Is It Possible to Correlate Age Related Time-Dependent Micro Mechanical Properties of Cortical Bone and Its Physico-Chemical Data?

Sebastián Jaramillo-Isaza[1](✉), Pierre-Emmanuel Mazeran[2], Karim El-Kirat[2], and Marie-Christine Ho Ba Tho[2]

[1] Antonio Nariño University, Bogotá, Colombia
sebastian.jaramillo@uan.edu.co
[2] Sorbonne Universities – University of Technology of Compiègne, Compiègne, France

Abstract. Correlational models between mechanical and physico-chemical properties are of interest for bone modeling and numerical simulations of bone growth and remodeling. Therefore, the aim of this work was to determine if it is possible to correlate mechanical and physic-chemical properties of cortical bone.

Micro-mechanical and physico-chemical data were assessed in a rat cortical bone life-span model including samples aged of 1, 4, 9, 12, 18 and 24 months old. Time-dependent mechanical properties at the micro scale including elasticity, viscous properties and hardness were determined using nanoindentation tests. Meanwhile, physico-chemical properties include tissue micro-porosity, mineral, phosphates, carbonates and collagen content were determined using specific techniques for each property. Then, both properties were correlated and the highest matched were used to propose correlational models using multivariable-linear regressions.

These initial results show good determinant and correlation coefficients in high agreement with the experimental data. These results could provide new evidences of how physico-chemical properties affect the mechanical response of bone material.

Keywords: Bone biomechanics · Nanoindentation · Physico-chemical properties · Mechanical properties · Correlations

1 Introduction

Ageing is a natural process inducing variation of bone properties and is widely investigated in literature [1–7]. However, assessments on human bone models are rarely reported mostly due to ethical issues. Therefore, animal models such as rabbit or rat models are commonly used to investigate bone structural, mechanical and physico-chemical variations. Most of those studies include the effects of diseases, dietary conditions or single physical activity [8–10]. Rat and rabbit models are useful in such

© Springer Nature Switzerland AG 2019
J. C. Figueroa-García et al. (Eds.): WEA 2019, CCIS 1052, pp. 345–356, 2019.
https://doi.org/10.1007/978-3-030-31019-6_30

investigations thanks to the availability of young specimens (with only a few days of birth) and to the possibility to include the complete life span from growth to senescence.

Mechanical properties at the micro-scale are commonly assessed using nanoindentation. Mazeran et al. [11] proposed a new four stages protocol (load-hold-unload-hold) combined with a time-dependent mechanical model allowing the calculation of the elastic, viscoelastic, plastic and viscoplastic properties from a single nanoindentation test. This method has been successfully tested on polymers [11] and cortical bone [12, 13].

In order to describe the evolution of the structural and physic-chemical properties through ageing, it is necessary to perform measurements i.e. tissue micro-porosity, mineral, phosphates, carbonates and collagen content over a longer period (from birth to death). For this aim, a previous study performed in our laboratory by Vanleene et al. [14, 15] provides the data of the micro structural and physico-chemical properties.

To have a better understanding of how mechanical and physico-chemical properties are related and how they can affect each other, different correlations can be performed. In this work, linear regressions between physico-chemical properties and mechanical properties are quantified allowing the construction of correlational models for the evolution of mechanical properties due to bone ageing from growth to senescence.

2 Materials and Methods

2.1 Bone Samples

Femoral cortical bones of male rats RJHan:WI Wistar covering ages from growth to senescence (ages 1, 4, 9, 12, 18, 24 months old) were used. All the samples were cut transversely at the proximal and distal end of the femoral diaphysis. It is important to highlight that each bone sample coming from different specimens. Physico-chemical and mechanical properties were assessed at different moments of this study. Thus, two different samples size per age was used for each assessment. The first group of six samples per age was used for physico-chemical analyses. Meanwhile a second group of five samples per age was used to determine mechanical properties.

2.2 Mechanical Properties

Mechanical properties were assessed using nanoindentation tests. The time-dependent mechanical properties have been computed according to the model proposed by Mazeran et al. [11]. This model composed of different mechanical elements (spring for elasticitic modulus (E_{elast})), two Kelvin-Voigt elements for viscoelasticity (E_{ve1} and E_{ve2}; η_{ve1} and η_{ve2}), slider for plasticity-hardness (H) and dashpot for viscoplasticity (η_{vp})) is used to fit the experimental indentation depth versus time curves. All these elements have a quadratic response (square root of the load proportional to displacement and/or displacement velocity). Then the experimental nanoindentation curves have been correctly fitted by the mechanical model.

2.3 Physico-Chemical Properties

Physical and chemical characteristics were measured using different methods that are described here below.

ESEM Images and Micro-Porosities

Micro porosities were analyzed from complete images of proximal cross sections of femurs which were first reconstructed from about 10 partial images (pixel resolution: 2 μm) using an ESEM (Environmental Scanning Electron Microscope) (Philips XL30 ESEM-FEG, Royal Philips Electronics, the Netherlands). Then, ESEM images were analyzed using QWinStandard image analysis software V2.7 (Leica Microsystems Imaging Solution Ltd., UK). Percentage of micro porosity (% porosity) was calculated from the total pore area, including canals and lacunae, divided by the total cross sectional area.

Fourier Transformed InfraRed (FTIR)

Spectroscopy analyses were performed on six samples per age group (1760- X FTIR Spectrometer, PerkinElmer Inc., MA, USA). The spectra were curve-fitted in the ν4 PO_4, ν2 CO_3 and collagen amide band domains (Galactic GRAMS software, NH, USA).

Chemical Test for Carbonates and Nitrogen

Two chemical analyses were performed on samples. Carbonates weight percentage (CO3W%) was measured on six samples per age group using a CO2 Coulometer (Coulometrics Inc., Co, USA). Protein nitrogen weight content was analyzed on three samples per age group using an Elemental Analyzer EA 1110 CHNS (Thermo Fisher Scientific Inc., MA, USA).

X-ray Diffraction

As previous analyses consumed most of sample powder, specimens were pooled in each age group. X-ray diffraction was recorded with a X-ray diffractometer (Inel CPS 120, Enraf Nonius SA, France) using Co radiation (X-ray wave length = 1789 Å). Two peaks at 30° and 45° (2θ) were identified respectively as [002] (c-axis of apatite lattice) and [310] diffraction planes of the apatite crystals.

2.4 Correlational Models

The correlation and the determination coefficients were computed for both mechanical and physico-chemical properties using the statistical analysis and graphics software SYSTAT version 2012 (SYSTAT Software Inc.). Multivariable regressions were computed in the non-normalized experimental data. These regressions were carried out using the physico-chemical properties with high correlation coefficients and could fit very well the mechanical response. In the present study, all physico-chemical variables were considered as independent even if they are strongly correlated. They are different only when multicollinearity is detected.

These regressions were performed to obtain the best fit of the experimental data and to assess the relevance of each physico-chemical property to increase or decrease the mean value of the mechanical response. The Eq. 1 represents the model used for these regressions:

$$M_{property} = Constant \times \left(1 + A \times X_{Property} + B \times Y_{property} \ldots \ldots\right) \quad (1)$$

Where, M is the mechanical property, A, B, are coefficients and X, Y are the higher correlated physico-chemical properties and the sign positive or negative indicates their effect in the mechanical response.

The model considers linear relationships between the different physico-chemical properties and the different mechanical properties. This simple model is sufficient to generate very good correlation between most of the mechanical properties.

3 Results and Discussion

3.1 Time-Dependent Mechanical Properties

The mean values and standard deviation of the mechanical properties are summarized in Table 1.

Table 1. Values of the mechanical properties computed from the nanoindentation experiments in the longitudinal direction of the rat femoral cortical bone [12] (Copyright© Reprinted with permission). Mean ± standard deviation

Age (Months)	E_{elast} (GPa)	E_{ve1} (GPa)	$\eta_{ve1} \times 10^2$ (GPa.s)	E_{ve2} (GPa)	$\eta_{ve2} \times 10^3$ (GPa.s)	H (GPa)	η_{vp} (GPa.s)
1	26.4 ± 3.4	43.2 ± 6.1	17.6 ± 3.3	79.0 ± 12.3	48.0 ± 9.9	0.70 ± 0.09	250.9 ± 28.8
4	40.7 ± 6.7	57.6 ± 16.9	19.3 ± 4.6	114.8 ± 15.8	64.0 ± 13.6	0.93 ± 0.06	334.6 ± 27.9
9	35.9 ± 3.8	75.6 ± 17.7	28.3 ± 8.2	140.1 ± 23.0	73.6 ± 12.1	0.97 ± 0.10	364.3 ± 43.0
12	39.8 ± 6.3	78.2 ± 18.4	23.6 ± 9.6	150.1 ± 25.9	57.4 ± 13.5	1.04 ± 0.12	357.6 ± 45.5
18	38.4 ± 6.8	75.1 ± 19.1	28.2 ± 8.4	150.4 ± 25.9	71,5 ± 15.1	1.06 ± 0.10	381.6 ± 35.8
24	34.6 ± 4.6	71.4 ± 15.4	22.6 ± 9.6	146.0 ± 18.6	68.6 ± 18.2	1.13 ± 0.09	408.5 ± 43.1

3.2 Physico-Chemical Properties

The values of the micro structural and physico-chemical properties are summarized in Table 2. They were reported for a similar set of samples by Vanleene et al. [14, 15].

Table 2. Variation of the physico-chemical properties of Wistar rat femoral cortical bone with age

Age (Months)	Porosity%	CO_3W%	PO_4%	Ca%	N%	Collagen%
1	8.1	4.1	20.7	42.2	4	21.4
4	3.1	4.9	18.2	39.2	3.6	19
9	3.3	6.1	17.9	39.4	3.3	17.6
12	2.6	6.1	18	39.7	3.3	18.3
18	3.6	6	18	39.3	3.3	17.8
24	4	6	18	39.3	3.3	17.4

3.3 Correlations Between the Mechanical and Physico-Chemical Properties

Mechanical properties obtained by nanoindentation tests were correlated with microstructural and physico-chemical properties. The correlational coefficients (R) are presented in Table 3.

Table 3. Simple correlation coefficient R for the mechanical and physicochemical properties of rat bone

Mechanical properties	Porosity%	CO_3W%	PO_4%	Ca%	N%	Collagen%
E_{elast}	−0.949	0.608	−0.867	−0.878	−0.703	−0.673
E_{ve1}	−0.828	0.992	−0.885	−0.780	−0.981	−0.919
η_{ve1}	−0.576	0.842	−0.682	−0.597	−0.810	−0.778
E_{ve2}	−0.842	0.981	−0.914	−0.837	−0.991	−0.948
η_{ve2}	−0.679	0.744	−0.822	−0.844	−0.794	−0.884
H	−0.799	0.900	−0.899	−0.864	−0.937	−0.940
η_{vp}	−0.781	0.903	−0.908	−0.886	−0.942	−0.974

3.4 Correlational Models

The correlational models were computed using the multivariable linear regressions method and the highest coefficients calculated in Sect. 3.3. Then, each equation was compared and analyzed to its corresponding experimental mechanical property. The equations proposed for each mechanical property are described hereafter.

Elastic Response

The equation that relies the elastic modulus to the structural and physico-chemical properties obtained from the multivariable regression is:

$$E_{elast} = 73.7 \times (1 + (0.109 \times PO_4\%) - (0.06 \times Porosity) - (0.0571 \times Ca\%))$$
$$R^2 = 0.971 \tag{2}$$

The values obtained from the experimental data and by means of the correlational equation are presented in Fig. 1.

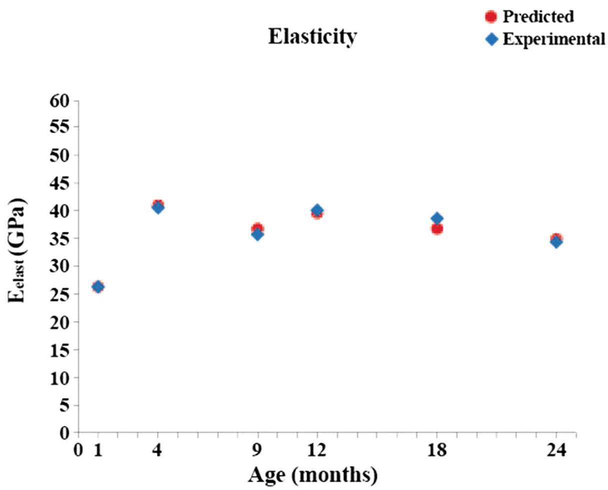

Fig. 1. Values show the elastic modulus from the experimental data "♦" and the correlational model "•" computed as a function of bone porosity, phosphate and calcium content with $R^2 = 0.971$.

Elastic modulus reacts positively to the increase of phosphate, more precisely, a decrease of the phosphate composition during the life-span leads to decrease of the elastic modulus (of about 30%) but negatively to the increase of porosity (values vary by about 33%), and calcium (variation of about 17%). According to Vanleene et al. [15] porosity decrease with age and affects the elastic response of bone. In addition, excess of calcium is associated with bone pathologies [16] and could decrease the stiffness of bones.

Globally, porosity affects the elastic response of bone but not the properties linked to permanent deformation and viscosity. The porosity measured in this study is not the genuine microporisity but an apparent one. This fact could explain why R value for the elastic response is lower than values of other correlations. However, the results obtained here are in good agreement with previous works which have reported a strong relationship between this structural characteristics and the elastic response of bone [15, 17, 18].

Viscoelasticity

For the elastic component of the two viscoelasticities, the following equations were calculated:

$$E_{ve1} = 110 \times (1 - (0.5 \times N\%) + (0.0661 \times CO_3W\%) + (0.0524 \times Collagen\%))$$
$$R^2 = 0.997 \quad (3)$$

$$E_{ve2} = 1278 \times (1 - (0.31 * N\%) - (0.0471 \times CO_3W\%) + (0.0148 \times PO_4\%) + (0.00876 \times Collagen\%))$$
$$R^2 = 0.999 \quad (4)$$

The values obtained from the experimental data and by means of the correlational equations are presented in Fig. 2.

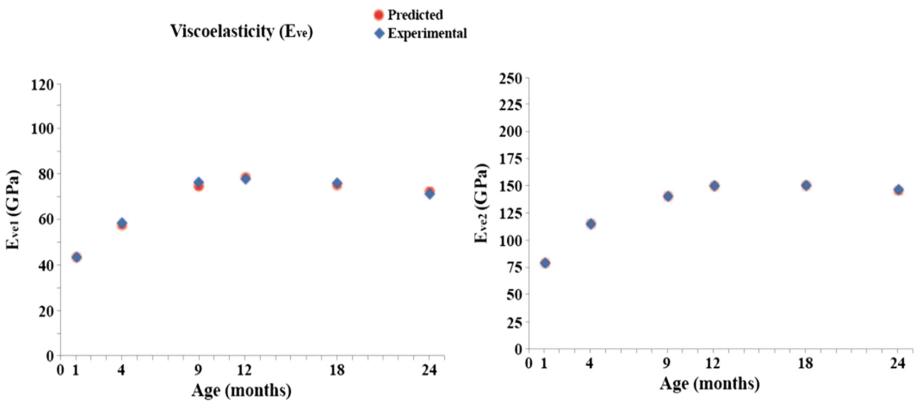

Fig. 2. Values show the elastic component of the viscoelastic response from the experimental data "♦" and the correlational model "•" computed for the elastic component E_{ve1} with bone carbonates, nitrogen, collagen and phosphates with $R^2 = 0.997$. and E_{ve2} with bone nitrogen, carbonates and collagen with $R^2 = 0.999$.

These results suggest that physico-chemical properties have not the same effects in the two elastic components. According to the correlational equation, the elastic component E_{ve1} reacts positively to the increase of carbonates (variation about 13%) and collagen (21%) but negatively to the increase of nitrogen (35%). Meanwhile the second elastic component reacts positively to the increase phosphate (variation close to 4%) and collagen (about 4%) but it is affected by the increase of nitrogen (22%) and carbonates (10%). Taking into account the information proposed by the correlational equations, nitrogen content is a critical parameter that affects the elastic response of viscoelasticity. According to Jarvis et al. [19], nitrogen content present in human bone decreases with burial age and acts as an inhibitor of decomposition during the initial period of interment.

For the viscous components of viscoelasticity, the determination coefficients were low for all the physico chemical properties. Nevertheless, the following equations were computed with those that are lightly correlated and are able to reproduce the chaotic aspect of the curve:

$$\eta_{ve1} = -10236 \times (1 + (0.267 \times N\%) + (0.107 \times CO_3W\%) - (0.0156 \times Collagen\%))$$
$$R^2 = 0.740 \tag{5}$$

$$\eta_{ve2} = 420 \times (1 + (0.0412 * P0_4\%) - (0.0307 \times Ca\%) - (0.0205 \times Collagen\%))$$
$$R^2 = 0.874 \tag{6}$$

The values obtained from the experimental data and by means of the correlational equations are presented in Fig. 3.

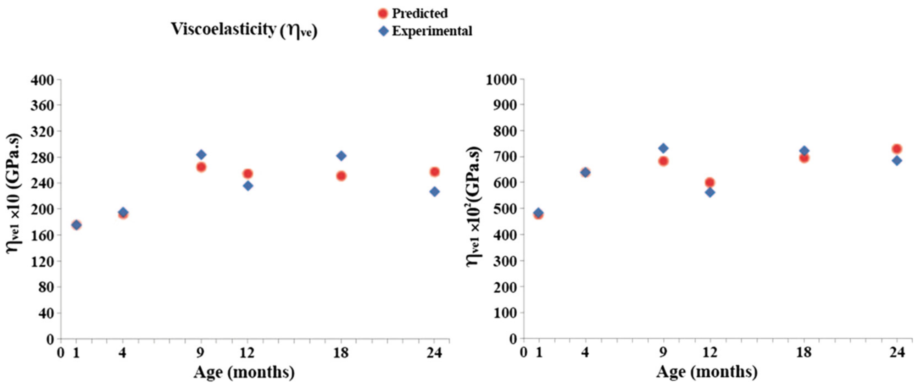

Fig. 3. Values show the viscous component of the viscoelastic response from the experimental data "♦" and the correlational model "•" computed by multivariable regression analyses: for the viscous component η_{ve1} bone nitrogen, carbonates and collagen with R^2 = 0.740. For the second viscous component η_{ve2} bone phosphate, calcium and collagen with R^2 = 0.874.

According to these results, the first viscous component, η_{ve1} of viscoelasticity reacts positively to the increase of nitrogen (19%) and carbonates (21%) and negatively to collagen (6%). Meanwhile the second viscous component η_{ve2} reacts positively to the increase of phosphate (12%) and negatively to the increase of calcium (9%) and collagen (8%).

Hardness
For hardness of bone material, the following equation was computed:

$$H = 18.3 \times (1 - (0.337 * N\%) - (0.0902 \times CO_3W\%) + (0.0201 \times Ca\%) - (0.00426 \times Collagen\%))$$
$$R^2 = 0.991 \tag{7}$$

The values obtained from the experimental data and by means of the correlational equation are presented in Fig. 4.

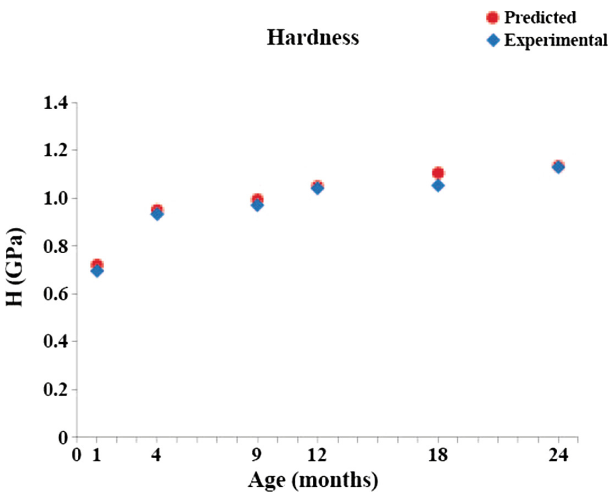

Fig. 4. Hardness response from the experimental data "♦" and the correlational model "•" computed as a function of bone carbonates, nitrogen, calcium and collagen with R^2 = 0.991.

Hardness is related to bone strength. According to our results, this property reacts positively to the increase of calcium (6%). This calcium is an essential mineral involved in bone mass and structure. Meanwhile the increase of nitrogen (24%), carbonates (18%) and collagen (2%) seem to have a negative effect in bone toughness.

Viscoplasticity
The viscoplastic response of bone could be described the following equation as:

$$\eta_{vp} = 4810 \times (1 - 0.303 * N\% - 0.0869 \times CO_3W\% + 0.0203 \times Ca\% - 0.0111 \times Collagen\%)$$

$$R^2 = 0.999 \tag{8}$$

The values for Viscoplasticiy (η_{vp}) obtained from the experimental data and by means of the correlational equation are presented in Fig. 5.

Viscoplasticity is related to the evolution of permanent deformation of the bone structure when a constant stress is hold during a given time. According to our results, this property reacts positively to the increase of calcium (6%); meanwhile it is affected negatively by the increase of nitrogen (21%), carbonates (17%) and collagen (4%).

It could be notice that hardness and viscoplasticity are correlated to the same properties (CO3W%, PO4%, nitrogen% and collagen %) and they are poorly correlated to porosity. The organic components of bone such as collagen fibrils take part in the elastic recovery of bone. This could justify why the increase of collagen can negatively affect the viscoplastic response of bone. It is clear from Eqs. (7) and (8) that the evolution of hardness and viscoplasticity obeys to the similar laws suggesting that these two properties are intrinsically dependent.

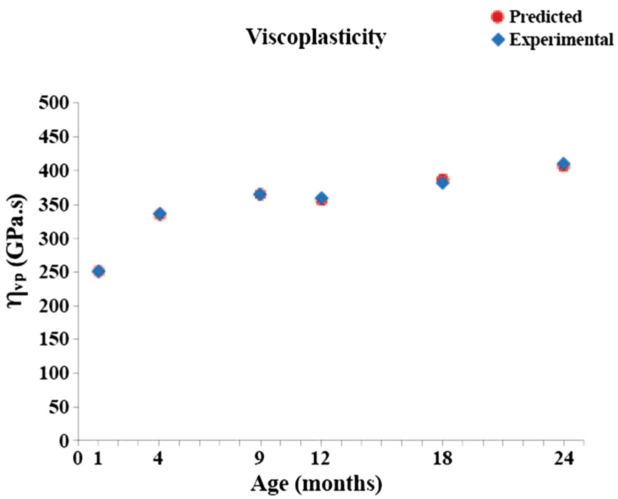

Fig. 5. Visco-plastic response from the experimental data "♦" and the correlational model "•" computed as a function of bone carbonates, nitrogen, calcium and collagen with R^2 = 0.999.

Table 4 summarizes the influence of the physical properties on the different mechanical properties. It appears from the analysis of the data that the different behaviors are sensitive to different physical properties. Indeed, Elastic modulus is mainly sensitive to porosity, phosphate and calcium, whereas plasticity and visco-plasticity are mainly sensitive to carbonate, nitrogen and calcium. Considering the visco-elastic behaviors, the two elastic components are mainly sensitive to carbonates, nitrogen and collagen composition. Nevertheless, the influence is different: an increase of carbonates percentage generates an increase of the first elastic component, whereas it generates a decrease of the second elastic component. Plasticity and viscoplasticity have very similar behavior and could be considered as similar.

Table 4. Influence of the physical properties on the different mechanical properties.

Mechanical properties	Porosity	$CO_3W\%$	$PO_4\%$	Ca%	N%	Collagen%
E_{elast}	−33%		31%	−17%		
E_{ve1}		13%			−35%	21%
η_{ve1}		11%			27%	−2%
E_{ve2}		−9%	4%		−22%	4%
η_{ve2}			12%	−9%		−8%
H		−18%		6%	−24%	−2%
η_{vp}		−17%		6%	−21%	−4%

The selected correlational variables were chose on the fact that they provide a good R^2 coefficient. This does not mean that other physico-chemical variables cannot affect the mechanical properties of bone. In fact, even if the selection of the variables was extensive, there is always the probability of new variables that have not been considered or even defined yet as being critical to the outcome. Nevertheless, this information could be useful to understand how and which mechanical properties of bone are affected by the variation of some physico-chemical properties. Most of the mechanical properties were negatively correlated with the compositional properties. The Pearson's correlation show that all the high correlated physico-chemical properties have significant statistical level ($p < 0.05$).

Finally, it is important to notice that physico-chemical properties vary with aging, the correlational model proposed in this work does not predicts the variation of the mechanical properties with aging but the value of mechanical properties for specific physico-chemical conditions.

4 Conclusions

The correlational method used in this study allows one to identify the influence of dietary or metabolic factors, diseases or other variables in the structural and material properties of bone. This information could be useful to better understand the variation of the mechanical properties of cortical bone with aging.

Lineal regressions are a simple way to determine connections among the data. However, if more information, for example new physicochemical data, is added to the equations proposed here, those equations could change. This is why, if others computational methods or data processing techniques are used, they could be providing more information about how these properties are linked.

References

1. Currey, J.D., Brear, K., Zioupos, P.: The effects of ageing and changes in mineral content in degrading the toughness of human femora. J. Biomech. **29**, 257–260 (1996)
2. Bailey, A.J., Paul, R.G.: The mechanisms and consequences of the maturation and ageing of collagen. Proc. Indian Acad. Sci. Chem. Sci. **111**, 57–69 (1999)
3. Mosekilde, L.: Age-related changes in bone mass, structure, and strength–effects of loading. Z. Rheumatol. **59**(1), 1–9 (2000)
4. Danielsen, C.C., Mosekilde, L., Svenstrup, B.: Cortical bone mass, composition, and mechanical properties in female rats in relation to age, long-term ovariectomy, and estrogen substitution. Calcif. Tissue Int. **52**, 26–33 (1993)
5. Fukuda, S., Iida, H.: Age-related changes in bone mineral density, cross-sectional area and the strength of long bones in the hind limbs and first lumbar vertebra in female Wistar rats. J. Vet. Med. Sci. **66**, 755–760 (2004)
6. Willinghamm, M.D., Brodt, M.D., Lee, K.L., Stephens, A.L., Ye, J., Silva, M.J.: Age-related changes in bone structure and strength in female and male BALB/c mice. Calcif. Tissue Int. **86**, 470–483 (2010)
7. Burket, J., Gourion-Arsiquaud, S., Havill, L.M., Baker, S.P., Boskey, A.L., van der Meulen, M.C.H.: Microstructure and nanomechanical properties in osteons relate to tissue and animal age. J. Biomech. **44**, 277–284 (2011)

8. Indrekvam, K., Husby, O.S., Gjerdet, N.R., Engester, L.B., Langeland, N.: Age-dependent mechanical properties of rat femur. Measured in vivo and in vitro. Acta Orthop. Scand. **62**, 248–252 (1991)
9. Akkus, O., Adar, F., Schaffler, M.B.: Age-related changes in physicochemical properties of mineral crystals are related to impaired mechanical function of cortical bone. Bone. **34**, 443–453 (2004)
10. Isaksson, H., Malkiewicz, M., Nowak, R., Helminen, H.J., Jurvelin, J.S.: Rabbit cortical bone tissue increases its elastic stiffness but becomes less viscoelastic with age. Bone **47**, 1030–1038 (2010)
11. Mazeran, P.-E., Beyaoui, M., Bigerelle, M., Guigon, M.: Determination of mechanical properties by nanoindentation in the case of viscous materials. Int. J. Mater. Res. **103**, 715–722 (2012)
12. Jaramillo Isaza, S., Mazeran, P.-E., El Kirat, K., Ho Ba Tho, M.-C.: Time-dependent mechanical properties of rat femoral cortical bone by nanoindentation: an age-related study. J. Mater. Res. **29**, 1135–1143 (2014)
13. Jaramillo Isaza, S., Mazeran, P.-E., El-Kirat, K., Ho Ba Tho, M.-C.: Heterogeneity of time-dependent mechanical properties of human cortical bone at the micro scale. J. Musculoskelet. Res. **18**, 1550017 (2015)
14. Vanleene, M., Mazeran, P.-E., Ho Ba Tho, M.-C.: Influence of strain rate on the mechanical behavior of cortical bone interstitial lamellae at the micrometer scale. J. Mater. Res. **21**, 2093–2097 (2006)
15. Vanleene, M., Rey, C., Ho Ba Tho, M.C.: Relationships between density and Young's modulus with microporosity and physico-chemical properties of Wistar rat cortical bone from growth to senescence. Med. Eng. Phys. **30**, 1049–1056 (2008)
16. Dent, C.E., Smellie, J.M., Watson, L.: Studies in osteopetrosis. Arch. Dis. Child. **40**, 7–15 (1965)
17. Currey, J.D.: The mechanical consequences of variation in the mineral content of bone. J. Biomech. **2**, 1–11 (1969)
18. Ho Ba Tho, M.-C., Mazeran, P.-E., El-Kirat, K., Bensamoun, S.: Multiscale characterization of human cortical bone. Comput. Model. Eng. Sci. **87**, 557–577 (2012)
19. Jarvis, D.R.: Nitrogen levels in long bones from coffin burials interred for periods of 26–90 years. Forensic Sci. Int. **85**, 199–208 (1997)