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EFFECT OF BONE COMPOSITION AND APPARENT DENSITY ON LOCATIONAL VARIATION IN ELASTIC AND PLASTIC MODULI OF CORTICAL BONE

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ABSTRACT

This study is focused on the locational variation in deformational behaviour of cortical bone at different locations of cortical bone diaphysis during elastic as well as plastic deformation. The elastic and plastic moduli of cortical bone are estimated with the help of uniaxial tensile testing in longitudinal direction and correlated with bone composition and density. The elastic and plastic moduli were found to be significantly correlated with different compositional parameters. The study shows that the variations in apparent density, percentage of minerals, organics and water content along the bone diaphysis are responsible for locational variation in elastic as well as plastic deformational behaviour of cortical bone.

Keywords: cortical bone, bone diaphysis, elastic modulus, plastic modulus.

INTRODUCTION

Bone is a highly complex and hierarchical composite material made of assemblies of collagenous protein molecules, water and carbonated hydroxyapatite minerals. Bone tissue is also considered as a heterogeneous material due to local variation in its compositional parameters such as porosity, density, minerals etc (Biltz, 1969; Ingram, 1993). This is evident from various studies that the variation in compositional parameters may lead to variation in mechanical properties of cortical bone (Carter, 1976; Currey, 1969, 1975, 1996; Dong, 2004; Hasegawa, 1994; Keller, 1994; Saha, 1977; Sharma, 2012). The relationship between variation in compositional parameters and mechanical properties is useful for the design of orthopedic implants and study of adoptive bone remodeling. Since 1951, various investigations have been conducted to analyze the mechanical heterogeneity in cortical bone and most of them were focused on elastic inhomogeneity in bone material (Amtmann, 1968; Evans, 1951; Hernandez, 2001; Martin, 1998; Orias, 2009; Orr, 1990; Reilly, 1974). However, in order to understand the overall deformation behavior of bone material, it is essential to investigate the elastic as well as plastic inhomogeneity in bone material and role of compositional parameters to this inhomogeneity as bone is supposed to have lot of toughening mechanisms and non-linearity during deformation (Courtney, 1996; Fondrk, 1999; Sharma, 2011; Yan, 2007). In the present study the overall deformation behavior of bone material was examined with the help of elastic and plastic moduli with an objective to investigate the locational variation in elastic, plastic stiffness, hardening rate and modulus degradation during post-yield deformation along the cortical bone diaphysis. The apparent density and compositional parameters were further evaluated for the corresponding locations of bone diaphysis in order to analyze the influence of these parameters on elastic and plastic inhomogeneity in cortical bone.

MATERIALS AND METHOD

The present study has been conducted in the tibiae cortical bones obtained from young bovine of age about 36 months. After removal of bone tissue from the body the surrounding soft tissue was removed and bone tissue was wrapped in gauze, soaked in normal saline, wrapped with plastic wrap and placed in sealed, airtight plastic bags. These plastic bags were placed in freezer and stored at -20°C within 1 hr after the bone tissues had been harvested. The bones were kept hydrated in saline upon removal from the freezer and during all stages of tissue preparation. For specimen preparation the epiphyses ends of the long bone were removed using vertical band saw leaving only the diaphysis section. The round cylindrical edges of the diaphysis were flattened into flat rectangular prismatic edges with the help of a belt sander. After flattening, the whole diaphysis of the cortical bone was sectioned into three equal segments namely; upper, middle and lower parts of the bone diaphysis. Different anatomic quadrants (A = anterior, M = medial, P = posterior and L = lateral) of bone diaphysis were identified and marked accordingly on each one third segment of the diaphysis. Each segment of the diaphysis was then subsequently sectioned into number of specimens according to different anatomic quadrants. The preparation of specimens from different anatomic locations of the bone diaphysis is shown in Fig. 1. In all 15 dumbbell shape stripe type longitudinal tensile specimens were prepared from different locations (upper, middle and lower bone diaphysis) of bovine tibiae cortical bone with thickness 2.5 mm, gauge length 25 mm, gauge width 4 mm and total length 80 mm. All these specimens were stored at room temperature in a solution of 50% saline and 50% ethanol at all time until testing. In order to keep the specimens wet and to avoid heating during cutting and polishing a constant spray of water was supplied. The uniaxial tensile tests were performed on MTS 858 Table Top Universal Testing Machine and a miniature extensometer of gauge length 5 mm was used to measure strain during testing. These tensile tests were performed at a low displacement rate of 1.8 mm/min.

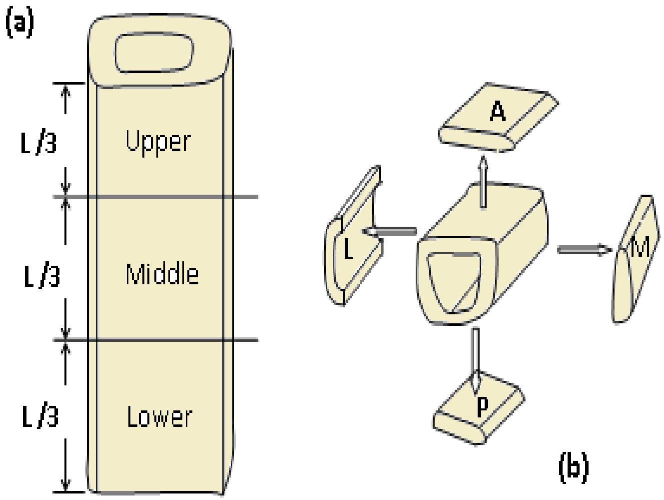


Fig.1 Schematic diagram showing sectioning of cortical bone diaphysis for preparation of tensile test specimens from different anatomic locations of the bone diaphysis (a) The flattened cortical bone diaphysis was sectioned into three equal length (L/3) segments. (b) Each segment of the diaphysis was subsequently sectioned into four parts according to different anatomic quadrants (A = anterior, M = medial, P = posterior, and L = lateral) for further preparation of samples.

The stress-strain curves in case of uniaxial tensile test for longitudinal specimens obtained from different locations of the bone diaphysis are shown in Fig. 2. The yield strength values were obtained corresponding to 0.2% permanent set. The values of plastic modulus for different locations of bone diaphysis were calculated using linear curve fitting of true stress vs plastic strain curve as shown in Fig. 3.

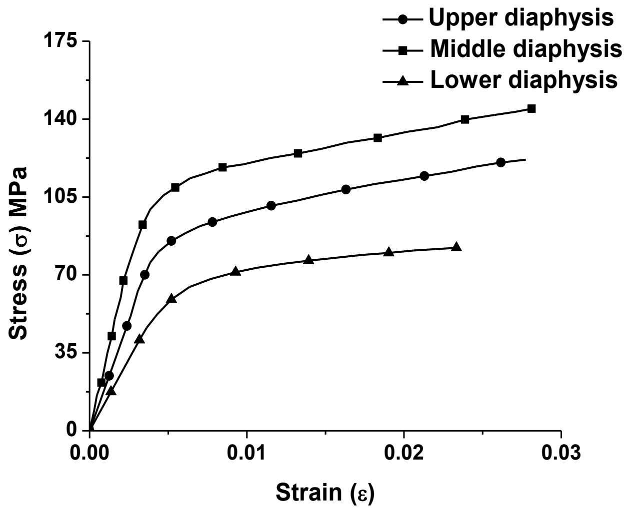


Fig.2 Stress-strain curve for different locations of bone diaphysis

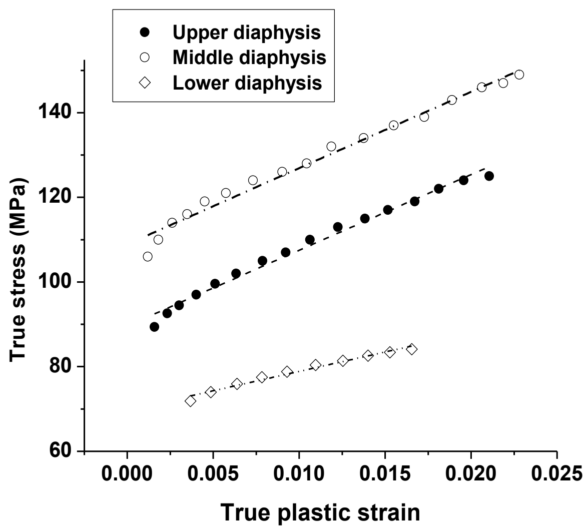


Fig.3 Linear regression fitting of the true stress (σ^t) vs true plastic strain (ε^t_{pl}) curves for the evaluation of plastic modulus (H) for upper ($\sigma^t = 1786.35\ \varepsilon^t_{pl} + 89.64$, $r^2 = 0.99$ and $p < 1.14 \times 10^{-14}$), middle ($\sigma^t = 1801.10\ \varepsilon^t_{pl} + 108.89$, $r^2 = 0.98$ and $p < 1.13 \times 10^{-14}$) and lower ($\sigma^t = 916.30\ \varepsilon^t_{pl} + 69.71$, $r^2 = 0.98$ and $p < 6.60 \times 10^{-8}$) diaphysis of cortical bone

The values of tangent modulus (E_t) were calculated using equation (1), whereas percentage of modulus degradation (% E_d) was calculated using equation (2) as given below;

$$E_t = \frac{E.H}{E+H} \qquad (1)$$

$$\%E_d = 100X\left(\frac{E - E_t}{E}\right) \tag{2}$$

To analyze the apparent density and composition of cortical bone at different anatomic locations of bone diaphysis, rectangular samples were cut from different test specimens using diamond cutter (Isomet 4000). From each tested specimen, three samples were randomly selected and analyzed to obtain the values of bone composition parameters and apparent density for that particular specimen. The dimensions of these samples were measured using a digital caliper to calculate their volumes. The samples were hydrated overnight and after weighing the samples the wet weight was recorded. After measuring the wet weight these specimens were placed in acetone for overnight and then placed in an oven at 60° C for 24 hrs along with silicate gel to remove the remaining moisture. These samples were then weighed to measure the dry weight. The samples were then placed in a furnace at 600° C for 24 hrs. After removing the samples from the furnace, they were placed in the desiccator to reach the room temperature and finally the weight of residue i.e ash was recorded. For these samples different compositional parameters were calculated using the following equations (Yeni, 1998);

Wet density, $$\rho_w = \frac{\text{Wet weight}}{\text{Volume}} \tag{3}$$

Dry density, $$\rho_d = \frac{\text{Dry weight}}{\text{Volume}} \tag{4}$$

% Mineral, % Min = $$\frac{\text{Ash weight}}{\text{Dry weight}} \times 100 \tag{5}$$

% Organic (dry), % Org_d $$= \frac{\text{Dry weight} - \text{Ash weight}}{\text{Dry weight}} \times 100 \tag{6}$$

% Ash = $$\frac{\text{Ash weight}}{\text{Wet weight}} \times 100 \tag{7}$$

% Organic (wet), % Org_w $$= \frac{\text{Dry weight} - \text{Ash weight}}{\text{Wet weight}} \times 100 \tag{8}$$

% H_2O = $$\frac{\text{Wet weight} - \text{Dry weight}}{\text{Wet weight}} \times 100 \tag{9}$$

In equation (7), % Ash is normalized with wet weight which can be referred as apparent mineral content, whereas as per equation (5), % Mineral can be referred to as material mineral content and is independent of porosity. The same concept is applied for % Org_d and % Org_w.

RESULTS

The values of elastic and plastic moduli along with the yield strength, tangent modulus and percentage of modulus degradation for different locations of bone diaphysis are reported in Table 1, whereas the corresponding values of compositional parameters and densities are reported in Table 2. A paired t-test analysis was conducted to compare the results obtained for different locations of bone diaphysis. Analysis of variance (ANOVA) test was also conducted to analyze the variation in mean values of compositional parameters and densities for different diaphysis locations.

Table 1 Values of elastic modulus (E), yield strength (σ_{ys}), plastic modulus (H), tangent modulus (E_t) and % of modulus degradation ($\% E_{deg}$) for upper, middle and lower bone diaphysis

	Upper	**Middle**	**Lower**
***E* (GPa)**	19.8 ± 1.35	30.3 ± 1.33	12.9 ± 0.72
σ_{ys} (MPa)	90.1 ± 3.05	111.0 ± 7.05	67.0 ± 4.52
***H* (MPa)**	1786.4 ± 80.25	1801.1 ± 22.34	916.3 ± 29.27
E_t (MPa)	1637.8 ± 68.47	1699.9 ± 20.69	855.4 ± 27.04
$\% E_{deg}$	91.70 ± 0.58	94.38 ± 0.23	93.35 ± 0.32

Table 2 Values of compositional parameters and density for upper, middle and lower bone diaphysis

	Upper	**Middle**	**Lower**	**ANOVA**
ρ_{wet} (g/cm^3)	2.09 ± 0.01^b	2.19 ± 0.04^a	2.05 ± 0.05^b	$p < 0.05$
P_{dry} (g/cm^3)	1.89 ± 0.02^b	2.01 ± 0.02^a	1.86 ± 0.05^b	$p < 0.05$
%Ash	63.63 ± 0.40^b	64.87 ± 0.70^a	62.28 ± 0.66^{a,b}	$p < 0.05$
% Min	70.38 ± 0.17	70.63 ± 0.76	68.83 ± 0.30^{a,b}	$p < 0.05$
$\% Org_w$	26.77 ± 0.18	26.98 ± 0.77	28.20 ± 0.33^{a,b}	$p < 0.05$
$\% Org_d$	29.61 ± 0.17	29.37 ± 0.76	31.16 ± 0.30^{a,b}	$p < 0.05$
$\% H_2O$	9.59 ± 0.48^b	8.14 ± 0.61^a	9.52 ± 0.80^b	$p < 0.05$

[a]Indicates a statistically significant difference compared with upper diaphysis ($p < 0.05$)
[b]Indicates a statistically significant difference compared with middle diaphysis ($p < 0.05$)

The locational variations in compositional parameters and densities for different locations of bone diaphysis are shown in Figs. 4 and 5 respectively.

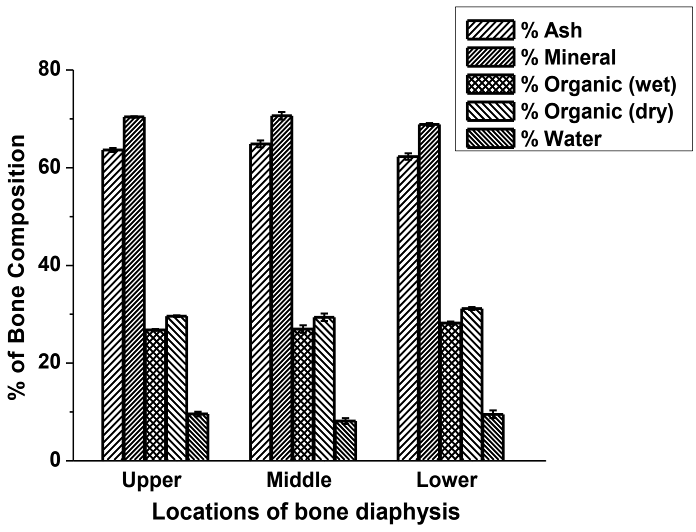


Fig.4 Variation in bone compositional parameters along the bone diaphysis

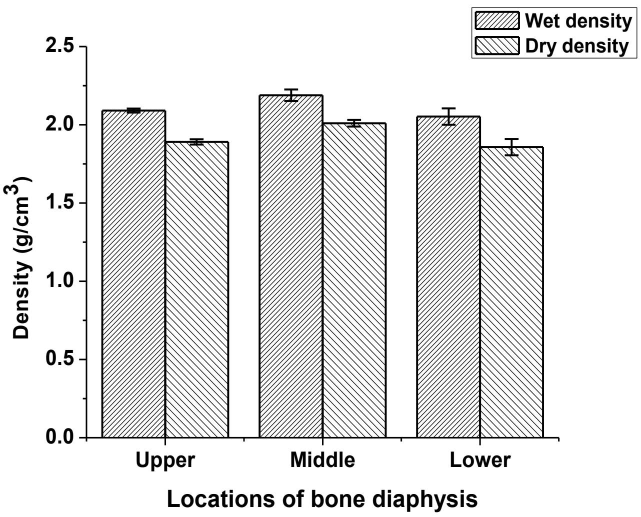


Fig.5 Variation in bone density along the bone diaphysis

The values of elastic modulus and yield strength were found to be significantly greater ($p < 0.0001$ for E and $p < 0.001$ for σ_{ys}) for the middle location as compared to the upper location of bone diaphysis. The latter values for upper location were observed to be significantly greater ($p < 0.01$) as compared to the lower location of bone diaphysis. No significant differences were found in plastic as well as tangent moduli values of upper and middle locations. However, the values of plastic and tangent moduli were noticed to be significantly less ($p < 0.0001$) for the lower location as compared to the other locations of bone diaphysis. The percentage of modulus degradation was found to be significantly greater ($p < 0.05$) for middle location as compared to the lower location of diaphysis, whereas for lower location it was found to be significantly greater ($p < 0.05$) as compared to that of the upper location of diaphysis.

The ANOVA test results reported in Table 2 shows that one or more mean values of the reported parameters for different diaphysis locations are significantly different. As per the paired t-test analysis wet density, dry density and % Ash of the middle diaphysis were significantly greater ($p < 0.01$, $p < 0.0001$ and $p < 0.05$ respectively) than those of the upper diaphysis. No significant difference in wet and dry density was found between the upper and lower diaphysis locations. % Ash of upper location, however, was significantly greater ($p < 0.05$) than that of the lower location. % Min, % Org_w and % Org_d were not found to be significantly different for middle and lower locations. % Min of upper location was significantly greater ($p < 0.001$) than that of lower location, whereas % Org_w and % Org_d of lower location were significantly greater ($p < 0.0001$ and $p < 0.001$ respectively) than those of upper location. % H_2O of upper location was noticed to be significantly higher than that of middle location, whereas no significant difference in % H_2O was found between upper and lower locations.

The compositional parameters and apparent density of bovine tibia along its diaphysis were also compared with the help of corresponding coefficients of variation (standard deviation/mean) as shown in Fig. 6. The maximum (10.2%) and minimum (1.3%) degrees of variation were observed in the values of % H_2O and % Min respectively. The coefficient of variation of wet density (3.2%), % Org_w (2.9%) and % Org_d (3.1) were found to be almost in the same range as compared to slightly higher and lower coefficients of variation of dry density (3.8) and % Ash (1.9) respectively.

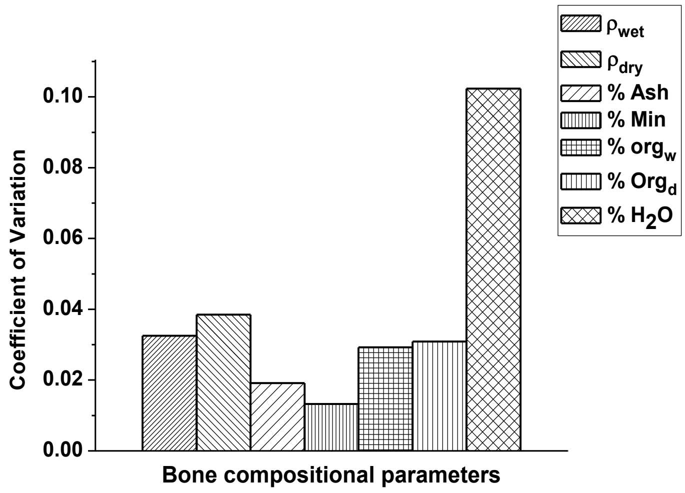


Fig.6 Comparison of the coefficient of variation of the various compositional parameters and densities along the bone diaphysis

Correlations between compositional parameters and different mechanical properties are presented in Table 3. Significant positive correlations between elastic, plastic and tangent moduli were found for bovine tibia bone (Table 3, Fig. 7). Both elastic and plastic moduli increased with increasing density (wet and dry), % Ash and % Min (Table 3, Figs. 8 to 10), whereas decreased with increasing % Org_w and % H_2O (Table 3, Figs. 11 and 12). The correlation between plastic modulus and % H_2O, however, was nonsignificant.

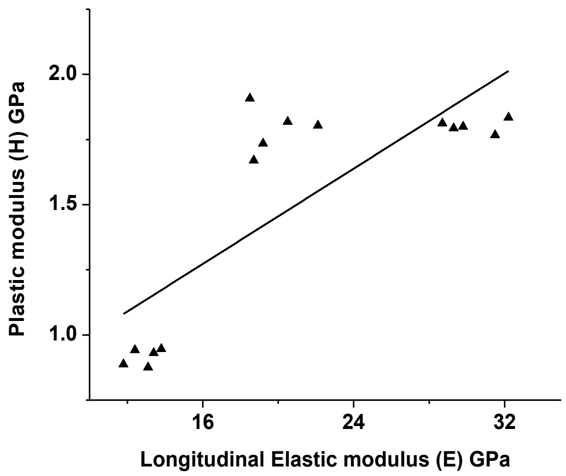


Fig.7 Variation in plastic modulus (*H*) with elastic modulus (*E*) for bovine tibia. The data indicates that *H* increases with increasing *E* ($r^2 = 0.60$, $p < 0.0004$). $H = 0.046\ E + 0.541$.

Wet and dry densities were found to be highly correlated to each other. They were also significantly correlated with % Ash and % Min. However, no significant correlation was found between densities and % Org_w. Both densities decreased with increasing % H_2O. However, the correlation between wet density and % H_2O was nonsignificant. % Ash was observed to be negatively correlated with %Org_w and % H_2O, whereas the correlation between % Min and H_2O was nonsignificant.

Table 3 Correlations (*r*) among compositional parameters and mechanical properties

	E	σ_{ys}	H	E_t	ρ_w	ρ_d	% Ash	% Min	%Org_w	% H_2O
E	1	0.93[a]	0.79[a]	0.83[a]	0.82[a]	0.86[a]	0.83[a]	0.73[a]	-0.56[a]	-0.61[a]
σ_{ys}	0.93[a]	1	0.85[a]	0.87[a]	0.79[a]	0.82[a]	0.84[a]	0.76[a]	-0.6[a]	-0.59[a]
H	0.79[a]	0.85[a]	1	0.99[a]	0.59[a]	0.58[a]	0.75[a]	0.85[a]	-0.78[a]	-0.31
E_t	0.83[a]	0.87[a]	0.99[a]	1	0.62[a]	0.61[a]	0.77[a]	0.85[a]	-0.77[a]	-0.34
ρ_w	0.82[a]	0.79[a]	0.59[a]	0.62[a]	1	0.97[a]	0.64[a]	0.54[a]	-0.4	-0.49
ρ_d	0.86[a]	0.82[a]	0.58[a]	0.61[a]	0.97[a]	1	0.74[a]	0.54[a]	-0.34	-0.68[a]
% Ash	0.83[a]	0.84[a]	0.75[a]	0.77[a]	0.64[a]	0.74[a]	1	0.86[a]	-0.65[a]	-0.75[a]
% Min	0.73[a]	0.76[a]	0.85[a]	0.85[a]	0.54[a]	0.54[a]	0.86[a]	1	-0.94[a]	-0.32
% Org_w	-0.56[a]	-0.6[a]	-0.78[a]	-0.77[a]	-0.4	-0.34	-0.65[a]	-0.94[a]	1	-0.01
% H_2O	-0.61[a]	-0.59[a]	-0.31	-0.34	-0.49	-0.68[a]	-0.75[a]	-0.32	-0.01	1

[a]Statistical significance $p < 0.05$

Multiple regression models were formed using more than one compositional parameter for estimating elastic and plastic moduli. The ones with largest coefficient of multiple determination (R^2) and adjusted R^2 values are given in Tables 4 and 5 for elastic and plastic moduli respectively. The model with all five parameters considered in this study (dry density, % Ash, % Min, % Org_W, and % H_2O) has $R^2 = 0.84$, adjusted $R^2 = 0.75$ ($p < 0.0021$) for elastic modulus and $R^2 = 0.83$, adjusted $R^2 = 0.74$ ($p < 0.0027$) for plastic modulus.

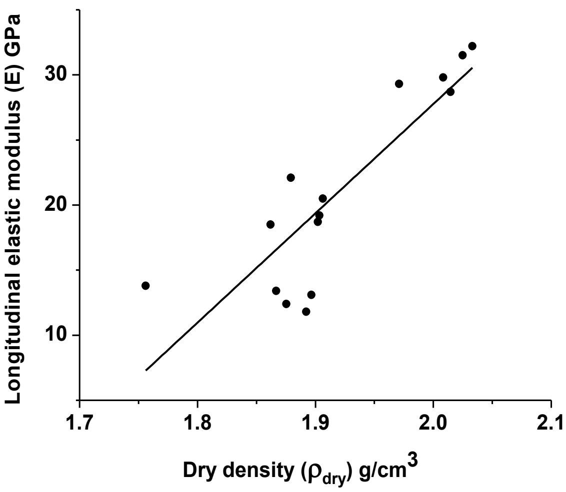


Fig.8 Variation in elastic modulus (E) with dry density (ρ_{dry}) for bovine tibia. The data indicates that E increases with increasing ρ_{dry} ($r^2 = 0.73$, $p < 0.00005$).
$E = 83.99\ \rho_{dry} - 140.21$.

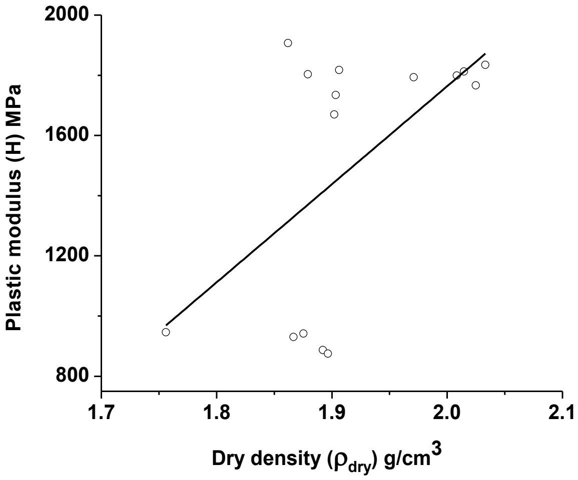


Fig.9 Variation in plastic modulus (H) with dry density (ρ_{dry}) for bovine tibia. The data indicates that H increases with increasing ρ_{dry} ($r^2 = 0.33$, $p < 0.0240$). $H = 3258.64\ \rho_{dry} - 4753.56$.

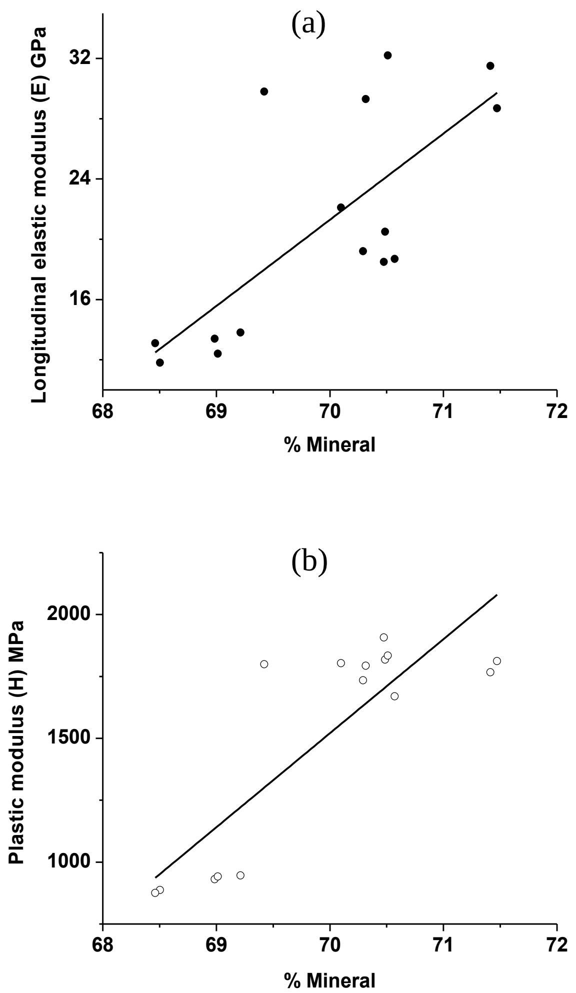
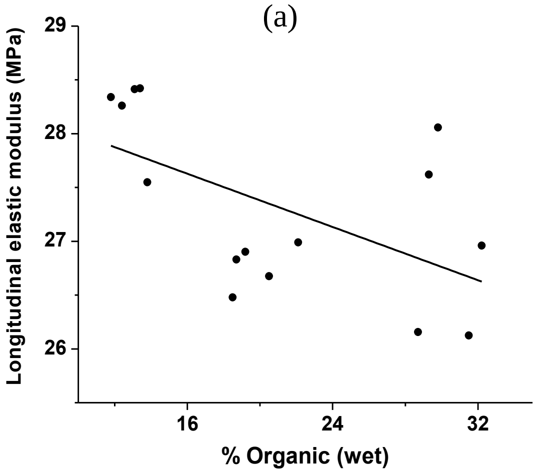


Fig.10 Variation in (a) elastic and (b) plastic moduli with % Mineral for bovine tibia. The data indicates that both E ($r^2 = 0.54$, $p < 0.0019$) and H ($r^2 = 0.72$, $p < 0.0001$) increases with increasing % Mineral. E = 5.72 % Min – 379.45. H = 379.87 % Min – 25070.26.



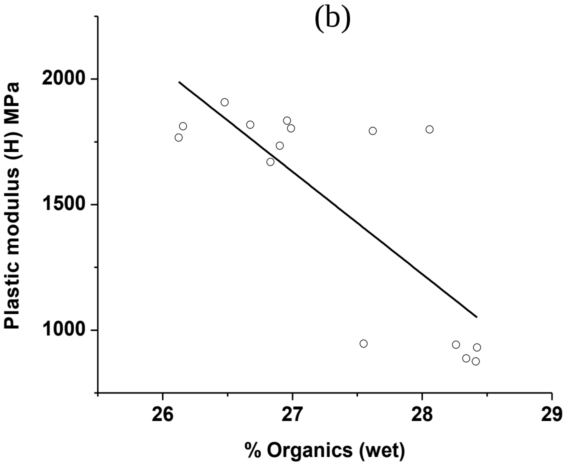


Fig.11 Variation in (a) elastic and (b) plastic moduli with % Organic (wet) for bovine tibia. The data indicates that both E ($r^2 = 0.31$, $p < 0.0294$) and H ($r^2 = 0.61$, $p < 0.0006$) decreases with increasing % Org_w.
E = - 0.062 % Org_w + 28.62. H = - 408.11 % Org_w + 12650.11.

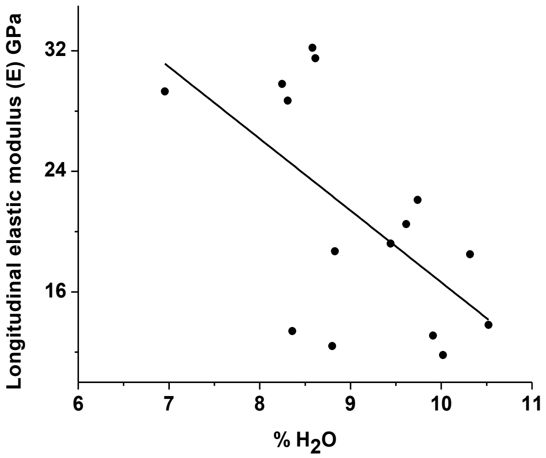


Fig.12 Variation in elastic modulus with % H_2O for bovine tibia. The data indicates that E decreases with increasing % H_2O ($r^2 = 0.61$, $p < 0.0155$). E = - 4.77 % H_2O + 64.29.

Table 4 Multivariable models[a] for elastic modulus of cortical bone.

Model	Parametrs	R^2	Adj R^2	*p*- Value
E1	A, B	0.82	0.79	3.13×10^{-5}
E2	A, C	0.84	0.81	2.04×10^{-5}
E3	A, B, C	0.84	0.79	0.0001
E4	A, B, D	0.84	0.79	0.0001
E5	A, B, E	0.84	0.79	0.0001
E6	A, C, E	0.84	0.79	0.0001
E7	A, D, E	0.84	0.79	0.0001
E8	A, B, C, D, E	0.84	0.75	0.0021

Table 5 Multivariable models[a] for plastic modulus of cortical bone.

Model	Parametrs	R^2	Adj R^2	*p*- Value
H1	B, C, D	0.78	0.72	0.0007
H2	C, D, E	0.78	0.72	0.0007
H3	B, C, E	0.78	0.72	0.0007
H4	A, B, C, D	0.82	0.75	0.0009
H5	A, B, C, E	0.82	0.75	0.0009
H6	A, C, D, E	0.82	0.75	0.0009
H7	A, B, C, D, E	0.83	0.74	0.0027

[a]Symbols A, B, C, D and E in Table 4 and 5 represents respectively the dry density, % Ash, % Min, % Org_w and % H_2O of cortical bone.

DISCUSSION

The compositional parameters used in this study were apparent quantities and evaluated by assuming bone material to be homogeneous mixture of minerals, organics and water components at different locations of bone diaphysis. Therefore the limitation of this study was that the structural organization of different components was not considered. Further the values of elastic and plastic moduli were considered to be homogeneous for each one third diaphysis location and around the circumferential direction.

In the present investigation a correlation between compositional and stiffness parameters was established for different locations of a bone diaphysis, whereas, most of the earlier reports were based on the correlation of these parameters obtained from different groups of bone. Therefore it is difficult to make a direct comparison between other studies and the current study.

The percentage of water was observed to be the main cause of compositional heterogeneity (Fig. 6) along the bone diaphysis. Note that % H_2O represents the amount of water in all cavities and may be considered as an indicator of porosity and mineralization. A noticeable variation in density was also observed along the diaphysis and since bone is composed of organic and inorganic constituents along with water fractions, variation in density should be associated with change in some or all of these components (Biltz, 1969).

Plastic modulus values determined in this study may be used to analyze the hardening rate at different locations of bone diaphysis, whereas, the modulus degradation during plastic deformation may be analyzed with the help of tangent modulus. A positive correlation between elastic and plastic moduli (Fig. 7) shows that the elastic modulus and hardening rate are also somehow related to each other for bone material. The higher hardening rate at middle diaphysis indicates higher amount of energy consumption at this location during plastic deformation that occurs from multiple, concurrent deformation mechanisms (breaking of H bonds, intermolecular sliding etc.) at different hierarchical levels of bone (Ritchie, 2009). The amount of modulus degradation was also noticed to be higher at middle location, indicating higher amount of energy dissipation at this location during yielding.

A positive correlation obtained in this study between elastic modulus, apparent density, % Ash, and % Minerals supports the positive relationship between stiffness, apparent density and mineralization observed in earlier reports (Carter, 1977, 1978; Martin, 1989, 1993; Nazarian, 2011). The study shows that plastic modulus also has a positive correlation with the latter compositional parameters similar to elastic modulus.

The elastic and plastic moduli were found to have a negative relationship with percentage of organic (wet). The effect of percentage of organic (wet) as compared to percentage of mineral was observed to be very less on elastic modulus and comparatively higher on plastic modulus (Figs. 10 and 11). This shows that as compared to minerals, percentage of organics along bone diaphysis have less effect on stiffness and more effect on post-yield parameters. This observation is consistent with the findings of earlier reports (Burr, 2002; Wang, 2001)

A significant correlation was also found between percentage of water and elastic modulus along bone diaphysis, whereas for plastic modulus this correlation was not significant. This study shows a negative relationship between elastic modulus and percentage of water. Although direct comparison of earlier finding and present work is not possible, the results of previous work (Dong; 2004; Nyman, 2006) also indicate a similar relationship of elastic modulus to water content and porosity.

The multiple regression analysis carried out with five compositional parameters (dry density, % Ash, % Min, % Org_w, and % water) achieved statistically significant multiple determination coefficients for the determination of both elastic and plastic moduli. For the estimation of elastic modulus a multivariable model (E2, Table 4) with two compositional parameters (dry density and % Mineral) was found to be the best model. However, for the case of plastic modus, a multivariable model (H7, Table 5) with all five compositional parameters was found to the best fit model.

CONCLUSION

The elastic and plastic moduli of bovine tibia cortical bone were evaluated at three different locations of bone diaphysis with the help of uniaxial tensile test. The compositional parameters of cortical bone are also determined for the corresponding locations of the diaphysis to observe there correlation with elastic and plastic moduli. The percentage of water was found to be having maximum variability along the bone diaphysis and therefore was considered to be the main cause of heterogeneity in the compositional parameters. Plastic modulus was observed to be having positive correlation with elastic modulus. The percentage of modulus degradation and hardening rate were higher at the middle location of bone diaphysis. Different compositional parameters (apparent density, % Ash and % Mineral) were found to be positively correlated with elastic and plastic moduli, whereas, percentage of Organic (wet) was negatively correlated to the latter mechanical properties. Multiple regression analysis was also carried out to show the effect of more than one compositional parameters on elastic and plastic moduli.

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