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Nonlinear ultrasound can detect accumulated damage in human bone

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Abstract

Bone micro-damage is commonly accepted as a relevant parameter for fracture risk assessment, but there is no available technique for its non-invasive characterization. The objective of this work is to study the potential of nonlinear ultrasound for damage detection in human bone. Ultrasound is particularly desirable due to its non-invasive and non-ionizing characteristics. We show results illustrating the correlation of progressive fatigue of human bone samples to their nonlinear dynamical response. In our experiments, damage was induced in 30 samples of diaphyseal human femur using fatigue cycling. At intervals in the cycling, the nonlinear response of the samples was assessed applying Nonlinear Resonant Ultrasound Spectroscopy (NRUS). The nonlinear parameter α, which in other materials correlates with the quantity of damage, dramatically increased with the number of mechanical testing cycles. We find a large spread in α in the pristine samples and infer that the spread is due to damage differences in the sample population. As damage accumulates during cycling, we find that α is much more sensitive to damage than other quantities measured, including the slope and hysteresis of the load/displacement curve, and the dynamic wavespeed. To our knowledge, this study represents the first application of the concept of nonlinear dynamic elasticity to human bone. The results are promising, suggesting the value of further work on this topic. Ultimately, the approach may have merit for *in vivo* bone damage characterization.

Keywords: Micro-damage assessment; Nonlinear ultrasound; Cortical bone; Fracture risk; Fatigue testing

1. Introduction

Fracture risk prediction is of great interest due to the fact that osteoporosis and bone fragility are increasingly widespread diseases. Fracture risk and bone fragility are commonly assessed through the measurement of parameters related to bone density applying X-ray densitometry (1993), peripheral quantitative computed tomography (Hudelmaier et al., 2004), and/or Quantitative Ultrasound (QUS) (Hans et al., 2003). These techniques measure either bone density or ultrasonic characteristics, which correlate to bone density and/or fracture risk, but show limited ability to accurately predict the observed variations in bone strength. Research in fracture risk prediction is currently directed towards the assessment of other parameters such as bone micro-architecture, material properties, and micro-damage. Among them, it is now widely accepted that micro-damage has significant consequences on bone mechanical properties (Burr et al., 1998; Zioupos, 2001; Martin, 2003).

Micro-damage in bone is induced by daily cyclic loading due to walking, lifting, etc. triggering the remodeling process specifically designed to heal micro-damage (Martin, 2003; Burr and Turner, 2003; Burr et al., 1997; O'Brien et al., 2000; O'Brien et al., 2003) Typical crack lengths are of 5–500 μm and crack density increases exponentially with age, with a significant increase corresponding to the beginning of menopause (Schaffler et al., 1995). Micro-damage has important consequences on bone mechanical properties such as toughness (Zioupos, 2001), stiffness (Schaffler et al., 1989) and strength (Burr, 2003). To date, no techniques are available for bone micro-damage assessment *in vivo*.

In comparison to X-ray absorptiometry, QUS potentially provides a multitude of variables that should provide

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the means for bone strength assessment, since ultrasound is a mechanical wave, whose propagation characteristics are determined by medium structural and material properties (Laugier, 2006). Several QUS approaches have already been developed for bone strength assessment and fracture risk clinical prediction. These techniques measure linear ultrasonic characteristics such as bone frequency-dependent attenuation or wavespeed. Despite promising developments, it is known that linear QUS mostly reflects bone mineral density, and provides limited information on bone biomechanical competence beyond that conveyed by X-ray techniques. In particular, these techniques have been shown to be insensitive to mechanically induced damage in human cancellous bone (Nicholson and Bouxsein, 2001). In this work, we present results of a novel nonlinear ultrasonic method for assessing cortical bone micro-damage. A brief background describing nonlinear dynamics is presented, followed by materials and methods, results, discussion, and conclusions.

2. Background

Nonlinear acoustical methods, known as Nonlinear Elastic Wave Spectroscopy (NEWS), are widely used in materials non-destructive evaluation, as a very sensitive tool to investigate materials mechanical damage (Abeele et al., 2000). Over the previous decade, it has been shown that a large class of materials exhibit nonlinear, non-equilibrium dynamics, related to material integrity (Guyer and Johnson, 1999). Materials in the class include earth materials—rock, sediments—granular media, some ceramics and metal alloys, and, seemingly, *all damaged solids*. The physical basis of the behavior is related to damage features (dislocations, cracks) at scales apparently ranging from 10^{-9} to 10^{-1} m. All materials in the class exhibit universal scaling relations (Johnson and Sutin, 2005). For instance, if these materials are perturbed by dynamic wave excitation, the scaling between harmonic growth with the fundamental frequency amplitude is identical. Further, under resonance conditions, the materials exhibit a universal scaling of change in resonance frequency with vibration amplitude. These materials exhibit classical Landau-type elastic scaling behavior at low excitation amplitudes (Tencate et al., 2004); however, the physical origin is *due to the mechanical damage state* rather than anharmonicity. The resulting nonlinear parameters are one or more orders of magnitude larger than intact materials. As wave amplitudes increase, this behavior transitions to a regime where hysteresis in the dynamic and quasistatic pressure–strain response becomes important, simultaneously with strained state memory. The memory is termed *slow dynamics*. The combination of fast and slow nonlinear dynamics in these materials is known as non-equilibrium, nonlinear dynamics. There are similarities to other highly nonlinear dynamical systems, such as a bubble oscillated in liquid (Lauterborn and Koch, 1987); however, the response diverges in that hysteresis in pressure–strain response and slow dynamics are exhibited. In their quasistatic behavior, these materials manifest memory by exhibiting hysteresis in stress–strain, and end-point memory (Guyer and Johnson, 1999). A sensitive relation between nonlinear response and mechanical damage has been consistent in all materials interrogated to date. Most measurements show this relation qualitatively due to the difficulty of extracting crack density from a solid, especially if the cracks are distributed. Some studies show a quantitative relation. For instance, progressive tension fatigue studies of a single crack in "dogbone"-shaped samples of metal conducted by us show direct correlation between crack length and nonlinear response.

A preliminary study suggested a qualitative correlation of increasing nonlinear dynamic response to increasing damage in bovine samples, manifested by a change in resonance frequency with increasing vibration amplitude, while linear ultrasound speed measurements remained insensitive to accumulated damage (Muller et al., 2005, 2006). That work and the other works in numerous non-biological samples, are the basis of this study.

The nonlinear dynamic response is exploited in this study to infer human bone integrity at different damage steps induced by fatigue cycling. We extract the nonlinear parameter α reflecting damage intensity, from the change in resonance frequency as a function of wave amplitude, by employing Nonlinear Resonant Ultrasound Spectroscopy (NRUS) (Abeele et al., 2000).

3. Materials and methods

3.1. Samples

The mid-distal parts of 30 fresh human femurs (11 female and 19 male donors, of mean age 81 years, SD = 13 years, range 47–100 years) were assessed in this study. Diaphyseal samples were 6 cm long. Soft tissue was removed and the specimens were kept frozen at $-20\,^{\circ}$C before measurement sessions. Specimens were measured at room temperature, and kept hydrated during experiments. Ethical approval for specimen collection was granted by the Human Ethics Committee of the Institute of Anatomy at the University René Descartes (Paris, France). The tissue donors or their legal guardians provided informed written consent to provide their tissues for investigation, in accord with legal clauses stated in the French Code of Public Health (Code de la Santé Publique Français).

3.2. Fatigue

Fatigue damage was progressively induced in the specimens applying compressive mechanical testing at 4 Hz (INSTRON 8500, Instron Corporation, Norwood, MA, USA). Samples were held in place by the force imposed from plates located at each end of the sample in the testing device. The cycling load was determined for each sample in order to induce a maximum strain amplitude of 0.5% for the samples from female donors older than 81, and 0.6% for all samples from the other donors. The age of 81 was selected as the median age for female hip fracture (Cummings and Melton, 2002; Eastell et al., 2001) and the smaller strain was used as a precaution against early failure. Samples were submitted to 10 successive cycling sessions although several samples failed before the final session completion. Cycling sessions were 15,000 cycles in duration. We note that, due to small variations in the sample surface area ends and the fact that smaller strains were applied to the female donors older than 81, the applied stress was slightly different for each sample. As we are interested in the *relative* change of linear and nonlinear parameters as a function of fatigue cycling, this does not influence our conclusions.

3.3. Biomechanical parameters

During the cycling sessions, load/displacement curves were recorded. Biomechanical parameters were extracted from the slope and hysteresis of these curves. The slope, reflecting the sample stiffness, was measured as the slope of the tangent to the load/displacement curve. The hysteresis, reflecting the energy viscoelastically dissipated during each cycle, was measured from the area contained by the upward and the downward load/displacement curves, normalized to the area below the downward curve (Koeller et al., 1986). These parameters are known to be relevant for damage characterization in that the stiffness decreases and viscous loss increases as damage increases (Zioupos et al., 1996; Carter et al., 1981).

3.4. Nonlinear resonant ultrasound spectroscopy

Before and between each cycling session, the nonlinear parameter α was measured using NRUS (Abeele et al., 2000). Samples were glued to a large piezoceramic source using phenyl salicylate. The source was attached to an 8 cm thick steel backload using epoxy, to increase the wave strain amplitude. Frequency sweeps at progressively increasing amplitude were performed on the specimens (Dynamic Resonance Systems, Inc.) in order to measure the frequency response as function of amplitude. Dynamic displacements at the surface of the sample were measured using a laser interferometer [BMI, SH120] (Fig. 1). The nonlinear parameter α was derived from the resonance frequency shift as a function of strain,

$$\frac{\Delta f}{f_0} = \frac{f - f_0}{f_0} \approx \alpha \Delta \varepsilon, \quad (1)$$

where $\Delta\varepsilon$ is the strain amplitude, f the resonance frequency, and f_0 the resonance frequency at the lowest (linear) drive level for a given resonance mode. The parameter α is a measure of the hysteresis contained in a dynamical wave oscillation. In the broad class of materials studied, Eq. (1) holds approximately true. Reproducibility (precision) measurements were conducted on three samples by repeating the NRUS measurement five times on each sample, in constant damage state. The transducer was removed and replaced between each reproducibility measurement. The error was defined as twice the standard deviation about the mean of the five values of $\alpha\cdot$ Note α is a unitless number.

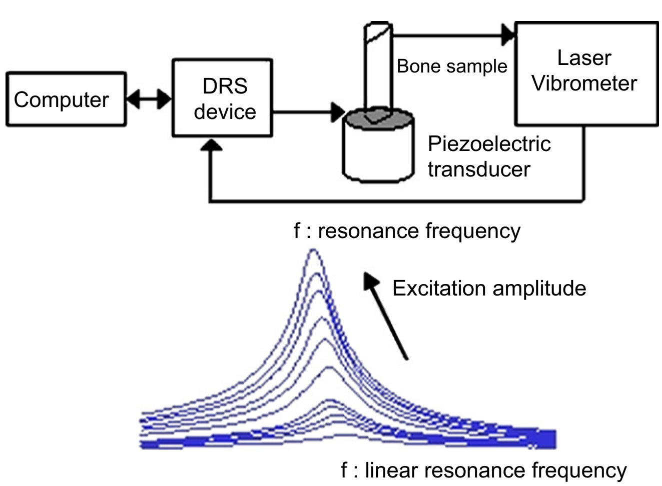

Fig. 1. Top: Nonlinear Resonant Ultrasound Spectroscopy setup. Bottom: frequency response of one sample for increasing excitation amplitudes. The resonance frequency decreases as drive amplitude increases in damaged samples.

3.5. Synchrotron micro-computed tomography

After the fatigue process and NRUS measurements were completed, the samples that produced, respectively, the largest and the smallest absolute values of nonlinear parameter α were cut for imaging ($4 \times 4 \times 8\,mm^3$). These samples were imaged applying Synchrotron radiation micro-computed tomography (SR μ-CT). The SR μ-CT experiments were performed at the ID19 beamline of the European Synchrotron Radiation Facility in Grenoble, France. The photon energy was 28 keV and voxel size was 5.3 μm.

3.6. Histological measurements

Post-cycling, bone samples were stained with xylenol orange, a fluorescent chelating agent selectively labeling bone micro-damage by binding calcium. The samples were immersed in a 5×10^{-4} M solution for 8 h, in a vacuum dessicator. They were then rinsed using de-ionized water and cut into slices using a micrometric water-cooled diamond-tipped saw (Isomet® 4000, Buehler Ltd.). Microscope observation of xylenol orange fluorescence allowed a histological observation of micro-damage.

4. Results

Among the 30 samples tested, six failed before (within the first three hours of mechanical testing) and were rejected. Six other samples did not provide good reproducibility of the dynamical measurements. These samples exhibited significant curvature at the measurement point, leading to difficulties in measuring with the laser interferometer. (Note all samples were measured at the identical sample point.) Meaning, as sample curvature increases, the return beam from the laser becomes more scattered, and signal-to-noise ratio increases, making sample placement a serious issue. It was determined prudent to eliminate this group. The results presented here are those obtained from the remaining 18 samples, sufficient to ensure the statistical validity of the study.

Fig. 2a, b shows the raw and normalized evolution of α with damage cycle for all samples. Significant scatter was observed in the initial, pre-cycling values of the nonlinear parameter α. For all samples, α increased significantly with the number of mechanical testing cycles. For clarity, error bars were not added on each data point, but their value is $\delta\alpha = 200$. Fig. 2c compares α to the measured (linear) velocity and slope of the stress–strain curves. Note that the velocity is related to the dynamic stiffness [Young's velocity $C = \text{sqrt}\ (E/\rho)$, where E is the Young's modulus (or average stiffness) and ρ is the density]. The velocity is extracted from the lowest amplitude resonance peak (assumed elastically linear). The slope and hysteresis of the load/displacement curve remained essentially unchanged contrary to what one might expect from previous studies on bone and other materials (Carter et al., 1981). As an example from one sample, Fig. 2c shows that α changes significantly while the wavespeed, and the slope and hysteresis of the load/displacement, curve remain unchanged through all the fatigue sampling. Indeed, a decrease of the slope in the load/displacement curve was observed only very close to failure. On the contrary,

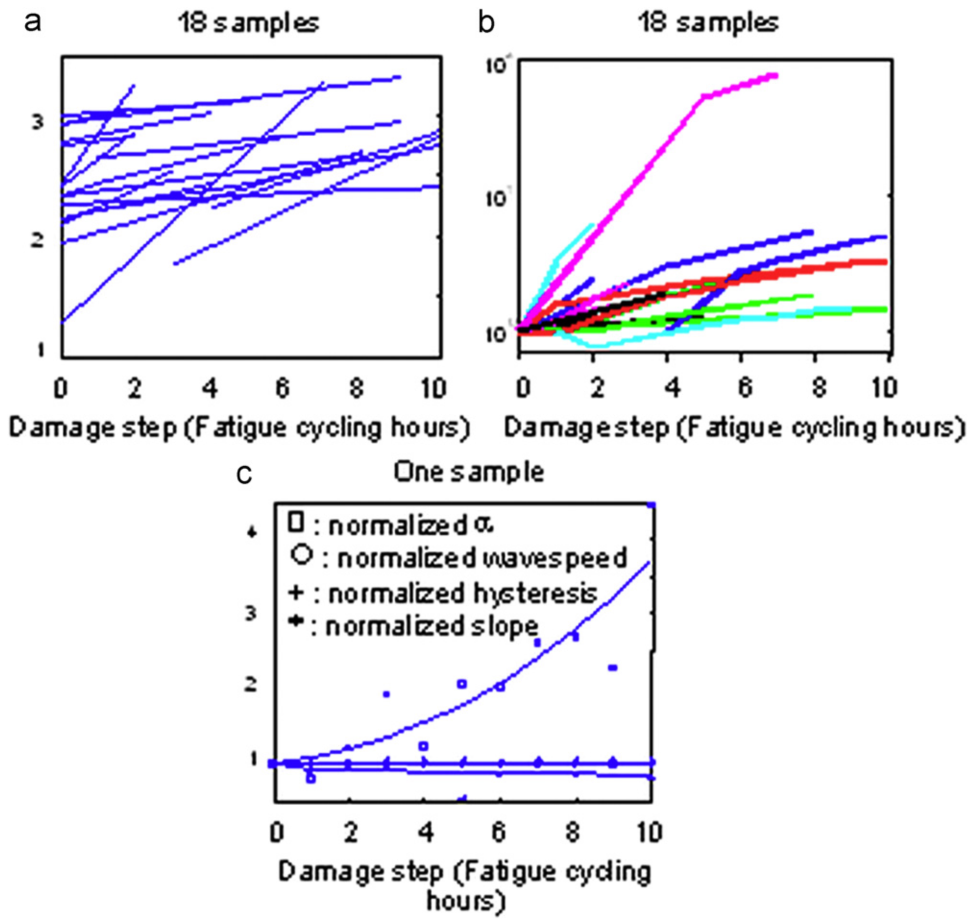

Fig. 2. Sensitivity of the nonlinear parameter α to fatigue cycling. (a) evolution of the nonlinear parameter α as a function of mechanical cycling time for all the tested samples. Note the values of α at the first damage step (before any mechanical cycling) are sample dependent; (b) Evolution of the nonlinear parameter α, normalized to its value at the first damage step. Note the response of α to given progressive damage protocol is sample dependent. The observation of Fig. 1(a, b) provides a measure of scatter in the population; (c) comparison of the evolution of the nonlinear parameter, the wavespeed, the slope and the hysteresis of the load/displacement curve for one of the samples. Note there is no change in the normalized wavespeed, and no significant change of the biomechanical parameters, when taking into account the measurement errors. On the other hand, α is extremely sensitive even very early on in the damage process.

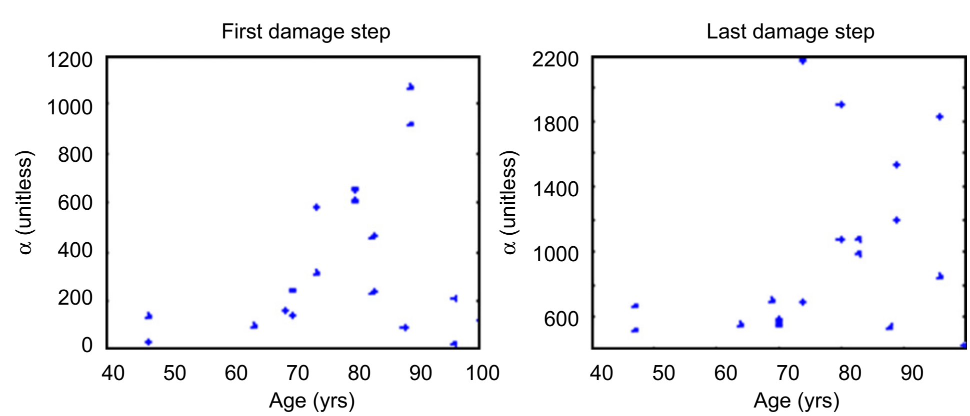

Fig. 3. Evolution of the nonlinear parameter α as a function age for all the tested samples. An exponential relationship was found between the two parameters. Note that an exponential relationship had also been found in a previous study between age and micro-crack density (Schaffler et al., 1995).

the measured α shows change immediately, after the first cycling in most cases, and changes significantly over the duration of cycling in most cases.

In Fig. 3 we show α versus age at the beginning of the experiment, before fatigue cycling commenced, and post-cycling. An approximate exponential relationship was

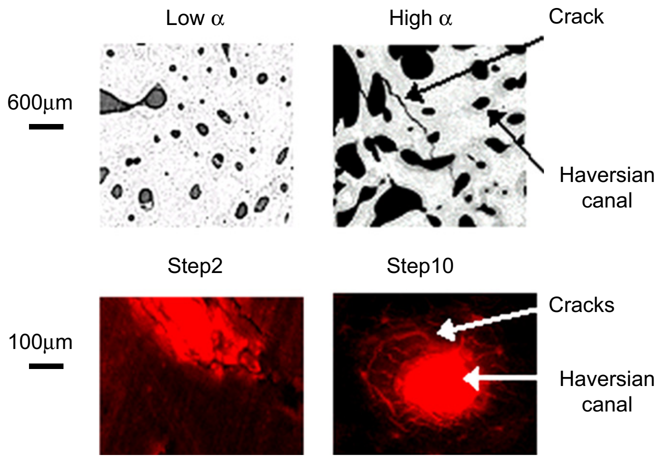

Fig. 4. Independent evaluation of damage using Synchrotron micro-computed tomography and histomorphometry. Bottom: Histological image of two samples. Top left: the sample was subjected to 2 h of fatigue cycling. Top right : the sample was subjected to 10 h of fatigue cycling. A large crack is visible (500 μm long and 30 μm large), propagating across the porous network. Top: Synchrotron image of two samples. Bottom left: sample with a relatively low α (α≅200). Bottom right: sample with a relatively high α (α≅2000). The crack density around the Haversian canal is higher in the sample that went through 10 h of fatigue cycling.

found between α and the age of the donors (caveat: other nonlinear functions could fit these data equally well, and due to the fact that we have a limited sample number for young donors, the functionality inference should be treated with caution). The scatter in α distribution was larger for older donor samples than for young donor samples, due to a real effect or due to skewed age sampling. Of significance is that the overall change in α increased after fatigue cycling.

The Synchrotron μ-CT imaging and histology results are shown in Fig. 4. Some cracks with a typical length of 500 μm could be observed in the sample exhibiting a high α (2000) after the last damage step. A sample exhibiting a smaller α (200) at the last damage step showed no sign of damage (it is presumed too small to measure). In addition, histological observation of other samples qualitatively showed greater micro-crack density in samples at later damage steps than in samples at early damage steps.

5. Discussion

The scatter observed in the nonlinear parameter distribution at damage step 0 (before fatigue cycling) is similar to the strong variability of the nonlinear parameter in some materials. Large variability occurs in rocks, for example, in which the nonlinear parameter has been widely studied, even rocks of the same type (Johnson and Sutin, 2005; Johnson et al., 2004). The scatter in the α parameter distribution ought to reflect the initial mechanical damage state rather than the sample porosity. Thinking simply, the nonlinear hysteretic behavior reflects the ability of elements in the material to open and close during excitation by an acoustic wave. Pores and voids remain rigid, and therefore do not behave as soft inclusions as cracks do (Ostrovsky and Johnson, 2001). Therefore, we assume that the measured scatter reflects the initial, relative scatter in the damage state of the "intact" samples, being an indication of the sensitivity of α to damage state, and reflects the variations in the "damage state" of the population, which would logically be large.

The fact that no significant evolution of the hysteresis in the cyclic loading measurements could be observed as the samples were progressively mechanically cycled may be surprising: it should increase, based on observations in bone and other materials. We believe that accurate hysteresis measurement was limited due to inherent noise in the cyclic loading experiment. In short, the dynamic nonlinearity is by far the most sensitive measure of progressive mechanical damage. Wavespeed, modulus, and hysteresis fail to show any change until very near to failure.

The behavior of α with donors age (Fig. 3) is difficult to interpret due to the relative number of samples for older donors. We will note, however, that the behavior of micro-damage measured directly as a function of age exhibits an exponential relationship (Schaffler et al., 1995). A larger scatter of α was observed for older ages, and values tend to be larger with age in general. This observation is consistent with the distribution of damage accumulation across ages reported by (Schaffler et al. (1995) and with the fact that the scatter of fracture occurrence is larger for an aged population than for a young population. The similarity of the behaviors of the nonlinear parameter and micro-damage accumulation as a function of age must be verified with further study.

While only qualitative and not statistically significant, the morphological analysis provided some level of reassurance. It is known that typical micro-crack lengths are in a range of 5–500 μm. Hence, the 5.3 μm voxel size available from the Synchrotron μ-CT facility did not provide the resolution necessary for smaller micro-crack detection. However, larger micro-cracks could be observed. A larger number of cracks were observed in the samples that experienced a longer fatigue cycling time. In addition, large cracks (500 μm long) were detected in samples exhibiting high values of α, while no such features could be observed in samples exhibiting lower values of α. These qualitative results could be additional indication of the fact that damage was actually induced by mechanical testing, and that increased values of α were associated to the increase of damage.

There are several limitations to this study. First, no quantitative assessment of micro-damage was achieved. Therefore, no quantitative relationship could be derived between the parameter α and the degree of damage. Furthermore, it would be of great importance to elucidate the relationships between α and micro-crack characteristics such as location, length, orientation, and surface area. Additional work needs to be done to combine nonlinear ultrasonic measurements with proper analysis techniques that would allow micro-damage quantification and damage morphology characterization. The systematic use of a high-resolution imaging modality, such as SR-μCT, with resolution capabilities of a few μm would be helpful. Another limitation is related to mechanical properties measurement. In the configuration chosen, end-artifacts occurred with the compression platens and damage accumulated preferentially at the extremities of the specimens. The specimens were not glued or embedded in a polymer due to the constraints of the NRUS measurements, performed at each step. Moreover, the experiments were conducted on diaphysis cylinders exhibiting various cross-sectional areas. The input data for the fatigue testing (e.g. minimum and maximum input strains) could therefore not be adapted to each specimen, leading to small differences in applied stresses between samples. The number of cycles until failure would have been easier to predict if calibrated specimens had been used. However, calibrated specimens of human bones would have been too small to perform the NRUS experiments in the current configuration of the setup. Other nonlinear methods may work for smaller samples and are investigated. Finally, mechanical parameter accuracy could be improved, avoiding end-artifacts and using calibrated specimens. Nonetheless, the study clearly shows that the nonlinear response is a very sensitive indicator of damage accumulation, and suggests that the method is promising.

6. Conclusion and perspectives

In this work, human femoral samples were progressively damaged using compressional mechanical testing. As damage accumulated in the samples, the nonlinear parameter α measured using NRUS increased. The nonlinear parameter turned out to be much more sensitive early on in the fatigue testing than biomechanical parameters such as the slope and hysteresis of the load/displacement curve, as well as linear soundspeed. In addition, an exponential correlation between donors age and the nonlinear parameter was found. We infer that the correlation is due to the different "damage states" of individuals. We believe that the lack of correlation with fatigue loading and other dynamic measures is due to the fact that these measures are far less sensitive to the presence of mechanical damage that the nonlinear response. To our knowledge, this study represents the first application of the concept of nonlinear dynamic elasticity applied to human bone. In particular, it demonstrates for the first time the great sensitivity of the nonlinear parameter to *early* damage in human bone. These results are promising because of the potential of the nonlinear dynamical methods to eventual application to *in vivo* bone fragility assessment. A suite of experiments for the development of an *in vivo* protocol is currently being conducted.

Conflict of interest statement

All authors disclose any financial and personal relationships with other people or organizations that could inappropriately influence their work.

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