Electronic Supplementary Information.

Experimental

Eight femora, (three right femora and five left), and their corresponding acetabula were harvested from genetically identical 12 week old Wistar rats and all soft tissue (including periosteum) was carefully removed. The femora and acetabula were stored at -20ºC until testing. One day prior to testing they were slowly thawed at room temperature. Prior to the experiment a hole was carefully drilled in the distal femur, as far away from the proximal end where the femur will be loaded, to enable access to the trabecular bone in the proximal femur. Fig. 1 is an example of one of the harvested femora and acetabula, showing their orientations.

Experimental set-up

Prior to the experiment each intact femur was scanned by a micro-CT scanner (GE Healthcare eXplore Locus SP). For ESPI measurements, each femur was mounted up to its mid-shaft in an upright position in a drilled stainless-steel ball (16 mm in diameter) using dental acrylic composite (JET acrylic, Lang Dental MFG), with its cranial aspect facing the CCD camera of the ESPI. The steel ball and femur were placed and secured inside the test chamber, on the lower stationary anvil attached to the bottom of the chamber. The corresponding acetabulum was attached to a stainless-steel rod which served as an upper compressing anvil, using dental composite (Z250, 3M-ESPE). This was polymerized using a hand-held blue light-cure device for 120 seconds (LITEX 682, Dentamerica CA, USA). Then, by moving the upper anvil down and adjusting the lower anvil, the head of the femur was placed within its acetabulum, just above the contact zone. Next, the test chamber was sealed with a high-grade glass window (BK-7 $\lambda/10$ grade) and filled with water to immerse the samples. Finally, the upper anvil was lowered further until a pre-load compression of ~7N was obtained (Fig. 2).

Each femur was loaded incrementally within its elastic regime in an axial direction by the upper anvil via its acetabulum, using a custom-designed mechanical tension-compression device with stainless-steel parts[37, 44] (SS 316). The upper anvil was attached to an immersible load-cell (AL311BN,I6, Honeywell-Sensotec, OH, USA) capable of measuring loads up to 250N in tension or compression with an

accuracy of 0.15% of the full scale. The upper anvil was moved by a sub-micrometer high-precision DC motor and controller (PI M-235.5DG, Physik Instrumente (PI) GmbH, Germany) in order to compress the femur against the lower stationary anvil. Force measurements were collected and stored on a computer using an A/D converter (Omega DAQP-308 PCMCIA 16-bit Analogue I/O) and further analyzed by custom-written software (National Instruments Labview v 7.0 and Matlab v 6.0). For a detailed description see *Shahar et al*. 2007[44].

The loading sequence consisted of 30 increments of 2 micrometers each to a total of 60 micrometers, reaching a force of up to 23N. Just prior to and directly after each 2 micrometer increment, the force was determined by averaging 20,000 readings (taken at 80 kHz), and surface deformations were determined by ESPI. In order to compare the deformational behavior of different femora and also the same bone in the intact and manipulated state (see below) we used the displacements measured at the same end-load of around 15N. It should be noted that the absolute vertical displacements measured below the femoral head, where load was applied, are much smaller than the movement of the upper anvil. This difference is due to the compliance of the load cell and of the composite used to conduct the load from the anvil to the bone, as well as the elastic interaction at the interface between the cartilage of the femoral head and the pelvic acetabulum. ESPI measurements are however insensitive to these differences, since displacements are measured relative to a reference point located on the femur itself[39]. This reference point was chosen to be located just above the lower anvil, at the region of interest (ROI) mid-bottom, where minimal displacement was expected.

A 1.5 second interval was required for ESPI measurements between successive increments; therefore, dynamic strain is not measured. A deformation map is obtained after each loading increment for each of the 30 increments of the loading sequence. The loading sequence was repeated four times for each femur. At the end of each compression series the upper anvil automatically returned to its original pre-load position (~7N). By repeating the loading sequence four times a robust and reproducible data set was obtained. Moreover, each loading sequence itself was composed of 30 individual increments, each of which was evaluated independently.

Upon the completion of four sets, the test chamber was opened and the upper anvil with the acetabulum attached to it was lifted away from the femoral head. The

femur was then released from its metal ball and the chamber was resealed again to enable the acetabulum to be immersed in water. A small dental root-canal probe was introduced through the pre-drilled hole at the distal end of the femur. By gently manipulating the probe, some trabecular bone tissue in the distal femoral neck was broken and removed. Next, the femur was scanned again by micro-CT, to semi-quantitatively evaluate the damage to the trabecular bone. The manipulated femur was then returned and remounted in the test chamber and submerged in water. The upper anvil with the acetabulum was lowered onto the femoral head and the loading sequence was repeated. Special care was taken to keep the entire set-up as similar as possible to the measurement conditions of the intact femur; the femur was remounted in the same anatomical orientation and the same contact between the femoral head and the acetabulum was reestablished.

Optical measurements

Surface deformations of the cranial face of the proximal femur were measured as the bones were loaded, using an electronic speckle pattern-correlation interferometry (ESPI) device (Q300, Dantec-Ettemeyer, Ulm, Germany). The submerged femur surface was illuminated by a coherent laser light source (780 nm wavelength) from two symmetrically opposite angles relative to the plane normal to the surface. As the light scatters from the optically rough surface of the bone, speckles are formed due to local interference. These speckles are imaged onto a CCD detector array. Any sub-micrometer scale displacement on the bone surface will affect the optical path of the light propagating towards the CCD detector array, changing the detected interference pattern. The differences between interference intensity patterns of successive speckle images taken prior to and right after each loading increment are used to detect shifts in the phases of the speckles. These phase shifts correspond to surface displacements along the measurement axes (X, Y or Z) and their magnitudes can be determined using a phase-shifting algorithm combined with phase unwrapping. Thus it is possible to measure displacements as small as 25 nm along three orthogonal axes (X, Y, and Z) and generate displacement maps.

In our experiments, the femora were grouped into two groups. Surface deformations were recorded for the entire upper part of the femur for samples belonging to the first group, while in the second group we focused on the proximal

femur, mostly the femoral neck, in order to achieve higher resolution. Consequently, for the first group (femora #2, #4, #5 and #6) each detector on the CCD detector array covered a sub-area of approximately 19 x 19 micrometers on the sample surface, and for the second group (femora #8, #9, #10 and #11) each detector on the CCD detector array covered a sub-area of approximately 11 x 11 micrometers on the surface of the sample. Due to the small dimensions of the samples relative to the distance to the ESPI lens (3-5 mm versus 220-270 mm respectively), spherical aberrations could be neglected.

Micro-CT imaging

For each of the eight femora the proximal part of the bone was scanned twice: once in the intact state and then after removing part of the trabecular bone. Scans were carried out at a resolution of 8 micrometers and morphometric analyses of the neck region were performed, using the GE Healthcare eXplore Locus SP micro-CT instrument. Evaluation of the scanned volumes was carried out using GE Healthcare eXplore MicroView v. 2.0 3D volume viewer software. A region of interest (ROI) was selected within the region of the femoral neck. This region was defined by the boundary between the widening of the proximal neck towards the femoral head and the opening of the distal neck to the intertrochantric region (see Fig. 3 for nomenclature and Fig. 4c for an illustration of the neck ROI). The selected neck ROI was identical for each femur in the intact and manipulated states up to a difference of 2%. For each femur the following parameters were calculated and compared: volume of ROI, average trabecular number per cubic millimeter and average trabecular thickness per cubic millimeter. Trabecular number was calculated by MicroView software using a stereology algorithm which is inherently 2D and works by a point-intercept approach. Test lines are drawn within the ROI and the number of cross-points between each line and the surrounding trabeculae are counted. Trabecular thickness was calculated by MicroView software using the direct measurement algorithm which operates in a 3D environment. The software finds the maximum sphere size that fits within each trabecula in the selected ROI; the accuracy of the trabecular thickness calculation is 0.01 voxel. The micro-CT scans which were taken after manipulation of the bone also served as confirmation that no other part for the trabecular tissue in the femoral neck was damaged.

Data analysis

Our data consist of deformation maps representing measured displacements along the X- and Y- axes inside a pre-chosen border, relative to an arbitrarily-selected reference point. Fig. 3 shows the outer border of the proximal femur region of interest (ROI). The ROI border was identical for each bone in the intact and manipulated states. Displacement difference maps were produced by subtracting the displacement magnitudes for each pixel within the proximal femur ROI in the manipulated femoral map from the same pixel value in the intact femoral displacement map.

In order to determine the behavior of different regions within the proximal femur, the proximal femur was further sub-divided into five sub-regions which were analyzed separately. Fig. 3 defines these five regions within the proximal femur. For each region the following parameters were calculated: minimum and maximum values of displacement magnitudes (*i.e.* displacement range), average and median displacement magnitudes and standard deviations. Average and median values correlated well with each other, and hence we present only the average values.

Statistical analysis was performed using JMP software (SAS Institute. 2005. JMP Version 6. SAS Inst. Inc.,Cary, NC.). For each femur, all group pairs of displacement values (same sub-region in the intact and manipulated states) were tested for equal means by t-test (assuming un-equal variances) and by Welch ANOVA. $P < 0.05$ was considered significant. All calculated mean differences but one (Y-direction Proximo-lateral sub-region in femur #6) were found to be significantly different.

Femur #	Intact	Manipulated
2-Left	20 (18-21)	15 (13-16)
4-Left	25 (24-28)	15 (14-15)
5- Right	30 (29-30)	17 (16-17)
6-Left	20 (18-21)	18 (17-19)
8-Right	19 (18-20)	18 (17-19)
9-Left	20 (15-23)	19 (16-21)
10-Right	17 (16-17)	15 (15)
11-Left	25 (25)	23 (23)

Table S1. The number of compression steps needed to achieve ~15N of load in the intact and manipulated femora; in parentheses is the range of values in the different sets of each experiment. In all eight femora the number of steps was reduced after manipulation.

Femur #		Neck		Greater trochanter		Intertrochantric		Proximo-lateral		Lateral	
		Intact	Manipulated	Intact	Manipulated	Intact	Manipulated	Intact	Manipulated	Intact	Manipulated
2	Y	-5.8 (1.2) -11.3; -1.5	-3.9 (1.2) -16.6; -0.2	-0.5 (0.3) -6.3; 1.7	-0.3 (0.1) -2.1; 1.4	-1.6 (0.1) -5.8; 0.7	-0.8 (0.1) -3.1; 1.1	0.0 (0.2) -1.2; 1.2	0.4 (0.1) -0.4; 1.5	-0.3 (0.1) -1.2; 1.0	0.4 (0.1) -0.7; 2.1
	X	-7.2 (0.9) -14.6; -2.6	-5.2 (0.6) -13.5; -1.3	-9.4 (1.2) -11.1; -4.8	-8.1 (1.1) -9.8; -3.2	-6.6 (0.1) -9.8; -2.2	-5.3 (0.1) -8.3; -2.0	-6.5 (0.8) -9.3; -2.2	-5.4 (0.9) -8.0; -2.0	-2.9 (0.4) -5.2; -0.5	-2.5 (0.3) -4.3; -0.8
4	Y	-6.3 (1.1) -12.3; 0.15	-4.3 (1.0) -13.9; -0.1	-2.4 (0.6) -6.6; 0.3	-0.6 (0.3) -4.5; 2.9	-2.6 (0.1) -6.3; -0.2	-1.2 (0.1) -4.8; 1.2	-0.7 (0.1) -2.2; 1.0	0.5 (0.1) -1.1; 1.8	-0.1 (0.1) -1.5; 0.2	0.5 (0.1) -0.4; 1.4
	X	-9.3 (1.0) -15.5; -3.4	-6.2 (1.0) -13.6; -0.8	-14.2 (1.9) -17.6; -8.1	-8.9 (1.4) -13.4; -5.0	-9.3 (0.2) -16.6; -3.5	-6.1 (0.4) -12.7; -2.1	-9.0 (0.5) -14.2; -3.3	-5.8 (0.1) -8.9; -2.0	-4.1 (0.1) -7.0; -1.3	-2.4 (0.1) -4.5; -0.5
5	Y	-5.4 (0.9) -10.5; 3.1	-6.8 (1.1) -13.8; 3.3	-1.4 (0.4) -5.2; 3.0	-1.1 (0.5) -6.9; 3.1	-1.7 (0.1) -5.4; 2.0	-2.3 (0.1) -6.8; 1.6	0.4 (0.3) -2.5; 3.1	-0.5 (0.3) -3.1; 1.7	0.5 (0.2) -2.2; 2.7	-0.6 (0.3) -3.1, 2.4
	X	9.3 (1.0) 3.7; 16.8	7.8 (0.9) 2.9; 15.7	12.9 (1.7) 6.4; 16.0	10.2 (1.3) 3.7; 15.1	8.9 (0.1) 3.5; 13.3	6.9 (0.2) 2.7; 11.9	8.4 (1.3) 3.8; 12.3	6.3 (1.0) 2.7; 10.0	4.4 (0.5) 1.52; 7.62	3.7 (0.4) 2.4; 7.0
6	Y	-6.5 (1.3) -16; 0.1	-6.0 (1.1) -20.5; 3.2	-1.6 (0.5) -7.7; 1.4	-1.4 (0.5) -6.4; 2.0	-2.3 (0.1) -6.0; 0.7	-2.0 (0.1) -6.0; 1.1	0.1* (0.2) -2.2; 1.7	0.1* (0.3) -2.0; 2	0.3 (0.1) -1.0; 1.4	0.3 (0.1) -1.0; 1.4
	X	-8.8 (1.0) -16.9; -1.4	-8.1 (1.1) -13.7; -0.8	-12.8 (1.6) -15.5; -6.5	-11.6 (1.2) -15.2; -5.3	-8.8 (0.2) -12.9; -3.0	-7.6 (0.2) -12.8; -2.8	-8.7 (0.9) -13.1; -3.5	-7.6 (0.9) -12.5; -3.0	-4.2 (0.5) -7.0; -1.3	-3.5 (0.4) -6.2; -0.9
8	Y	-4.6 (0.9) -11.7; 0.5	-4.7 (0.8) -14.0; 0.1	0.0 (0.2) -2.7; 2.3	-0.4 0.2) -3.8; 2.4	-0.5 (0.1) -3.5; 2.0	-0.9 (0.1) -4.4; 1.3	1.0 (0.2) -0.6; 2.4	0.9 (0.1) -0.5; 2.5	1.1 (0.1) 0.1; 2.6	1.0 (0.1) -0.2; 2.5
	X	4.7 (0.5) -0.7; 15.7	5.1 (0.6) 0.3; 17.0	6.6 (0.8) 2.4; 9.8	8.1 (1.1) 3.3; 10.4	4.3 (0.1) 1.4; 7.4	5.2 (0.1) 1.8; 8.9	4.0 (0.4) 1.5; 6.9	4.5 (0.3) 1.4; 8.2	1.1 (0.2) -1.2; 3.2	1.5 (0.3) -1.6; 3.8
9	Y	-3.0 (0.7) -14.5; 4.4	-2.0 (0.4) -7.4; 3.3	0.6 (0.2) -2.5; 2.7	0.7 (0.2) -2.4; 2.6	0.0 (0.1) -3.4; 2.4	0.3 (0.1) -2.2; 1.9	1.5 (0.2) -0.3; 3.6	1.4 (0.2) 0.4; 2.4	1.8 (0.2) 0.5; 3.0	1.5 (0.1) 0.4; 2.1
	X	-3.5 (0.4) -13.2; 2.3	-2.7 (0.4) -10.9; 1.4	-7.7 (1.0) -12.4; -2.8	-5.2 (0.7) -6.8; -1.6	-4.9 (0.1) -8.5; -1.9	-3.4 (0.1) -6.1; -0.9	-5.0 (0.5) -8.0; -1.8	-3.3 (0.4) -5.4; -1.2	-1.7 (0.2) -3.9; 0.2	1.0 (0.2) -2.4; 0.7
10	Y	-4.5 (1.0) -12.0; 2.9	-3.3 (0.7) -11.4; 0.2	0.4 (0.2) -5.0; 2.4	-0.1 (0.2) -4.1; 1.9	0.1 (0.2) -4.76; 1.85	-0.3 0.1) -3.1; 1.2	1.6 (0.2) 0.4; 2.6	1.0 (0.1) -0.1; 1.9	1.6 (0.1) 0.6; 2.7	1.0 (0.1) 0.1; 2.0
	X	3.5 (0.4) -6.0; 9.1	2.4 (0.4) -5.9, 9.1	5.8 (0.7) 2.53; 8.1	4.4 (0.6) 1.8; 5.8	3.3 (0.1) 0.3; 7.0	2.5 (0.1) 0.4; 5.2	3.0 (0.3) 0.6; 5.4	2.3 (0.2) 0.5; 4.2	0.5 (0.2) -1.0; 1.8	0.4 (0.1) -0.5; 1.6
11	Y	-4.4 (0.9) -12.4; 2.6	-3.6 (0.7) -11.0; 7.2	0.8 (0.3) -3.0; 3.6	0.9 (0.3) -3.9; 3.6	-0.6 (0.1) -3.3; 1.9	-0.2 (0.1) -3.4; 2.2	1.5 (0.2) -0.3; 3.3	1.2 (0.2) -0.2; 3.2	1.4 (0.2) 0.1; 2.7	1.0 (0.1) 0.1; 2.1
	X	-6.5 (0.7) -16.6; 1.1	-3.9 (0.7) -11.5; 17.1	-10.7 (1.4) -13.4; -6.2	-6.6 (0.9) -9.5; -3.2	-6.8 (0.1) -10.8; -2.3	-4.0 (0.1) -7.4; -1.1	-6.5 (0.7) -10.1; -2.4	-3.8 (0.4) -6.6; -1.0	-1.8 (0.5) -4.5; 0.9	-0.9 (0.3) -2.7; 0.8

Table 3. Upper row in each cell gives the average displacements value for each region at a load of 15N, standard deviation is in parenthesis; lower row in each cell gives the displacement ranges. All values are in micrometers. Pair values (except Femur #6, Proximo-lateral region; marked with *) are statistically significantly different. Dark shaded pairs (femur #5 and femur #8) show an opposite trend and have higher average displacements in the manipulated state as compared to the intact state; these results are consistent with the results shown in Fig. 6.