Fatigue in osteoporotic human trabecular bone Comparison between genders

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Abstract

In the present work, the fatigue behaviour of human trabecular bone of samples proceeding from 2 genders (male and female) was studied. The samples were obtained from patients that underwent hip arthroplasty due to femoral neck's fracture, related to osteoporosis (OP). From the femoral heads, 25 cylindrical trabecular bone specimens were obtained. The samples were structurally characterized with scanning electron microscopy (SEM). Initially, the specimens were submitted to 10 pre-conditioning cycles with a frequency of 2 Hz to calculate the initial modulus, E_0. The specimens were submitted to 1000 compressive fatigue cycles at a frequency of 1/3 Hz. They were compressed between a small pre-load and a maximum stress corresponding to a normalized stress, by the initial modulus, (σ_{max}/E_0^{trav}) equal to 0,002 (23 samples) or a stress corresponding to a normalized stress amplitude ($\Delta\sigma/E_0^{trav}$) of 0,002 (2 samples). The results show an evolution of the maximum and residual strains (ε_{max} and ε_{res}, respectively) in accordance to what is described in the literature. The normalized secant modulus (E_{sec}/E_0) remains relatively constant, which allow us to conclude that the fatigue tests were performed with very small deformations. No significant differences were found between the fatigue behaviour of the two groups in study. The analysis of microscopic images of non-tested samples shows a similar structure between both genders. The analysis of the SEM images of tested samples suggests that, with the test conditions used (small deformations and a low number of cycles) there is no damage formation.

Introduction

The understanding of the bone's structural and mechanical proprieties is relevant from the biologic and clinic points of view. Almost all *in vivo* loading acting on the living body is cyclic by nature [1]. Repetitive, cyclic loading of bone during daily course of activities is one of the primary causes of bone fracture in humans [2-5]. These fractures occur in the elderly people, with bone of compromised quality, and are known as age-related fragility [1, 3-5]. Nonetheless, there is few data available about the fatigue strength of trabecular bone [1, 6, 7] and only a small number of these studies were based on human trabecular bones [1, 6]. The few data available imply that the influence of some diseases like osteoporosis is yet to be known.

The fatigue tests are usually made in a servo-hydraulic machine, using cylindrical specimens [1-8]. The specimen preparation often includes a initial step of de-fatting or narrow removal [1-6]. The specimens are press-fit into brass end caps and glued in place to avoid crushing of the specimen ends during testing [1-8]. The extension is usually measured with an extensometer attached to the end caps, using elastic bands [2-7]. The specimens are tested in compressive fatigue loading to the load corresponding to a normalized stress level ($\Delta\sigma/E_0$). The use of $\Delta\sigma/E_0$ has the purpose of reducing the scatter in the results caused by large variations in the initial Young's modulus (E_0) associated to density variations in the trabecular bone [1-7]. The control of the $\Delta\sigma/E_0$ level allows us study low-cycle fatigue (high $\Delta\sigma/E_0$) or high-cycle fatigue (low $\Delta\sigma/E_0$) [2-5].

The fatigue behaviour of trabecular bone is characterised by an increase of the residual strain (ε_{res}) and plastic strain at each cycle ($\Delta\varepsilon_{pl}$) and by a decrease of the normalized secant modulus (E_{sec}/E_0) [1, 3, 4, 7, 8]. The strains follow the three classical stages of fatigue: a transient behaviour characterised by rapid strain increase within the first load cycles, a saturation of strains and a fast increase of strains near catastrophic failure [1].

The number of cycles until failure (N_f) decreases with an increase of $\Delta\sigma/E_0$ [3, 4, 7] such as the accumulation rate of ε_{res} in the saturation stage does [4]. The E_{sec}/E_0 is better predicted by the ε_{max} [3].

During the fatigue tests, microdamage is generated and is presented in form of different patterns. The microdamage accumulation follows the reduction in the E_{sec}/E_0 [2, 3].

Some of these results were obtained using bovine trabecular bone [1-5, 8]. There are evidences of similarity in the fatigue behaviour of human and bovine trabecular bone [1, 7], therefore these results should be observed for human trabecular bone.

This study's goal is to increase the knowledge about the fatigue behaviour of the osteoporotic human trabecular bone and characterise the differences between genders concerning fatigue properties. Another objective is to analyse the structure of the bone with scanning electron microscopy (SEM) images.

Materials and Methods

In this work, there were retrieved 25 femoral heads from patients who had a total hip arthroplasty because of a fracture in the femoral neck, attributed to osteoporosis (OP). The samples were assembled by gender in 2 groups: male (M) and female (F).

After the medical procedure, the femoral epiphyses were immediately stored at -80ºC. Before testing, this material was defrosted at room temperature and measurements of the diameter were performed in three different axis.

Bone cylinders were obtained by drilling in the highest load direction with a perforating drill with a diameter of 15 mm (approximately). The cylinders extremities were polished with 600 sandpaper under flowing water (Surface Polishing Machine Struers DAP-V) to make them parallel. Bone cylinders were de-fatted during three hours, using a chloroform and methanol solution (1:1 ratio), and were hydrated overnight in phosphate-buffered saline (PBS) solution.

The fatigue tests were performed in a universal testing machine (Instron 5566™, Instron Corporation, Canton, USA) with a load cell of 10kN. Prior to fatigue testing, the specimens were preconditioned by loading for 10 cycles in strain by means of a triangular waveform from 0.5% compressive strain to 1% compressive strain at a frequency of 2 Hz. The modulus E_0 was measured by taking the slope of the best linear fit of the 10th loading cycle.

Fatigue tests were performed under load control at a frequency of 1/3 Hz (triangular waveform). Specimens were loaded from a small preload (20N or 40N) to the load corresponding to σ/E_0 equal to 0.002 or to $\Delta\sigma/E_0$ equal to 0.002. During the tests, the modulus ε^{trav} was measured and the E_{sec} was calculated using the equation 1.

$$E_{\text{sec}}(c) = \frac{\sigma_{\max}(c) - \sigma_{\min}(c)}{\varepsilon_{\max}(c) - \varepsilon_{res}(c)} \quad (1)$$

The strains measurement was based on the displacement of the cross-head.

Small slices of bone were extracted and prepared for SEM analysis. The slices were placed in a mixture of *Résine Mecaprex MA2* (04008) and *Durisseur pour résine Mecaprex MA2* (Presi SA. Tavernolles, 38320 Brie & Angonnes, France) in a 100:12 ratio. When the resin dried, the samples were polished to expose the trabecular bone. For this purpose, five different granulometries were used. The granulometries are designated by 240, 320, 600, 800 and 1000. For each granulometry, samples were polished with variations of 90 degrees, until reaching a smooth and uniform surface. As the bone isn't a conductor, a thin layer of gold was deposited in their surface before the examination in a scanning electron microscope (Hitachi S-2400). The images were obtained at 25 keV accelerating voltage with secondary electrons and with a magnification of 20x. The analysis was preformed in 23 non-tested samples and 10 tested samples.

The maximum possible number of images was taken for each sample with the purpose of covering the largest surface area. The images were analysed with the software Image J. The measured parameters were: percentage of trabecular area, inter-trabecular distance and trabecular thickness. For the measurement of the trabecular area, only the trabecular bone that was on the first plan of the image was included. To acquire inter-trabecular distance, it was necessary three measurements in vertical and horizontal directions in all the inter-trabecular spaces. The trabecular thickness was obtained through three perpendicular measurements (to each trabecula) in each strut.

Results

Fatigue Tests

Figure 1 presents the 10 cycles of the preconditioned stage. Table 1 shows the resume of the age of the patients, the diameter specimens and the modulus E_0. There are no significant differences between the E_0 of the two groups.

The stress-strain curve obtained during the tests is confuse so, as represented in figure 2, the choice was to represent only some cycles (number 1, 200, 400, 600, 800 and 1000). The evolution of $\varepsilon_{\max}$, ε_{res} and E_{sec}/E_0 with the number of cycles (figure 3) occurred as expected. It is clear that there is a fast increase of the strains in the first cycles and it is suggested that the strain saturation is

Table 1 – Age of the patients, diameter specimens, E_0 and comparison between groups

Group	Number of samples	Age (years)	Specimen's height (mm)	E_0^{trav} (GPa)
M	11	85,45 ± 4,03	27,74 ± 4,42	171,37 ± 56,86
F	14	80 ± 7,71	25,69 ± 3,42	179,29 ± 83,87
p-value	-	0,045 *	0,204 *	0,791 *

* t-student test

** Mann-Whitney test

Table 2 – Means of ε_{max}^{trav}, ε_{res}^{trav}, E_{sec}^{trav} e $E_{sec}^{trav}/E_0^{trav}$ at the 1000th cycle and comparison between groups

Group	ε_{max}^{trav} (%)	ε_{res}^{trav} (%)	E_{sec}^{trav} (GPa)	$E_{sec}^{trav}/E_0^{trav}$
M	0,272 ± 0,122	0,188 ± 0,126	214,46 ± 82,6	1,219 ± 0,223
F	0,203 ± 0,061	0,11 ± 0,076	189,8 ± 94,22	1,042 ± 0,111
p-value	0,075 **	0,075 **	0,5 *	0,016 *

* t-student test

** Mann-Whitney test

achieved with constant rate of strain accumulation.

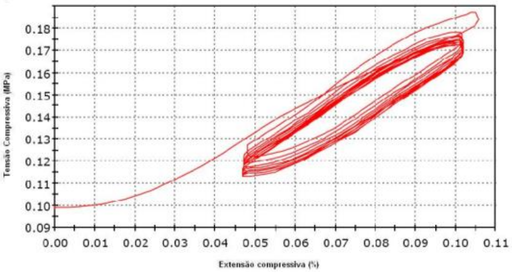

Figure 1 –Example of stress-strain curve of the preconditioning stage (group M)

Only the characteristics of the last cycle were used to compare the two genders. Table 2 resumes the observations at the last cycle and shows the results of the comparison. The only parameter where a significant difference is observed is the E_{sec}/E_0.

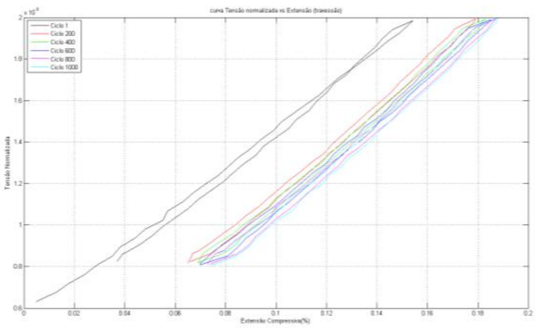

Figure 2 – Example of σ/E_0 vs. ε curve (group F).

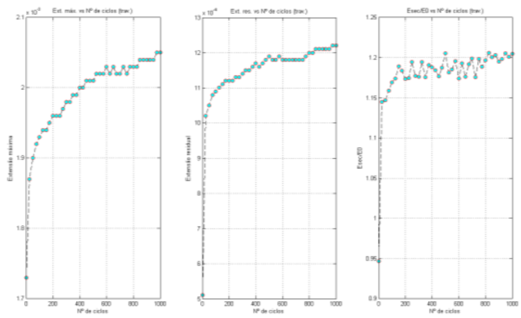

Figure 3 – Evolution of ε_{max}, ε_{res} and E_{sec}/E_0 with the number of cycles (group M).

Structural analysis

Figure 4 is an image of a non-tested sample. Both non-tested (table 3) and tested samples didn't show significant differences between genders. A comparison between the non-tested and the tested samples showed no significant differences (table 4). The qualitative analysis of tested images indicates no evidence of damage.

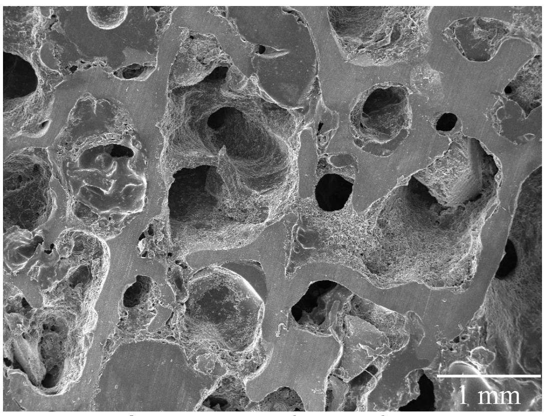

Figure 4 – SEM image of group M.

Table 3 – Trabecular area, inter-trabecular distance and trabecular thickness of non tested samples

Group	Age (years)	Trabecular area (%)	Inter-trabecular distance (µm)	Trabecular thickness (µm)
M	85,70 ± 4,17	25,05 ± 6,02	715,326 ± 114,676	199,964 ± 46,189
F	80,31 ± 7,93	21,49 ± 3,97	791,901 ± 160,154	174,115 ± 25,624
p-value	0,065 *	0,102 *	0,215 *	0,131 **

* t-student test
** Mann-Whitney test

Table 4 – Means of ε_{max}^{trav}, ε_{res}^{trav}, E_{sec}^{trav} e $E_{sec}^{trav}/E_0^{trav}$ at the 1000th cycle and comparison between groups

Samples	Trabecular area (%)	Inter-trabecular distance (µm)	Trabecular thickness (µm)
Non-tested	23,58 ± 6,73	729,232 ± 155,522	182,586 ± 53,184
tested	22,93 ± 3,71	802,9 ± 196,994	178,119 ± 22,195
p-value	0,102 *	0,215 *	0,131 **

* t-student test
** Mann-Whitney test

Discussion

This study examined the fatigue properties of osteoporotic trabecular bone and the differences in the properties between genders. The observations taken from the tests are that the human trabecular bone removed from the femoral head has similar behaviour to that described in the literature. The only parameter where a significant difference exists is in the E_{sec}/E_0 at the 1000th cycle. But the mean of the value for the two groups is superior to one, making the comparison irrelevant once this value should be equal or inferior to 1. The structural analysis showed that the structure is similar in both genders.

The absence of significant differences between genders in the measured parameters suggests that the trabecular bone's fatigue behaviour is equal in male and in female samples.

The comparison between tested and non tested samples may be biased. The slices are obtained from different sources (tested samples are from the highest load direction of the bone but the non tested samples are not), making the quantitative comparison meaningless. Nonetheless, the evolution of E_{sec}/E_0 and the absence of damage evidence in the SEM analysis of the tested samples suggest that there isn't damage formation after testing the specimens in the presented conditions ($\Delta\sigma/E_0 < 0.002$).

In fact, the compliancy of the machine makes the E_0 underestimated. The consequence is a maximum load also underestimated when compared to the maximum load hat would be obtained if the real E_0 was used. But, this study wasn't preformed in a servo-hydraulic machine. On the contrary, it was preformed in an universal testing machine that is not the ideal instrument for fatigue testing. If the real E_0 was used, the loads of the cyclic compression would be too high for the machine to handle. If the loads are too high, the machine starts to shake and it is impossible to get a good stress-strain curve.

Conclusions

These results show that these tests were made under the limit ($\Delta\sigma/E_0$ = 0.0035) of damage threshold [3]. This means that, under this limit, there is no difference in the fatigue properties between genders and that, at these low loads, even osteoporotic bone has an infinite fatigue life ($N_f > 10^6$).

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