bone is considered to be important for the evaluation of success in treatment. For the experimental measurement of biomechanic parameters bone samples are required. But, for the sake of treatment it is not possible to obtain bone samples from patients. Developments in computer technologies bring us some opportunities such as Finite Element Analysis (FEM) used especially in the field of engineering. This analysis provides the determination of biomechanical properties of bones in the absence of their samples. By this method, stress and torsion distributions can be measured and by means of modeling the bone in small pieces,[6] points where these parameters reach critical values can be observed.

The aim of this study was the determination of biomechanical properties of normal and osteoporptic rat cortical femur both by using experimental biomechanical analyses and by using FEDA, and comparison of results obtained. Ovariectomised rat model was used to constitute exprimental osteoporosis.

Material and methods

Fourteen young adult female Sprague-Dawley rats weighing 225-250 g were used in the experiments. The rats were housed in galvanized cages and maintained with 12-h dark/light cycle in a controlled atmosphere of 21 oC temperature and 50 % humidity with free access to pelleted feed and water ad libitum. The animals were assigned randomly into two groups: the control group (n=7) and ovariectomy (OV) group (n=7). Then, OV rats were anesthetized with ketamine (Ketalar; Eczacibasi Pharmaceutical Co.) and underwent bilateral ovariectomy via ventral incision. The Institutional Animal Care and Use Committee at Mersin University Medical Faculty approved the experiments described in this study.

Twelve weeks after the ovariectomy, bone mineral density of rats (BMD) were measured at mid-diaphysis femoral region by dual-energy X-ray absorbsiometry (DEXA; Norland 45 XR) adapted to the measurement of BMD in small animals. The rats were sacrificed by under high-dose ketamine anesthesia. The bilateral femur of each animal was harvested. The left femora were subjected to biomechanical analysis and were stored at _20 °C until mechanical testing. The right femora were subjected to imaging cortical bone cross-section.

Biomechanical measurements were performed at the mid-diaphysis of the left femur. After thawing at room temperature, samples were tested using biomaterial testing machine (MAY 03; USA). For the tensile test, the femur bone was mounted horizontally in the machine by using colacryl. Distance between the two ends was 3 mm. The tensile loading speed in all tests was 1 mm/s. Data were transferred to the computers translating the numerical signals by 16-bit A/D converter for off line analysis. The chosen sampling rate was 1000 sample/s and force was 5g/s. Load-displacement data were recorded using BIOPAC MP 100 Acquisition System Version 3.5.7 (Santa Barbara, USA). Load-displacement curve was normalized by cross-sectional area and this curve was converted to a stress–strain curve. Ultimate tensile strength and displacement were determined from load-displacement curve. Stress and strain were determined from the stress-strain curve. Stress represents the maximum

Table 1. Biomechanics variables in the normal and osteoporotic rat femora

Variables	Normal rat femur		Osteoporotic rat femur	
	Experimental biomechanics	Finite element analysis	Experimental biomechanics	Finite element analysis
Deformation (mm)	0.21±0.019	0.19±0.022	0.13±0.043	0.09±0.057
Stress (MPa)	39.46±5.68	41.3±7.96	19.40±3.96	21.77±3.46
Strain	0.13±0.05	0.11±0.033	0.048±0.0013	0.049±0.0021

stress before fracture occurred. The ultimate stress was calculated from that equation: s=F/A. In this equation, s_ is the ultimate stress (MPa), F is the failure load (N), A is the cortical area of the specimen (m2). The ultimate strain was calculated from the that equation: e= DL/L0. In this, e _is the strain, DL is the change in the length (mm) and L0 is the original length.

Theoretical calculation - Finite element analysis

Right femora of control and OV groups were scanned at 1mm resolution by computerized tomography (ARSTAR 40, Erlangen, German). These images were transferred to the personal computer and boundary points were generated using the AUTOCAD software. Three dimensional model of cortical femora was designed using solid model programme (SOLIDWORKS). Finite element analysis was performed on computer-generated three-dimensional images using the ANYSIS 9.0 software. For this analysis bone was assumed to be an isotropic linearly elastic solid. Cortical bone elastic modulus was assumed to be 0.5 GPa and Poisson's ratio 0.3. A 4- node quadrilateral solid element was used for modeling the cortical bone. Same protocol was used for force magnitude and direction in experimental and finite element analysis.

Statistical analysis

Statistical analysis was performed by using SPSS v. Statistical software. 10.0. Data were given as means ± SD and significance was set at $p<0.05$. After documenting normal distribution (Kolmogorov-Smirnov), the data were analyzed using Student's t-test.

Results

In the control group and OV group, mean BMD was 0.14+0.32 g/cm2 and 0.10+0.037 g/cm2, respectively. Compared to the control group, bone mineral density decreased by 29% in the OV group and this decrease was statistically significant ($p<0.05$). Similary, decreases in deformation, stress and strain values were 39%, 51% and 64 %, respectively, in the OV group in comparison to controls ($p<0.05$) and these reductions were statistically significant. These results were accepted as indicators for osteoporosis

The results of biomechanical tests and FEM for normal and osteoporotic bone were summarized in Table 1. The FEM deformation, stress and strain distribution of normal and osteoporotic bones were shown in Figure 1. No significant differences were found between the results of biomechanical measurements and finite element analysis ($p>0.05$).

Discussion

In this study the quality of cortical femoral bone was investigated by biomechanical analysis and FEA at normal and osteoporotic rats and found that a consistensy at two analysis according to distribution of deformation, stress and strain.

Finite element analysis was firstly used by Brekelmans for bone analysis at 1972. Brekelmans investistigated the distribution of stres at human bone with physiological loading.[8] Then, this analysis was used commonly for the biomechanical properties of orthopedical implants.[9-12]

The authors suggested that bone biomechanical properties were more important than BMD for the prevention and treatment of osteoporotic fractures. In clinical practice, the FEA was feasible method for bone biomechanical quality, but some parameters might be affected by the results. One of these parameters was the restoration of three dimension bone structure. The structure model must be similar to the original. For these reasons, the computerized tomography images of bone transverse section was trans-

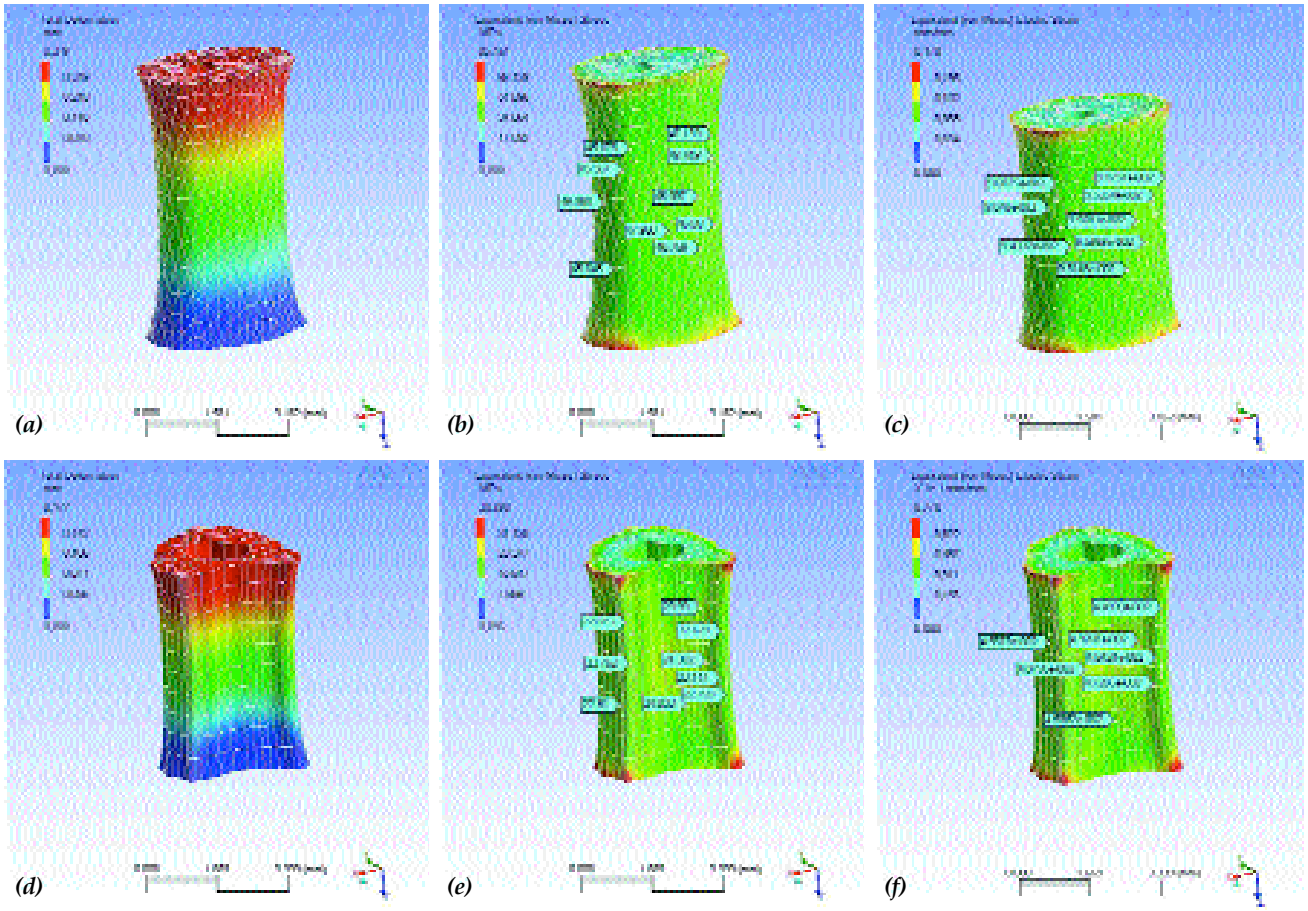

Figure 1. Distrubution of deformation, stres and strain in the **(a-c)** normal and osteoporotic **(d-f)** rat femur

ferred to the solid model program and the new 3D cortical bone model was generated. Bone material properties were assumed as homogenous and isotropic for the finite element analysis, based on the other studies.[9-12] This assumption was used for simplification of analysis.[13] We did not found significant differences between the biomechanical results and finite elemental analysis.

Recently, the probability of restoration of advanced model was increased with the technological development and scanning of microstructure. The other important parameter was the exact definition of bone material properties. Elastic module and Poisson ratio were important parameters. The force and its direction were also definite exactly.

The results of this study showed that finite elemental analysis might be used for the biomechanical quality of bone in vivo. The studies are still undergone for the use of this technique in clinical practice, particularly in the treatment of osteoporosis and the determination of fracture risk.

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